

# Experimental and modeling assessment of the main bio-physical-chemical mechanisms and kinetics in high-solids anaerobic digestion of organic waste

Vicente Pastor Poquet

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Dottore di Ricerca in Tecnologie Ambientali



Degree of Doctor in Environmental Technology



Thesis for the degree of Doctor of Philosophy in Environmental Technology

## Tesi di Dottorato – Thèse – PhD thesis – Väitöskirja Vicente Pastor Poquet

Experimental and Modeling Assessment of the Main Bio-physical-chemical Mechanisms and Kinetics in High-solids Anaerobic Digestion of Organic Waste

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Defended in front of the evaluation committee

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### Abstract

The organic fraction of municipal solid waste (OFMSW) includes readily biodegradable wastes such as food waste, and slowly biodegradable wastes such as lignocellulosic materials. Anaerobic digestion (AD) is a mature treatment biotechnology in which OFMSW is decomposed to a mixture of methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>), known as biogas. Due to the elevated CH<sub>4</sub> content (50 - 70 %), biogas can be used as a source of renewable energy. Moreover, AD yields also a partially stabilized digestate, allowing the recycle of nutrients back to agriculture.

High-solids anaerobic digestion (HS-AD) is a well-suited strategy to enhance the overall AD efficiency for OFMSW treatment. HS-AD is operated at a total solid (TS) content  $\geq 10$  %, permitting to reduce the reactor size and overall operational costs. Nonetheless, the TS increase can result into biochemical instability, and even reactor failure by acidification. Both the high organic load and the buildup of inhibitors can be responsible for the HS-AD instability. The most notable inhibitor in HS-AD of OFMSW is the free ammonia nitrogen (NH<sub>3</sub>). Therefore, a balance is often required between enhancing the HS-AD economy and the 'undesired' instability for OFMSW treatment.

This PhD research investigated the main bio-physical-chemical mechanisms and kinetics in HS-AD of OFMSW, with the aim to optimize the industrial application and maximize the kinetic rates. Laboratory-scale batch and semi-continuous experiments highlighted the main strengths and weaknesses of HS-AD. Simultaneously, the development of a HS-AD model permitted to condense the experimental knowledge about the main bio-physical-chemical effects occurring when increasing the TS content in HS-AD.

HS-AD batch experiments required a tradeoff between the initial TS, the inoculum-tosubstrate ratio (ISR), the alkalinity and the nitrogen content, to assess the effects of increasing the initial TS content upon the methane yield, TS removal and chemical oxygen demand conversion. Particularly, a low ISR led to acidification, whereas the NH<sub>3</sub> buildup led to volatile fatty acid (VFA) accumulation, reducing the methane yield, whether or not codigestion of OFMSW with beech sawdust was used.

In semi-continuous experiments, HS-AD of OFMSW required a reduced effluent compared to the influent to counterbalance the organic mass removal associated to the biogas production. Nonetheless, mono-digestion of readily-biodegradable OFMSW could not sustain a TS  $\geq$  10 % without exacerbating the risk of substrate overload. Overloading was associated to the high biodegradability of OFMSW and the NH<sub>3</sub> buildup. Thus, adding sawdust to OFMSW permitted to operate the reactors up to 30 % TS, due to the lower biodegradability and nitrogen content of lignocellulosic substrates.

As the main novelty of this PhD research, a HS-AD model based on the Anaerobic Digestion Model No.1 (ADM1) was developed. This model simulates the reactor mass and TS in HS-AD, in contrast of models focusing on 'wet' AD simulations (TS < 10 %). Moreover, the HS-AD model considers also the TS concentration effect on soluble species. A 'non-ideal' bio-physical-chemical module, modifying predominantly the acid-base equilibrium constants, was subsequently coupled to the HS-AD model. Noteworthy, HS-AD is often characterized by a high ionic strength ( $I \ge 0.2$  M), affecting the pH, NH<sub>3</sub> concentration and CO<sub>2</sub> liquid-gas transfer, as the most important triggers for HS-AD inhibition.

The HS-AD model calibration required multiple experimental datasets to circumvent parameter non-identifiability. The model calibration showed that HS-AD of OFMSW might be operated at I up to 0.9 M and NH<sub>3</sub> concentrations up to 2.3 g N/L, particularly at higher TS contents (25 - 30 %). Moreover, the model calibration suggested that a reversible non-competitive NH<sub>3</sub> inhibition should be further tested. Further HS-AD model developments (e.g. precipitation) were also recommended. All these results might aid in the optimization of HS-AD for organic waste treatment, renewable energy and nutrient recovery.

## Sommario

La frazione organica dei rifiuti solidi urbani (FORSU) comprende rifiuti facilmente biodegradabili, come rifiuti alimentari, e rifiuti lentamente biodegradabili, come i materiali lignocellulosici. La digestione anaerobica (DA) è una biotecnologia di trattamento in cui la FORSU è decomposta in una miscela di metano (CH<sub>4</sub>) e anidride carbonica (CO<sub>2</sub>), nota come biogas. Grazie all'elevato contenuto di CH<sub>4</sub> (50 - 70 %), il biogas può essere utilizzato come fonte di energia rinnovabile. Inoltre, la DA comporta la produzione di un digestato parzialmente stabilizzato che permette, nel caso venisse utilizzato in agricoltura, un riutilizzo dei nutrienti in esso contenuti.

L'utilizzo di un elevato tenore di solidi consente di migliorare l'efficienza globale della DA per il trattamento della FORSU. A partire da un contenuto di solidi totale  $(TS) \ge 10$  %, si ha una riduzione delle dimensioni del digestore e dei costi operativi complessivi. Ciononostante, l'aumento di TS può comportare instabilità biochimica e persino il fallimento del digestore per acidificazione. Sia l'alto carico organico che l'accumulo di sostanze inibenti possono essere responsabili dell'instabilità della DA ad alto contenuto di solidi. La sostanza inibente più importante è sicuramente l'azoto ammoniacale nella sua forma indissociata (NH<sub>3</sub>). Pertanto, è spesso richiesto un equilibrio tra il miglioramento dell'economia della DA e l'instabilità "indesiderata" per il trattamento della FORSU.

La ricerca condotto nell'ambito del presente lavoro di dottorato si è incentrata sullo studio dei principali meccanismi bio-fisico-chimici e cinetici che avvengono durante la DA della FORSU ad alto contenuto di solidi, con l'obiettivo di ottimizzare l'applicazione industriale e massimizzare le cinetiche biologiche. Esperimenti batch e semi-continui a scala di laboratorio hanno evidenziato i principali punti di forza e di debolezza del processo. Allo stesso tempo, lo sviluppo di un modello matematico ha permesso di condensare le conoscenze sperimentali sugli effetti bio-fisico-chimici che si verificano quando si aumenta il contenuto di TS nella DA.

Gli esperimenti batch hanno richiesto un compromesso tra il TS iniziale, il rapporto inoculo-substrato, l'alcalinità e il contenuto di azoto, per valutare gli effetti dell'aumento del contenuto iniziale di TS sulla resa di produzione di metano, sulla rimozione di TS e la conversione della sostanza organica espressa come domanda chimica di ossigeno (COD). In particolare, un rapporto inoculo-substrato basso ha comportato l'acidificazione della miscela, mentre l'aumentare della concentrazione di NH<sub>3</sub> ha portato all'accumulo di acidi grassi volatili (VFA), riducendo la resa di metano, indipendentemente dal fatto che sia stata utilizzata la co-digestione di FORSU con segatura di faggio.

I reattori eserciti in modalità semi-continua hanno richiesto la riduzione della portata effluente rispetto all'influente per controbilanciare la rimozione di massa organica associata alla produzione di biogas. Si è osservato che la digestione di sola FORSU (più rapidamente biodegradabile) è avvenuta efficientemente solo per valori di TS  $\leq 10$  % perché, data l'elevata biodegradabilità, un sovraccarico di substrato ha comportato l'accumulo eccessivo di NH<sub>3</sub>. Pertanto, l'aggiunta di segatura alla FORSU ha permesso di operare i reattori fino al 30 % di TS, a causa della minore biodegradabilità e minore contenuto di azoto dei substrati lignocellulosici.

Come principale novità di questa ricerca di dottorato, è stato sviluppato un modello per la DA ad alto contenuto di solidi, basato sul classico ADM1. Questo modello ha permesso di simulare la massa del reattore e l'andamento dei TS durante la DA, al contrario dei modelli che si concentrano su simulazioni di DA "ad umido" (TS < 10 %). Inoltre, il modello considera anche l'effetto delle alte concentrazioni di TS sulle specie solubili. Un modulo biofisico-chimico 'non ideale', che modifica prevalentemente le costanti di equilibrio acido-base, è stato successivamente accoppiato al modello. Infatti, la DA ad alto contenuto di solidi è spesso caratterizzata da un'elevata forza ionica ( $I \ge 0.2$  M) che influenza il pH, la concentrazione di NH<sub>3</sub> e il trasferimento liquido-gas di CO<sub>2</sub>, che sono i principali fattori scatenanti l'inibizione del processo.

La calibrazione del modello ha richiesto vari set di dati sperimentali per aggirare la non-identificazione dei parametri. La calibrazione del modello ha dimostrato che la DA ad alto contenuto di solidi di FORSU può essere utilizzata fino a valori di *I* pari a 0.9 M e concentrazioni di NH<sub>3</sub> pari a 2.3 g N/L, con particolare riferimento ai contenuti di TS più elevati (25 - 30 %). Inoltre, la calibrazione del modello ha suggerito di testare ulteriormente l'inibizione reversibile non-competitiva di NH<sub>3</sub>. Infine, sono stati consigliati ulteriori sviluppi per il modello della DA ad alto contenuto di solidi (ad esempio, includendo il processo di precipitazione chimica). Tutti questi risultati permetteranno di aiutare i ricercatori e i gestori di impianti reali verso l'ottimizzazione della DA ad alto contenuto di solidi per il trattamento dei rifiuti organici, il recupero d'energia rinnovabile e dei nutrienti.

### Résumé

La fraction fermentescible des ordures ménagères (FFOM) comprend des déchets facilement biodégradables (alimentaires), et des lentement biodégradables (lignocellulosiques). La digestion anaérobie (DA) est une biotechnologie dans laquelle la FFOM est décomposé dans biogaz (CH<sub>4</sub> + CO<sub>2</sub>). En raison de la teneur élevée en CH<sub>4</sub> (50 - 70 %), le biogaz pouvant être utilisée comme source d'énergie. En outre, DA produit un digestat partiellement stabilisé, riche d'éléments nutritifs.

La DA à haute teneur en solides est une stratégie pour l'amélioration de l'efficacité. Elle correspond à une opération avec une teneur en matières sèches  $(MS) \ge 10$  %, qui permet de réduire la taille du réacteur et les coûts de fonctionnement. Toutefois, l'augmentation de la MS peut entraîner une instabilité biochimique, et même une défaillance par acidification, à cause de la forte charge organique et l'accumulation d'inhibiteurs. L'inhibiteur le plus notable est l'azote ammoniacal libre (NH<sub>3</sub>). Par conséquent, un équilibre entre l'amélioration de l'économie et l'instabilité est requis pour le traitement de la FFOM par DA à haute teneur en solides.

Cette thèse de doctorat porte sur les principaux mécanismes e cinétiques bio-physiqueschimiques mis en jeu lors de la DA à haute teneur en solides, dans le but d'optimiser son application. Des expériences de laboratoire ont mis en œuvre pour élucider les principales forces et faiblesses de ce procédé. Simultanément, le développement d'un modèle spécifique à la DA à haute teneur en solides a permis de condenser les connaissances expérimentales sur les effets qui se produisent lors de l'augmentation de la teneur de la MS.

Les expériences en réacteur batch ont nécessité un compromis entre la teneur initiale en MS, le rapport entre l'inoculum et le substrat (X/S), l'alcalinité et la teneur en azote, afin d'évaluer les effets de l'augmentation de la teneur initiale en MS sur le rendement en CH<sub>4</sub>, l'élimination de la MS et la conversion de la demande chimique en oxygène. En particulier, des ratios X/S bas ont conduit à l'acidification, tandis que l'accumulation de NH<sub>3</sub> a conduit à une accumulation d'acides gras volatils (AGV).

Dans des expériences en semi-continue, la DA à haute teneur en solides nécessitait de diminuer le débit de l'effluent pour contrer l'élimination de la masse. Cependant, la monodigestion de la FFOM facilement biodégradable ne peut pas supporter  $MS \ge 10$  % sans augmenter le risque de surcharge organique. La surcharge était associée à la forte biodégradabilité et à l'accumulation de NH<sub>3</sub>. Par conséquent, l'ajout de sciure de bois à FFOM a permis à des réacteurs semi-continus de fonctionner jusqu'à 30 % de MS, en raison de la biodégradabilité et de la teneur d'azote plus faibles ce substrat. La principale nouveauté de cette thèse est le développement d'un modèle pour la DA à haute teneur en solides. Ce modèle permet de simuler la dynamique masse et de MS dans des digesteurs, contrairement aux modèles sur des simulations de MS < 10 %. Ce modèle prend également en compte l'effet de la concentration en MS sur les espèces solubles. Un module bio-physico-chimique « non idéal », modifiant les constantes d'équilibre acide-base, a été couplé ensuite au modèle. Il est à noter que la DA à haute teneur en solides est souvent caractérisée par une force ionique élevée ( $I \ge 0.2$  M), affectant le pH, la concentration en NH<sub>3</sub> et le transfert de CO<sub>2</sub> liquide-gaz.

L'étalonnage du modèle a montré que la DA à haute teneur en solides requis plusieurs jeux de données expérimentaux pour contourner la « non-identifiabilité » des paramètres. La DA à haute teneur en solides pouvait fonctionner à une *I* allant jusqu'à 0.9 M et NH<sub>3</sub> allant jusqu'à 2.3 g N/L, à des teneurs en MS élevées (25 - 30 %). En outre, l'étalonnage a suggéré que l'utilisation d'une inhibition non-compétitive de NH<sub>3</sub> devrait être testée plus avant. Il a également été recommandé de mettre au point d'autres développements du modèle. Ces résultats pourraient aider à l'optimisation de la DA à haute teneur en solides.

## Tiivistelmä

To be added.

viii

# Samenvatting

To be added.

х

### Preface

This thesis condenses the effort of three years of research in anaerobic digestion of solid waste. The research was carried out between l'Università di Cassino e del Lazio Meridionale (UNICLAM, Italy), l'Institut National de la Recherche Agronomique (INRA, France) and the Tampere University of Technology (TUT, Finland), as part of the Marie Skłodowska-Curie Joint PhD Program in Advanced Biological Waste-to-Energy Technologies (ABWET). During these years, multitude of ups and downs resulted in this document, the publication of different articles in prestigious scientific journals, and some presentations at important international conferences. Needless to say, this research is the result of the close collaboration with all these superb people I have been fortunate to know. both within and outside the scientific world. Without all of them, this research would have been impossible. Among them, I would like to highlight my parents, my grandparents and my family in general, as a source of inspiration and tireless effort; Angela Andreella who closely followed each of my steps during these three years, in the good and not so good times; my PhD supervisors Giovanni Esposito, Stefano Papirio, Eric Trably, Jukka Rintala and Renaud Escudié, and especially also Jean-Philippe Stever, for their invaluable help and sharp advice; all my ABWET, UNICLAM, TUT and INRA colleagues, with whom I had the opportunity to work; the international PhD reviewers Katerina Stamatelatou, Baris Calli and Paolo Calabro; and all those anonymous reviewers who significantly contributed to improve the quality of the scientific manuscripts. Undoubtedly also to acknowledge Xavier Flotats and Belén Hernández from l'Universitat Politècnica de Catalunya (UPC) and l'Institut de Recerca i Technologia Agroalimentària (IRTA), for introducing me to the world of anaerobic digestion, and helping me to get this scholarship; José M<sup>a</sup> Baldasano for fueling my interest in mathematical modeling; and all those magnificent people who dedicated me their time, conversations, smiles and incredible memories all throughout these years.

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Vicente Pastor Poquet Naples, 5<sup>th</sup> December 2018 xii

### **Prólogo**

Esta tesis condensa el esfuerzo de tres años de investigación en el campo de la digestión anaerobia de residuos sólidos. La investigación se ha llevado a cabo entre l'Università di Cassino e del Lazio Meridionale (UNICLAM, Italia), l'Institut National de la Recherche Agronomique (INRA, Francia) y the Tampere University of Technology (TUT, Finlandia), como parte del Marie Skłodowska-Curie Joint PhD Program in Advanced Biological Wasteto-Energy Technologies (ABWET). Durante estos años, multitud de altibajos han dado como resultado el presente documento, así como la publicación de varios artículos en prestigiosas revistas científicas, y también algunas presentaciones en importantes conferencias internacionales. Ni que decir tiene que los resultados de esta investigación son fruto de la estrecha colaboración junto a todas aquellas grandes personas que he tenido la suerte de conocer, tanto dentro como fuera del ámbito científico. Sin ellos, esta investigación no hubiese sido posible. Entre todas esas personas me gustaría destacar a mis padres, a mis abuelos y a mi familia en general, todos ellos fuente de inspiración e incansable trabajo; a Angela Andreella que ha seguido de cerca cada uno de mis pasos durante estos tres años, en los buenos y no tan buenos momentos; a mis supervisores de doctorado Giovanni Esposito, Stefano Papirio, Eric Trably, Jukka Rintala y Renaud Escudié, y en especial a Jean-Philippe Steyer, por su inestimable ayuda y consejos; a todos mis compañeros ABWET, UNICLAM, TUT e INRA con los que he tenido la oportunidad de trabajar; a los revisores internacionales de esta tesis Katerina Stamatelatou, Baris Calli y Paolo Calabrò; y a todos aquellos revisores anónimos que han contribuido a mejorar notablemente la calidad de los manuscritos. Sin duda también a Xavier Flotats y a Belén Hernández de l'Universitat Politècnica de Catalunya (UPC) y l'Institut de Recerca i Technologia Agroalimentària (IRTA) por introducirme en el mundo de la digestión anaerobia, y ayudarme a conseguir la beca para este doctorado; a José M<sup>a</sup> Baldasano por alimentar mi creciente interés hacia la modelización matemática; y a todas aquellas magníficas personas que me han dedicado su tiempo, sonrisas e increíbles recuerdos a lo largo de estos años.

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> Vicente Pastor Poquet Nápoles, 5 de Diciembre de 2018

xiv

# **Table of Contents**

| Abstract              | i      |
|-----------------------|--------|
| Sommario              | iii    |
| Résumé                | v      |
| Tiivistelmä           | vii    |
| Samenvatting          | ix     |
| Preface               | xi     |
| Prólogo               | xiii   |
| Table of Contents     | xv     |
| List of Abbreviations | xxi    |
| List of Symbols       | xxiii  |
| List of Tables        | xxix   |
| List of Figures       | xxxi   |
| List of Publications  | xxxv   |
| Author's Contribution | xxxvii |

## Chapter 1

| General | Introduction and Thesis Outline | 1 |
|---------|---------------------------------|---|
| 1.1.    | General Introduction            | 3 |
| 1.2.    | Objectives                      | 4 |
| 1.3.    | Thesis Structure                | 6 |

| Theoretical Background11 |                        |    |
|--------------------------|------------------------|----|
| 2.1.                     | Anaerobic Digestion    | 13 |
| 2.2.                     | Main Biochemical Steps | 13 |
| 2.3.                     | Dark Fermentation      | 15 |

| 2.4 | •     | Mair  | n Substrates                                         | 16 |
|-----|-------|-------|------------------------------------------------------|----|
|     | 2.4.1 |       | Organic Fraction of Municipal Solid Waste            | 16 |
| -   | 2.4.2 |       | Co-digestion                                         | 17 |
| 2.5 | •     | Nutr  | ient Content                                         | 18 |
|     | 2.5.1 |       | Macro-Nutrients                                      | 18 |
|     | 2.5.2 |       | Micro-Nutrients                                      | 19 |
| -   | 2.5.3 |       | Trace Elements                                       | 19 |
| 2.6 | •     | Inhit | pitory Substances                                    | 19 |
| 2.7 | •     | Mair  | n Operational Parameters                             | 21 |
|     | 2.7.1 |       | Anaerobic Biodegradability                           | 22 |
| -   | 2.7.2 |       | Retention Time and Organic Load                      | 22 |
|     | 2.7.3 |       | Temperature                                          | 23 |
|     | 2.7.4 |       | pH, Buffering Capacity and Alkalinity                | 24 |
| ,   | 2.7.5 |       | Total Solid Content                                  | 25 |
| 2.8 | •     | High  | -Solids Anaerobic Digestion                          | 26 |
| 2.9 | •     | The   | Role of Water Deficiency and Substrate Complexity    | 27 |
| 2.1 | 0.    | The   | Importance of the Substrate Composition in HS-AD     | 29 |
| 2.1 | 1.    | Rheo  | blogy Complexity and Mass Transfer Effects           | 29 |
| 2.1 | 2.    | High  | n-solids Anaerobic Digestion Reactors                | 31 |
|     | 2.12. | 1.    | Batch-Type (Leach-Bed) Configuration                 | 32 |
| 4   | 2.12. | 2.    | Continuous-Type (Plug-Flow) Configuration            | 33 |
| ,   | 2.12. | 3.    | Examples of Operative Parameters at Industrial Scale | 34 |
| 2.1 | 3.    | Need  | l for Optimization                                   | 35 |
| 2.1 | 4.    | HS-A  | AD Models                                            |    |

### Chapter 3

| Assessing th<br>Ammonia In | e Tradeoff between Total Solids, Inoculum-to-Subst<br>hibition in High-Solids Anaerobic Digestion Batch E | rate Ratio an xperiments .4 | 1d<br>49 |
|----------------------------|-----------------------------------------------------------------------------------------------------------|-----------------------------|----------|
| Abstract                   |                                                                                                           |                             | 51       |
| 3.1. Intr                  | oduction                                                                                                  |                             | 52       |
| 3.2. Mat                   | terials and Methods                                                                                       |                             | 54       |
| 3.2.1.                     | Organic Substrates and Inoculum                                                                           |                             |          |
| 3.2.2.                     | Batch Experiments                                                                                         |                             |          |
| 3.2.3.                     | Biomethane potential of OFMSW and beech sawdust                                                           |                             |          |
| 3.2.4.                     | Physical-Chemical Analyses                                                                                |                             |          |
| 3.2.5.                     | Calculations                                                                                              |                             |          |
| 3.3. Res                   | ults and Discussion                                                                                       |                             | 59       |
| 3.3.1.                     | Bio-Physical-Chemical Characterization of Substrates and I                                                | noculum59                   |          |
| 3.3.2.                     | Batch Experiments                                                                                         |                             |          |
| 3.4. Cor                   | clusions                                                                                                  | ,                           | 72       |

| Abstract |                                |    |
|----------|--------------------------------|----|
| 4.1. Int | roduction                      |    |
| 4.2. Ma  | aterials and Methods           |    |
| 4.2.1.   | Substrates and Inoculum        |    |
| 4.2.2.   | Experimental Setup             |    |
| 4.2.3.   | Operation Strategy             |    |
| 4.2.4.   | Bio-Physical-Chemical Analyses |    |
| 4.2.5.   | Statistical Analyses           |    |
| 43 Re    | sults and Discussion           | 86 |

#### **Chapter 5**

| High-Solids | Anaerobic Digestion Model for Homogenized Reactors | .103  |
|-------------|----------------------------------------------------|-------|
| Abstract    |                                                    | . 105 |
| 5.1. Intr   | oduction                                           | .106  |
| 5.2. Mat    | erials and Methods                                 | . 108 |
| 5.2.1.      | High-Solids Model Implementation 108               |       |
| 5.2.2.      | Verification of the Model Implementation           |       |
| 5.2.3.      | Experimental Data and Data Recalculation           |       |
| 5.2.4.      | Model Calibration                                  |       |
| 5.3. Res    | ults and Discussion                                | .118  |
| 5.3.1.      | Model Implementation Verification                  |       |
| 5.3.2.      | Model Calibration                                  |       |
| 5.4. Cor    | clusions                                           | .127  |

| Coupling | g a Non-Ideal   | <b>Bio-Physical-Chemical</b> | Module | to    | the   | <b>High-Solids</b> |
|----------|-----------------|------------------------------|--------|-------|-------|--------------------|
| Anaerob  | ic Digestion Mo | del                          | •••••  | ••••• | ••••• | 131                |
| Abstrac  | et              |                              |        |       |       | 133                |
| 6.1.     | Introduction    |                              |        | ••••• |       | 134                |
| 6.2.     | Methodology     |                              |        | ••••• |       | 137                |

|    | 6.2.1.  | Activity Coefficients and Modified Equilibrium Constants |     |
|----|---------|----------------------------------------------------------|-----|
|    | 6.2.2.  | Model Implementation Verification                        |     |
|    | 6.2.3.  | Experimental Data and Model Calibration 144              |     |
| 6. | 3. Resu | Its and Discussion                                       | 146 |
|    | 6.3.1.  | Verification of the 'Non-Ideal' Model Implementation 146 |     |
|    | 6.3.2.  | HS-AD Calibration under 'Non-Ideal' Conditions 155       |     |
| 6. | 4. Conc | clusions                                                 | 158 |

## Chapter 7

| <b>High-Solids</b> | Anaerobic Digestion Model Calibration and Cross-Validation               | 163   |
|--------------------|--------------------------------------------------------------------------|-------|
| Abstract           |                                                                          | 165   |
| 7.1. Intr          | roduction                                                                | 166   |
| 7.2. Me            | ethodology                                                               | 169   |
| 7.2.1.             | Experimental Data 169                                                    |       |
| 7.2.2.             | HS-AD Model 170                                                          |       |
| 7.2.3.             | Model Calibration and Validation                                         |       |
| 7.3. Res           | sults and Discussion                                                     | 179   |
| 7.3.1.             | GSA – Selecting the Most Influencing Input Parameters for Calibra<br>179 | ition |
| 7.3.2.             | Parameter Optimization                                                   |       |
| 7.3.3.             | Main Effects of Increasing the TS Content in HS-AD of OFMSW 190          |       |
| 7.4. Co            | nclusions                                                                | 191   |

| General Con | nclusions and Future Perspectives       |     |
|-------------|-----------------------------------------|-----|
| 8.1. Ge     | neral Conclusions                       |     |
| 8.1.1.      | Chapter 3 – Batch Experiments           | 197 |
| 8.1.2.      | Chapter 4 – Semi-continuous Experiments | 198 |

|     | 8.1.3.  | Chapter 5 – Model Development            | 199 |  |
|-----|---------|------------------------------------------|-----|--|
|     | 8.1.4.  | Chapter 6 – 'Non-Ideal' Effects          | 200 |  |
|     | 8.1.5.  | Chapter 7 – Model Calibration/Validation | 201 |  |
| 8.: | 2. Futu | re Perspectives                          |     |  |
|     | 8.2.1.  | Experimental Data Collection             | 204 |  |
|     |         |                                          |     |  |

#### Annex

| Thesis O        | verview:              | From th       | e Experir     | nental Setu      | ıp to    | Further     | Model         |
|-----------------|-----------------------|---------------|---------------|------------------|----------|-------------|---------------|
| Requirem        | ents                  |               | •••••         |                  |          | •••••       | 211           |
| Abstract        |                       |               |               |                  |          |             |               |
| A.1. I          | ntroduction           |               |               |                  |          |             | 214           |
| A.2. N          | Methodology           | <i>.</i>      |               |                  |          |             | 216           |
| A.2.1.          | Experimen             | ntal Section. |               |                  |          |             | 16            |
| A.2.2.          | HS-AD M               | odel          |               |                  |          |             | 18            |
| A.2.3.          | Kinetic Ra            | ates          |               |                  |          |             | 18            |
| A.2.4.          | Model Ver             | rification    |               |                  |          |             | 20            |
| A.2.5.          | Model Cal             | libration     |               |                  |          |             | 20            |
| A.3. F          | Results and D         | Discussion    |               |                  |          |             |               |
| A.3.1.          | Bio-Physic            | cal-Chemica   | Characteriza  | ation of Substr  | ates and | Inoculum 22 | 23            |
| A.3.2.          | Batch Exp<br>224      | periments –   | Dealing wi    | th Acidification | on and . | Ammonia I   | nhibition     |
| A.3.3.<br>accum | Semi-conti<br>ulation | inuous Expe   | riments – C   | ounterbalancin   | g the T  | S removal a | ind VFA<br>25 |
| A.3.4.          | HS-AD M               | odel – Cond   | ensing the Le | essons Learnt    |          |             | 26            |
| A.3.5.          | Future HS-            | -AD Model     | and Data Rec  | uirements        |          |             | 33            |
| A.4. C          | Conclusions           |               |               |                  |          |             | 234           |

## List of Abbreviations

| Abbreviation     | Definition                                |  |
|------------------|-------------------------------------------|--|
| AA               | Amino Acid                                |  |
| ABC              | Approximate Bayesian Computation          |  |
| AD               | Anaerobic Digestion                       |  |
| ADM1             | Anaerobic Digestion Model No. 1           |  |
| ALK              | Alkalinity                                |  |
| ALKI             | Intermediate Alkalinity                   |  |
| ALK <sub>P</sub> | Partial Alkalinity                        |  |
| ALK <sub>T</sub> | Total Alkalinity                          |  |
| BD               | Anaerobic Biodegradability                |  |
| BMP              | Biomethane Potential                      |  |
| CFD              | Computational Fluid Dynamics              |  |
| COD              | Chemical Oxygen Demand                    |  |
| CODbd            | Biodegradable Chemical Oxygen Demand      |  |
| CSTR             | Continuously Stirred Tank Reactor         |  |
| DF               | Dark Fermentation                         |  |
| FW               | Food Waste                                |  |
| GE               | Global Effect                             |  |
| GHG              | Greenhouse Gases                          |  |
| GSA              | Global Sensitivity Analysis               |  |
| HRT              | Hydraulic Retention Time                  |  |
| HS-AD            | High-Solids Anaerobic Digestion           |  |
| HS-DF            | High-Solids Dark Fermentation             |  |
| IE               | Individual Effect                         |  |
| ISR              | Inoculum-to-Substrate Ratio               |  |
| LCA              | Life Cycle Assessment                     |  |
| LCFA             | Long Chain Fatty Acids                    |  |
| LHS              | Latin Hypercube Sampling                  |  |
| MSW              | Municipal Solid Waste                     |  |
| OFMSW            | Organic Fraction of Municipal Solid Waste |  |
| OL               | Organic Load                              |  |
| OLR              | Organic Loading Rate                      |  |
| OS               | Organic Substrate                         |  |
| PHA              | Poly-hydroxy-alkanoates                   |  |
| SS-AD            | Solid-State Anaerobic Digestion           |  |
| Т                | Temperature                               |  |
| TAN              | Total Ammonia Nitrogen                    |  |
| TE               | Trace Element                             |  |
| TKN              | Total Kjeldahl Nitrogen                   |  |
| TS               | Total Solids                              |  |
| VFA              | Volatile Fatty Acids                      |  |
| VS               | Volatile Solids                           |  |
| WW               | Wastewater                                |  |
| WWS              | Wastewater Sludge                         |  |

xxii

# List of Symbols

#### Table S.1 Biochemical Symbols

| Symbol                          | Name                                                               | Units                                          |
|---------------------------------|--------------------------------------------------------------------|------------------------------------------------|
| Ci                              | Inert Compound Carbon Content                                      | kg COD m <sup>-3</sup>                         |
| $\mathbf{f}_{\mathrm{ac,su}}$   | Acetate from Sugars                                                | kg COD kg COD <sup>-1</sup>                    |
| $\mathbf{f}_{bu,su}$            | Butyrate from Sugars                                               | kg COD kg COD <sup>-1</sup>                    |
| $f_{ch,xc}$                     | Carbohydrates from Composite                                       | kg COD kg COD <sup>-1</sup>                    |
| $f_{h2,su}$                     | Hydrogen from Sugars                                               | kg COD kg COD <sup>-1</sup>                    |
| $\mathbf{f}_{\mathrm{i,j}}$     | Chemical Oxygen Demand Fractioning of (j) into (i)                 | kg COD kg COD <sup>-1</sup>                    |
| $\mathbf{f}_{\text{pr,subs}}$   | Protein from Substrate-Inoculum Mixture                            | kg COD kg COD <sup>-1</sup>                    |
| $\mathbf{f}_{\text{pro,su}}$    | Propionate from Sugars                                             | kg COD kg COD <sup>-1</sup>                    |
| $\mathbf{f}_{si,subs}$          | Soluble Inerts from Substrate-Inoculum Mixture                     | kg COD kg COD <sup>-1</sup>                    |
| f <sub>si,xc</sub>              | Soluble Inert from Composite                                       | kg COD kg COD <sup>-1</sup>                    |
| $\mathbf{f}_{\mathrm{xi,subs}}$ | Particulate Inert from Substrate-Inoculum Mixture                  | kg COD kg COD <sup>-1</sup>                    |
| $\mathbf{f}_{xi,xc}$            | Particulate Inert from Composite                                   | kg COD kg COD <sup>-1</sup>                    |
| $I_{h2}$                        | Hydrogen Inhibition Term                                           | kg COD kg COD-1                                |
| Iin                             | Nitrogen Secondary Substrate Term                                  | kmol N kmol N <sup>-1</sup>                    |
| Inh3                            | Free Ammonia Inhibition Term                                       | kmol N kmol N <sup>-1</sup>                    |
| $I_{pH}$                        | pH Inhibition Term                                                 |                                                |
| $\mathbf{k}_{d}$                | Decay Constant                                                     | d <sup>-1</sup>                                |
| k <sub>dis</sub>                | Composite Disintegration Constant                                  | d <sup>-1</sup>                                |
| $\mathbf{k}_{\mathbf{h}}$       | Hydrolysis Constant                                                | d <sup>-1</sup>                                |
| $k_{h,ch}$                      | Hydrolysis Constant for Carbohydrates                              | $d^{-1}$                                       |
| $\mathbf{k}_{\mathrm{h,li}}$    | Hydrolysis Constant for Lipids                                     | $d^{-1}$                                       |
| k <sub>h,pr</sub>               | Hydrolysis Constant for Proteins                                   | $d^{-1}$                                       |
| $\mathbf{K}_{i}$                | Half-Inhibition Constant                                           | kg COD m <sup>-3</sup> or kmol m <sup>-3</sup> |
| $\mathbf{K}_{i,Sh2}$            | Hydrogen Half-Inhibition Constant                                  | kg COD m <sup>-3</sup>                         |
| K <sub>i,Snh3</sub>             | Free Ammonia Half-Inhibition Constant                              | kmol N m <sup>-3</sup>                         |
| Ki,Snh3,Xac                     | Free Ammonia Half-Inhibition Constant for Acetoclastic Methanogens | kmol N m <sup>-3</sup>                         |
| Ki,Snh3,Xc4                     | Free Ammonia Half-Inhibition Constant for Butyrate Degraders       | kmol N m <sup>-3</sup>                         |
| $K_{i,Snh3,Xc5}$                | Free Ammonia Half-Inhibition Constant for Valerate Degraders       | kmol N m <sup>-3</sup>                         |
| $K_{i,Snh3,Xh2}$                | Free Ammonia Half-Inhibition Constant for Hydrogen Degraders       | kmol N m <sup>-3</sup>                         |
| Ki,Snh3,Xpro                    | Free Ammonia Half-Inhibition Constant for Propionate Degraders     | kmol N m <sup>-3</sup>                         |
| km                              | Maximum Growth Constant                                            | d-1                                            |
| k <sub>m,aa</sub>               | Maximum Growth Constant for Amino Acid Degraders                   | d-1                                            |
| k <sub>m,ac</sub>               | Maximum Growth Constant for Acetoclastic Methanogens               | d-1                                            |
| k <sub>m,c4</sub>               | Maximum Growth Constant for Butyrate Degraders                     | d-1                                            |
| k <sub>m,c5</sub>               | Maximum Growth Constant for Valerate Degraders                     | $d^{-1}$                                       |
| k <sub>m,fa</sub>               | Maximum Growth Constant for LCFA Degraders                         | $d^{-1}$                                       |
| k <sub>m,h2</sub>               | Maximum Growth Constant for Hydrogenotrophic Methanogens           | $d^{-1}$                                       |

| Symbol                         | Name                                                      | Units                            |
|--------------------------------|-----------------------------------------------------------|----------------------------------|
| k <sub>m,pro</sub>             | Maximum Growth Constant for Propionate Degraders          | d-1                              |
| k <sub>m,su</sub>              | Maximum Growth Constant for Sugar Degraders               | d-1                              |
| $\mathbf{K}_{\mathrm{pH}}$     | Proton Inhibition Constant                                | kmol m <sup>-3</sup>             |
| Ks                             | Substrate Half-Saturation Constant                        | kg COD m <sup>-3</sup> or kmol m |
| K <sub>S,Sin</sub>             | Inorganic Nitrogen Half-Saturation Constant               | kg COD m <sup>-3</sup> or kmol m |
| K <sub>S,Xaa</sub>             | Amino Acid Half-Saturation Constant                       | kg COD m <sup>-3</sup> or kmol m |
| K <sub>S,Xac</sub>             | Acetate Half-Saturation Constant                          | kg COD m <sup>-3</sup> or kmol m |
| K <sub>S,Xc4</sub>             | Butyrate Half-Saturation Constant                         | kg COD m <sup>-3</sup> or kmol m |
| K <sub>S,Xc5</sub>             | Valerate Half-Saturation Constant                         | kg COD m <sup>-3</sup> or kmol m |
| K <sub>S,Xfa</sub>             | LCFA Half-Saturation Constant                             | kg COD m <sup>-3</sup> or kmol m |
| K <sub>S,Xh2</sub>             | Hydrogen Half-Saturation Constant                         | kg COD m <sup>-3</sup> or kmol m |
| K <sub>S,Xpro</sub>            | Propionate Half-Saturation Constant                       | kg COD m <sup>-3</sup> or kmol m |
| K <sub>S,Xsu</sub>             | Sugar Half-Saturation Constant                            | kg COD m <sup>-3</sup> or kmol m |
| N <sub>aa</sub>                | Proteins/Amino Acids Nitrogen Content                     | kmol N kg COD-1                  |
| N <sub>bac</sub>               | Nitrogen Content of Microorganisms                        | kmol N kg COD-1                  |
| Ni                             | Inert Compound Nitrogen Content                           | kmol N kg COD-1                  |
| Ni,subs                        | Inert Nitrogen Content of the Substrate-Inoculum Mixture  | kmol N kg COD-1                  |
| $\mathbf{N}_{\mathrm{pH}}$     | Proton Inhibition Exponent                                |                                  |
| $\mathbf{N}_{\text{subs}}$     | Global Nitrogen Content of the Substrate-Inoculum Mixture | kmol N kg COD-1                  |
| $pH_{LL,ac}$                   | Lower pH Level for Acetate Degraders                      |                                  |
| $pH_{UL,ac}$                   | Upper pH Level for Acetate Degraders                      |                                  |
| $\mathbf{S}_{aa}$              | Soluble Amino Acids                                       | kg COD m <sup>-3</sup>           |
| $\mathbf{S}_{ac}$              | Soluble Acetate                                           | kg COD m <sup>-3</sup>           |
| San                            | Soluble Anions                                            | kmol m <sup>-3</sup>             |
| $\mathbf{S}_{\mathrm{bu}}$     | Soluble Butyrate                                          | kg COD m <sup>-3</sup>           |
| S <sub>cat</sub>               | Soluble Cations                                           | kmol m <sup>-3</sup>             |
| $\mathbf{S}_{\mathrm{ch4}}$    | Dissolved Methane                                         | kg COD m <sup>-3</sup>           |
| $S_{co2}$                      | Dissolved Carbon Dioxide                                  | kmol C m <sup>-3</sup>           |
| $\mathbf{S}_{\mathrm{fa}}$     | Long Chain Fatty Acids                                    | kg COD m <sup>-3</sup>           |
| $\mathbf{S}_{h+}$              | Protons, H <sup>+</sup>                                   | kmol m <sup>-3</sup>             |
| $\mathbf{S}_{h2}$              | Soluble Hydrogen                                          | kg COD m <sup>-3</sup>           |
| $\mathbf{S}_{i}$               | Soluble Inert from Composite                              | kg COD m <sup>-3</sup>           |
| $\mathbf{S}_{\mathrm{i,subs}}$ | Soluble Inert from the Substrate-Inoculum Mixture         | kg COD m <sup>-3</sup>           |
| $\mathbf{S}_{\mathrm{ic}}$     | Soluble Inorganic Carbon                                  | kmol C m-3                       |
| $\mathbf{S}_{\mathrm{in}}$     | Soluble Inorganic Nitrogen                                | kmol N m <sup>-3</sup>           |
| S <sub>nh3</sub>               | Soluble Free Ammonia Nitrogen                             | kmol N m <sup>-3</sup>           |
| $S_{nh4+}$                     | Soluble Ammonium Ion Nitrogen                             | kmol N m <sup>-3</sup>           |
| $\mathbf{S}_{\mathrm{pro}}$    | Soluble Propionate                                        | kg COD m <sup>-3</sup>           |
| Sproton                        | Protons, H <sup>+</sup>                                   | kmol m <sup>-3</sup>             |

Table S.1 (Continuation) Biochemical Symbols

| Symbol                     | Name                                                  | Units                  |
|----------------------------|-------------------------------------------------------|------------------------|
| $\mathbf{S}_{su}$          | Soluble Sugars                                        | kg COD m <sup>-3</sup> |
| $\mathbf{S}_{\mathbf{va}}$ | Soluble Valerate                                      | kg COD m <sup>-3</sup> |
| X <sub>aa</sub>            | Amino Acid Degraders                                  | kg COD m <sup>-3</sup> |
| $X_{ac}$                   | Acetoclastic Methanogens                              | kg COD m <sup>-3</sup> |
| $X_{\text{Biomass}}$       | Sum of All Microorganisms Concentrations              | kg COD m <sup>-3</sup> |
| $X_{c}$                    | Composite                                             | kg COD m <sup>-3</sup> |
| $X_{c4}$                   | Butyrate Degraders                                    | kg COD m <sup>-3</sup> |
| Xc5                        | Valerate Degraders                                    | kg COD m <sup>-3</sup> |
| $X_{ch}$                   | Carbohydrates                                         | kg COD m <sup>-3</sup> |
| $X_{ch,fast}$              | Readily-Biodegradable Carbohydrates                   | kg COD m <sup>-3</sup> |
| $X_{ch,slow}$              | Slowly-Biodegradable Carbohydrates                    | kg COD m <sup>-3</sup> |
| $\mathbf{X}_{\mathrm{fa}}$ | Long Chain Fatty Acids Degraders                      | kg COD m <sup>-3</sup> |
| $\mathbf{X}_{g}$           | Lipids                                                | kg COD m <sup>-3</sup> |
| $X_{h2}$                   | Hydrogenotrophic Methanogens                          | kg COD m <sup>-3</sup> |
| $X_i$                      | Particulate Inert from Composite                      | kg COD m <sup>-3</sup> |
| $X_{i,subs}$               | Particulate Inert from the Substrate-Inoculum Mixture | kg COD m <sup>-3</sup> |
| $\mathbf{X}_{\mathrm{pr}}$ | Proteins                                              | kg COD m <sup>-3</sup> |
| $X_{pro}$                  | Propionate Degraders                                  | kg COD m <sup>-3</sup> |
| $\mathbf{X}_{\mathrm{su}}$ | Sugar Degraders                                       | kg COD m <sup>-3</sup> |
| $\mathbf{Y}_{b}$           | Biomass Yield                                         | kg COD kg COD-1        |

Table S.1 (Continuation) Biochemical Symbols

| Symbol                                 | Name                                         | Units                                                                          |
|----------------------------------------|----------------------------------------------|--------------------------------------------------------------------------------|
| C <sub>T,i</sub>                       | Total Concentration (Soluble or Particulate) | kg COD m <sup>-3</sup> or kmol m <sup>-3</sup>                                 |
| m <sub>Biogas</sub>                    | Biogas Mass Flow (Including Vapor)           | kg d <sup>-1</sup>                                                             |
| mEffluent                              | Effluent Mass Flow                           | kg d <sup>-1</sup>                                                             |
| $M_{Global}$                           | Reactor Content Mass                         | kg                                                                             |
| mi                                     | Individual Mass Flow or Mass Fraction        | kg d <sup>-1</sup> or kg kg <sup>-1</sup>                                      |
| $M_i$                                  | Individual Mass Content                      | kg                                                                             |
| MInerts                                | Inert Material Mass                          | kg                                                                             |
| mInfluent                              | Influent Mass Flow                           | kg d <sup>-1</sup>                                                             |
| Mri                                    | Individual Molecular Weight                  | g kmol <sup>-1</sup>                                                           |
| M <sub>Solids</sub>                    | Solid Material Mass                          | kg                                                                             |
| $M_{Solvent}$                          | Liquid-Solvent (Water) Content Mass          | kg                                                                             |
| $m_{Vapor}$                            | Water Vapor Mass Flow                        | kg d <sup>-1</sup>                                                             |
| Mvolatiles                             | Mass of Volatile Substances at 105 °C        | kg                                                                             |
| P <sub>T</sub>                         | Global Pressure                              | bar                                                                            |
| $P_{v}$                                | Vapor Pressure                               | bar                                                                            |
| Q                                      | Volumetric Flow                              | $m^3 d^{-1}$                                                                   |
| Q <sub>Effluent</sub> , Q <sub>e</sub> | Effluent Volumetric Flow                     | $m^3 d^{-1}$                                                                   |
| $Q_{g}$                                | Biogas Volumetric Flow (Including Vapor)     | $m^3 d^{-1}$                                                                   |
| QInfluent, Q0                          | Influent Volumetric Flow                     | $m^3 d^{-1}$                                                                   |
| R                                      | Ideal Gas Constant                           | bar m <sup>3</sup> kmol <sup>-1</sup> K <sup>-1</sup>                          |
| rj                                     | Reaction Term                                | kg COD m <sup>-3</sup> d <sup>-1</sup> or kmol m <sup>-3</sup> d <sup>-1</sup> |
| <b>r</b> <sub>j,ADM1</sub>             | Reaction Term                                | kg COD m <sup>-3</sup> d <sup>-1</sup> or kmol m <sup>-3</sup> d <sup>-1</sup> |
| $\mathbf{S}_{\mathrm{T,i}}$            | Total Soluble Concentration                  | kg COD m <sup>-3</sup> or kmol m <sup>-3</sup>                                 |
| $S_{T,i,App}$                          | Total Apparent Concentration                 | kg COD m <sup>-3</sup> Solvent or kmol m <sup>-3</sup> Solvent                 |
| Т                                      | Temperature                                  | K                                                                              |
| TS                                     | Total Solids                                 | kg kg <sup>-1</sup>                                                            |
| $TS_{Recalc}$                          | Total Solids Recalculated                    | kg kg <sup>-1</sup>                                                            |
| V                                      | Volume                                       | m <sup>3</sup>                                                                 |
| $V_{g}$                                | Reactor Headspace Gas Volume                 | m <sup>3</sup>                                                                 |
| $V_{\mathrm{Global}}$                  | Reactor Content Volume                       | m <sup>3</sup>                                                                 |
| V <sub>Reactor</sub>                   | Reactor Design Volume                        | m <sup>3</sup>                                                                 |
| VS                                     | Volatile Solids                              | kg kg <sup>-1</sup>                                                            |
| V <sub>Setpoint</sub>                  | Reactor Volume to be Maintained Constant     | m <sup>3</sup>                                                                 |
| $VS_{Recalc}$                          | Volatile Solids Recalculated                 | kg kg <sup>-1</sup>                                                            |
| $x_i$                                  | Individual Molar Fraction                    | kmol kmol <sup>-1</sup>                                                        |
| $X_{T,i}$                              | Total Particulate Concentration              | kg COD m <sup>-3</sup> or kmol m <sup>-3</sup>                                 |
| 3                                      | Gaseous Porosity                             | $m^{3}m^{-3}$                                                                  |
| ρ                                      | Specific Weight                              | kg m <sup>-3</sup>                                                             |
| ρο                                     | Specific Weight of the Influent              | kg m <sup>-3</sup>                                                             |
| $ ho_{Global}$                         | Reactor Content Specific Weight              | kg m <sup>-3</sup>                                                             |
| $\rho_i$                               | Individual Specific Weight                   | kg m <sup>-3</sup>                                                             |
| $\rho_{Solids}$                        | Solid Materials Specific Weight              | kg m <sup>-3</sup>                                                             |
| $\rho_{Solvent}$                       | Solvent Specific Weight                      | kg m <sup>-3</sup>                                                             |

Table S.2 Mass Balances Symbols

| Symbol                      | Name                                                              | Units                                  |
|-----------------------------|-------------------------------------------------------------------|----------------------------------------|
| А                           | Temperature-dependent Constant                                    |                                        |
| $a_{h+}$                    | Proton Activity                                                   | kmol m <sup>-3</sup>                   |
| ai                          | Activity                                                          | kmol m <sup>-3</sup>                   |
| ai <sup>o</sup>             | Ions Size-dependent Parameter                                     |                                        |
| anh3                        | Free Ammonia Activity                                             | kmol m <sup>-3</sup>                   |
| $a_{nh4+}$                  | Ammonium Ion Activity                                             | kmol m <sup>-3</sup>                   |
| В                           | Temperature-dependent Constant                                    |                                        |
| bi                          | Non-Charged Coefficient                                           |                                        |
| Ι                           | Ionic Strength                                                    | kmol m-3                               |
| $\mathbf{K}_{\mathrm{a,i}}$ | Equilibrium/Dissociation Constant                                 | kmol m <sup>-3</sup>                   |
| $\mathbf{K}_{\mathrm{a,i}}$ | Modified Equilibrium/Dissociation Constant                        | kmol m <sup>-3</sup>                   |
| $\mathbf{K}_{a,in}$         | Equilibrium/Dissociation Constant for Inorganic Nitrogen          | kmol m <sup>-3</sup>                   |
| K <sub>a,in</sub> '         | Modified Equilibrium/Dissociation Constant for Inorganic Nitrogen | kmol m <sup>-3</sup>                   |
| $K_{H,co2}$                 | Henry's Constant for Carbon Dioxide                               | kmol m <sup>-3</sup>                   |
| $K_{\mathrm{H,i}}$          | Henry's Constant                                                  | kmol m <sup>-3</sup> bar <sup>-1</sup> |
| $\mathbf{K}_{\mathrm{H,i}}$ | Modified Henry's Constant                                         | kmol m <sup>-3</sup> bar <sup>-1</sup> |
| $\mathbf{S}_{i}^{Zi}$       | Ion Concentration                                                 | kmol m <sup>-3</sup>                   |
| $\mathbf{Z}_{i}$            | Ion Charge                                                        |                                        |
| γο                          | Activity Coefficient for Non-Charged Species                      |                                        |
| $\gamma_{h+}$               | Activity Coefficient for Protons                                  |                                        |
| $\gamma_{i}$                | Activity Coefficient                                              |                                        |
| $\gamma_{nh3}$              | Activity Coefficient for Free Ammonia                             |                                        |
| $\gamma_{nh4+}$             | Activity Coefficient for Ammonium Ion                             |                                        |

Table S.3 Physical-Chemical Symbols

xxviii

| Symbol               | Name                                        |
|----------------------|---------------------------------------------|
| D                    | Number of Experimental Datasets             |
| 3                    | Tolerance Coefficient                       |
| $J(\theta)$          | Objective Funtion                           |
| Ν                    | Number of Input Parameters                  |
| p(y)                 | Normalizing Constant                        |
| $p(y \theta)$        | Likelihood Function                         |
| $p(\theta)$          | Prior Distribution                          |
| $p(\theta y)$        | Posterior Distribution                      |
| $p(	heta_{opt}   y)$ | Optimal Input Parameter Distribution        |
| R                    | Number of Batch Simulations                 |
| $t_{exp}$            | Experimental Time                           |
| W                    | Weighting Coefficient                       |
| у                    | Experimental Data                           |
| $y^{sim}(\theta)$    | Model Output                                |
| $\theta$             | Input Parameters                            |
| heta'                | 'Potentially Identifiable' Input Parameters |
| $	heta_B$            | Initial/Influent Conditions                 |
| $	heta_{opt}$        | Optimal Input Parameters                    |
| $	heta_P$            | Structural/Biochemical Parameters           |
| $\bar{y}$            | Average Experimental Data                   |

 Table S.4 Calibration Symbols

## List of Tables

| Table S.1 Biochemical Symbols                                                                                                                                          |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Table S.2 Mass Balances Symbolsxxvi                                                                                                                                    |
| Table S.3 Physical-Chemical Symbols         xxvii                                                                                                                      |
| Table S.4 Calibration Symbols                                                                                                                                          |
| Table 2.1 Characterization of the organic fraction of municipal solid waste. TS – Total                                                                                |
| Solids, VS – Volatile Solids, TKN – Total Kjeldahl Nitrogen, TP – Total Phosphorous, BMP – Biomethane Potential, Adapted from Campuzano et al. (2016)                  |
| Table 2.2 Main acid-base equilibriums in anaerobic disestion     24                                                                                                    |
| Table 2.3 Main Operative Parameters and Performance Framples of the Main High-Solids                                                                                   |
| Anaerobic Disection Applications at Commercial Scale Adapted from Karthikevan et al.                                                                                   |
| (2013)                                                                                                                                                                 |
| Table 3.1 Summary of high-solids batch experiments and biomethane potential tests (BMP)                                                                                |
| Table 3.2 Bio-physical-chemical characterization of substrates and inoculum                                                                                            |
| <b>Table 3.3</b> Effect of total solids on the performances of high-solids anaerobic digestion of the                                                                  |
| organic fraction of municipal solid waste using an inoculum-to-substrate ratio of 1.5 g VS/g                                                                           |
| <i>VS</i> ( <i>Test 3</i> )                                                                                                                                            |
| Table 4.1 Bio-physical-chemical characterization of substrates                                                                                                         |
| Table 4.2 Physical-chemical characterization of inoculums                                                                                                              |
| Table 5.1 Biochemical kinetics used for model implementation verification and calibration         113                                                                  |
| Table 5.2 Influent and initial conditions used for model implementation verification and                                                                               |
| model calibration 116                                                                                                                                                  |
| Table 5.3 Summary of steady state results for model implementation varification       110                                                                              |
| Table 5.5       Summary of steady-state results for model implementation verification         Table 5.4       Main parameters modified for model calibration       119 |
| Table 6.1 Biochemical rates used for model implementation verification and model                                                                                       |
| calibration 141                                                                                                                                                        |
| <b>Table 6.2</b> Influent conditions used for model implementation verification at different total                                                                     |
| solid (TS) contents and initial conditions used for model calibration 142                                                                                              |
| <b>Table 6.3</b> Biochemical parameters modified for model calibration at thermophilic (55°C)                                                                          |
| conditions                                                                                                                                                             |
| <b>Table 6.4</b> Summary of steady-state results (i.e. day 365) for model implementation                                                                               |
| verification at different influent total solid (TS) contents Roth the ADM1 and the HS-AD                                                                               |
| requestion in aggreent nyment total solar (15) contents, both the ADMI and the H5-AD                                                                                   |
| Table 7.1 Batch experiments and initial conditions used for HS-AD model call                     | libration and |
|--------------------------------------------------------------------------------------------------|---------------|
| cross-validation                                                                                 |               |
| Table 7.2 Biochemical rates used for the HS-AD model in this study                               |               |
| Table 7.3 Initial conditions used for all batch simulations in this study                        |               |
| <i>Table 7.4</i> Main input parameters ( $\theta$ ) used for global sensitivity analysis (GSA),  | including the |
| initial values, lower and upper thresholds                                                       |               |
| <b>Table 7.5</b> Global sensitivity analysis (GSA) of input parameters ( $\theta$ ): Individu    | ual (IE) and  |
| global (GE) effects upon the objective function, $J(\theta)$ , variance                          |               |
| <b>Table 7.6</b> Calibration of potentially-identifiable input parameters ( $\theta$ '): Prior a | and posterior |
| distributions                                                                                    |               |

| Table A.1 High-solids anaerobic digestion (HS-AD) batch experiments an            | different   |
|-----------------------------------------------------------------------------------|-------------|
| inoculum-to-substrate ratio (ISR) and initial total solids (TS) for the organic p | raction of  |
| municipal solid waste (OFMSW) including in some cases beech sawdust as a co       | -substrate. |
| Adapted from [Chapter 3 – Batch Manuscript]                                       |             |
| Table A.2 Biochemical rates used in the high-solids anaerobic digestion model     |             |
| Table A.3 Modified parameters in this study                                       |             |
| Table A.4 Initial and influent conditions used for simulations                    |             |
| Table A.5 Physical-chemical characterization of organic wastes and inoculum       | . Adapted   |
| from [Chapter 3 – Batch Manuscript] and (Pastor-Poquet et al., 2018, In Press)    |             |

# **List of Figures**

| Figure 1.1 Overview of this PhD thesis | 7 |
|----------------------------------------|---|
|----------------------------------------|---|

| Figure 2.1 Schematic representation of anaerobic digestion                              | 14         |
|-----------------------------------------------------------------------------------------|------------|
| Figure 2.2 Schematic representation of dark fermentation                                | 15         |
| Figure 2.3 Concentration of hydrogen sulfide (H <sub>2</sub> S) – left panel – and free | ammonia    |
| nitrogen $(NH_3)$ – right panel – as a function of temperature $(T)$ and pH             | 21         |
| Figure 2.4 Schematic representation of total solid increase - left panel - and the      | otal solid |
| concentration effect – right panel – in high-solids anaerobic digestion                 |            |
| Figure 2.5 Example of the total liquid, including the global, liquid-filled and         | gas-filled |
| porosities, as a function of increasing total solids in high-solids anaerobic digestion | ı 30       |
| Figure 2.6 Schematic representation of high-solids anaerobic digestion reactors         |            |
| Figure 2.7 COD flow in ADM1                                                             |            |

# 

 Figure 5.1 High-solids vs. 'wet' anaerobic digestion
 107

 Figure 5.2 Schematic representation of the high-solids anaerobic digestion model
 111

 Figure 5.3 Hydraulic retention time and organic loading rate in model implementation
 111

 Figure 5.3 Hydraulic retention time and organic loading rate in model implementation
 111

 Figure 5.3 Hydraulic retention time and organic loading rate in model implementation
 111

 Figure 5.3 Hydraulic retention time and organic loading rate in model implementation
 111

 Figure 5.4 Batch mono-digestion of OFMSW at 15 % total solids: a) accumulated methane
 120

 Figure 5.4 Batch mono-digestion of OFMSW at 15 % total solids: a) accumulated methane
 123

 production and reactor mass content; b) volatile fatty acids; c) total and free ammonia
 123

Figure 6.1 Schematic representation of the 'ideal' or 'non-ideal' physical-chemical implementation used for all ADM1-based models in this study. t refers to the simulation time-step. Tol refers to tolerance (in this study  $Tol = 10^{-6}$ ). I is the ionic strength; while  $S_{T,i}$  is the global concentration;  $K_{a,i}$  is the dissociation equilibrium constant; and  $y_i$  is the activity Figure 6.2 Summary of results for model implementation verification as a function of influent total solids (TS). Comparison between standard ADM1, ADM1 Non-Ideal, HS-AD Model and HS-AD Model Non-Ideal outputs: a) Total acetate concentration ( $S_{ac}$ ) vs. initial TS; b) total acetoclastic methanogens to biomass ratio (Xac/Xbiomass) vs. initial TS; c) total acetate concentration ( $S_{ac}$ ) vs. total NH<sub>3</sub> concentration ( $S_{nh3}$ ); and d) Henry's constant difference for  $CO_2$  ( $K_{H,co2}$ ) vs. ionic strength. The global and apparent concentrations are interrelated by TS, and the specific weight of reactor content ( $\rho_{Global}$ ) and aqueous solvent  $(\rho_{Solvent} = 1000 \text{ kg/m}^3)$ . The NH<sub>3</sub> half-inhibition constant for acetoclastic methanogens Figure 6.3 Contour plots for the relative difference between the 'ideal' and 'non-ideal' implementations of both ADM1 and the HS-AD model at different influent total solid (TS) contents: a) Acetate (Sac) difference [Equation 6.14]; and b) NH<sub>3</sub> (Snh3) difference [Equation 6.15]. Values in parentheses show the NH<sub>3</sub> half-inhibition constants used for acetoclastic methanogens ( $K_{i,Snh3,Xac}$ , kmol N/m<sup>3</sup>). Positive values over the 'inversion' threshold in panel b represent the influent TS at which the steady-state NH<sub>3</sub> concentration is higher for the 'non-Figure 6.4 Effect of 'non-ideality' during the initial 40 days of HS-AD model simulations at 30 % influent TS. Comparison between 'ideal' and 'non-ideal' conditions, including the Phreeqc engine: a) Acetoclastic methanogens concentration  $(X_{ac})$ ; b) total acetate concentration  $(S_{ac})$ ; c) pH; d) apparent NH<sub>3</sub> concentration  $(S_{nh3,App})$ ; e) total ammonia

#### xxxii

Figure A.1 Batch experiment results obtained during the mono-digestion of the organic fraction of municipal solid waste (OFMSW) using an inoculum-to-substrate ratio of 1.5 g VS/g VS: Chemical oxygen demand (COD) conversion, and total (TAN) and free ( $NH_3$ ) ammonia nitrogen at the end of the experiment (day 92) ......224 Figure A.2 Experimental and simulated values for the sacrifice test using mono-digestion of the organic fraction of municipal solid waste (Test 6): a) Cumulative biogas production; b) total (TS) and volatile (VS) solids; c) total ammonia nitrogen (TAN), ammonium ion  $(NH_{4^+})$ and free ammonia  $(NH_3)$ ; d) total volatile fatty acids (VFA), in chemical oxygen demand Figure A.3 Experimental and simulated values for the semi-continuous mono-digestion of the organic fraction of municipal solid waste: a) Cumulative biogas production; b) total (TS) and volatile (VS) solids; c) total ammonia nitrogen (TAN), ammonium ion  $(NH_{4^+})$  and free ammonia  $(NH_3)$ ; d) total volatile fatty acids (VFA), in chemical oxygen demand (COD) Figure A.4 Experimental and simulated values for the semi-continuous co-digestion of the organic fraction of municipal solid waste and beech sawdust: a) Cumulative biogas production; b) total (TS) and volatile (VS) solids; c) total ammonia nitrogen (TAN), ammonium ion  $(NH_4^+)$  and free ammonia  $(NH_3)$ ; d) total volatile fatty acids (VFA), in 

xxxiv

# **List of Publications**

- I. Pastor-Poquet, V., Papirio, S., Trably, E., Rintala, J., Escudié, R., & Esposito, G. (*submitted*). High-Solids Anaerobic Digestion requires a tradeoff between Total Solids, Inoculum-to-Substrate Ratio and Ammonia Inhibition.
- II. Pastor-Poquet, V., Papirio, S., Trably, E., Rintala, J., Escudié, R., & Esposito, G. (2018, *In Press*). Semi-continuous Mono-digestion of OFMSW and Co-digestion of OFMSW with Beech Sawdust: Assessment of the Maximum Operational Total Solid Content. *Journal of Environmental Management*, XXX, XXX-XXX
- III. Pastor-Poquet, V., Papirio, S., Steyer, J.-P., Trably, E., Escudié, R., & Esposito, G. (2018). High-solids anaerobic digestion model for homogenized reactors. *Water Research*, 142, 501-511.
- IV. Pastor-Poquet, V., Papirio, S., Steyer, J.-P., Trably, E., Escudié, R., & Esposito, G. (*submitted*). Non-Ideal Bio-Physical-Chemical Effects on High-Solids Anaerobic Digestion of the Organic Fraction of Municipal Solid Waste.

In this thesis, the candidate also presents unpublished data and results in Chapter 7. These unpublished results will be later submitted for publication (Pastor-Poquet, V., Papirio, S., Harmand, J., Steyer, J.-P., Trably, E., Escudié, R., & Esposito, G. (*in preparation*). Assessing Practical Identifiability during Calibration and Cross-Validation of a Structured Model for High-Solids Anaerobic Digestion [2019]).

In this thesis, the candidate also presents unpublished data and results as an Annex. These unpublished results will be later submitted for publication (Pastor-Poquet, V., Papirio, S., Steyer, J.-P., Trably, E., Rintala, J., Escudié, R., & Esposito, G. (*in preparation*). Developing a High-Solids Anaerobic Digestion Model: From the Experimental Setup to Further Model Requirements [2019]). xxxvi

# **Author's Contribution**

- Paper I, In this study, Vicente Pastor Poquet planned and performed the batch experiments, carried out the bio-physical-chemical analyses, and wrote the manuscript. The other co-authors suggested the manuscript structure, helped with the results discussion and the correction of the manuscript. The authors thank Luca Cioci and Gelsomino Monteverde for helping with the batch preparation and physical-chemical analyses.
- Paper II, In this study, Vicente Pastor Poquet planned and performed the semi-continuous experiments, carried out the bio-physical-chemical analyses, and wrote the manuscript. The other co-authors suggested the manuscript structure, helped with the results discussion and the correction of the manuscript.
- Paper III,In this study, Vicente Pastor Poquet developed the HS-AD model, performedChapter 5:In this study, Vicente Pastor Poquet and Jean-<br/>Philippe Steyer proposed, discussed and evaluated the model simulations. The<br/>other co-authors helped with the results discussion and the correction of the<br/>manuscript.
- Paper IV, In this study, Vicente Pastor Poquet developed the HS-AD model upgrade, performed the simulations, and wrote the manuscript. Vicente Pastor Poquet and Jean-Philippe Steyer proposed, discussed and evaluated the model simulations. The rest of co-authors helped with the results discussion and the correction of the manuscript.
- **Chapter 7:** In this study, Vicente Pastor Poquet performed the HS-AD model simulations and wrote the manuscript. Vicente Pastor Poquet, Jean-Philippe Steyer and Jérôme Harmand proposed the methodology, discussed and evaluated the model simulations. The rest of co-authors helped with the correction of the manuscript. Angela Andreella is gratefully acknowledged for revising all the statistical concepts.
- Annex: This study revisits some of the concepts presented in Chapters 3 to 6, includes further model simulations and also some guidelines to further extend/develop the HS-AD model. In this study, Vicente Pastor Poquet performed the HS-AD model simulations and wrote the manuscript. The rest of co-authors helped with the results discussion and the correction of the manuscript.

Chapter 1

**General Introduction and Thesis Outline** 

#### 1.1 General Introduction

In absence of oxidative species (i.e.  $O_2$ ,  $NO_3^-$ , and/or  $SO_4^{2-}$ ), the decomposition of organic matter leads to a mixture of methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>), as a result of the biochemical activity of microorganisms in anaerobic conditions. This natural process is known as anaerobic digestion (AD), whereas the gas mixture of CH<sub>4</sub> and CO<sub>2</sub> is known as biogas. Both CH<sub>4</sub> and CO<sub>2</sub> are greenhouse gases (GHG), contributing to global warming when released to the atmosphere. In particular, CH<sub>4</sub> shows approximately 35-times stronger greenhouse effect than CO<sub>2</sub> (IPCC, 2013; Viéitez et al., 2000).

Anaerobic zones within landfills embedding organic materials are prone to biogas production. Particularly, the uncontrolled GHG emissions from landfilling the organic fraction of municipal solid waste (OFMSW) account up to 5 % of the global anthropogenic emissions (Hoornweg et al., 2012). On top of that, a heterogeneous and water deficient environment as a landfill results into an inefficient AD conversion and prolonged GHG emissions (i.e. up to 40 years) (Mora-Naranjo et al., 2004; Viéitez et al., 2000).

Landfilling OFMSW has another important disadvantage in terms of organic N and P sequestration. N is one of the main building blocks of amino-acids, whereas P is a principal constituent of the cellular wall in all living organisms (Gerardi, 2003; Madigan et al., 2012). Thus, N and P are ubiquitous in all organic materials within OFMSW. Meanwhile, both N and P are essential nutrients for plant growth and need to be added in agriculture by means of organic or inorganic fertilizers. Noteworthy, after the Green Revolution (back to the 60's), the great majority of chemical fertilizers worldwide result from industrial synthesis, being the synthesis of fertilizers another important source of anthropogenic GHG emissions. For example, the only production of N-based fertilizers accounts for around 0.8 % of the global GHG emissions (Brentrup, 2009). Therefore, landfilling OFMSW is also associated to indirect GHG emissions from fertilizer production.

Due to the important contribution of landfilling to the global anthropogenic pool of GHG emissions, legislative initiatives are being implemented worldwide to divert OFMSW from landfills. Particularly, the European Waste Framework Directive (Directive 2008/98/EC) aims the reuse and recycling, including biochemical treatments, of at least 50 % of the produced OFMSW by 2020. Similarly, the Landfill Directive (1999/31/EC) and the European Renewable Energy directive (2009/28/EC) contribute also towards the reduction of OFMSW landfilling, while promoting the use of sustainable treatment technologies.

AD can be easily optimized within industrial reactors (digesters). More in particular, AD of OFMSW at industrial scale is a well-established technology allowing the recovery of biogas as a source of renewable energy and the recycling of nutrients (i.e. N and P) back to the environment. Importantly, AD shows the best life cycle assessment (LCA) of all renewable and non-renewable technologies for OFMSW treatment (Baldasano et al., 2000; Edelmann et al., 2005). Moreover, the increasing acceptance of recycling in Europe during the last 30 years has led to a progressive decrease of the OFMSW impurities (i.e. metals, plastics), enhancing the suitability of this waste for renewable energy production and nutrient recovery by AD (Campuzano et al., 2016; Clarke, 2018; De Baere, 2006; Jokela et al., 2003; Pavan et al., 2000).

Nowadays, the major interest lies on enhancing the overall efficiency and economy of AD for OFMSW treatment. High-solids anaerobic digestion (HS-AD) is a well-suited technology in this regard. HS-AD is a particular AD operation at a total solid (TS) content  $\geq$  10 %. Increasing the TS permits to reduce the reactor size and the overall operational costs. However, the TS increase might also lead to biochemical instability. Both the high organic load and the buildup of biochemical inhibitors can be responsible for the HS-AD instability. The free ammonia nitrogen (NH<sub>3</sub>), resulting from the protein and amino acid decomposition, is one of the most important biochemical inhibitor in HS-AD of OFMSW (Gerardi, 2003; Jokela et al., 2003). Therefore, a balance is often required between enhancing the process economy against the 'undesired' instability, to foster the advantages of HS-AD for OFMSW treatment.

The mathematical models are invaluable tools to understand and optimize biochemical processes (Eberl et al., 2006; Lauwers et al., 2013). The Anaerobic Digestion Model No.1 (ADM1) (Batstone et al., 2002) is one of the most important theoretical models for AD. ADM1 gathers together the main biochemical and physical-chemical mechanisms in AD. Thus, ADM1 can be used for HS-AD optimization at industrial scale (Batstone, 2006; Batstone et al., 2015). However, ADM1 was primarily conceived for 'wet' AD (i.e.  $TS \le 5$ %), while the higher TS content in HS-AD (i.e.  $TS \ge 10$ %) strongly influences the AD biochemistry and physical-chemistry. For example, the ionic interactions are exacerbated as a consequence of the low 'free' water available in HS-AD. Therefore, HS-AD simulations with ADM1 need to be approached differently than 'wet' AD simulations.

#### 1.2 Objectives

This PhD research investigated the main bio-physical-chemical mechanisms and kinetics in HS-AD of organic waste, with the aim to optimize the industrial application and maximize the process rates. OFMSW was used as main substrate due to its high biodegradability, TS and nutrient (i.e. nitrogen and phosphorus) content. The objective was subdivided into:

- Understand the effects of increasing the initial TS content in HS-AD batch experiments, in terms of methane yield, TS removal, and chemical oxygen demand (COD) conversion;
- 2) Understand the main operational parameters, advantages and limitations of semicontinuous HS-AD;
- 3) Develop a HS-AD model based on ADM1;
- 4) Understand the effects of a 'non-ideal' bio-physical-chemistry in HS-AD; and
- 5) Calibrate and cross-validate the HS-AD model to assess the risk of acidification and free ammonia (NH<sub>3</sub>) inhibition in HS-AD of OFMSW.

The main novelty of this PhD research is the development of a robust HS-AD model for homogeneized reactors, as an outstanding tool to evaluate and foresee the effect of the main operational variables (e.g. substrate composition and organic load), in the overall set of HS-AD bio-physical-chemical mechanisms and kinetic rates. In this scheme, the preliminary assessment of experimental data was essential to pose realistic hypotheses to restrain the model complexity, but also to calibrate the main model parameters. For example, the relatively low TS content of OFMSW (e.g.  $\leq$  30 %) and the important TS removal observed in the experimental setups for HS-AD of OFMSW (e.g. > 50 %), indicated that a zerodimensional model (i.e. homogeneized conditions) could be adequated for these simulations, instead of a much more complex approach (e.g. computational fluid dynamics).

With all the above, this PhD research deepens into the inherent complexity of the HS-AD bio-physical-chemistry and its mathematical modeling. Particularly, the research aimed to further understand and simulate the NH<sub>3</sub> inhibition and acidification mechanisms in HS-AD, as the main limitations for OFMSW treatment. To this aim, the inclusion of 'non-ideal' corrections in the bio-physical-chemistry, as a function of the ionic strength (I), was considered crucial. The overall set of PhD results contribute towards the industrial optimization of HS-AD, as a sustainable technology for organic waste treatment, renewable energy and nutrient recovery.

#### **1.3** Thesis Structure

This PhD thesis is divided into seven additional chapters. Chapter 2 presents the theoretical background about HS-AD, including the use of OFMSW as main substrate. Chapters 3 and 4 focus on the HS-AD experimental setups in batch and semi-continuous mode used in this research. Chapters 5 to 7 present the HS-AD model development, the inclusion of 'non-ideal' bio-physical-chemical corrections, and the model calibration/cross-validation strategy based on the previous experimental data. The general conclusions are presented in Chapter 8, where some guidelines are also suggested to benefit future HS-AD experimental designs and/or model developments.

Noteworthy, both the batch/semi-continuous experiments (Chapters 3 & 4) and the HS-AD model development (Chapter 5 & 6) were performed simultaneously in this study. Thus, the preliminary experimental results served to highlight the main strengths and weaknesses in HS-AD of OFMSW, to be prioritarily addressed by the HS-AD model, as highlighted before. In this line, it was soon realized the need to adequately assess the risk of NH<sub>3</sub> inhibition and digester acidification in HS-AD, including the importance of 'non-ideal' corrections upon the main bio-physical-chemical mechanisms (i.e. acid-base equilibrium and liquid-gas transfer). On the other hand, all experimental setups and bio-physical-chemical analyses performed were preliminarily conceived with the final aim of fully calibrate the HS-AD model during the last steps of the research (Chapter 7).

Finally, it must be remarked that the development of a mathematical model is an inherently continuous process of refuting hypotheses, where the correct design and evaluation of the experimental setup become crucial. More in particular, the experimental assessment requires a mathematical model to evaluate all the non-linear patterns, not easily observable from experimental results. Simultaneously, the mathematical model requires multiple and target-oriented experimental data to validate the preliminary hypotheses and calibrate the model parameters. Only after calibration, a mathematical model can be used for prediction and optimization, potentially highlighting some of the aspect to be further developed regarding the experimental assessment (Batstone et al., 2015; Donoso-Bravo et al., 2009; Gupta et al., 2008; Lauwers et al., 2013; Saltelli et al., 2006). In other words, the continuous development of a HS-AD model and the experimental setups should be considered circular, as exemplified in the thesis outline [Figure 1.1].



Figure 1.1 Overview of this PhD thesis

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Chapter 2

# **Theoretical Background**

#### 2.1 Anaerobic Digestion

Anaerobic digestion (AD) is a biochemical treatment technology in which an organic waste (OW) is decomposed in absence of oxidative species (i.e.  $O_2$ ,  $NO_3^-$ ,  $SO_4^{2-}$ ) to a highenergy content biogas and a partially stabilized organic material known as digestate. Biogas is mainly composed of methane (i.e. 60 - 70 %) and carbon dioxide (i.e. 30 - 40 %), showing potential applications as a source of heating power and/or electricity production (Gerardi, 2003; Ward et al., 2008). However, biogas includes traces of other gases, such as hydrogen sulfide (H<sub>2</sub>S) and free ammonia nitrogen (NH<sub>3</sub>), often requiring purification and/or upgrading to bio-methane, before being used as a source of renewable energy. Meanwhile, digestate can be used in agriculture with or without previous conditioning, due to the adequate composition for improving the soil characteristics (i.e. carbon content, pH, moisture retention) and nutrient content (i.e. N and P) (De Baere et al., 2013; Jokela et al., 2003). Importantly, the recovery of renewable energy and nutrients makes AD the most costeffective and environmental-friendly technology for OW treatment, in comparison to landfilling, composting and incineration (Baldasano et al., 2000; Mata-Alvarez et al., 2000).

#### 2.2 Main Biochemical Steps

The main biochemical steps in AD are hydrolysis, acidogenesis, acetogenesis and methanogenesis [Figure 2.1]. The hydrolysis and acidogenesis steps are carried out by acidogenic bacteria. Particularly, acidogenic microorganisms release extracellular enzymes to hydrolyze complex substrates (i.e. carbohydrates, proteins and lipids) into more simple ones (i.e. sugars; amino acids, AA; and long chain fatty acids, LCFA) that can be easily transferred inside the cytoplasm (Vavilin et al., 2007). The end-product of acidogenesis consist of a mixture of volatile fatty acids (VFA) and hydrogen (H<sub>2</sub>). VFA in AD include acetic, propionic, butyric, and valeric acids as major constituents, though formic, lactic and hexanoic acids can be also detected (Gerardi, 2003). Subsequently, acetogenic microorganisms degrade the VFA into acetate. During methanogenesis, the acetic acid and H<sub>2</sub> are consumed by acetoclastic and hydrogenotrophic archaea, respectively, to produce CH<sub>4</sub>. Importantly, the activity of acetoclastic methanogens yields simultaneously soluble inorganic carbon (i.e. bicarbonate, HCO<sub>3</sub><sup>-</sup>), as an important source of pH buffering capacity against the VFA accumulation in AD.



Figure 2.1 Schematic representation of anaerobic digestion

Many diverse microbial populations are capable of carrying out the different biochemical steps in AD (Vanwonterghem et al., 2014). However, due to the low energy released by the anaerobic metabolism, anaerobic microorganisms crucially rely on syntrophic relationships, where the lower degradation steps consume the organic products – intermediates – of the immediately-higher degradation steps (Morris et al., 2013; Westerholm et al., 2016). Particularly, the acetic acid and H<sub>2</sub> concentrations must be maintained low by methanogens to favor the thermodynamic feasibility of acetogenesis (McInerney et al., 2009). Similarly, either hydrolysis and/or acidogenesis might become inhibited by high concentrations of VFA, H<sub>2</sub>, or other organic intermediates (i.e. sugars, AA and LCFA) (Cazier et al., 2015; Vavilin et al., 2008).

The biochemical step 'controlling' AD is substrate-specific. Noteworthy, the methanogenic populations (usually archaea) are characterized by a considerably slower growth than the acidogenic bacteria, but also by specific nutrient requirements, and an extreme sensitivity towards the modification of environmental factors such as pH, temperature (T) and chemical substances (De Vrieze et al., 2012; Ferry, 1993; Hori et al., 2006). Thus, whether methanogens suffer from inhibitory compounds, usually associated to the organic substrate decomposition, VFA accumulate in AD, exacerbating the risk of bioreactor (digester) failure by acidification (i.e.  $pH \le 6.0$ ). On the other hand, whether hydrolysis is considerably faster than methanogenesis, VFA and H<sub>2</sub> may accumulate in AD, potentially leading to also acidification (Angelidaki et al., 1999; Vavilin et al., 2004). This phenomenon is known as organic overload.

With all the above, methanogenesis is usually the rate limiting step in AD, though hydrolysis can be also the rate limiting step, particularly in AD of complex (slowlybiodegradable) substances (Demirel et al., 2008; Pavlostathis et al., 1991). Meanwhile, acidification is the most common reason for AD failure, and can be either related to an excessively high organic load (OL), inhibitory substances, and/or poor digester management. When acidification occurs, the AD process might need to be restarted with an external source of methanogens. The reason lies on the incapability of methanogens to sporulate under stressing conditions, in contrast to acidogenic/acetogenic species (Fricke et al., 2007; Gerardi, 2003).

# 2.3 Dark Fermentation

Dark fermentation (DF) is a biochemical process where an OW is transformed to biohydrogen (H<sub>2</sub>) and a mixture of organic intermediates (i.e. VFA, alcohols) (Ghimire et al., 2015). DF can be considered as a 'transitional' AD process, where methanogenesis is (un)intentionally inhibited [Figure 2.2]. H<sub>2</sub> is a highly energy compound to be used as an energy vector, among many industrial applications. Moreover, since DF releases little energy to the biochemistry, some organic intermediates with industrial applications can be also recovered from the resulting liquid media. For example, the DF end-products poly-hydroxyalkanoates (PHA) can be recovered to produce bio-plastics (Rodriguez et al., 2006).



Figure 2.2 Schematic representation of dark fermentation

DF occurs by different biochemical pathways depending on the microorganism involved and the reactor conditions (i.e. pH, temperature, substrate composition). H<sub>2</sub> is produced in some fermentative pathways, while other pathways yield low or no H<sub>2</sub>, depending on the thermodynamics and environmental variables at a molecular level (Kleerebezem et al., 2015; Motte et al., 2015; Rodriguez et al., 2006). This characteristic of fermentative media is known as variable stoichiometry. Moreover, in absence of acetoclastic methanogens, the buffering capacity of DF is compromised and the accumulation of organic intermediates can lead to an acidification state (i.e.  $pH \le 5.0$ ) where biochemistry collapses (Saady, 2013). Therefore, DF requires external buffering addition to maintain an adequate pH. On the other hand, the low activation energy of H<sub>2</sub> also favors the development of opportunistic H<sub>2</sub>-degrading communities (i.e. homoacetogens) lowering the H<sub>2</sub> yield in DF.

With all the above, DF is a more complex system to be operated than AD, and industrial implementations are rare (Ghimire et al., 2015). However, DF and AD can be operated in tandem to benefit simultaneously from the production of H<sub>2</sub> and CH<sub>4</sub>, the recovery of nutrients (i.e. N and P), and organic intermediates (i.e. VFA). For example, a two-step process with DF followed by AD can lead to the production of bio-hythane (i.e. a mixture of H<sub>2</sub>, CH<sub>4</sub> and CO<sub>2</sub>) from OW (Cavinato et al., 2012). The main disadvantage to couple both biochemical processes is the greater number of operational parameters involved (e.g. pH, recirculation).

#### 2.4 Main Substrates

Among the most important substrates to be treated by AD are OFMSW, food waste (FW), wastewater (WW) or WW sludge (WWS), animal manure, lignocellulosic materials, including agricultural (AW) and green (GW) waste, algal biomass, and industrial waste (IW; i.e. slaughterhouse and food processing) (Mata-Álvarez, 2003; Mata-Alvarez et al., 2014). All of these OW show a high anaerobic biodegradability (BD), yielding an economical-suitable AD process. However, each OW requires an optimal AD setup to address the challenges posed by the particular biochemical and physical-chemical characteristics of the substrate, since the OW composition (i.e. nutrient content) influences the microbial community and stability of AD (Climenhaga et al., 2008; De Vrieze et al., 2015; Lerm et al., 2012). Moreover, the OW determines the reactor design and main operational variables in AD (Christensen, 2011; Karthikeyan et al., 2013; Kothari et al., 2014).

#### 2.4.1 Organic Fraction of Municipal Solid Waste

This study focuses mainly on OFMSW, since this OW is particularly suited for AD due to the high biodegradability and high nutrient content, but also the minimal need for pretreatment (e.g. particle reduction) (De Baere et al., 2013; Lissens et al., 2001; Mata-Álvarez, 2003). Moreover, the high OFMSW production worldwide, and the resulting greenhouse gases (GHG) emissions from landfilling OFMSW, are of particular concern nowadays (Clarke, 2018; De Baere et al., 2013). In this line, important legislative efforts are required to divert OFMSW from landfills, permitting to take advantage of the biochemical energy and nutrients content of this OW. Recycling is particularly beneficial in this regard. Recycling separates the different fractions of municipal solid waste (MSW), including organics, plastics, paper and cardboard, glass, metals, bulking materials, and/or hazardous substances. Therefore, recycling reduces significantly the impurities of OFMSW, enhancing not only the biodegradability potential and nutrient content, but also the quality of the digestate (Campuzano et al., 2016; Christensen, 2011; Jokela et al., 2003; Mata-Álvarez, 2003).

The OFMSW composition, and particularly the addition of GW to OFMSW, depends on regional, seasonal, and cultural factors, as well as the waste management strategy (Campuzano et al., 2016; Christensen, 2011; Tchobanoglous et al., 2002). Table 2.1 shows some examples of OFMSW. In general, the high TS content of OFMSW permits to maintain a high organic concentration within AD, minimize the reactor size, and speed up the biochemical rates. Moreover, OFMSW shows a well-balanced nutrient content for the anaerobic biomass.

 Table 2.1 Characterization of the organic fraction of municipal solid waste. TS – Total

 Solids, VS – Volatile Solids, TKN – Total Kjeldahl Nitrogen, TP – Total Phosphorous, BMP

 – Biomethane Potential. Adapted from Campuzano et al. (2016)

| Country           | Year | TS<br>(%) | VS<br>(%) | VS/TS<br>(%) | TKN<br>(g/kg) | TP<br>(g/kg) | BMP<br>(NL/kg VS) | Reference                |  |
|-------------------|------|-----------|-----------|--------------|---------------|--------------|-------------------|--------------------------|--|
| Finland           | 2010 | 27.0      | 24.9      | 92.3         | 6.5           | 0.7          | -                 | (VALORGAS, 2010)         |  |
| Portugal          | 2010 | 33.8      | 27.6      | 81.7         | 5.1           | 1.7          | -                 | (VALORGAS, 2010)         |  |
| Czech<br>Republic | 2011 | 32.5      | 23.1      | 71.0         | 4.5           | 0.7          | -                 | (Hanc et al., 2011)      |  |
| Spain             | 2011 | 29.0      | 22.3      | 77.0         | 5.3           | -            | 382               | (Ponsá et al., 2011)     |  |
| Greece            | 2012 | 46.3      | 34.9      | 75.3         | 6.9           | -            | -                 | (Komilis et al., 2012)   |  |
| China             | 2013 | 21.2      | 19.7      | 92.8         | -             | -            | 465               | (Dai et al., 2013)       |  |
| France            | 2013 | 21.3      | 17.5      | 82.1         | 4.5           | -            | -                 | (Adhikari et al., 2012)  |  |
| Belgium           | 2013 | 25.5      | 24.0      | 94.0         | 11.9          | 0.7          | 319               | (De Vrieze et al., 2013) |  |
| Italy             | 2015 | 30.5      | 28.1      | 92.0         | 7.7           | 1.2          | 490               | (Alibardi et al., 2015)  |  |
| Mexico            | 2015 | 29.7      | 22.3      | 75.1         | 5.4           | 1.8          | 545               | (Campuzano et al., 2015) |  |

#### 2.4.2 Co-digestion

Co-digestion of OFMSW and lignocellulosic materials (i.e. sawdust) was used in this study to increase the TS content and understand the effects of high TS on the AD of OFMSW. Co-digestion of two or more OW benefits from the synergism of the environmental, technological, and/or economic characteristics of different substrates (Garcia-Gen et al., 2015; Mata-Álvarez, 2003). Thus, co-digestion may enhance the methane production, modify the overall biodegradability, maximize the pH buffering capacity, improve the rheological performance, and/or dilute inhibitory compounds in comparison to a single OW. Particularly, co-digestion can be used to increase the nitrogen content of carbonaceous wastes or to reduce the nitrogen content of highly proteinaceous wastes (Mata-

Alvarez et al., 2014; Ward et al., 2008). Importantly, co-digestion is highly dependent on the availability of co-substrates and/or the overall OW treatment economy (Ortner et al., 2014).

#### 2.5 Nutrient Content

Microbial cells are highly complex structures composed by carbohydrates, proteins and lipids, and a great range of elements such as carbon (C), nitrogen (N), hydrogen (H), oxygen (O), phosphorus (P), sulfur (S), iron (Fe), and other metals and metalloids (e.g. cobalt, Co; nickel, Ni; selenium, Se). Microorganisms require all these elements as nutrients for catabolism/anabolism (Rittman et al., 2001). Interestingly, the elemental composition of microorganisms usually reflect the most favorable environmental conditions for growth (Fagerbakke et al., 1996).

With all the above, AD is tightly dependent on the availability and optimal supply of nutrients for methanogens, as the most sensitive step, though the optimum nutrient concentration is difficult to determine (Kayhanian et al., 1995). Nutritional deficiencies may result in the VFA accumulation and eventual digester acidification (Bryant, 1979; Demirel et al., 2011; Kayhanian et al., 1995). Depending the relative AD requirements (i.e. in orders of magnitude), nutrients are divided into macro-nutrients (i.e. C and N), micro-nutrients (i.e. S, P and Fe) and trace elements (TE).

# 2.5.1 Macro-Nutrients

Carbon (C) and nitrogen (N) are the main substrates for microorganisms. Thus, the carbon-to-nitrogen ratio (C:N) is one of the most important operational variables in AD, since low N substrates (high C:N) result in an inefficient conversion of the organic matter, while an excess of N (low C:N) potentially results on AD inhibition by NH<sub>3</sub> accumulation (Gerardi, 2003; Kayhanian et al., 1994). An optimum C:N ratio for AD microorganisms is around 20 - 30 kmol/kmol (Brown et al., 2013). However, the chemical oxygen demand (COD)-to-nitrogen ratio (COD:N) and/or the anaerobically biodegradable COD (CODbd)-to-nitrogen ratio (CODbd:N) are more appropriate to be evaluated than the C:N ratio in AD, since not all the C is available for biomass uptake – C can be also present as inorganic carbon or highly-recalcitrant compounds (e.g. lignin) – in contrast to the N content (Kayhanian et al., 2007). Both the optimum C:N and COD:N ratios depend on the OS characteristics (i.e. BD) and the operational parameters (e.g. retention time) (Henze et al., 1997).

#### 2.5.2 Micro-Nutrients

Phosphorous (P), sulfur (S) and iron (Fe) are the main micro-nutrients for AD. P is a key element in nucleic acids and phospholipids, while S is included in the AA cysteine and methionine, as well as a great range of vitamins (Madigan et al., 2012). Meanwhile, Fe is present in many enzymes mainly as catalytic center. Among these elements, methanogens require S and Fe in higher quantities than the rest of AD microorganisms (Gerardi, 2003; Henze et al., 1997). Furthermore, iron addition either as Fe, Fe<sup>2+</sup> or Fe<sup>3+</sup> might be used to precipitate hydrogen sulfide (H<sub>2</sub>S) to mitigate inhibition in AD and/or to avoid a high H<sub>2</sub>S concentration in biogas (Drosg, 2013; Fermoso et al., 2015).

Anaerobic microorganisms also require sodium (Na<sup>+</sup>), potassium (K<sup>+</sup>), magnesium (Mg<sup>2+</sup>), and calcium (Ca<sup>2+</sup>) for growth, though these micro-nutrients are normally present in adequate amounts in organic substrates (Chen et al., 2008; Rittman et al., 2001). Importantly, the calcium (Ca<sup>2+</sup>) and magnesium (Mg<sup>2+</sup>) alongside phosphate (i.e. H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, HPO<sub>4</sub><sup>2-</sup>, PO<sub>4</sub><sup>3-</sup>), sulfide (i.e. HS<sup>-</sup>, S<sup>2-</sup>), iron (i.e. Fe<sup>2+</sup>, Fe<sup>3+</sup>), and carbonate (i.e. HCO<sub>3</sub><sup>-</sup>, CO<sub>3</sub><sup>2-</sup>) ions dominate the physical-chemical mechanisms (i.e. ion pairing and precipitation) in AD (Batstone et al., 2015; Callander et al., 1983; Fermoso et al., 2015; Gerardi, 2003).

#### 2.5.3 Trace Elements

TE play a crucial role in the metabolism of anaerobic microorganisms, mainly as catalytic centers in enzymes (Banks et al., 2012). Methanogens are associated to specific requirements of essential TE as nickel (Ni), cobalt (Co), molybdenum (Mo) and selenium (Se) (Deublein et al., 2008; Zupančič et al., 2011). Therefore, TE are often added to AD reactors to minimize the risk of nutrient deficiency and bioprocess failure. The TE addition can decrease the VFA content, increase the methane yield and/or the biomass concentration in AD (Lindorfer et al., 2012). However, the specific AD requirements for TE are not yet fully understood, since the bioavailability of TE might be related to complexation and/or precipitation mechanisms, among other highly-complex biochemical and physical-chemical processes (Banks et al., 2012; Callander et al., 1983).

#### 2.6 Inhibitory Substances

Some of the elements/compounds required as nutrients for AD might show inhibitory effects due to an excessive accumulation in the digester. Among the most relevant AD

inhibitors are the free ammonia (NH<sub>3</sub>), H<sub>2</sub>S, VFA, LCFA (i.e. palmitic and stearic acids), inorganic cations (i.e. Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>) and anions (i.e. Cl<sup>-</sup>, F<sup>-</sup>, PO<sub>4</sub><sup>3-</sup>), and complex organic compounds (e.g. alcohols, phenolic rings, pesticides) (Chen et al., 2008).

Inhibitory substances are normally associated to the OW composition and may lead to VFA accumulation and even reactor failure. For example,  $H_2S$  results from protein degradation and from OW showing a high content of sulfate ( $SO_4^{2-}$ ) (Higgins et al., 2006). Therefore, the risk of AD inhibition is also substrate-specific. On the other hand, inhibitors can be also introduced (un)intentionally in AD. For example, sodium-containing substances (i.e. NaHCO<sub>3</sub>) are usually added to AD as a source of pH buffering, though an excessive Na<sup>+</sup> accumulation may result in methanogenic inhibition (Chen et al., 2008; Feijoo et al., 1995).

Importantly, the inhibitory potential of individual substances can be partially and/or totally compensated by the biomass acclimation, the right combination of operational variables (i.e. pH, T, co-digestion), and/or an adequate reactor design. Some of the most common strategies to minimize inhibition in AD are the reactor content dilution or the addition of mitigating substances to reduce the inhibitory concentration, the biomass immobilization using carrier materials to shield microorganisms against inhibitory 'shocks', and/or the use of a longer retention time (RT) inside the reactor to promote methanogenic adaptation (Chen et al., 2008; Drosg, 2013; Kayhanian et al., 2007; Rajagopal et al., 2013).

#### Ammonia Inhibition

Ammonia is the main inhibitor in AD of OFMSW, but also of many other OW, such as animal manure or slaughterhouse waste. The ammonia buildup affects all the microorganisms within AD, but particularly the acetoclastic and hydrogenotrophic methanogens. A high total ammonia nitrogen (TAN) results in a reduced biogas yield, a progressive VFA accumulation, and/or an eventual digester failure (Angelidaki et al., 1993; De Vrieze et al., 2012; Drosg, 2013; Jokela et al., 2003). Ammonia inhibits AD microorganisms either by the ammonium ion (NH4<sup>+</sup>) blocking essential enzymes and/or by free ammonia nitrogen (NH<sub>3</sub>) diffusing passively into the cell, and causing proton (H<sup>+</sup>) imbalance or potassium (K<sup>+</sup>) deficiency (Astals et al., 2018; Riggio et al., 2017; Sprott et al., 1986). Nonetheless, NH<sub>3</sub> is considered the most important source of inhibition, since digesters showing an NH<sub>3</sub> concentration  $\geq 1$  g N/L are often unable to efficiently convert organic substances into biogas (Gallert et al., 1997; Jewell et al., 1999; Kayhanian, 1999).

Acetoclastic methanogens might be more sensitive than hydrogenotrophic methanogens to high NH<sub>3</sub> concentrations, though some controversy still exists. Moreover, an excessive NH<sub>3</sub> accumulation eventually affects all the degradation pathways in AD, either by direct NH<sub>3</sub> inhibition and/or by intermediate (i.e. VFA, H<sub>2</sub>) accumulation (Angelidaki et al., 1993;

Banks et al., 2012; Poggi-Varaldo et al., 1997; Vavilin et al., 2008; Westerholm et al., 2016). For example, high concentrations of propionic, butyric and valeric acids are normally observed when operating AD at high ammonia contents (i.e.  $TAN \ge 4 \text{ g N/L}$ ). The eventual TAN or NH<sub>3</sub> concentration showing inhibition in AD depends on the microbial community/adaptation, the OW characteristics, and the operational parameters (Fricke et al., 2007; Rajagopal et al., 2013). For example, the thermophilic microorganisms (i.e. T = 55 °C) might tolerate as much as twice the NH<sub>3</sub> concentration of the mesophilic (i.e. T = 35 °C) counterparts (Gallert et al., 1997).

## 2.7 Main Operational Parameters

The most important operational parameters are BD, T, pH, TS, OL, and RT. All these parameters strongly determine the stability and economy of AD. Moreover, these parameters are tightly interrelated among themselves but also to the OW characteristics, and the main inhibitors in AD. For example, both pH and T define the NH<sub>3</sub> and H<sub>2</sub>S concentration [Figure 2.3]. On the other hand, the operational variables are also tightly related to the reactor design, the maximum methane yield, and the overall OW stabilization (Karthikeyan et al., 2013; Kothari et al., 2014; Mata-Álvarez, 2003). Therefore, no general rules are available for AD design and operation, since a specific tradeoff must be found among the operational parameters for each particular substrate or mixture of substrates (Christensen, 2011).



Figure 2.3 Concentration of hydrogen sulfide  $(H_2S)$  – left panel – and free ammonia nitrogen  $(NH_3)$  – right panel – as a function of temperature (T) and pH

#### 2.7.1 Anaerobic Biodegradability

AD reduces the organic content yielding a volatile solid (VS; i.e. 50 - 80 %) and/or a COD (i.e. 60 - 70 %) content lower than the original OW. Thus, BD assesses the AD potential to reduce the organic content of an OW in terms of added VS and/or COD. In this line, BD strongly determines the suitability/economy of AD for OW treatment. Three main indicators of BD in AD are the VS/TS ratio, the methane yield (i.e. NmL CH<sub>4</sub>/g VS), and/or the quotient between CODbd and the total COD. The biomethane potential (BMP) test assesses the maximum methane yield of an OW. The biodegradability rate determines treatment length and/or the risk of overload in AD. A more complete description of these indicators and the evaluation methodologies can be found, for example, in (Angelidaki et al., 2004; Drosg, 2013; Holliger et al., 2016; Lissens et al., 2001; Mata-Álvarez, 2003; Rittman et al., 2001; Tchobanoglous et al., 2002).

BD is also related to the need for OW pretreatment. Some OW require pre-treatments to enhance the BD, eventually increasing the overall treatment costs. For example, since the complex polymeric structure of lignin prevents the enzymatic attack to the cellulose and hemicellulose fractions, lignocellulosic materials (e.g. straw, wood chips) normally require particle size reduction and/or other physical-chemical pretreatments (e.g. acid hydrolysis) to enhance the biodegradability during AD (Barakat et al., 2013; Barlaz et al., 1990; Monlau et al., 2012).

#### 2.7.2 Retention Time and Organic Load

RT is a measure of the AD treatment length. The AD reactor design and operation needs to ensure an adequate RT for the methanogenic growth. Noteworthy, methanogenic doubling time is around 20 days, though it may extend considerably in the presence of inhibitory compounds (Drosg, 2013; Henze et al., 1997; Jokela et al., 2003; Kayhanian et al., 1994). RT should also ensure a maximum biogas yield and an adequate OW stabilization in terms of organic removal (i.e. VS, COD, VFA), while minimizing the cost of digester heating and digestate disposal. In batch reactors, RT is the total AD duration. In continuous reactors, the feedstock is continuously loaded, while a similar amount of digestate is removed to maintain constant the reactor content volume. Thus, the hydraulic retention time (HRT) assesses the average time a water particle remains inside a continuous reactor. Whether the solid digestate is subsequently recovered (e.g. by settling) and recirculated within the continuous reactor, the solid retention time (SRT) is extended compared to the HRT. This operation is known as decoupling, and permits to increase the biomass retention inside the reactor. In both batch and continuous operation, the re-use/recirculation of

digestate permits to reduce the reactor size, preventing overload and speeding up the organic conversion.

OL is understood as the OW fed per unit of reactor volume, and is a measure of the AD performance and/or capacity to treat a particular OW. Therefore, OL must be maximized to enhance the process economy. However, an excessively high OL – higher than the maximum OL methanogens are able to withstand – may result in overload, as mentioned before. In batch systems, both the initial OL (i.e. kg VS/m<sup>3</sup>) and/or the inoculum-to-substrate ratio (ISR; i.e. g VS/g VS) need to be adapted to the specific substrate (Schievano et al., 2010). In continuous reactors, the organic loading rate (OLR) is normally assessed as the daily average organic mass entering in the system per unit of reactor content volume (e.g. kg VS/m<sup>3</sup>·d). The size of a continuous reactor is determined by the HRT and OLR that ensure a desired organic removal. Noteworthy, both the feeding and/or recirculation patterns are crucial to determine the microbial community in AD (De Vrieze et al., 2015; Kayhanian et al., 2007).

## 2.7.3 Temperature

AD biochemistry is favored within four main T ranges: psychrophilic (i.e. -4 - 10 °C); mesophilic (i.e. 10 - 40 °C); thermophilic (i.e. 40 - 60 °C); and hyper-thermophilic (i.e.  $\geq$  60 °C) (Higgins et al., 2006; Madigan et al., 2012). A higher T is normally associated to enhanced biochemical rates, though a T greater than 60 °C decreases the AD efficiency. T also influences all the physical-chemical mechanisms in AD (i.e. precipitation, gas-liquid transfer). Moreover, T determines the overall AD economy by affecting the reactor operation (i.e. heat exchange) and the eventual digestate properties (i.e. viscosity).

Mostly mesophilic (i.e. T = 35 °C) and thermophilic (i.e. T = 55 °C) conditions are used at industrial scale. The main advantages of thermophilic in contrast to mesophilic AD include higher biogas yields, biomass production, pathogen destruction and organic solids removal efficiencies. Other thermophilic advantages include the faster hydrolysis, less reactor foaming, reduced effluent viscosity, and better digestate dewaterability (Angelidaki et al., 2006; Hartmann et al., 2006; Khalid et al., 2011; Mata-Álvarez, 2003; Moen et al., 2003). Moreover, thermophilic is considered superior to mesophilic AD at industrial scale, since more energy is produced than consumed (Cecchi et al., 1991; Lepistö et al., 1995; Pavan et al., 2000). However, the main disadvantages of thermophilic AD include the higher chances of NH<sub>3</sub> inhibition, the difficulties of process startup, the process instability, particularly due to the 'chronically' high propionic acid concentrations, and the increased odor emission (Angelidaki et al., 2006; Kayhanian et al., 2007; Zitomer et al., 2008). Summarizing, the operational T requires a tradeoff between the biochemical aspects and the overall AD economy.

#### 2.7.4 pH, Buffering Capacity and Alkalinity

pH, the buffering capacity and alkalinity are three of the most important indicators of the AD performance, and they are tightly interrelated among themselves: pH is the negative logarithm of the proton ( $H^+$ ) concentration, pH =  $-\log_{10}([H^+])$ ; the buffering capacity is the potential of an AD solution to withstand changes in pH; and alkalinity (ALK) is a measurement of the buffering capacity, particularly against  $H^+$  addition.

pH strongly determines the biochemical rates and kinetics in AD mainly due to the effects upon the metabolic enzymes of all microorganisms (Madigan et al., 2012; Rittman et al., 2001). Moreover, the acid-base equilibrium of ionic species depends on the H<sup>+</sup> concentration. In AD, the main ionic species include the inorganic carbon, ammonia, sulfide, phosphate, and VFA. The balance between the protonated and deprotonated species in an acid-base equilibrium in solution is assessed by the acid dissociation constant (K<sub>a</sub>). Table 2.2 shows the main acid-base equilibriums in AD. Noteworthy, the K<sub>a</sub> logarithm shows pH units,  $pK_a = -log_{10}(K_a)$ . Therefore, pH is tightly linked also to all the physical-chemical mechanisms (i.e. acid-base dissociation, ion pairing and precipitation) in AD.

| Equilibrium                               | Protonated<br>Species                                                    |                   | Proton                              |   | Deprotonated<br>Species                                                              | pKa<br>(20 °C) | рК <sub>а</sub><br>(35 °С) | рК <sub>а</sub><br>(55 °С) |
|-------------------------------------------|--------------------------------------------------------------------------|-------------------|-------------------------------------|---|--------------------------------------------------------------------------------------|----------------|----------------------------|----------------------------|
| Acetic Acid/Acetate                       | CH <sub>3</sub> COOH                                                     | $\leftrightarrow$ | $\mathrm{H}^{+}$                    | + | CH <sub>3</sub> COO <sup>-</sup>                                                     | 4.76           | 4.77                       | 4.82                       |
| Propionic<br>Acid/Propionate              | CH <sub>3</sub> CH <sub>2</sub> COOH                                     | $\leftrightarrow$ | $\mathrm{H}^{\scriptscriptstyle +}$ | + | CH <sub>3</sub> CH <sub>2</sub> COO <sup>-</sup>                                     | 4.81           | 4.85                       | 4.93                       |
| Butyric Acid/Butyrate                     | CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> COOH                     | $\leftrightarrow$ | $\mathrm{H}^{\scriptscriptstyle +}$ | + | CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub><br>-COO <sup>-</sup>                 | 4.88           | 4.90                       | 4.95                       |
| Valeric Acid/Valerate                     | CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub><br>-COOH | $\leftrightarrow$ | $\mathrm{H}^{+}$                    | + | CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub><br>-COO <sup>-</sup> | 4.88           | 4.90                       | 4.95                       |
| Carbonic<br>Acid/Bicarbonate              | $H_2CO_3$                                                                | $\leftrightarrow$ | $\mathrm{H}^{+}$                    | + | HCO <sub>3</sub> -                                                                   | 6.39           | 6.33                       | 6.34                       |
| <b>Bicarbonate/Carbonate</b>              | HCO3 <sup>-</sup>                                                        | $\leftrightarrow$ | $H^+$                               | + | CO3 <sup>2-</sup>                                                                    | 10.38          | 10.25                      | 10.15                      |
| Phosphoric Acid/Di-<br>hydrogen Phosphate | $H_3PO_4$                                                                | $\leftrightarrow$ | $\mathrm{H}^{\scriptscriptstyle +}$ | + | $H_2PO_4^-$                                                                          | 2.11           | 2.15                       | 2.20                       |
| Di-/Mono-Hydrogen<br>Phosphate            | $H_2PO_4$                                                                | $\leftrightarrow$ | $\mathrm{H}^{\scriptscriptstyle +}$ | + | HPO <sub>4</sub> <sup>2-</sup>                                                       | 7.21           | 7.17                       | 7.13                       |
| Mono-Hydrogen<br>Phosphate/Phosphate      | HPO4 <sup>2-</sup>                                                       | $\leftrightarrow$ | $\mathrm{H}^{\scriptscriptstyle +}$ | + | PO4 <sup>3-</sup>                                                                    | 12.66          | 12.66                      | 12.66                      |
| Hydrogen<br>Sulfide/Sulfide               | $H_2S$                                                                   | $\leftrightarrow$ | $\mathrm{H}^{\scriptscriptstyle +}$ | + | HS <sup>-</sup>                                                                      | 7.12           | 6.93                       | 6.71                       |
| Ammonium/Ammonia                          | $\mathrm{NH_{4}^{+}}$                                                    | $\leftrightarrow$ | $\mathrm{H}^+$                      | + | $NH_3$                                                                               | 9.39           | 8.92                       | 8.37                       |
| Water Dissociation                        | $H_2O$                                                                   | $\leftrightarrow$ | $H^+$                               | + | OH                                                                                   | 14.15          | 13.64                      | 13.07                      |

Table 2.2 Main acid-base equilibriums in anaerobic digestion

25

A pH decrease in AD indicates VFA accumulation (Drosg, 2013; Kayhanian et al., 2007). VFA accumulation may be related to the presence of inhibitory compounds and/or an imbalance between the growth of methanogens and acidogenic/acetogenic microorganisms, as mentioned before. Importantly, pH  $\leq$  6.5 might result in a strong methanogenesis inhibition (Switzenbaum et al., 1990). Meanwhile, pH  $\geq$  8.0 exacerbates the chances of NH<sub>3</sub> inhibition [Figure 2.3b]. Therefore, industrial digesters are normally operated at pH between 7.3 and 7.5, either by controlling operative parameters (i.e. organic load, retention time) and/or by adding alkaline/buffering substances as hydroxides (i.e. NaOH, Ca(OH)<sub>2</sub>) or carbonates (i.e. NaHCO<sub>3</sub>, Na<sub>2</sub>CO<sub>3</sub>) (Drosg, 2013; Zupančič et al., 2011).

The buffering capacity is associated to all the acid-base equilibria of the aforementioned ionic species [Table 2.2]. Particularly, the inorganic carbon and/or nitrogen contents are the main buffers in AD permitting to maintain the pH within a suitable range for methanogens (i.e.  $7.0 \le pH \le 8.0$ ). The reason is related to the high amounts of these compounds normally found in well-operated digesters, but also to the fact that the pK<sub>a</sub> of bicarbonate (H<sub>2</sub>CO<sub>3</sub>/HCO<sub>3</sub><sup>-</sup>) and ammonia (NH<sub>4</sub><sup>+</sup>/NH<sub>3</sub>) equilibriums is around 6.3 and 8.5, respectively. In the same line, the VFA accumulation potentially shifts the pH towards lower values, since the pK<sub>a</sub> of acetic, propionic, butyric and valeric acids is around 4.9.

ALK is the proton accepting capacity of an AD solution due to the presence of different acid-base buffers (i.e. inorganic carbon, ammonia nitrogen, VFA). The use of ALK is motivated by the difficulties to measure the total carbonate in an AD solution (Moosbrugger et al., 1993). Instead, using the interdependency of the bicarbonate equilibrium with pH, the carbonate concentration can be approximated by acid titration. Among the most implemented methods for alkalinity measurement, Lahav et al. (2002) proposed a double titration of an AD sample to the pH endpoints of 5.75 and 4.30 for the partial/carbonate alkalinity (ALK<sub>P</sub>) and total alkalinity (ALK<sub>T</sub>), respectively. The intermediate alkalinity (ALK<sub>I</sub>) is the difference between ALK<sub>T</sub> and ALK<sub>P</sub>, and roughly indicates the concentration of VFA in the digester. The experimental measurement of ALK is also subjected to important uncertainties in AD. However, the alkalinity ratio between ALK<sub>I</sub> and ALK<sub>P</sub> is an outstanding indicator of the VFA accumulation and loss of carbonate buffering capacity in AD (Drosg, 2013; Gerardi, 2003).

# 2.7.5 Total Solid Content

Three main AD strategies are differentiated according to the operative TS content: 'wet' (W-AD; i.e. TS < 10 %), 'semi-solid' (i.e.  $10 \le TS < 20$  %) and 'dry' or 'solid-state' (SS-AD; i.e. TS  $\ge 20$  %) (Abbassi-Guendouz et al., 2012; De Baere et al., 2013). High-solids anaerobic digestion (HS-AD) includes the two latter cases. The operational TS content in AD is strongly determined by the OW being treated (i.e. initial TS and biodegradability). Therefore, W-AD is mostly used for liquid wastes (i.e. WWS), while HS-AD is a more common strategy to treat high-solid wastes (i.e. OFMSW and lignocellulosic biomass) (Henze et al., 1997; Tchobanoglous et al., 2002). The operational TS is also influenced by the inhibitory content, since counteracting measurements might be needed to reduce inhibition (e.g. dilution), as mentioned before. Moreover, a high TS content might be inhibitory itself at concentrations around 40 % TS, as a consequence of water deficiency and/or a compromised mass transfer (De Baere et al., 1984a; Staley et al., 2011). Importantly, the specific role of a high TS upon the biochemistry is not yet fully understood (Xu et al., 2015).

The above characteristics of HS-AD (i.e. substrate, inhibition and TS content) can be extrapolated to high-solids dark fermentation (HS-DF) (Motte et al., 2015). However, due to the fact that DF is a more complex biotechnology than AD, as previously highlighted in section 2.3. Fortunately, the further understanding about HS-AD will doubtless contribute towards the simultaneous understanding about HS-DF, due to their close bio-physical-chemical interrelationship. At this point, it is important to mention that both acidified and non-acidified zones might co-exist within HS-AD reactors (Staley et al., 2011; Xu et al., 2014), being therefore crucial to understand the transitional pathways from the intermediate biochemical steps (i.e. acidogenesis/acetogenesis) to methanogenesis, in order to enhance the HS-AD performance. Furthermore, it must be noted that the coupling between both biotechnologies (i.e. HS-DF and HS-AD in tandem) might allow the recovery of renewable energy (i.e.  $H_2 + CH_4$ ) and nutrients (i.e. N, P), alongside other valuable organic intermediates (i.e. VFA), enhancing considerably the overall economy of the OW treatment.

#### 2.8 High-Solids Anaerobic Digestion

HS-AD was conceived to treat solid wastes 'as such' (or 'as received'), minimizing the waste pretreatment and dilution, and reducing the overall treatment costs (Jewell et al., 1981). Since the HS-AD reactor design and operational parameters are strongly influenced by the OW used as feedstock, HS-AD is adequate to treat organic wastes with TS  $\geq$  15 %, including OFMSW, GW, FW, AW and IW (Bolzonella et al., 2006; Brown et al., 2013; Liao et al., 2014; Lissens et al., 2001; Oleszkiewicz et al., 1997). In this line, HS-AD of OFMSW is operated within a range between 10 and 50 % TS (Brown et al., 2013).

HS-AD shows many advantages in comparison to W-AD including the possibility to use a smaller reactor, reduce the water addition and heating requirements, increase the organic conversion rate, maximize the organic load, minimize the substrate pre-treatment, and reduce the need for digestate dewatering and digestate post-treatment (Benbelkacem et al., 2015; De Baere, 2006; De Baere et al., 1984a; Drosg, 2013; Jewell et al., 1981; Kayhanian et al., 2007). In short, HS-AD permits to reduce the initial investment and operational costs of the OW treatment facilities. Moreover, HS-AD is more robust and flexible than W-AD in terms of operational variables (i.e. TS and organic load), due to the higher biomass concentration, simpler design, and absence of reactor stirrer (Cysneiros et al., 2012; Lissens et al., 2001). Similarly, other operational problems such as settling, foaming or flotation, normally encountered during W-AD, can be circumvented at much higher TS contents (Brown et al., 2013).

However, some drawbacks limit also the applicability of HS-AD, as the reduced kinetic rates and/or reduced methane yield associated to mass transfer effects, the need for longer retention times, the higher requirements for pumping and mixing of high-solid substrates, and the process instability (Benbelkacem et al., 2015; Climenhaga et al., 2008; Lei et al., 2015). Particularly, HS-AD instability is associated to the risk of reactor overload and/or the buildup of biochemical inhibitors. The risk of overload requires to increase the digestate re-use/recirculation, reducing the reactor utilization efficiency, and increasing the overall treatment costs (Brown et al., 2013; De Baere, 2006). On the other hand, the organic degradation in HS-AD potentially lowers the C:N ratio exacerbating the chances of NH<sub>3</sub> inhibition (Pognani et al., 2015). The stability issues still limit a wider acceptance/implementation of HS-AD at industrial scale, particularly for OFMSW treatment (Schievano et al., 2010). Therefore, increasing the HS-AD robustness against instability is crucial to enhance the industrial applicability of HS-AD.

## 2.9 The Role of Water Deficiency and Substrate Complexity

The two main sources of HS-AD instability (i.e. overloading and NH<sub>3</sub> inhibition) are common for any OW used as feedstock, mainly because of the low 'free' water available as TS increases. Therefore, understanding the main biochemical and physical-chemical processes occurring as water is reduced becomes compulsory to optimize HS-AD. In particular, water deficiency affects the soluble (substrates, nitrogen and other nutrients) concentration, the buffering capacity, and the liquid-gas transport processes in HS-AD.

A simple example might help to understand the effects of decreasing the water content in HS-AD. Consider a solution composed of solids, ammonia, bicarbonate and water with masses S, A, B, and W, respectively. The mass balance is: S + A + B + W = 1. Assume that these compounds have the same density. S is relatively lower than W, while A and B are much lower than S – case of HS-AD. In such a system, the molal concentration of ammonia
and bicarbonate are A/W and B/W, respectively, while TS can be approximated as: (S + A + B)/(S + A + B + W). Therefore, increasing the TS content by increasing S to S', while accordingly reducing W to W', the new concentration of ammonia and bicarbonate are A/W' and B/W', respectively. Since A/W < A/W' and B/W < B/W', both the buffering capacity and the ammonia inhibition in HS-AD were affected without modifying neither the ammonia, nor bicarbonate content. A schematic representation of this example is shown in Figure 2.4.



Figure 2.4 Schematic representation of total solid increase – left panel – and total solid concentration effect – right panel – in high-solids anaerobic digestion

The above example highlights the importance of the water deficiency to increase the solute concentration in HS-AD, in contrast to 'wet' AD (i.e.  $TS \le 5$  %). Equation 2.1 shows the apparent concentration [S<sub>App</sub>] of a soluble compound "S" in HS-AD. Unfortunately, HS-AD is much complex than the above example and many other mechanisms must be taken into account to correctly understand the HS-AD biochemistry and physical-chemistry. These mechanisms include strongly 'non-linear' effects upon the physical-chemistry, rheology, biodegradability, biochemical rates, and the buildup of inhibitory compounds, when increasing the TS content.

$$[S_{App}]\left(\frac{kg \ Solute}{kg \ Solvent}\right) = \frac{[S]\left(\frac{kg \ Solute}{kg \ Global}\right)}{(1 - TS)\left(\frac{kg \ Solvent}{kg \ Global}\right)}$$
(2.1)

#### 2.10 The Importance of the Substrate Composition in HS-AD

OFMSW is likely the most complex substrate for HS-AD due to the solid nature, elevated biodegradability, and high nutrient content. The OFMSW composition varies among sources, though the biodegradability and nitrogen content is always high [Table 2.1]. In this line, no operational parameters can be reliable determined for HS-AD of OFMSW. For example, ISR is not a useful parameter to predict HS-AD acidification in batch using OFMSW as a substrate (Schievano et al., 2010). Instead, ISR – and the OL in general – depend on the OW biodegradability rate, that is unknown *a priory*, but also many other parameters as, for example, the alkalinity, microbial adaptation or biochemical activity, and/or the presence of inhibitory compounds. NH<sub>3</sub> inhibition is another important burden when operating HS-AD of OFMSW (De Baere et al., 2013; Kayhanian, 1999). The high risk of acidification and/or inhibition implies that HS-AD of OFMSW needs to be normally operated within 'conservative' limits, at the expense of the process economy.

Many chemical processes occur simultaneously in HS-AD solutions (i.e. ion interaction, ion pairing, precipitation, mineral adsorption, solubilization). As the concentration gradually increases, highly 'non-linear' effects among those processes are exacerbated. This is known as 'non-ideal' bio-physical-chemistry and is often the case of HS-AD for OFMSW treatment due to the high organic concentration (Batstone et al., 2012; Solon et al., 2015). Therefore, an adequate bio-geochemical approach is required to correctly understand HS-AD of OFMSW. Furthermore, since HS-AD of OFMSW includes solids, liquids and gases, hydrodynamic/rheological effects need also to be considered, since these strongly affect the liquid-gas transfer, but also the whole set of biochemical and physical-chemical mechanisms. To end up, studying a highly-complex substrate as OFMSW might permit to extrapolate the gathered knowledge to other high-solids substrates, showing either high biodegradability, a solid or 'semi-solid' nature, and/or high risk of NH<sub>3</sub> inhibition (e.g. agricultural waste, manure).

### 2.11 Rheology Complexity and Mass Transfer Effects

The HS-AD rheology and transport processes are considerably more complex than in W-AD. Thus, HS-AD rarely takes place in a homogeneous medium due to the high viscosity, the particular rheology of the solid or semi-solid substrate, and the absence of reactor stirrer (García-Bernet et al., 2011; Rivard et al., 1990). Instead, an important spatial variability can be often observed within HS-AD reactors, where an organic solid is being biochemically degraded, a liquid solvent percolates through the porous matrix by gravity, and biogas flows upwards due to the lower density ( $\rho$ ). Moreover, depending the specific weight ( $\rho$ s), porosity

( $\epsilon$ ) and/or structural properties of the different solid compounds, both flotation and/or sedimentation effects might occur in 'semi-solid' HS-AD (i.e.  $10 \le TS < 20$  %).

Importantly, as TS increases, the diffusive transport mechanisms in HS-AD rise in importance, in contrast to convective mechanisms (Bollon et al., 2011). Noteworthy, the diffusive transport is considerably slower than the convective transport, consequently lengthening the HS-AD degradation. Moreover, above the SS-AD threshold (i.e.  $TS \ge 20$  %), the solid matrix porosity ( $\varepsilon$ ) becomes partially or totally filled with biogas and the HS-AD degradation further slows down, since diffusion rates in gases are various orders of magnitude slower than in water (Bollon et al., 2013; Xu et al., 2014). Figure 2.5 shows an example of the porosity dynamics as a function of TS in HS-AD.



*Figure 2.5 Example of the total liquid, including the global, liquid-filled and gas-filled porosities, as a function of increasing total solids in high-solids anaerobic digestion* 

Diffusive mechanisms are beneficial to avoid acidification of all the methanogenic centers in case of HS-AD overload (Vavilin et al., 2005; Xu et al., 2014). However, the heterogeneous HS-AD medium might also prevent the access of microorganisms to great portions of the substrate, reducing the methane yield and potentially compromising the adequate organic removal. Therefore, digestate recirculation is normally used in HS-AD to partially homogenize the digester and speedup the biochemical rates (De Baere et al., 1984b; Lissens et al., 2001).

The complex hydrodynamics/rheology in HS-AD, where both convective and diffusive transport mechanisms need to be jointly considered, also affect biochemistry. Particularly, the organic degradation occurs either in the liquid body and/or the solid surface in HS-AD, being associated to different pathways/mechanisms in these two mediums (Kothari et al., 2014; Martin et al., 2003). More in particular, during SS-AD, the spatially-distributed methanogenic aggregates serve as initiation centers from where the organic degradation expands (Martin et al., 2003; Staley et al., 2011). This is known as the frontier hypothesis. In such systems, both methanogenic (i.e. AD) and acidogenic (i.e. DF) environments, characterized by high and low pH values, respectively, simultaneously co-exist.

### 2.12 High-solids Anaerobic Digestion Reactors

HS-AD reactors share a simple design in absence of stirrer, together with low construction and operational costs (Karthikeyan et al., 2013; Kothari et al., 2014; Patinvoh et al., 2017). Two main HS-AD reactor configurations are predominantly used: batch and plug-flow type [Figure 2.6]. Within this classification, many different HS-AD setups are commercialized based on minor differences in the design and/or operation strategy. The number of stages and the operational TS content are crucial variables to minimize the cost and enhance the HS-AD performance (Lissens et al., 2001). In general, multistage reactors increase the stability of HS-AD, though also increase the operational costs. In this line, one phase systems are clearly predominant in HS-AD (Kothari et al., 2014; Lei et al., 2015; Mata-Alvarez et al., 2000).



Figure 2.6 Schematic representation of high-solids anaerobic digestion reactors

## 2.12.1 Batch-Type (Leach-Bed) Configuration

An industrial batch reactor, also known as garage-type reactor, consist of a sealed chamber with a perforated floor, where a mixture of organic waste and previous operation digestate is discontinuously loaded/unloaded [Figure 2.6a]. Batch reactors are highly influenced by the unfavorable mass transfer properties of the high-solids medium (Lei et al., 2015). However, the relatively simple design permits to use OW with great amounts of lignocellulosic (i.e. paper, cardboard, wood) and/or inert materials (i.e. plastic bags, stones, glass and metals) (Kothari et al., 2014; Pognani et al., 2015). To reduce the initial mixture heterogeneity and to enhance the overall waste degradation in absence of external mixing, the liquid percolate (leachate) flowing out through the perforated floor is collected and continuously recirculated to the top of the solid mixture.

The high risk of acidification is one of the main drawbacks of the batch configuration. Meanwhile, the initial conditions (i.e. ISR, TS and  $\varepsilon$ ) in these systems are crucial to minimize the risk of overloading and to ensure an adequate percolation (Schievano et al., 2010; Ward et al., 2008). The initial conditions also influence to a great extent the overall treatment economy. Thus, the initial batch mixture usually contains around 40 - 50 % (v/v) spent digestate as a source of methanogenic inoculum, whereas bulking/lignocellulosic materials might be added to the substrate-inoculum mixture to enhance the porosity (Brown et al., 2013; Di Maria et al., 2012) However, these last two strategies considerably reduce the operational AD volume. To circumvent this drawback, many industrial (batch) systems are usually operated in parallel, recirculating the leachate from previously-operated reactors to recently-set reactors as a source of alkalinity (Lissens et al., 2001). Simultaneously, the high-VFA-content percolate of newly-set reactors is recirculated to more mature reactors. This

sequential batch operation minimizes the overall risk of acidification and speeds up the OW treatment.

Noteworthy, batch-type reactors simply consisting of a sealed bottle and a gas output might be also used at laboratory-scale, due to the simplicity of the design and operation. Nonetheless, these setups might suffer from mass-transfer limitations as TS increases, due to the particular rheology of high-solid substrates and the absence of stirrer, as mentioned before and also explained in Chapter 3. In either case, the laboratory-scale sealed bottles can be considered as an adequate compromise between experimental setup complexity and informativeness, and as a previous step to the implementation of most complex leach-bed reactors.

# 2.12.2 Continuous-Type (Plug-Flow) Configuration

Continuously-fed reactors are normally preferred in HS-AD due to the enhanced conversion rates. In this configuration, a plug-flow type might be more adequate than a continuous stirred tank reactor (CSTR), since methanogens at the opposite side of the feeding port (within a plug-flow type) are better shielded in case of overload (Vavilin et al., 2004). In these HS-AD reactors, the volumetric effluent must be also relatively smaller than the volumetric influent to compensate the organic removal and maintain constant the reactor volume and HRT (Kayhanian et al., 1994; Rivard et al., 1990).

In the vertical plug-flow configuration, OW is thoroughly mixed with digestate obtained at the bottom of the reactor, and then fed to the reactor top [Figure 2.6b]. In absence of reactor stirrer, some vertical digesters use additionaly the recirculation of biogas as a source of partial mixing. In the horizontal configuration, OW is fed in one side of the reactor, previously mixed with digestate obtained from the opposite side [Figure 2.6c]. In such configuration, a series of rotating paddles degas the reactor content and minimize stratification. Some examples of inclined configurations also exist, aiming to simultaneously benefit from the main advantages of the vertical and horizontal configurations. All these reactors can be fed with OW showing a TS content up to 40 - 50 %. However, due to clogging, sedimentation, and inhibitory problems, the operational TS content might be substantially lower (i.e. 20 - 30 %) (Edelmann et al., 2005; Kothari et al., 2014; Lissens et al., 2001).

#### 2.12.3 Examples of Operative Parameters at Industrial Scale

Table 2.3 contains some of the main comercial applications for HS-AD of OFMSW at industrial scale, their main operative parameters, and their performance in terms of methane yield. Nonetheless, it must be mentioned that the main operational parameters and the overall performance of industrial HS-AD reactors strongly depend on the specific substrate characteristics, which might be slightly different among these applications, as mentioned before, but also on the overall configuration of the OW treatment facilities. For example, the overall HS-AD reactor efficiency and even the operational TS content can be influenced by the existence of pretreatments and/or solid-liquid recirculations. Therefore, the performance of the industrial HS-AD reactor should be always evaluated with care, paying attention to the integral role of the reactor within the industrial facilities. Some examples of guidelines to further evaluate the performance of industrial HS-AD of OFMSW can be found in (Clarke, 2018; De Baere et al., 2013; Drosg, 2013; Edelmann et al., 2005; Hartmann et al., 2006; Karthikeyan et al., 2013; Kothari et al., 2014; Lei et al., 2015; Lissens et al., 2001; Mata-Álvarez, 2003).

| Industrial<br>System | Substrate     | Reactor Type             | T<br>(°C) | OLR<br>(kg<br>VS/m <sup>3</sup> ·d) | RT or<br>HRT<br>(d) | VS<br>Removal<br>(%) | Methane<br>Yield<br>(Nm <sup>3</sup> /kg<br>VS <sub>removed</sub> ) |
|----------------------|---------------|--------------------------|-----------|-------------------------------------|---------------------|----------------------|---------------------------------------------------------------------|
| DiCOM                | SS-OFMSW      | Batch-Type               | 55        | NA                                  | 12                  | NA                   | 0.17-0.44                                                           |
| SEBAC                | OFMSW +<br>GW | Batch-Type               | NA        | NA                                  | 21                  | NA                   | 0.34                                                                |
| BEKON                | Biowaste      | Batch-Type               | NA        | NA                                  | 28-35               | NA                   | 0.17-0.37                                                           |
| ATF                  | Biowaste      | Batch-Type               | NA        | NA                                  | 15-25               | NA                   | 0.10-0.32                                                           |
| KOMPOGAS             | OFMSW         | Horizontal<br>Continuous | 55        | 4.3                                 | 29                  | NA                   | 0.39-0.58                                                           |
| ITDAR                | SS-OFMSW      | Inclined<br>Continuous   | 55        | 2.5-10.6                            | 17-25               | 59-65                | 0.17-0.36                                                           |
| DRANCO               | SS-OFMSW      | Vertical<br>Continuous   | 50-<br>55 | 10-15                               | 20                  | 40-70                | 0.21-0.30                                                           |
| VALORGA              | SS-OFMSW      | Vertical<br>Continuous   | 37-<br>55 | 10-15                               | 20                  | 60                   | 0.21-0.30                                                           |

 Table 2.3 Main Operative Parameters and Performance Examples of the Main High-Solids

 Anaerobic Digestion Applications at Commercial Scale. Adapted from Karthikeyan et al.

 (2013)

NA: Non-available; SS-OFMSW: Source-sorted OFMSW; Noteworthy, 'biowaste' might be also used to express a mixture of OFMSW and GW; while OLR is predominantly used for continuous reactors.

### 2.13 Need for Optimization

HS-AD is a mature technology with many industrial applications available (De Baere et al., 2013). However, the HS-AD design and operation are mainly based on empirical evidence, since the process operation is more complex than W-AD (De Baere, 2006; Guendouz et al., 2010). Therefore, further research is needed to understand the most important operational parameters of HS-AD, in order to enhance the applicability and/or acceptability of these OW treatment technologies. Particularly, further research must focus on understanding the role of TS upon the biochemistry and physical-chemistry, the role of the OW characteristics to maximize the TS content, the risk of overload, and the NH<sub>3</sub> inhibition in HS-AD.

Understanding the high level of interrelationships among the inner HS-AD mechanisms requires a straight but powerful approach that could be summarized as follows: 1) understand the substrate; 2) the geo-physical-chemistry; 3) the biochemistry; and 4) the rheology; and eventually 4) optimize the reactor design and operation. The objective is to enhance the HS-AD robustness against instability, while enhancing simultaneously the process economy. Fulfilling this broad objective would doubtless contribute to the wider acceptance of HS-AD as a remarkable biotechnology to treat OW. To this aim, a robust HS-AD model is required.

# 2.14 HS-AD Models

Mathematical models enhance our understanding about the inner HS-AD dynamics permitting to validate hypotheses, predict the process behavior under different operational conditions, and reveal opportunities for reactor design and optimization (Donoso-Bravo et al., 2011; Kothari et al., 2014; Lauwers et al., 2013). HS-AD models are divided into theoretical, empirical, and statistical models (Xu et al., 2015). Theoretical models (e.g. the Anaerobic Digestion Model No.1, ADM1 (Batstone et al., 2002)) assess simultaneously the various interdependences between solids, liquids, gases, and microorganisms within HS-AD, in a structured way (i.e. substrates-to-products). Empirical models (e.g. Gompertz) consist on mathematical equations than describe the experimental data without the need to understand the inner mechanisms. Statistical models (e.g. neuronal networks) take advantage of the patterns within the experimental data to obtain statistical relationships between the HS-AD inputs and outputs.

The most important aspect of mathematical models is the pursued objective, since the model objective and the experimental data available determine the required model complexity (Batstone, 2006; Batstone et al., 2015; Steyer et al., 2006). Understanding and

minimizing the HS-AD instability is yet strongly required, as mentioned before. Moreover, a HS-AD model must correlate adequately the biogas production with the reactor content mass and TS removal (Richards et al., 1991). For these aims, a theoretical model as ADM1 may be particularly well suited. ADM1 is a structured model gathering together the main biochemical and physical-chemical mechanisms in AD. ADM1 includes 7 microbial, 12 soluble and 12 particulate compounds. The COD flow in ADM1 is shown in Figure 2.7. Thus, ADM1 is particularly useful tool to perform AD dynamic simulations, while allowing the operational analysis and technology development (Batstone et al., 2006; Donoso-Bravo et al., 2011). However, ADM1 was primarily conceived for 'wet' AD (i.e. TS  $\leq$  5%) while some modifications might be required to simulate adequately HS-AD. Importantly, ADM1 can be easily adapted to simulate further mechanism and/or dynamic variables.

Among the ADM1 upgrades required to simulate HS-AD of OFMSW, an adequate 'non-ideal' bio-physical-chemical approach is needed. 'Non-ideality' strongly affects the ionic equilibrium, the pH, and the liquid-gas transfer in AD (Batstone et al., 2015; Tait et al., 2012). Therefore, 'non-ideality' affects also the NH<sub>3</sub> inhibition and risk of overload. However, 'non-ideal' mechanisms were not originally included in ADM1. Accurate HS-AD rheology and mixing regimes might be also needed to predict the biogas production in large scale bioreactors (Batstone, 2006; Van Hulle et al., 2014). Nonetheless, simulating the rheology require to solve the mass balances and transport equations as a function of the spatial coordinates, increasing the model complexity and computational time. Importantly, to keep the HS-AD simulations 'tractable', particularly regarding the great number of variables involved, a complexity reduction is needed during preliminary steps of HS-AD model development. In other words, some HS-AD mechanisms and/or mechanisms need to be 'purposely' disregarded. Noteworthy, a golden rule of structured models is: "the model must be always kept as simple as possible, and only as complex as needed" (Eberl et al., 2006).



Figure 2.7 COD flow in ADM1

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# **Chapter 3**

Assessing the Tradeoff between Total Solids, Inoculum-to-Substrate Ratio and Ammonia Inhibition in High-Solids Anaerobic Digestion Batch Experiments

This chapter has been submitted as:

Pastor-Poquet, V., Papirio, S., Trably, E., Rintala, J., Escudié, R., & Esposito, G. (*submitted*). High-Solids Anaerobic Digestion requires a tradeoff between Total Solids, Inoculum-to-Substrate Ratio and Ammonia Inhibition.

#### Abstract

Seven batch experiments were conducted at 55°C to investigate the effects of increasing the initial total solids (TS) content on high-solids anaerobic digestion (HS-AD) of the organic fraction of municipal solid waste (OFMSW) and beech sawdust. With an inoculumto-substrate ratio (ISR) = 1.5 g VS/g VS and a maximum TS = 19.6 %, mono-digestion of OFMSW showed a methane yield of 174-236 NmL CH<sub>4</sub>/g VS<sub>subs</sub>. With an ISR  $\leq$  1.0 g VS/g VS and a maximum TS  $\leq$  24.0 %, mono-digestion of OFMSW resulted in acidification. Codigestion of OFMSW and sawdust permitted to reduce the ISR to 0.16 g VS/g VS while increasing TS up to 30.2 %, though achieving a lower methane yield (i.e. 117-156 NmL CH<sub>4</sub>/g VS<sub>subs</sub>). At each ISR, a higher TS corresponded a to higher ammonia and volatile fatty acid accumulation. Thus, a 40 % lower methane yield of OFMSW was observed at a NH<sub>3</sub> concentration  $\geq$  2.3 g N/kg and TS = 15.0 %. Meanwhile, the addition of sawdust to OFMSW lowered the nitrogen content, being the risk of acidification exacerbated only at TS  $\geq$  20.0 %. Therefore, the biodegradability of the substrate, as well as the operational TS and the ISR, are closely-interrelated parameters determining the success of methanogenesis, but also the risk of ammonia inhibition during HS-AD.

**Keywords**: HS-AD of OFMSW and Sawdust; Thermophilic; Ammonia Inhibition; Inoculum-to-substrate Ratio; Methanogenesis; Acidification.



### **Graphical Abstract**

# 3.1 Introduction

Anaerobic digestion (AD) is a biochemical treatment technology in which an organic waste (OW) is decomposed to a mixture of gases – mainly CH<sub>4</sub> and CO<sub>2</sub> – known as biogas, and a partially stabilized organic material known as digestate. Biogas shows a high calorific content, while the nutrient-concentrated digestate has the potential to be used as a soil amendment (De Baere et al., 2013). AD takes place by a sequential set of fermentative steps carried out symbiotically by different microbial consortia (Gerardi, 2003). The main AD steps are hydrolysis, acidogenesis, acetogenesis and methanogenesis, while the AD biochemistry lies on a balance between volatile fatty acid (VFA) production by acidogens/acetogens and VFA consumption by methanogens. When an imbalance occurs, VFA and/or H<sub>2</sub> accumulate, potentially leading to AD failure by acidification (i.e.  $pH \le 6.0$ ) (Motte et al., 2014; Staley et al., 2011). Other inhibitory substances may also accumulate during AD, such as free ammonia (NH<sub>3</sub>) and cations (e.g. Na<sup>+</sup>, K<sup>+</sup>) (Chen et al., 2008; Riggio et al., 2017).

Depending on the total solid (TS) content, AD can be operated under 'wet' (i.e. TS < 10 %), 'semi-solid' (i.e.  $10 \le TS < 20$  %) and 'dry' (i.e.  $TS \ge 20$  %) conditions (Abbassi-Guendouz et al., 2012; Pastor-Poquet et al., 2018). High-solids AD (HS-AD) includes the two last cases, and has some advantages such as the use of a smaller reactor volume, and a reduced need for water addition and dewatering operations, enhancing the process economy (André et al., 2018; Kothari et al., 2014). However, HS-AD also shows some drawbacks such as a high risk of reactor acidification by substrate overload, and a reduced mass transfer associated to the low content of free water in the system (Benbelkacem et al., 2015; Bollon et al., 2013; García-Bernet et al., 2011). Moreover, as the TS content is rather high in HS-AD, a lower amount of water is available to dilute potential inhibitors (i.e. NH<sub>3</sub>) than during 'wet' AD.

HS-AD of the organic fraction of municipal solid waste (OFMSW), including food waste (FW) and green/lignocellulosic waste (GW), is widely used. Indeed, the high TS content (i.e. 20-50 %) and the high biodegradation potential of OFMSW are particularly favorable to lower the operational costs of HS-AD (De Baere et al., 2013). In this line, batch systems for OFMSW treatment at industrial scale can be operated up to 40 % TS, provided that leachate is continuously recirculated as a source of microorganisms and partial mixing (André et al., 2018; Riggio et al., 2017).

The operational TS of HS-AD mainly depends on the TS and volatile solid (VS) of the OW, but also its biodegradability under anaerobic conditions, since AD of OFMSW might yield a 30-80 % reduction of the substrate TS (Pastor-Poquet et al., 2018). On the other hand, the presence of lignocellulosic substrates (i.e. GW or paper/cardboard) in OFMSW might

reduce the overall biodegradability rate due to their slower hydrolysis, as well as the chances of NH<sub>3</sub> inhibition in HS-AD due to their lower protein content (Brown et al., 2013; Mancini et al., 2018).

Laboratory-scale batch experiments are normally used to obtain valuable information about the main operating parameters and/or the AD dynamics for a given OW. One of the main parameters is the inoculum-to-substrate ratio (ISR) to be used avoiding acidification. For example, when assessing their maximum methane yield of highly biodegradable substrates (i.e. FW) during a biomethane potential (BMP) test, a relatively high ISR (i.e. 2-4 g VS/g VS) is recommended (Holliger et al., 2016). However, as a sole parameter, the ISR is inadequate to avoid HS-AD acidification (Schievano et al., 2010). Indeed, a given mixture substrate-inoculum sets simultaneously the ISR (i.e. g VS/g VS) and the maximum TS, according to the VS and TS mass balances, respectively. Therefore, adapted combinations of ISR (i.e. 0.25-4 g VS/g VS) and FW:GW ratio (i.e. 0-100 %) are required to circumvent acidification, while maximizing the TS content in HS-AD experiments (Brown et al., 2013; Capson-Tojo et al., 2017; Schievano et al., 2010).

The effects of increasing the initial TS content on HS-AD in laboratory-scale batch tests are not yet fully understood, since a higher initial TS has been reported to reduce the methane yield of substrates such as cardboard (Abbassi-Guendouz et al., 2012) and OFMSW (Forster-Carneiro et al., 2008b; Liotta et al., 2014), but not of lignocellulosic substrates (Brown et al., 2012). Importantly, whether the TS increase lowers the methane yield, the overall HS-AD efficiency decreases, potentially compromising the OFMSW treatment economy (Fernández et al., 2010; Mata-Álvarez, 2003).

This study evaluates the effects of increasing the initial TS content on the methane yield, TS removal and chemical oxygen demand (COD) conversion in HS-AD laboratory-scale batch bioassays at 55°C, using mono-digestion of OFMSW and co-digestion of OFMSW and beech sawdust. Sawdust simulates the addition of biodegradable GW (e.g. branches and leaves) to OFMSW, permitting to stabilize HS-AD at high TS (i.e.  $\geq 20$  %). To maximize TS while avoiding acidification, different ISR and/or co-digestion ratios were used. Furthermore, this study highlights the important interrelationship between the initial conditions (i.e. TS and ISR) and the main AD inhibitors (i.e. NH<sub>3</sub>) in HS-AD of OFMSW, by evaluating the pH, TS, VFA and ammonia dynamics during sacrifice experiments.

# 3.2 Materials and Methods

# 3.2.1 Organic Substrates and Inoculum

OFMSW consisted of a mixture of household waste, restaurant waste, spent coffee collected and GW (i.e. organic soil, small branches and leaves) collected in Cassino (Italy). The wastes were gathered independently during one month while stored in buckets at 4°C, and eventually mixed into a 100 L barrel. In total, 60 kg of waste were collected with an approximated weight proportion of 45, 35, 15 and 5 % (w/w) for household waste, restaurant waste, spent coffee and GW, respectively. The mixed waste was minced twice to a pastry material with a particle size smaller than 5-10 mm by means of an industrial mincer (REBER 9500NC), fully homogenized and stored in 5 L buckets at -20°C, aiming to minimize the composition fluctuations during the experimental period.

To increase the TS content in the batch experiments, 1-2 kg of OFMSW were dried for 7-10 days at 55°C until constant weight right before each experiment. The resulting agglomerate was further minced with mortar and pestle, homogenized to a flour-like material with a particle size  $\leq 2$  mm, and stored in air-tight containers until use. Goldspan<sup>®</sup> beech sawdust with a 1.0-2.8 mm particle size was used as co-substrate.

Three 'wet' and six high-solids inocula were used in this study, since different experiments were started at different periods. All inocula were sampled from a 30 L methanogenic reactor fed with OFMSW under thermophilic (55°C) conditions. Prior to being used in the experiments, all inocula were degassed for 7-10 days at 55°C and subsequently filtered through a 1 mm mesh to remove coarse materials. These inoculums were considered 'wet' since TS was  $\leq 5$  %. To increase simultaneously the TS and ISR of batch experiments, the 'wet' inoculums were centrifuged at 6000 rpm for 10 min with a bench-scale centrifuge (REMI XS R-10M, India), right before each experiment – high solids inoculum. The supernatant was separated and the remaining viscous material was manually homogenized. Finally, micronutrients were added to each inoculum as recommended by Angelidaki et al. (2004).

## **3.2.2 Batch Experiments**

# 3.2.2.1. Experimental Setup

Seven batch experiments were performed to evaluate the effects of increasing the initial TS from 10.0 to 33.6 % in HS-AD. Dried OFMSW and/or sawdust were used as organic

substrates under different mono- and co-digestion conditions [Table 3.1]. Because of availability, experiments were performed in 160 or 280 mL serum bottles (Wheaton, USA), all incubated at 55°C. The different TS were obtained by an adequate combination of substrate, inoculum and distilled water addition. To minimize the occurrence of experimental biases, each bottle contained exactly the same amount of substrate and inoculum, while the amount of distilled water depended on the desired TS. Thus, different medium volumes were obtained within the same set of batch experiments [Table 3.1].

The bottles were sealed with butyl rubber stoppers and aluminum crimps, and flushed with inert gas (helium or nitrogen), before adding 0.2 mL of 10 g/L Na<sub>2</sub>S to guarantee an adequate redox potential (Angelidaki et al., 2004). All batch assays lasted until the gas production was negligible (i.e. < 1 mL/d) during three consecutive measurements. The bottles were manually agitated when the gas production was measured. For each experiment, blank assays were conducted in triplicate to evaluate the biomethane production of the sole inoculum. Blank assays contained the same amount of inoculum, while further distilled water was used to compensate for the absence of substrate [Table 3.1].

| No. | Objective                     | Substrate                        | Inoculum* | ISR<br>(g VS/g VS) | Initial TS Content (%)                       | Substrate<br>Added (g) | Inoculum<br>Added (g) | Water Added<br>(mL)         | water<br>Added to the<br>Blank (mL) | Replicates | Volume<br>(mL) |
|-----|-------------------------------|----------------------------------|-----------|--------------------|----------------------------------------------|------------------------|-----------------------|-----------------------------|-------------------------------------|------------|----------------|
| -   | Study the Main                | 55°C-dried                       |           | 0.50               | 10.2, 12.6, 15.6, 19.2, 23.3,<br>28.3 & 33.6 | 4.4                    | 15                    | 45, 33, 23, 15,<br>9, 4 & 0 | 50                                  | 3          | 280            |
| 2   | Biodegradability              | OFMSW                            | SH        | 1.00               | 9.5, 13.6, 18.4 & 24.0                       | 2.2                    | 15                    | 27, 14, 6 & 0               | 27                                  | 3          | 280            |
| ŝ   | CH <sub>4</sub> vield TS      |                                  |           | 1.50               | 10.8, 13.4, 16.4 & 19.6                      | 1.2                    | 15                    | 13, 8, 3 & 0                | 14                                  | 3          | 160            |
| 4   | Removal and                   | Sawdust                          | W         | 0.04               | 9.8, 14.6, 19.3 & 24.1                       | 6.5                    | 20                    | 39, 18, 7 & 0               | 44                                  | ŝ          | 160            |
| 5   | COD<br>Conversion)            | 55°C-dried<br>OFMSW +<br>Sawdust | HS        | 0.16<br>(1.0:4.0)  | 10.0, 15.0, 20.0, 24.7 &<br>30.2             | 12.5                   | 15                    | 110, 65, 42, 29<br>& 19     | 120                                 | 3          | 280            |
| 9   | Study the Main                | 55°C-dried<br>OFMSW              |           | 1.00               | 15.0                                         | 2.6                    | 20                    | 14                          | 16                                  | 15         | 280            |
| 7   | Dynamics (i.e.<br>TS and VFA) | 55°C-dried<br>OFMSW +<br>Sawdust | SH        | 0.60<br>(1.0:1.1)  | 19.4                                         | 4.2                    | 20                    | 10                          | 13                                  | 15         | 280            |
|     | Determine the<br>Maximum      | OFMSW                            | W         | 2.00               | 2.9                                          | 3.0                    | 50                    | 40                          | 43                                  | 9          | 280            |
|     | Methane Yield<br>(i.e. BMP)   | Sawdust                          | W         | 1.00               | 4.1                                          | 1.0                    | 50                    | 0                           | 0                                   | ç          | 160            |

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| Table 3.1          |

HS: High-solids inoculum; W: 'Wet' inoculum. Parenthesis refer to the ratio between organic fraction of municipal solid waste (OFMSW) and sawdust (i.e. g TS:g TS). \*Inoculums were different for each experimental setup.

#### 3.2.2.2. HS-AD Biodegradability Indicators

Five out of seven batch experiments were aimed to evaluate the effects of increasing the initial TS on the HS-AD methane yield, TS removal and COD conversion, using initial TS contents from 'wet' (i.e. TS = 10 %) to 'dry' conditions (i.e.  $TS \ge 20$  %) [Test 1-5, Table 3.1]. Mono-digestion experiments were run with a homogeneous mixture of dried OFMSW and high-solids inoculum at an ISR of 0.50, 1.00 and 1.50 g VS/g VS, for Test 1, 2 and 3, respectively. The ISR increase resulted in lower initial TS [Table 3.1]. In the fourth experiment (Test 4), HS-AD of sawdust was investigated by using a mixture of beech sawdust and 'wet' inoculum at an ISR = 0.04 g VS/g VS. In the fifth experiment (Test 5), co-digestion of dried OFMSW and sawdust was performed with high-solids inoculum. The OFMSW:sawdust ratio was 1:4 g TS:g TS and the overall ISR was 0.16 g VS/g VS. All TS conditions were evaluated in triplicate.

### 3.2.2.3. Sacrifice Tests

To evaluate the main dynamics (i.e. TS, VFA, ammonia nitrogen and COD conversion) during HS-AD, two batch experiments were performed as sacrifice tests [Tests 6 and 7, Table 3.1]. 15 replicates were used in each test. After measuring the gas volume and composition, a single bottle was emptied and the content was analyzed (i.e. for VS, VFA and ammonia) every 3 to 5 days during the first two weeks, and every 7 to 10 days until the end of the experiment. In Test 6, dried OFMSW was used as the sole substrate in presence of high-solids inoculum. The initial TS and ISR were 15.0 % and 1.00 g VS/g VS, respectively. Test 7 was performed to study the co-digestion of OFMSW and beech sawdust with an initial TS = 19.4 % and an ISR = 0.60 g VS/g VS. The ratio OFMSW:sawdust was 1.0:1.1 g TS:g TS.

# 3.2.3 Biomethane potential of OFMSW and beech sawdust

The individual BMP of the raw OFMSW and beech sawdust at 55°C was estimated according to Angelidaki et al. (2004) and Holliger et al. (2016). The BMP assay with OFMSW was performed in 280 mL bottles using 6 replicates and an ISR of 2.00 g VS/g VS, whereas the BMP of sawdust was assessed in 160 mL bottles using 3 replicates and an ISR of 1.00 g VS/g VS [Table 3.1]. In the BMP test for OFMSW, the distilled water addition served to minimize the chances of ammonia inhibition. In contrast, ammonia build-up was not expected in the BMP test of sawdust, due to the low nitrogen content of this substrate, as shown in next section. The lower biodegradability of sawdust permitted to use also a lower ISR.

### 3.2.4 Physical-Chemical Analyses

The pH and alkalinity were measured right after 1) diluting the (semi-)solid sample with distilled water, 2) homogenization, 3) centrifugation at 6000 rpm for 15 min and 4) supernatant titration to a pH of 5.75 and 4.3 for the carbonate (ALK<sub>P</sub>) and total (ALK<sub>T</sub>) alkalinity, respectively (Lahav et al., 2002). The intermediate alkalinity (ALK<sub>I</sub>) was the difference between ALK<sub>T</sub> and ALK<sub>P</sub>. The TS and VS, total Kjeldahl (TKN) and ammonia nitrogen (TAN), and specific weight ( $\rho_s$ ) analyses were carried out according to the standard methods (APHA, 1999; EPA, 2015).

The density ( $\rho$ ) – containing the air-filled porosity ( $\epsilon$ ) – was approximated using a 1-2 L calibrated cylinder and a ± 0.01 g precision scale. The NH<sub>3</sub> was approximated as in Capson-Tojo et al. (2017). The COD of (semi-)solid samples was determined as described by Noguerol-Arias et al. (2012). The soluble COD (CODs) was determined with the same method by immediately analyzing the supernatant filtered through a 0.45 µm polypropylene membrane. The VFA (acetic, propionic, butyric and valeric acids) analysis of 0.45 µm pre-filtered samples was conducted with a LC-20AD HPLC (Shimadzu, Japan) equipped with a Rezex ROA-Organic Acids 8+ column (Phenomenex, USA) coupled to a 210 nm UV detector. The column was maintained at 70°C with a 0.0065 M H<sub>2</sub>SO<sub>4</sub> mobile phase flowing at 0.6 mL/min.

The biogas production was evaluated with a two-vessel water displacement system. The first vessel contained 4 N NaOH to capture the produced CO<sub>2</sub>, while the second vessel was filled with distilled water to be 'displaced'. Once measured the biogas production, the reactor headspace was sampled with a 250  $\mu$ L pressure-lock syringe for the analysis of the biogas composition in terms of CH<sub>4</sub>, CO<sub>2</sub>, H<sub>2</sub>, O<sub>2</sub> and N<sub>2</sub> with a 3400 GC-TCD (Varian, USA) equipped with a Restek Packed Column. The carrier gas was argon.

## 3.2.5 Calculations

The methane yields obtained in the seven batch experiments, as well as the BMP values for OFMSW and for beech sawdust, were expressed as the normalized methane production (P = 1 bar, T = 0°C), excluding the endogenous methane production of the inoculum, divided by the added substrate VS (VS<sub>subs</sub>). The Dixon's test was applied as recommended by Holliger et al. (2016) to discard any outlier in the batch experiments or BMP tests. The overall methane or hydrogen production at the end of each experiment was expressed as a normalized volume of gas (P = 1 bar, T = 0 °C) measured by water displacement, divided by the VS added (VS<sub>added</sub>) – including the substrate and inoculum. The hydrogen production by the VS removed (VS<sub>removed</sub>) was also calculated in some acidified reactors. The TS removal was the difference between the initial and final TS contents, divided by the initial TS. The global COD conversion included the overall methane and/or hydrogen production and the VFA content at the end of each experiment, divided by VS<sub>added</sub>. In sacrifice tests [Tests 6 and 7, Table 3.1], the progressive COD conversion was evaluated as the produced methane, hydrogen and VFA at a specific time interval, divided by VS<sub>added</sub>. The reactor content volume (V<sub>Global</sub>) for each initial mixture was obtained as  $\sum (M/\rho)$ , being M the mass of each compound in the batch experiments (i.e. inoculum, substrate and water). The liquid-solid volume (V<sub>Real</sub>) for the inoculum-substrate mixture was obtained as  $\sum (M/\rho_s)$ .  $\varepsilon$  was obtained as 1 - V<sub>Real</sub>/V<sub>Global</sub>. In this study, all the initial batch configurations were designed to be porosity free (i.e.  $\varepsilon = 0$ ; V<sub>Global</sub> = V<sub>Real</sub>), since gas reduces the metabolite mass transfer in comparison to liquid media (Bollon et al., 2013).

# 3.3 Results and Discussion

### 3.3.1 Bio-Physical-Chemical Characterization of Substrates and Inoculum

Table 3.2 shows the average composition of the raw OFMSW, dried OFMSW and sawdust. The TS of the raw OFMSW was 26 %, in agreement with reported values for source-sorted OFMSW (Christensen, 2011; Schievano et al., 2010). The TS of the dried OFMSW was 92 %. A relatively lower TAN, CODs/COD and COD/TKN ratios were observed for the dried compared to the raw OFMSW, while the VS/TS was maintained approximately constant and ε increased [Table 3.2]. Therefore, some volatilization of organic material (e.g. VFA, TAN) occurred when drying OFMSW at 55°C. However, drying was an adequate conditioning for assessing the effect of TS increase in HS-AD of raw OFMSW, since the macroscopic composition was maintained relatively constant [Table 3.2]. A similar conditioning was used by Forster-Carneiro et al. (2008a) to increase the TS in HS-AD batch reactors. The TS of beech sawdust was 94 % [Table 3.2], similar to that obtained by Brown et al. (2013) for GW.

The BMP of the raw OFMSW and sawdust at 55°C was  $497 \pm 58$  NmL CH<sub>4</sub>/g VS<sub>subs</sub> [Figure 3.1a] and 161 ± 12 NmL CH<sub>4</sub>/g VS<sub>subs</sub> [Figure 3.1b], respectively, indicating the lower biodegradability of sawdust than of OFMSW under anaerobic conditions. Moreover, reaching the maximum methane yield took a considerably longer for sawdust than OFMSW (i.e. 130 and 56 days, respectively), suggesting also a reduced hydrolysis rate for lignocellulosic substrates (Mancini et al., 2018; Vavilin et al., 2008). The higher standard deviation in the BMP for raw OFMSW was attributed to the waste heterogeneity. The BMP values were equivalent to those observed for source-sorted OFMSW and GW (Brown et al., 2013; Schievano et al., 2010).

The average composition of the 'wet' and high-solids inocula is reported in Table 3.2. Only minor deviations in macroscopic characteristics (i.e. TS and TKN) were observed between 'wet' and high-solids inocula sampled at different times. Centrifugation increased the TS content, and ALK<sub>I</sub>/ALK<sub>P</sub>, COD/TKN and VS/TS ratios compared to the 'wet' inoculum [Table 3.2]. A similar inoculum conditioning was used by Brown et al. (2013) to increase the TS in 'dry' co-digestion. Other inoculum pretreatments to increase TS in HS-AD include inoculum filtration (Liotta et al., 2014) or drying at 105°C (Capson-Tojo et al., 2017), though heating the inoculum at 105°C might result in methanogenesis inhibition (Ghimire et al., 2015).

|                                            | Organic Substrates |                  |               | Inoculum        |                 |  |
|--------------------------------------------|--------------------|------------------|---------------|-----------------|-----------------|--|
|                                            | OFMSW              | Dried OFMSW      | Sawdust       | Wet             | High-Solids     |  |
| TS (%)                                     | $26.2\pm0.1$       | $92.2\pm1.7$     | $93.6\pm0.6$  | $3.1 \pm 1.0$   | $15.6\pm2.0$    |  |
| <b>VS</b> <sup>a</sup> (%)                 | $24.1\pm0.5$       | $85.7\pm1.7$     | $92.9\pm0.3$  | $2.2\pm0.8$     | $12.4\pm1.4$    |  |
| $COD (g O_2/g)$                            | $0.43\pm0.02$      | $1.38\pm0.09$    | $1.16\pm0.00$ | $0.04\pm0.02$   | $0.21\pm0.05$   |  |
| $COD_{S} (g O_{2}/g)$                      | $0.14\pm0.01$      | $0.38 \pm 0.01$  | < 0.01        | N.A.            | N.A.            |  |
| TAN (g N/kg)                               | $1.29\pm0.06$      | $3.45\pm0.09$    | $0.11\pm0.00$ | $3.23\pm0.59$   | $3.24\pm0.65$   |  |
| TKN (g N/kg)                               | $6.50\pm1.50$      | $25.45 \pm 1.12$ | $0.67\pm0.45$ | $4.40\pm0.75$   | $8.66 \pm 1.35$ |  |
| рН                                         | $4.43\pm0.11$      | $4.37\pm0.16$    | $5.65\pm0.06$ | $8.44\pm0.15$   | $8.42\pm0.52$   |  |
| ALK <sub>P</sub> (g CaCO <sub>3</sub> /kg) | N.A.               | N.A.             | N.A.          | $8.13\pm0.99$   | $5.90 \pm 1.34$ |  |
| ALK <sub>I</sub> (g Acetic/kg)             | $0.84\pm0.68$      | $0.67\pm0.62$    | $2.16\pm0.68$ | $4.13 \pm 1.31$ | $3.50\pm1.53$   |  |
| ALK <sub>I</sub> /ALK <sub>P</sub>         | N.A.               | N.A.             | N.A.          | $0.82\pm0.22$   | $0.99\pm0.54$   |  |
| $\rho_{s}\left(g/mL ight)$                 | $1.09\pm0.01$      | 1.43             | 1.30          | 1.00            | $1.08\pm0.02$   |  |
| ρ (g/mL)                                   | $1.08\pm0.00$      | 0.59             | 0.31          | 1.00            | $1.08\pm0.02$   |  |
| 3                                          | $0.01\pm0.01$      | 0.59             | 0.76          | 0.00            | $0.00\pm0.01$   |  |
| <b>VS/TS (%)</b>                           | $92\pm0$           | $93\pm2$         | $99\pm1$      | $71 \pm 1$      | $79\pm2$        |  |
| CODs/COD (%)                               | $32\pm2$           | $27\pm9$         | < 0.01        | N.A.            | N.A.            |  |
| COD/TKN<br>(g O <sub>2</sub> /g N)         | $67 \pm 2$         | $54 \pm 1$       | $1743\pm4$    | $10 \pm 1$      | $24 \pm 1$      |  |
| TAN/TS (g N/kg TS)                         | $4.9\pm0.1$        | $3.7\pm0.1$      | $0.1\pm0.0$   | $103.3\pm0.6$   | $20.7\pm0.6$    |  |
| BMP (NmL CH4/g VS)                         | $497\pm58$         | N.A.             | $161\pm12$    | N.A.            | N.A.            |  |

Table 3.2 Bio-physical-chemical characterization of substrates and inoculum



**Figure 3.1** Cumulative methane production: a) Biomethane potential (BMP) test for the organic fraction of municipal solid waste (OFMSW); b) BMP test for sawdust; c) monodigestion of 55°C-dried OFMSW at an ISR of 1.50 g VS/g VS (Test 3); d) mono-digestion of beech sawdust at an ISR of 0.04 g VS/g VS (Test 4); and e) co-digestion of 55°C-dried OFMSW and beech sawdust at an ISR of 0.16 g VS/g VS (Test 5)

# **3.3.2** Batch Experiments

### 3.3.2.1. Acidified Experiments

Mono-digestion of OFMSW with an ISR of 0.5 and 1.0 g VS/g VS (Test 1 and Test 2) allowed to increase the TS up to 33.6 and 24.0 %, respectively [Table 3.1]. However, all the TS conditions resulted in acidification (i.e.  $pH \le 6.0$ ), likely due to the low ISR used (Angelidaki et al., 2004). Methanogenesis inhibition led to H<sub>2</sub> production and VFA accumulation. The highest H<sub>2</sub> production with an ISR = 0.5 g VS/g VS (Test 1) was achieved at the lowest TS (i.e. 10.2 %) and progressively decreased with increasing TS [Figure 3.2b], likely due to the reduced mass transfer in high-solids conditions. The H<sub>2</sub> production (i.e. 2-20 NmL H<sub>2</sub>/g VS<sub>added</sub> = 7-60 NmL H<sub>2</sub>/g VS<sub>removed</sub>) was comparable to that reported by Valdez-Vazquez et al. (2009) for OFMSW (i.e. 10-50 NmL H<sub>2</sub>/g VS<sub>removed</sub>). With an ISR = 1.0 g VS/g VS (Test 2), the H<sub>2</sub> production was  $\le 1$  NmL H<sub>2</sub>/g VS<sub>added</sub>. A reduced H<sub>2</sub> production can be attributed to a higher ISR.

In both experiments, an inverse relationship between the TS removal and the initial TS was observed [Figure 3.2c]. Meanwhile, the global COD conversion described an average 0.35 g COD/g VS<sub>added</sub> at an initial TS of around 10 % and a similar downward trend with increasing TS in both experiments [Figure 3.2d]. The COD conversion in acidified reactors corresponded from 87 to 96 % of the VFA accumulation. This confirms that H<sub>2</sub> production and/or VFA accumulation potentially reduced the hydrolysis rate (Cazier et al., 2015; Vavilin et al., 2008), playing a major role on the organic degradation at higher TS, due to the low water available (García-Bernet et al., 2011).



●OFMSW (ISR=0.5) ■OFMSW (ISR=1.0) ▲OFMSW (ISR=1.5) □Sawdust (ISR=0.04) ♦Codigestion (ISR=0.16)

*Figure 3.2 Main anaerobic biodegradability indicators: a) methane yield; b) hydrogen yield; c) total solid removal; and d) total chemical oxygen demand (COD) conversion* 

# 3.3.2.2. Methane-Producing Experiments

Despite mono-digestion of OFMSW at an ISR = 0.5 g VS/g VS (Test 1) acidified at TS from 10.2 to 33.6 %, methanogenesis occurred in 2 out of 3 replicates performed at 28.3 % TS, leading to an average methane yield of  $64 \pm 6$  NmL CH4/g VS<sub>subs</sub> [Figure 3.2a] – 87 % lower than the BMP of raw OFMSW – and a 23 % TS removal, after 100 days [Figure 3.2c]. The methanogenic onset observed in the two bottles at 28.3 % TS might relate to a favorable
mass transfer in the high-solids mixture, as discussed in section 3.3.2.4, since all the bottles contained exactly the same amount of substrate and inoculum.

Methanogenesis succeeded in all TS contents with mono-digestion of OFMSW using an ISR of 1.5 g VS/g VS (Test 3), though only a maximum 19.6 % TS was reached under these conditions [Figure 3.2a]. A methane yield of  $236 \pm 5$ ,  $199 \pm 32$ ,  $174 \pm 47$  and  $222 \pm 62$ NmL CH<sub>4</sub>/g VS<sub>subs</sub> was observed at initial TS of 10.8, 13.4, 16.4 and 19.6 %, respectively [Figure 3.1c and 3.2a], i.e. 52-65 % lower than the BMP of OFMSW. These methane yields corresponded to a volumetric productivity of  $8.8 \pm 0.2$ ,  $9.3 \pm 1.5$ ,  $10.2 \pm 2.8$  and  $15.8 \pm 4.4$ NmL CH<sub>4</sub>/L Reactor Content (data not shown) at initial TS of 10.8, 13.4, 16.4 and 19.6 %, respectively, being the higher volumetric productivity at increasing TS one of the main advantages of HS-AD (Brown et al., 2012). Interestingly, the standard deviation of the methane yield increased alongside the TS [Figure 3.2a], likely due to mass transfer effects and/or a higher heterogeneity of the initial mixture, as discussed in section 3.3.2.4. In contrast, the TS removal decreased at increasing initial TS contents, being 24.7 and 40.6 % at 19.6 and 10.8 % TS, respectively [Figure 3.2c]. The global COD conversion was approximately 0.38  $\pm$  0.05 g COD/g VS<sub>added</sub> at all TS, but showing a higher standard deviation at an initial TS of 19.6 % [Table 3.3].

Mono-digestion of sawdust (Test 4) showed a methane yield of  $64 \pm 3$ ,  $92 \pm 3$ ,  $94 \pm 4$ ,  $81 \pm 32$  NmL CH<sub>4</sub>/g VS<sub>subs</sub> at initial TS of 9.8, 14.6, 19.3 and 24.1 %, respectively [Figures 3.1d and 3.2a]. The methane yield at 9.8 % TS was approximately 30 % lower than that obtained at higher TS. After 100 days, the methane yield was 55-70 % lower than the BMP of sawdust, probably due to the lower ISR (i.e. 0.04 g VS/g VS) slowing down the biochemistry (Holliger et al., 2016), and/or the higher TS used. An 8-fold-higher standard deviation was observed at 24.1 % TS, likely due to inaccessible substrate regions at high TS – mass transfer limitations. The TS removal at initial TS = 24.1 % was around 50 % lower than that obtained at lower TS [Figure 3.2c]. The global COD conversion showed a downward trend from 14.6 to 24.1 % TS [Figure 3.2d].

With co-digestion of dried OFMSW and sawdust (Test 5), methane was produced only at 10.0 and 15.0 % TS, while higher TS conditions acidified [Figure 3.2], potentially due to the higher organic content at higher TS. The methane yield reached  $138 \pm 1$  and  $156 \pm 19$  NmL CH<sub>4</sub>/g VS<sub>subs</sub> at 10.0 and 15.0 % TS, respectively [Figure 3.1e]. Interestingly, 1 out of 3 replicates performed at 30.2 % TS also showed methanogenesis likely due to mass transfer effects in HS-AD, reaching a methane production of 117 NmL CH<sub>4</sub>/g VS<sub>subs</sub>. The H<sub>2</sub> yield – during the first week – decreased with increasing TS, showing a maximum of 2.3 NmL H<sub>2</sub>/g VS<sub>added</sub> at 10.0 % TS [Figure 3.2b]. The TS removal was 73.6 ± 0.6, 44.1 ± 0.4 and 8.1 ± 3.4 % at an initial TS of 10.0, 15.0 and 30.2 %, respectively [Figure 3.2c].

|                        | Initia                              | d values         |                              |            |                 |                 |                                             | Values at 1                                    | he end of the                   | experiment (c                                | lay 100)                                       |                                           |                                                     |   |
|------------------------|-------------------------------------|------------------|------------------------------|------------|-----------------|-----------------|---------------------------------------------|------------------------------------------------|---------------------------------|----------------------------------------------|------------------------------------------------|-------------------------------------------|-----------------------------------------------------|---|
| TS <sub>0</sub><br>(%) | VS <sub>0</sub> <sup>a</sup><br>(%) | TKN0<br>(g N/kg) | TAN <sub>0</sub><br>(g N/kg) | pH         | TAN<br>(g N/kg) | NH3<br>(g N/kg) | Acetic<br>(g COD/g<br>VS <sub>added</sub> ) | Propionic<br>(g COD/g<br>VS <sub>added</sub> ) | Butyric<br>(g COD/g<br>VSadded) | Valeric<br>(g COD/g<br>VS <sub>added</sub> ) | Total VFA<br>(g COD/g<br>VS <sub>added</sub> ) | CH4<br>Production<br>(g COD/g<br>VSadded) | Global<br>COD<br>Conversion<br>(g COD/g<br>VSadded) |   |
| $10.8 \pm$             | 9.1 ±                               | $4.94 \pm$       | $1.65 \pm$                   | 8.38 ±     | $2.83 \pm$      | $1.35 \pm$      | $0.0058 \pm$                                | $0.0014 \pm$                                   | $0.0046 \pm$                    | $0.0106 \pm$                                 | $0.0224 \pm$                                   | $0.3584 \pm$                              | $0.3807 \pm$                                        | _ |
| 0.0                    | 0.0                                 | 0.00             | 0.01                         | 0.02       | 0.09            | 0.04            | 0.0016                                      | 0.0010                                         | 0.0046                          | 0.0092                                       | 0.0147                                         | 0.0031                                    | 0.0129                                              |   |
| $13.4 \pm$             | $11.4 \pm$                          | $6.14 \pm$       | $2.05 \pm$                   | $8.45 \pm$ | $3.79 \pm$      | $1.97 \pm$      | $0.0094 \pm$                                | $0.0048 \pm$                                   | $0.0038 \pm$                    | $0.0178 \pm$                                 | $0.0358 \pm$                                   | $0.3161 \pm$                              | $0.3519 \pm$                                        | _ |
| 0.0                    | 0.0                                 | 0.00             | 0.01                         | 0.01       | 0.12            | 0.06            | 0.0009                                      | 0.0003                                         | 0.0007                          | 0.0020                                       | 0.0022                                         | 0.0356                                    | 0.0335                                              |   |
| $16.4 \pm$             | $13.9 \pm$                          | $7.46 \pm$       | $2.48 \pm$                   | $8.43 \pm$ | $4.22 \pm$      | $2.14 \pm$      | $0.0141 \pm$                                | $0.0078 \pm$                                   | $0.0059 \pm$                    | $0.0303 \pm$                                 | $0.0582 \pm$                                   | $0.2892 \pm$                              | $0.3473 \pm$                                        | _ |
| 0.0                    | 0.0                                 | 0.01             | 0.01                         | 0.01       | 0.36            | 0.18            | 0.0008                                      | 0.0010                                         | 0.0005                          | 0.0082                                       | 0.0071                                         | 0.0522                                    | 0.0463                                              |   |
| $19.6 \pm$             | $16.6 \pm$                          | $8.98 \pm$       | $2.99 \pm$                   | $8.42 \pm$ | $5.39 \pm$      | $2.70 \pm$      | $0.0205 \pm$                                | $0.0276 \pm$                                   | $0.0058 \pm$                    | $0.0457 \pm$                                 | $0.0996 \pm$                                   | $0.3412 \pm$                              | $0.4408 \pm$                                        |   |
| 0.0                    | 0.0                                 | 0.00             | 0.00                         | 0.04       | 0.26            | 0.13            | 0.0018                                      | 0.0030                                         | 0.0012                          | 0 0047                                       | 0.0104                                         | 0.0678                                    | 0.0731                                              |   |

Table 3.3 Effect of total solids on the performances of high-solids anaerobic digestion of the organic fraction of municipal solid waste using an inoculum-to-substrate ratio of 1.5 g VS/g VS (Test 3)

<sup>a</sup> Per gram of raw sample on wet basis.

#### 3.3.2.3. Main Effects when Increasing the Initial TS in HS-AD

The TS increase in HS-AD led to an increased biomethane volumetric productivity with mono-digestion of OFMSW (Test 3), but also resulted in acidification by substrate overload at higher initial TS with co-digestion of OFMSW and sawdust (Test 5). Moreover, higher standard deviations in the methane yields at higher TS, as well as the occurrence of methanogenesis only in some of the replicates at 28.3 and 30.2 %, were observed. These last results were likely due to mass transfer effects in HS-AD experiments, which influenced the occurrence of acidification and/or inhibition.

The low water content of a high-solids mixture hinders the accessibility of microorganisms to large portions of the substrate (Bollon et al., 2013), possibly explaining the increasing standard deviation in the methane yield at TS  $\geq 10$  % [Figure 3.2a]. Particularly, 'dry' AD (i.e. TS  $\geq 20$  %) is associated to the presence of spatially-differentiated acidogenic/methanogenic centers (Staley et al., 2011; Xu et al., 2014). In such systems, the convective transport is minimum, while the metabolite diffusion increases in importance, since the free-to-bound water ratio is low (Bollon et al., 2013; García-Bernet et al., 2011). Besides limiting the organic degradation, this phenomenon also reduces the chances of acidification of all the methanogenic centers in case of overload, likely explaining the methanogenesis onset observed in 2 out of 3 replicates at 28.3 % TS (Test 1), and in 1 out of 3 replicates at 30.2 % TS (Test 5). Homogenization devices, such as reactor stirrer or leachate recirculation, might help to prevent the influence of mass transfer limitations in HS-AD (André et al., 2018; Kothari et al., 2014).

#### 3.3.2.4. Maximizing the TS in HS-AD of OFMSW by Sawdust Addition

In this study, the physical-chemical characteristics of the substrate and inoculum (e.g. VS/TS and biodegradability) and the operational TS and ISR were found closely interrelated parameters determining the methane production or acidification in HS-AD. The ISR and the maximum TS were simultaneously adjusted in mono-digestion experiments according to the TS and VS balances of the substrate-inoculum mixture, since only one degree of freedom is available in a binary mixture (i.e. TS or ISR). Particularly, whether TS are higher in the substrate than in the inoculum, higher initial TS contents of a given substrate-inoculum mixture are obtained by lowering the ISR [Tests 1-3, Table 3.1]. Nonetheless, the ISR must be sufficiently high to avoid acidification, as a function of the substrate biodegradability of OFMSW required a higher ISR (i.e. 1.5 g VS/g VS), yielding a lower maximum TS (i.e. 19.6 %) [Figure 3.2]. In contrast, the lower methane potential and biodegradability rate of sawdust – as an example of lignocellulosic substrate – allowed the use of an extremely low ISR (i.e. 0.04 g VS/g VS) and a higher TS (i.e. 24.1 %).

In the case of co-digestion, two degrees of freedom are available in a ternary mixture (i.e. TS, ISR or OFMSW:GW ratio). Thus, a great number of combinations exists depending on the particular substrate and/or inoculum characteristics (e.g. VS/TS), explaining the different TS, ISR and FW:GW ratios used in literature for co-digestion. In this line, Brown et al. (2013) showed that, for a fixed ISR in 'dry' AD, the acidification risk increases by increasing the FW:GW ratio, due to the higher biodegradability of the inoculum-substrate mixture. Moreover, a higher FW:GW exacerbates the risk of TAN buildup and NH<sub>3</sub> inhibition in HS-AD.

Summarizing, adding sawdust to OFMSW reduces the biodegradability and TAN content of the substrate-inoculum mixture in comparison to mono-digestion of OFMSW, favoring the simultaneous TS and ISR increase in HS-AD. Thus, a OFMSW:sawdust ratio of 1:4 g TS:g TS was chosen in this study mainly to increase the maximum TS of co-digestion up to 30 %, but reducing the chances of NH<sub>3</sub> inhibition and acidification. Nonetheless, the addition of GW to OFMSW in industrial applications depends on the availability of co-substrates, the reactor design and/or the overall process economy (Christensen, 2011; Kothari et al., 2014).

#### 3.3.2.5. HS-AD Dynamics and NH<sub>3</sub> Inhibition

During the sacrifice test for mono-digestion of OFMSW (Test 6) [Figure 3.3], the daily methane production peaked around day 28, while the cumulative methane yield stabilized by day 65 reaching a value of  $296 \pm 13$  NmL CH<sub>4</sub>/g VS<sub>subs</sub>, i.e. 40 % lower than the BMP of OFMSW. Because of the organic degradation, TS decreased from 15.0 (day 0) to 9.8 % (day 92), corresponding to a 34.7 % TS removal. Acetic acid peaked to 8.40 g/kg (day 8) and was extensively consumed (i.e. < 0.10 g/kg) within 30 days from the reactor startup. Propionic, butyric and valeric acids increased from 1.30, 0.26 and 0.36 g/kg (day 0) to 5.20, 0.86 and 2.20 g/kg (day 92), respectively. TAN started at 2.4 g N/kg (day 0) and reached 3.8 g N/kg around day 35. At the same period, pH started at 7.3 (day 0), decreased to a minimum of 6.3 (day 8) and increased above 8 (day 35). The TAN and pH increase resulted in a NH<sub>3</sub> concentration up to 2.5 g N/kg (day 92). The global COD conversion was 0.63 g COD/g VS<sub>added</sub>.



**Figure 3.3** Sacrifice test with mono-digestion of organic fraction of municipal solid waste (Test 6). a) Daily and cumulative methane production, and pH; b) volatile fatty acids; c) total (TS) and volatile (VS) solids, and total (TAN)and free (FAN) ammonia nitrogen; and d) chemical oxygen demand (COD) conversion

These results suggest that the high ammonia levels were responsible for the reduced methane yield, TS removal and COD conversion in HS-AD, since all biodegradability indicators significantly slowed down in the mono-digestion sacrifice (Test 6) as NH<sub>3</sub> reached 2.3 g N/kg from day 45 [Figure 3.3]. Depending on the methanogens acclimation, NH<sub>3</sub> concentrations of 0.2-1.4 g N/L have been reported inhibitory (Chen et al., 2008; Fricke et al., 2007; Prochazka et al., 2012). In this study, the NH<sub>3</sub> increase correlated well with the propionic/valeric accumulation in Test 6 [Figure 3.3], being the VFA buildup a likely consequence of methanogenic inhibition (Demirel et al., 2008).

The above results indicate that the ammonia buildup most probably hampered the methane production also in the mono-digestion experiment using an ISR = 1.5 g VS/g VS (Test 3) [Figure 3.2]. Thus, the nitrogen content (i.e. TKN, TAN and NH<sub>3</sub>) was observed to increase in Test 3 alongside the higher initial TS, potentially exacerbating the NH<sub>3</sub> inhibition and VFA accumulation at higher TS [Table 3.3]. With all the above, the NH<sub>3</sub> accumulation can determine the overall anaerobic degradation (i.e. methane yield, TS removal and COD conversion) during HS-AD, particularly at higher initial TS contents. With the aim to reduce the risk of NH<sub>3</sub> inhibition while increasing the TS content, a co-digestion sacrifice was performed.

# 3.3.2.6. Other Factors Influencing Acidification in HS-AD

In co-digestion sacrifice (Test 7) [Figure 3.4], methanogenesis was inhibited from day 3, linked to a pH drop from 7.4 (day 0) to 6.0 (day 3). TS decreased from 19.4 (day 0) to 17.4 % (day 64), while TAN increased from 1.5 to 3.0 g N/kg (day 49), and NH<sub>3</sub> was < 0.1 g N/kg. Acetic, propionic, butyric and valeric acids increased from 0.37, 0.80, 0.24 and 0.48 g/kg (day 0) up to 9.17, 1.52, 7.09 and 0.96 g/kg, respectively (day 64). The overall H<sub>2</sub> production was 0.18 NmL H<sub>2</sub>/g VS<sub>added</sub> and the global COD conversion was 0.18 g COD/g VS<sub>added</sub>.



Figure 3.4 Sacrifice test with co-digestion of organic fraction of municipal solid waste and beech sawdust (Test 7). a) Daily and cumulative methane production, and pH; b) volatile fatty acids; c) total (TS) and volatile (VS) solids, and total (TAN) and free (FAN) ammonia nitrogen; and d) chemical oxygen demand (COD) conversion

The pH drop observed right after starting the HS-AD batch experiments (initial 0-3 days) was crucial to discern about the potential acidification in Tests 6 and 7. The initial pH drop is normally observed in AD when acidogenic outcompetes methanogenic growth (Gerardi, 2003), and becomes particularly important in HS-AD of OFMSW due to the high organic content used. Both mono- (Test 6) and co-digestion (Test 7) sacrifice tests showed an initial pH  $\geq$  7.3 (day 0) that rapidly dropped due to the VFA accumulation. In mono-digestion (Test 6), the pH = 6.4 from day 3 to 11 likely determined the low cumulative methane production (i.e. 6.3 NmL CH<sub>4</sub>/g VS<sub>subs</sub>) observed during these days, whereas the pH = 6.0 in the co-digestion sacrifice (Test 7) potentially inhibited methanogenesis (Demirel et al., 2008; Staley et al., 2011).

The ALK<sub>P</sub> and likely also the acclimation of the inoculum used as a seed in a HS-AD reactor played a major role to determine the acidification or methanogenesis onset, since ALK<sub>P</sub> is the main pH buffer in AD (Holliger et al., 2016; Prochazka et al., 2012). These factors mainly depend on the source reactor performance and the degassing period. Thus, the ALK<sub>P</sub> of the inoculum in this study determined the initial ALK<sub>P</sub> of the inoculum-substrate mixture [Table 3.2], by the ALK<sub>P</sub> mass balance.

At high TS, external buffer addition might help to circumvent HS-AD acidification. For example, Liotta et al. (2014) added NaHCO<sub>3</sub> to stabilize the acidogenic stages in HS-AD. However, whether inorganic buffering is used, particular attention is needed to minimize the TS dilution, while maintain an optimal cationic (i.e. Na<sup>+</sup>) concentration for microorganisms (Chen et al., 2008). Moreover, both the NaHCO<sub>3</sub> concentration and the NaHCO<sub>3</sub>-to-organics ratio (i.e. g NaHCO<sub>3</sub>/g TS) need to be the same along different initial TS, to allow comparison among these. Thus, NaHCO<sub>3</sub> addition was not used in this study to reduce the 'external' influencers in HS-AD.

In either case, acidification in this study did not associate to a low ALK<sub>P</sub>, nor to a high ALK<sub>I</sub>/ALK<sub>P</sub> ratio – data not shown. For example, mono-digestion Test 1 acidified at an initial ALK<sub>P</sub> of 1.7-5.6 g CaCO<sub>3</sub>/kg and ALK<sub>I</sub>/ALK<sub>P</sub> = 0.88, whereas acidification was avoided in mono-digestion Test 6 with ALK<sub>P</sub> of 2.6 and ALK<sub>I</sub>/ALK<sub>P</sub> = 2.12. Similarly, methanogenesis failed to start in Test 2, operated at the same ISR than Test 6 (i.e. 1.0 g VS/g VS), though the initial ALK<sub>P</sub> and ALK<sub>I</sub>/ALK<sub>P</sub> ratio were 1.5-3.8 g CaCO<sub>3</sub>/kg and 1.51, respectively, in the acidified experiment (Test 2).

In conclusion, other factors related to the initial inoculum-substrate mixture, and not assessed here, influenced also the HS-AD acidification. Some of these might include the different (micro-)nutrient or inhibitory content, but also the mass transfer, reactor homogenization, reactor headspace volume, particle size and/or inoculum activity (André et al., 2018; Bollon et al., 2013; Chen et al., 2008; Holliger et al., 2016; Motte et al., 2014).

Therefore, all these factors should be considered alongside the TS, ISR, ALK<sub>P</sub> and nitrogen content to evaluate HS-AD experiments using OFMSW as substrate.

# 3.4 Conclusions

This study shows that both the initial TS and ISR determine the success of methanogenesis in HS-AD of OFMSW. During mono-digestion of OFMSW, increasing the maximum TS required a lower ISR, enhancing the risk of acidification. Meanwhile,  $NH_3 \ge 2.3$  g N/kg at 15.0 % TS resulted in VFA accumulation (i.e. 0.13-0.14 g COD/g VS<sub>added</sub>) and 40 % lower methane yield. Adding sawdust to OFMSW permitted to increase simultaneously the TS and ISR, by reducing considerably the biodegradability and nitrogen content of the mixture, in comparison to mono-digestion of OFMSW. This also led to acidification occurring only at higher TS (i.e.  $\ge 20$  %). Therefore, the initial inoculum-substrate mixture in HS-AD must result from a tradeoff between the maximum TS and the optimum ISR, but also the buffering capacity and the nitrogen content, to circumvent acidification and NH<sub>3</sub> inhibition.

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# **Chapter 4**

# Assessing the Maximum Operational Total Solid Content in Semi-Continuous Anaerobic Digestion

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#### Abstract

In this study, mono-digestion of the organic fraction of municipal solid waste (OFMSW) and co-digestion of OFMSW with beech sawdust, simulating green waste, were used to investigate the maximum operational total solid (TS) content in semi-continuous high-solids anaerobic digestion (HS-AD). To alleviate substrate overloading in HS-AD, the effluent mass was relatively reduced compared to the influent mass, extending the mass retention time. To this aim, the reactor mass was daily evaluated, permitting to assess the reactor content removal by biogas production. During mono-digestion of OFMSW, the NH<sub>3</sub> inhibition and the rapid TS removal prevented to maintain HS-AD conditions (i.e.  $TS \ge 10$ %), without exacerbating the risk of reactor acidification. In contrast, the inclusion of sawdust in OFMSW permitted to operate HS-AD up to 30 % TS, before acidification occurred. Therefore, including a lignocellulosic substrate in OFMSW can prevent acidification and stabilize HS-AD at very high TS contents (i.e. 20-30 %).

**Keywords**: High-Solids Anaerobic Digestion; Influent/Effluent Uncoupling; Substrate Overloading; Acidification; Ammonia Inhibition.



# **Graphical Abstract**

# 4.1 Introduction

Anaerobic digestion (AD) of the organic fraction of municipal solid waste (OFMSW), including food waste (FW) and green waste (GW), is a particularly suited treatment biotechnology for energy and by-product recovery (Clarke, 2018; Mata-Álvarez, 2003). In AD, an organic waste is degraded to biogas, mainly composed by CH<sub>4</sub> and CO<sub>2</sub>, and a partially stabilized organic digestate, by consortia of different microorganisms working in absence of oxidative species (i.e. O<sub>2</sub> and NO<sub>3</sub><sup>-</sup>) (Astals et al., 2015; Gerardi, 2003).

The sequential steps in AD include hydrolysis, acidogenesis, acetogenesis and methanogenesis, during which different inhibitory substances can be formed leading to inhibitory effects for the anaerobic microorganisms and/or even a complete AD failure. Depending on the concentration, free ammonia (NH<sub>3</sub>), hydrogen sulfide (H<sub>2</sub>S) and free ions (i.e. Na<sup>+</sup>) are some of the inhibitory substances in AD, affecting predominantly the methanogenic stage, either acetoclastic and/or hydrogenotrophic, and potentially resulting in the buildup of volatile fatty acids (VFA) and H<sub>2</sub> in the system (Astals et al., 2015; Chen et al., 2008). Meanwhile, the acetoclastic activity results into inorganic carbon (i.e. HCO<sub>3</sub><sup>-</sup>) release in AD, as an important source of pH buffering, minimizing the risk of reactor acidification (i.e. pH  $\leq$  6.0) by VFA accumulation (Gerardi, 2003).

The interrelationship between the organic waste characteristics, operational conditions and reactor design determines the AD potential (Karthikeyan et al., 2013; Mata-Álvarez, 2003). AD can be differentiated depending on the operational total solid (TS) content into 'wet' (i.e. TS < 10 %) and high-solids AD (HS-AD, i.e. TS  $\geq$  10 %) (Benbelkacem et al., 2015). HS-AD allows the use of a smaller reactor, reducing the need for water addition and minimizing the digestate production (Karthikeyan et al., 2013; Pastor-Poquet et al., 2018). However, HS-AD drawbacks include the pervasive chances of reactor acidification due to substrate overload (Benbelkacem et al., 2015). Overloading is the consequence of the slowgrowing methanogens being unable to cope with the rapid VFA and/or H<sub>2</sub> buildup resulting from acidogenesis/acetogenesis in HS-AD (Pavan et al., 2000). Furthermore, overloading is in many cases related to the presence of methanogenic inhibitors (Drosg, 2013), such as NH<sub>3</sub>, due to the high protein content of OFMSW (Kayhanian, 1999).

HS-AD of OFMSW is a mature technology, with most of the recently-constructed industrial plants targeting the semi-continuous HS-AD process (Mattheeuws, 2016). The focus of semi-continuous HS-AD lies on the maximization of the organic loading rate (OLR) that optimizes the methane yield and ensures an adequate organic removal at high TS contents (Benbelkacem et al., 2015; Hartmann et al., 2006). In this line, depending on the organic waste used in HS-AD, the operational TS content is substantially lower than the feed

TS, as the organic substrate is converted to biogas by methanogenesis (Pastor-Poquet et al., 2018).

Therefore, HS-AD lies on a balance between maximizing the OLR and TS content, while minimizing the chances of reactor failure. Particularly, in order to startup HS-AD, the OLR needs to be increased relatively slowly, permitting the methanogens to grow and adapt to the new conditions. The transient (non-steady) OLR modification in HS-AD aims to find an optimum stationary (steady-state) operation to be used, avoiding acidification and maximizing the economy of the process (Angelidaki et al., 2006; Bolzonella et al., 2003). However, the risk of inhibition and failure is undesirably high under HS-AD startup, potentially requiring the implementation of recovering strategies (i.e. reactor content dilution) to minimize the influence of inhibitory substances, or even restarting the process when a significant methanogenic imbalance occurs (Fricke et al., 2007; Kayhanian, 1999).

This study evaluated the highest tolerable TS content in semi-continuous HS-AD of OFMSW, by gradually increasing the OLR in semi-continuous reactors operated at 55°C, until process failure occurred by acidification. Two feeding strategies were used: monodigestion of OFMSW and co-digestion of OFMSW and beech sawdust – as a model lignocellulosic substrate, simulating the inclusion of GW in OFMSW. Aiming to minimize the risk of substrate overload, the mass retention time (MRT) was relatively extended by reducing the effluent compared to the influent mass, according to the daily mass content removal by biogas production observed in the semi-continuous reactors.

# 4.2 Materials and Methods

# 4.2.1 Substrates and Inoculum

The substrates used in this study were OFMSW and beech sawdust. OFMSW consisted of a mixture of household waste collected in Cassino (Italy), restaurant waste, spent coffee, and garden waste collected at the university facilities, with an approximated wet weight proportion of 45, 35, 15 and 5 % (w/w), respectively. OFMSW was minced twice to a particle size  $\leq 5$ -10 mm by an industrial mincer [REBER 9500NC, Italy], fully homogenized manually and stored in 5 L buckets at -20°C. During mincing and homogenization, no extra water was added to the raw substrate. A single 5 L bucket of OFMSW was thawed at room temperature overnight, as required to feed the semi-continuous reactors. Goldspan<sup>®</sup> beech sawdust with 1.0-2.8 mm particle size was used as co-substrate, to simulate biodegradable green/lignocellulosic waste.

The inoculum for semi-continuous experiments was obtained from a pre-adapted 'wet' AD (i.e.  $TS \le 5$  %) source reactor operated at 55°C. The pre-adaptation of 20 L sludge, collected from a mesophilic (35°C) digester treating buffalo manure and mozzarella whey (Capaccio, Italy), consisted of a 4-month progressive feeding of tap-water-diluted OFMSW at 55°C, in order to adapt the inoculum to the new substrate and temperature.

Prior to start the mono-digestion experiments, the source reactor was kept unfed for 1 month to consume/reduce the organic content, while continuing with the inoculum adaptation to the new substrate. Subsequently, the feeding with diluted OFMSW was resumed to recover methanogenesis. After 7 and 15 days from the feeding restart, 4 kg of sludge were taken from the source reactor, filtered through a 1 mm mesh and used to inoculate the mono-digestion reactors "A" and "B", respectively. Therefore, the inoculum was slightly different in reactors A and B, as shown in section 4.3.1.

During the mono-digestion experiments, the source reactor was periodically fed with diluted OFMSW and the mono-digestion reactors effluents, to maintain the reactor volume and methanogenic activity. Once the mono-digestion experiments ended, the source reactor was kept unfed for 1 month to serve as inoculum for the co-digestion experiments. Thus, 3.4 kg of reactor content were filtered through a 1 mm mesh and used to inoculate each co-digestion reactor "A", "B" and "C".

# 4.2.2 Experimental Setup

The laboratory-scale semi-continuous reactors consisted of 5 L polyethylene terephthalate (PET) bottles with a modified head allowing the (semi-)solid waste input, reactor content withdrawal and biogas measurement [Figure 4.1]. The reactor port was a polyvinyl chloride (PVC) flexible hosepipe with two valves, easing the reactor loading/unloading while avoiding air intrusion. The biogas output, containing a sampling septum, was connected to 5 L Tedlar® bags [Sigma-Aldrich, USA]. All reactors were maintained at 55°C within a temperature-controlled TCF 400 oven [ARGOLAB, Italy].



Figure 4.1. Experimental setup. 1) Reactor body; 2) reactor head; 3) feeding port; 4) gas output; 5) gas measuring port; and 6) opening valves

# 4.2.3 Operation Strategy

Two semi-continuous reactors for mono-digestion of OFMSW or three reactors for codigestion of OFMSW and sawdust were operated simultaneously in a drag-and-fill mode. The semi-continuous reactors (i.e. kg) and the reactor influents/effluents (i.e. g) were weighed on a  $\pm$  0.01 precision scale. The OLR was evaluated as the daily substrate addition in terms of volatile solids (VS) divided by the reactor mass content (i.e. g VS/kg·d), while the MRT was evaluated as the quotient between the reactor mass and the daily effluent mass (i.e. days). Since the reactors were fed a maximum of 5 days per week, 7-days moving average OLR and MRT were estimated. Moving-average operational variables are well suited indicators of the immediately preceding operations (i.e. feeding, dilution, reactor content removal) to discern about the risk of VFA buildup in semi-continuous digesters. Moreover, expressing the operational conditions as a moving-average eases the comparison of digesters, when feeding days are not the same or an important mass removal occurs.

During each drag-and-fill operation, the reactor content was 1) homogenized before opening the system, 2) sampled and 3) analyzed mainly for pH and alkalinity – since pH had to be maintained over 6.5, as an important methanogenic inhibition might take place below this threshold (De Vrieze et al., 2012; Gerardi, 2003). Depending on the pH and alkalinity, 4) the proper amount of substrate was used or diluted as needed, 5) prior to be fed to the

reactors. Finally, 6) the reactor content was homogenized once again, while the Tedlar<sup>®</sup> bags were checked for biogas production and subsequently emptied.

To increase the reactor TS content from 'wet' AD (i.e. TS  $\leq$  5 %) to HS-AD (i.e. TS  $\geq$ 10 %), the OLR was controlled by increasing/decreasing the daily amount of substrate and/or tap water addition based on the methanogenic activity, and aiming to minimize the substrate overload. To evaluate the differences in the reactor performance, mono-digestion reactors were fed in parallel using different OLR/MRT in each reactor, as shown in section 4.3.2. Subsequently, co-digestion reactors were also operated in parallel at three different OLR/MRT. In each reactor, the methanogenic activity was roughly associated with the relative increase of the pH and inorganic carbon alkalinity (ALK<sub>P</sub>), the reduction of the reactor mass content and the biogas production compared to previous operational values, as also mentioned in section 4.3.2. For example, a relative pH and ALK<sub>P</sub> increase of approximately 0.5 pH units and 0.3 g CaCO<sub>3</sub>/kg, respectively, alongside a reactor mass removal of about 30-50 g/d and a specific biogas production higher than 250 mL/kg reactor content d were associated with ongoing methanogenesis, indicating that the OLR could be maintained or relatively increased. Similarly, the relative increase of intermediate alkalinity (ALK<sub>I</sub>) (i.e. 0.5 g Acetic Acid/kg) was used as a preliminary indicator of the potential VFA buildup and risk of substrate overload (Lahav et al., 2002).

All these parameters were further complemented with the user's evaluation of the previous operation, in order to decide for the daily feed/dilution to be used. Thus, all reactors were started with a low OLR (i.e. 2 g VS/kg·d) that was gradually increased to increase the TS content. As reactor performance deteriorated with increasing OLR, the reactor feeding was reduced/stopped to prevent acidification (i.e.  $pH \le 6.0$ ).

The reactor mass was maintained constant by reducing the effluent compared to the influent mass, according to the observed reactor mass content removed by biogas production from the previous operation. With this strategy, the MRT was relatively extended, aiming to promote the methanogenic adaptation in case of overloading. Semi-continuous reactors were fed until acidification occurred. From this point, feeding was stopped and reactor dilution and/or inorganic salt addition (i.e. NaHCO<sub>3</sub> and FeCl<sub>2</sub>) were tested as recovering strategies. A summary of the weekly operational variables is presented as Supplementary Information.

# 4.2.4 **Bio-Physical-Chemical Analyses**

The pH, ALK<sub>P</sub> and ALK<sub>I</sub> were determined from the supernatant of solid and semi-solid samples (Lahav et al., 2002), after diluting the sample with distilled water, homogenization

and centrifugation at 6000 rpm for 15 min (EPA, 2015). The TS and VS content, total Kjeldahl (TKN) and ammonia (TAN) nitrogen, and the total H<sub>2</sub>S were determined by the standard methods (APHA, 1999). The NH<sub>3</sub> was approximated as a function of TAN and pH (Astals et al., 2015). The VFA (acetic, propionic, butyric and valeric acids) were measured with an LC-20AD HPLC [Shimadzu, Japan], mounting a Rezex ROA-Organic Acids 8+ column coupled to a 210 nm UV detector, and using 0.0065 M H<sub>2</sub>SO<sub>4</sub> at 0.6 mL/min as mobile phase. The biogas composition (CH<sub>4</sub>, CO<sub>2</sub> and H<sub>2</sub>) was analyzed with a 3400 GC-TCD [Varian, USA], using argon as carrier gas.

The biomethane potential (BMP) test for OFMSW used 3.0 g of substrate, 50.0 g of source inoculum, 40.0 g of distilled water and 0.10 g of NaHCO<sub>3</sub> in 280 mL bottles (6 replicates), with an inoculum-to-substrate ratio (ISR) of 2.0 g VS/g VS. The BMP test for sawdust used 1.0 g substrate and 50.0 g of inoculum in 160 mL bottles (3 replicates), with an ISR of 1.0 g VS/g VS. BMP tests were performed according to Angelidaki et al. (2004) and Holliger et al. (2016). In the BMP test for OFMSW, the distilled water and NaHCO<sub>3</sub> addition served to minimize the chances of inhibition (i.e. by NH<sub>3</sub>) and acidification, respectively. In contrast, NH<sub>3</sub> build-up and acidification were not expected in the BMP test of sawdust, due to the low nitrogen content and the reduced biodegradability of sawdust, as thoroughly discussed in next section, permitting also to use a lower ISR. Both BMP tests lasted longer than 100 days. Blank assays included the inoculum and further distilled water compensating for the absence of substrate, using three replicates in each BMP. Inoculum activity assays using a reference substrate were not performed.

The BMP was the normalized methane production (P = 1 bar,  $T = 0^{\circ}C$ ), excluding the methane production of the inoculum, per unit of substrate VS added. The gas production was evaluated with a two-vessel displacement system, with the first vessel containing 4 N NaOH to capture CO<sub>2</sub> and the second vessel containing water to be 'displaced'. The bottles were sealed with butyl rubber stoppers and aluminum crimps and flushed with helium, before adding 0.2 mL of 10 g/L Na<sub>2</sub>S piercing the septum to ensure an adequate redox potential (Angelidaki et al., 2004). All bottles were incubated at 55°C and agitated only while measuring the gas production.

#### 4.2.5 Statistical Analyses

The Dixon's test for BMP outliers was applied as recommended by Holliger et al. (2016). The unpaired t-test of Microsoft Excel 2016 (Microsoft, USA) was applied to determine the statistical significance of experimental data, using the two-tail p-value at 95 % confidence.

# 4.3 Results and Discussion

#### 4.3.1 Bio-Physical-Chemical Characterization of Substrates and Inoculum

OFMSW showed a TS of 26 %, a VS/TS ratio of 0.93 and a TKN of 24.8 g N/kg TS, in agreement with real source-sorted OFMSW (Angelidaki et al., 2006; Bolzonella et al., 2006; Jokela et al., 2003). The high VS/TS ratio of OFMSW (i.e. > 0.9) indicated minimal presence of inert materials (Pavan et al., 2000). Sawdust showed a TS of 94 % and a VS/TS ratio of 0.99, similar to those obtained by Brown et al. (2013) for 40°C-dried yard waste, suggesting that beech sawdust could simulate GW. The BMP of OFMSW and sawdust was 497 and 161 NmL CH4/g VS, respectively. Table 4.1 shows the bio-physical-chemical characterization of OFMSW and sawdust. Despite the thorough mincing and homogenization, minor modifications were observed in the OFMSW characterization (i.e. TS, TKN or BMP), mainly attributed to the substrate heterogeneity.

|                                | OFMSW            | Sawdust         |
|--------------------------------|------------------|-----------------|
| TS (%)                         | $26.52 \pm 1.35$ | $93.69\pm0.42$  |
| VS (%)                         | $24.62 \pm 1.27$ | $92.64\pm0.70$  |
| VS/TS                          | $0.93 \pm 0.02$  | $0.99\pm0.01$   |
| TKN (g N/kg TS)                | $24.78 \pm 1.50$ | $0.98 \pm 0.17$ |
| TAN (g N/kg TS)                | $4.92\pm0.06$    | $0.12\pm0.01$   |
| рН                             | $4.40\pm0.14$    | $5.65\pm0.06$   |
| ALK <sub>I</sub> (g Acetic/kg) | $1.17\pm0.82$    | $1.50\pm0.26$   |
| BMP (NmL CH4/g VS)             | $497\pm58$       | $161\pm12$      |

Table 4.1 Bio-physical-chemical characterization of substrates

The inoculum in mono-digestion reactors A and B showed a common TS and TKN of 2.8 % and 161 g N/kg TS, respectively. An initial acetic acid concentration of 2.30 and 3.30 g/kg was observed in reactors A and B, respectively, being this difference associated with the later inoculation of reactor B than reactor A. The inoculum used in co-digestion reactors showed a TS of 2.5 %, a TKN of 139 g N/kg TS and an acetic acid concentration of 0.02 g/kg. The inoculum compositions are shown in Table 4.2.

|                                            | Mono-digestion |           | Co-digestion      |
|--------------------------------------------|----------------|-----------|-------------------|
|                                            | Reactor A      | Reactor B | Reactors A, B & C |
| TS (%)                                     | 2.8            | 2.8       | 2.5               |
| VS (%)                                     | 1.9            | 2         | 1.6               |
| VS/TS                                      | 0.69           | 0.70      | 0.64              |
| TKN (g N/kg TS)                            | 161            | 161       | 139               |
| TAN (g N/kg TS)                            | 122            | 121       | 122               |
| рН                                         | 8.12           | 8.44      | 8.69              |
| ALK <sub>P</sub> (g CaCO <sub>3</sub> /kg) | 9.6            | 9.6       | 9.3               |
| ALK <sub>I</sub> (g Acetic/kg)             | 5.3            | 6.3       | 3.2               |
| Acetic (mg/kg)                             | 2260           | 3310      | 20                |
| Propionic (mg/kg)                          | 470            | 980       | 490               |
| Butyric (mg/kg)                            | 480            | 260       | 140               |
| Valeric (mg/kg)                            | 0              | 210       | 0                 |

Table 4.2 Physical-chemical characterization of inoculums

# 4.3.2 Semi-continuous Operation – Increasing the TS Content

#### 4.3.2.1 Mono-digestion of OFMSW

Mono-digestion results are summarized in Figures 4.2 and 4.3. The weekly-averaged results were also included as Supplementary Information. The 7-days average OLR in reactors A and B was varied from an initial 2.4 (day 6) and 6.0 (day 13) g VS/kg·d to 4.9 and 5.5 g VS/kg·d, respectively, on day 17 [Figure 4.2a]. Thus, a common OLR (i.e. around 5 g VS/kg·d) was achieved, aiming to compensate for the 1-week-lagged inoculation in reactor B. After two days with no feed, feeding was resumed in reactor B on day 20, but not in reactor A due to the low pH (i.e. 6.4) [Figure 4.3a]. As pH recovered in reactor A (i.e. from 6.4 on day 21, to 7.6 on day 29) due to methanogenesis activity, feeding was resumed. During the same period, ALK<sub>P</sub> in reactor A increased alongside pH from 1.1 to 2.7 g CaCO<sub>3</sub>/kg (data not shown), as an indicator of ongoing methanogenesis. By day 45, a maximum OLR of 6.8 and 8.5 g VS/kg·d was reached in reactors A and B, respectively. After day 48, the OLR required progressive reduction to minimize the risk of acidification. The last feeding in reactors A and B was implemented on days 78 and 73, respectively, as both reactors showed pH  $\leq$  6.5 and CH<sub>4</sub> content  $\leq$  40 % [Figure 4.2f]. From this point, mono-digestion reactors were left unfed aiming to promote the recovery of methanogenesis.



**Figure 4.2.** Mono-digestion of OFMSW: a) Organic loading rate; b) mass retention time; c) total solids; d) total and free ammonia nitrogen (NH<sub>3</sub>); e) cumulative biogas production; and f) methane content. Black arrows represent the NaHCO<sub>3</sub> addition in reactor A, while dotted arrows represent the FeCl<sub>2</sub> or inoculum addition in reactor B



Figure 4.3. Mono-digestion of OFMSW: Volatile fatty acids and pH in a) reactor A; and b) reactor B. Black arrows represent the NaHCO<sub>3</sub> addition in reactor A, while dotted arrows represent the FeCl<sub>2</sub> or inoculum addition in reactor B

The OLR in reactor B was averagely about 1.5 g VS/kg·d higher than that used in reactor A during the whole experiment (p < 0.001), explaining the relatively faster acidification observed in reactor B. Thus, prior to the occurrence of acidification, reactor B was fed with an average 35 g VS/d, significantly higher than the 26 g VS/d used for reactor A (p = 0.03). The initial MRT was 55 (day 6) and 29 days (day 13) for reactors A and B, respectively, and was gradually increased to maintain the methanogenic performance at higher OLR [Figure 4.2b]. Noteworthy, the MRT and OLR in these semi-continuous reactors did not show an inverse pattern, since the dilution as well as the influent and effluent mass flows used were different to account for the organic removal.

Uncoupling the influent and effluent mass flows in the semi-continuous reactors, based on the HS-AD reactor content removal by methanogenesis, permitted to increase the MRT and OLR simultaneously. In this study, the MRT was considered as a more suited indicator in HS-AD than the hydraulic retention time (HRT), since both the specific weight of the influent/effluent and the reactor mass content varied, in contrast to 'wet' AD (Pastor-Poquet et al., 2018). As an example, the occurrence of methanogenesis led to a 60 g removal of the reactor mass content in both mono-digestion reactors from day 37 to 41 (data not shown). Prior to the occurrence of reactor acidification, the weekly effluent mass was significantly higher than the influent (i.e. 18 %; p = 0.03) to maintain the mono-digestion reactors mass content constant.

The MRT-uncoupling concept was proposed by Richards et al. (1991) and was used by Kayhanian et al. (1995) to operate a pilot-scale semi-continuous HS-AD reactor fed with OFMSW. In this study, uncoupling the influent and effluent in HS-AD promoted the methanogenic adaptation to overloading conditions and/or the buildup of inhibitors (i.e. NH<sub>3</sub>) during the OFMSW degradation. Noteworthy, the MRT must be longer than the doubling time of methanogens (i.e. 20-30 days) to avoid their 'washout' from continuous HS-AD reactors, while the methanogenic doubling time might lengthen considerably in presence of inhibitory substances (i.e. NH<sub>3</sub>) (Drosg, 2013; Gerardi, 2003; Rittman et al., 2001). Therefore, extending the MRT resulted in a more stable HS-AD operation (Hartmann et al., 2006; Rajagopal et al., 2013), though the sole implementation of influent-effluent uncoupling was not sufficient to avoid HS-AD overloading and acidification during mono-digestion of OFMSW.

#### 4.3.2.2 Co-digestion of OFMSW and Sawdust

Co-digestion results are summarized in Figures 4.4 and 4.5. The 7-days average OLR was increased from 4.5-4.9 g VS/kg·d (day 6) up to 10.9, 12.1 and 12.6 g VS/kg·d (day 23) in reactors A, B and C, respectively. To avoid acidification, feeding was stopped in reactors A and B from day 26, while the OLR was only reduced to 5.0 g VS/kg·d in reactor C [Figure 4.4a]. As pH recovered (i.e.  $\geq$  7.0) [Figure 4.5], feeding was resumed in reactors A and B. A maximum OLR of 14.8 g VS/kg·d was reached in reactor C (day 47) using a sawdust/OFMSW ratio of 2.1 g VS/g VS, prior to the occurrence of reactor acidification (day 56). The maximum OLR in reactor B was 15.1 g VS/kg·d (day 55) using a sawdust/OFMSW ratio of 1.6 g VS/g VS, while an OLR of 16.0 g VS/kg·d was reached in reactor A during the same period, using a sawdust/OFMSW ratio of 1.3 g VS/g VS. The last feeding in reactors A and B was performed on day 76, as a slight but continued drop in pH [Figure 4.5] and CH4 [Figure 4.4f] was observed in both reactors.



**Figure 4.4.** Co-digestion of OFMSW and sawdust: a) Organic loading rate – parentheses indicate the sole addition of OFMSW; b) mass retention time; c) total solids; d) total and free ammonia nitrogen (NH3); e) cumulative biogas production; and f) methane content



*Figure 4.5. Co-digestion of OFMSW and sawdust: Volatile fatty acids and pH for a) reactor A; b) reactor B; and c) reactor C* 

The average OLR used for co-digestion was two times higher than that for monodigestion (i.e. 8.3 vs. 4.5 g VS/kg·d, respectively; p < 0.001), due to the lower biodegradability of sawdust, though the OLR only due to OFMSW was similar in both cases (i.e. 4.1 vs. 4.5 g VS/kg·d, respectively; p = 0.07). Thus, a maximum OLR of 7.5-8.0 g VS/kg·d related to the sole supplementation of OFMSW was used in the three co-digestion reactors on day 21, while the OLR solely due to OFMSW was subsequently maintained below 6.0 g VS/kg·d, as sawdust was increased in the feeding mixture [Figure 4.4a]. In terms of average VS fed, reactor C was operated under relatively more stressing feeding conditions than reactors A and B (i.e. 53 vs. 44 g VS/d, respectively; p = 0.15), being again the fastest occurrence of reactor acidification related to the highest VS fed.

The initial MRT was higher than 168 days (day 6) and was decreased to 30 days (day 17), similarly in the three reactors [Figure 4.4c]. From this point, the MRT reached an average of 85 days (day 35) and was subsequently reduced to an average of 37 days (day 53) in all reactors, before being progressively increased to minimize the substrate overload. The MRT was significantly lower in co-digestion than mono-digestion (i.e. 69 *vs.* 92 days, respectively; p < 0.001), as lower MRT were predominantly linked to the higher OLR used in co-digestion.

# 4.3.3 Influence of the Substrate Composition on the TS Increase

The OLR/MRT control in the mono-digestion reactors fed with OFMSW permitted to increase the TS content, balancing the VFA accumulation with the rapid organic degradation observed [Figures 4.2 and 4.3]. Reactors A and B were started at TS = 2.8 % and reached a maximum of 10.7 (day 79) and 11.7 % (day 69), respectively [Figure 4.2c], being these TS slightly higher than the lower HS-AD threshold (i.e. TS  $\ge$  10 %). The highest TS in the semicontinuous reactors did not coincide with the maximum OLR, but were predominantly associated with low pH (i.e.  $\le$  6.5), when methanogenesis was potentially inhibited. In this line, a gradual increase of the VS/TS ratio (data not shown) was observed in both reactors from 0.69 (day 0) to 0.82 (day 40), reaching a maximum value of 0.87, prior acidification occurred on days 79 and 76 in reactors A and B, respectively.

The highest TS and VS/TS observed in semi-continuous HS-AD of OFMSW were associated with acidification and indicate a reduced VS degradation alongside inhibitory conditions. Particularly, the lowest HS-AD threshold (i.e. TS = 10 %) using OFMSW was reached only under extreme overloading. A more stable HS-AD fed with an easily biodegradable OFMSW (i.e. FW) is also associated with a TS increase alongside overloading/inhibitory conditions. For example, Tampio et al. (2014) reported a TS increase from 7 to 8 % during 400 days of semi-continuous AD fed with FW, though TS rapidly reached 11 % during the next 50 days of operation, when reactor inhibition was likely occurring. In the same line, Bolzonella et al. (2003) reported a TS increase from 5 to 15 % during the initial 60 days of continuous AD pilot-scale startup fed with OFMSW, being the maximum TS associated with the highest total VFA observed (i.e. 2.8 g Acetic Acid/L). All these results were likely related to methanogenesis inhibition, since the VFA accumulation affects the hydrolysis/acidogenesis rates, hampering the organic removal in HS-AD (Vavilin et al., 2008).

The maximum TS obtained in this study for semi-continuous HS-AD of OFMSW should be considered as indicative (only) of those obtainable in steady-state digesters, since the transient/acidification conditions potentially reduced the VS removal. Thus, the operational TS content of stable digesters fed with the easily biodegradable content of OFMSW (i.e. FW) might be lower than those observed along non-steady-state conditions. This is a further indication that a steady-state semi-continuous reactor using an easily biodegradable OFMSW as a substrate might not be operated within the HS-AD threshold (i.e. TS  $\geq$  10 %).

Co-digestion permitted to increase TS from 2.5 % (day 0) up to a maximum of 33.2 (day 79), 26.7 (day 76) and 27.0 % (day 57) in reactors A, B and C, respectively [Figure 4.4c]. Hence, the maximum TS reached in co-digestion before the reactors acidified (i.e. 29.0  $\pm$  2.8 %) was considerably higher than the lower HS-AD threshold (i.e. TS  $\geq$  10 %) and the maximum TS of mono-digestion (i.e. 11.5  $\pm$  0.5 %), due to the addition of sawdust to OFMSW. The highest TS was related again to acidified (i.e. pH < 6.5) or acidifying (i.e. downward trend on pH/CH<sub>4</sub> content) conditions, as observed for mono-digestion. Thus, the VS/TS ratio in co-digestion (data not shown) increased from 0.65 (day 0) to 0.90 (day 40), reaching a maximum of 0.95 before reactors acidified (day 76), due to both the higher VS/TS ratio of sawdust and the reduced VS removal during inhibitory conditions.

These results showed that the particular characteristics of OFMSW determined the maximum operating TS content in semi-continuous HS-AD. Co-digestion of OFMSW and sawdust resulted in approximately three times higher TS than mono-digestion (i.e. 29.0 and 11.5 %, respectively). The inclusion of sawdust in OFMSW favored the rapid TS and OLR increase compared to mono-digestion due to the higher TS and the lower biodegradability of sawdust, as demonstrated by the substantially lower BMP of sawdust than that of OFMSW (i.e. 161 and 497 NmL CH<sub>4</sub>/g VS, respectively) [Table 4.1]. Indeed, lignocellulosic materials (i.e. GW) are normally associated with a reduced biodegradation rate, compared to more easily degradable substrates (i.e. FW), due to the high lignin content hampering hydrolysis (Brown et al., 2013; Mancini et al., 2018a; Vavilin et al., 2008), being also beneficial to limit the VFA buildup in HS-AD. On the other hand, TAN was 22 % lower during co-digestion than mono-digestion (i.e. 2.9 vs. 3.7 g N/kg, respectively; p < 0.001) [Figures 4.2 and 4.4], due to the lower TKN of sawdust [Table 4.1]. Noteworthy, the TAN accumulation was likely promoting methanogenic inhibition in this study, as further discussed in section 4.3.5. Therefore, using sawdust - as GW - was also adequate to adjust the carbon-to-nitrogen (C/N) ratio in HS-AD of OFMSW.

# 4.3.4 Main Indicators of Substrate Overload

#### 4.3.4.1 Evolution of pH and VFA

pH in mono-digestion reactor A decreased from 8.1 to 6.4 due to the rapid acetic acid buildup (i.e. from 3.00 to 9.00 g/kg) observed during the initial 20 days of operation [Figure 4.3a]. As feeding was stopped from day 20 to 29, pH reached 7.6, while acetic acid decreased below 0.70 g/kg right afterwards (day 34). Propionic, butyric and valeric acids gradually increased from < 0.15 g/kg (day 0) to 5.00, 4.00 and 1.10 g/kg (day 79), respectively. From day 79, pH dropped from 7.1 to 6.1, linked to a sudden acetic acid increase from 3.00 to 5.00 g/kg, and the subsequent CH<sub>4</sub> content drop from 56 to 37 % [Figure 4.2f].

Mono-digestion reactor B was relatively more stressed than reactor A, as indicated by the wider acetic acid fluctuations (i.e.  $\pm 4.00$  g/kg) and the rapid accumulation of propionic acid from 1.20 (day 7) to 5.80 g/kg (day 73) [Figure 4.3b]. The VFA fluctuation is in line with the fact that methanogens grow relatively slower than the hydrolytic/acidogenic microorganisms in AD (De Vrieze et al., 2012; Gerardi, 2003). Thus, the higher OLR used in reactor B led to a more pronounced methanogenic/acidogenic imbalance, exacerbating the VFA accumulation. The VFA buildup led to a pH decrease from 8.4 to 6.2 in reactor B during the whole experimental period, while a significant acetoclastic inhibition occurred from day 70 to 73, when acetic acid abruptly increased from 2.70 to 5.80 g/kg.

pH in co-digestion reactor A gradually decreased from 8.7 to 6.4 along the experimental period, showing a minimum of 6.1 associated with a peak of acetic acid of 8.30 g/kg (day 26) [Figure 4.5a]. Acetic acid was considerably consumed (i.e. < 0.36 g/kg) by day 47 due to ongoing methanogenesis, and progressively increased thereafter by overloading. Similarly, pH in reactor B showed a minimum of 6.3 when acetic acid peaked at 8.20 g/kg (day 26) [Figure 4.5b], while the acetic acid was extensively consumed (i.e. < 0.35 g/kg) by day 41 prior to increase again steadily. In reactor C, acetic acid had a similar evolution with a maximum of 7.20 g/kg (day 26) [Figure 4.5c], while pH dropped to 6.0 on day 57, associated with a sharp acetic acid build-up from 1.00 to 3.70 g/kg. Propionic, butyric and valeric acids increased from 0.50, 0.14 and 0.00 g/kg (day 0) to a maximum range of 3.00-3.50, 2.90-3.20 and 2.50-2.60 g/kg, respectively, obtained right after acidification occurred on day 79 in reactors A and B, and on day 56 in reactor C. The pH was relatively lower (i.e. 2 %; p = 0.13) and total VFA was relatively higher (i.e. 5 %; p = 0.25) during mono-digestion than co-digestion, likely due to the faster degradation rates but also the higher release of inhibitory compounds related to OFMSW than sawdust, as discussed in section 4.3.3.

Feeding the reactors a maximum of 5 days per week influenced the reactor dynamics, since pH increased and VFA – mainly acetic acid – decreased during the periods with no

feed. The pH and VFA modifications [Figures 4.3 and 4.5] were associated with the TS removal, as mentioned before, and also affected the biogas production/composition, and the TAN buildup [Figures 4.2 and 4.4]. As an example, in co-digestion reactor A, pH increased from 7.0 to 7.9 from day 37 to 41, while acetic acid decreased from 4.30 to 2.40 g/kg, triggering a biogas production of 0.6 L/kg reactor content and a methane content increase from 59 to 70 % [Figure 4.4 and 4.5].

#### 4.3.4.2 Biogas Production and Composition

Mono-digestion of OFMSW resulted in a cumulative biogas production of 65 and 66 L/kg reactor content in reactor A and B, respectively [Figure 4.2e]. Biogas production was mainly correlated to the acetic acid consumption [Figure 4.3a], as mentioned in the previous subsection. For example, 21 L/kg reactor content of biogas were measured during the initial 20 days of reactor A, before acetic acid accumulated and biogas production slowed down.

Biogas composition measurements started on day 60 showing an average of 63 % CH<sub>4</sub> in both mono-digestion reactors [Figure 4.2f], which subsequently fluctuated showing a downward trend alongside the VFA accumulation. The CH<sub>4</sub> content dropped below 40 % in both reactors right after biogas production definitely ceased on days 78-79. The reduction of CH<sub>4</sub> content in the biogas is also an indicator of AD imbalance, though it might be inappropriate to assess rapid changes in the reactor performance (Drosg, 2013). The highest H<sub>2</sub> concentration (data not shown) was 1.8 and 1.1 % on day 59 in reactors A and B, respectively, while H<sub>2</sub> remained below 0.8 % in both reactors during the rest of the experiment. The presence of H<sub>2</sub> indicated that the hydrogenotrophic methanogens were unable to cope with the rapid H<sub>2</sub> production from acidogenesis, since H<sub>2</sub> higher than 1-2 % in the gas phase is normally associated with AD overloading (Drosg, 2013; Molina et al., 2009).

Co-digestion in reactor A led to a cumulative biogas production of 48 L/kg reactor content, while 49 and 27 L/kg reactor content were observed in reactors B and C, respectively [Figure 4.4e]. In spite of the higher OLR used in co-digestion, the biogas production was considerably lower than that obtained with mono-digestion (i.e. 65 L/kg reactor content). Thus, the specific biogas production was  $229 \pm 20$  L/kg VS added in mono-digestion and  $86 \pm 18$  L/kg VS added in co-digestion (i.e. 62 % lower), due to the reduced biodegradability of sawdust.

The CH<sub>4</sub> content [Figure 4.4f] reached a peak of 75 % during the first two weeks of operation in the three co-digestion reactors, but it decreased subsequently as VFA accumulated [Figure 4.5]. A minimum 43 % CH<sub>4</sub> was detected in reactor A associated with the last biogas production observed (day 82), while a sharp drop from 60 to 29 % CH<sub>4</sub> was

observed in reactor C right after day 60.  $H_2$  was detected at 0.3 % in the three co-digestion reactors on day 23 (data not shown). Thereafter,  $H_2$  was not detected in reactor A, while reactor B showed a single  $H_2$  peak of 1.5 % on day 70, right after the reactor was accidentally opened to the atmosphere. In reactor C,  $H_2$  peaks of 1.7, 1.2 and 1.6 % were observed on days 41, 47 and 58, respectively, supporting the occurrence of a more extensive overload in this reactor.

#### 4.3.5 Testing Recovering Strategies

Once acidification occurred, feeding was stopped and some recovering strategies were tested to resume methanogenesis. In mono-digestion reactor A, a 3 M NaHCO<sub>3</sub> buffer solution was added on days 83 and 84 to raise the pH (i.e. from 6.2 to 6.8) within a suitable range for methanogens (i.e. 6.5-7.0). Adding NaHCO<sub>3</sub> is normally used to counteract acidification when digesters show a reduced ALK<sub>P</sub> (Chen et al., 2008; Holliger et al., 2016). However, methanogenesis did not recover after more than 20 days.

On day 76, FeCl<sub>2</sub> was supplemented to mono-digestion reactor B in a higher amount than the stoichiometric, to precipitate the total H<sub>2</sub>S in the system (i.e. 30 mg H<sub>2</sub>S/kg, data not shown). However, FeCl<sub>2</sub> overdosing resulted in a pH drop from 6.3 to 5.7 (days 76-77). Thus, 2 M NaHCO<sub>3</sub> solution was rapidly added to recover the pH to 6.6 (day 77). Both Fe<sup>2+</sup> and/or Fe<sup>3+</sup> can be used to precipitate sulfide in AD, but Fe<sup>2+</sup> was preferred in this study to avoid the inclusion of a strong electron acceptor (i.e. Fe<sup>3+</sup>) that could react with organic compounds in the anaerobic digester (i.e. Fe<sup>3+</sup> + 1/2 H<sub>2</sub> -> Fe<sup>2+</sup> + H<sup>+</sup>,  $\Delta G^{o'} <<0$ ) (Fermoso et al., 2015; Rittman et al., 2001). After 2 weeks of methanogenic inhibition (day 90), 200 g of 'wet' AD inoculum from the source reactor were added to reactor B, allowing a gradual methanogenic recovery, associated with an increase of pH from 6.9 to 7.3 [Figure 4.3b] and CH<sub>4</sub> content from 20 to 52 % [Figure 4.2f], until the end of the reactor operation.

Aiming to recover methanogenesis in all co-digestion reactors, water was progressively added to dilute the effect of potential methanogenic inhibitor(s). The progressive addition of low amounts of water in co-digestion reactors permitted to maintain HS-AD conditions (i.e.  $TS \ge 10$  %), thanks to the elevated TS content reached before reactors acidified (i.e.  $TS \ge 30$  %). Dilution was performed in reactors A and B from day 79 and in reactor C from day 62. In reactor A and B an average of 180 and 170 mL of water was used, respectively, on days 79, 82, 84, 91 and 98, while an average of 160 mL of water was added to reactor C on days 62, 63, 68 and 91.

In conclusion, neither water, nor buffer addition permitted to recover acidified/acidifying HS-AD reactors, probably because of the important imbalance between methanogens and acid-producers in the system (Gerardi, 2003). In these conditions, inoculum addition might be the only way to recover an acidified HS-AD reactor, though emptying and re-inoculating the reactor might be necessary (Fricke et al., 2007). Another strategy to prevent reactor acidification and/or enhance the digester performance is trace element (i.e. Se, Ni, Co, Mo, W) addition (Fermoso et al., 2015; Mancini et al., 2018b). However, this was out of the scope of this manuscript.

# 4.3.6 Ammonia Buildup

In this study, overloading was associated with the high OLR used, but also with the NH<sub>3</sub> buildup, in the semi-continuous reactors. The high OLR and the degradation of the protein content of OFMSW increased the TAN content in both mono-digestion reactors [Figure 4.2d]. TAN ranged from 3.4 g N/kg (day 0) to a maximum of 4.8 and 4.9 g N/kg in reactors A and B (day 104), respectively, with both reactors showing a minimum TAN of 3.0 g N/kg around day 20. The initial NH<sub>3</sub> was 1.1 and 1.7 g N/kg in reactors A and B, respectively. Subsequently, NH<sub>3</sub> fluctuated with an overall decreasing trend along the pH modification in both reactors, showing peaks higher than 1.0 g N/kg mainly when pH was relatively high (i.e.  $\geq$  8.0) [Figure 4.3]. In reactor A, NH<sub>3</sub> reached peaks of 1.4 (day 7) and 1.5 g N/kg (day 34), while NH<sub>3</sub> higher than 1.5 g N/kg was repeatedly observed in reactor B (i.e. days 20, 27, 34 and 41).

In co-digestion, the initial TAN was 3.0 g N/kg and slightly increased to a maximum of 3.3, 3.6 and 3.3 g N/kg (day 61) in reactors A, B and C, respectively [Figure 4.4d]. TAN subsequently decreased due to the reduced OFMSW feeding and the progressive dilution used for HS-AD recovering, until a minimum of 1.9, 2.3 and 2.8 g N/kg was reached in reactors A, B and C, respectively (day 112). The initial NH<sub>3</sub> was 2.0 g N/kg and progressively decreased in the three reactors alongside pH. NH<sub>3</sub> peaked at 1.5 g N/kg (day 12) and 1.2-1.7 g N/kg (day 19), rapidly decreasing to  $\leq$  0.1 g N/kg (day 23), similarly in all reactors. From this point, NH<sub>3</sub> was maintained below 1.0 g N/kg in the three reactors. Thus, NH<sub>3</sub> was considerably reduced during co-digestion alongside the reduction of OFMSW in the feed, since peaks higher than 1.0 g N/kg were not observed from day 20 onwards, in contrast to mono-digestion reactors.

NH<sub>3</sub> inhibition was likely one of the main triggers of overloading in this study, since the high NH<sub>3</sub> levels observed (i.e.  $\geq 1.0$  g N/kg) are normally associated with methanogenic inhibition and VFA accumulation in AD (Drosg, 2013; Rajagopal et al., 2013). Thus, despite each AD system might show particular NH<sub>3</sub> inhibition thresholds depending on the anaerobic consortia (Fricke et al., 2007; Westerholm et al., 2016), a gradual methanogenic adaptation to high levels of TAN (i.e.  $\geq 4.0$  g N/kg) might be crucial to increase OLR in semicontinuous HS-AD of OFMSW (Hartmann et al., 2006; Rajagopal et al., 2013).

In this study, a tradeoff was needed between the 'undesired' TAN buildup and the rapid TS removal observed, to reach HS-AD conditions (i.e.  $TS \ge 10$  %) with mono-digestion of OFMSW. For example, the different TS and TAN dynamics can be appreciated in monodigestion reactor A from day 30, when TS fluctuated while TAN steadily increased [Figure 4.2]. Potential ammonia contingency strategies in AD, as increasing the substrate dilution, reducing the OLR, and/or increasing the MRT (Kayhanian, 1999; Rajagopal et al., 2013), would have lengthened considerably the experimental time, or even prevented to achieve HS-AD conditions (i.e.  $TS \ge 10$  %) with mono-digestion of OFMSW.

#### 4.4 Conclusions

In this study, reducing the effluent compared to the influent mass (i.e. 18 %) permitted to extend the MRT in semi-continuous mono-digestion of OFMSW, and obtain a specific biogas production of 229 L/kg VS added, due to the high biodegradability of OFMSW. However, the sole implementation of influent/effluent uncoupling was not sufficient to avoid reactor overload and acidification when reaching HS-AD conditions (i.e. TS  $\geq$  10 %). The average OLR was 4.5 g VS/kg·d, whereas a maximum 11.5 % TS was reached. In contrast, the addition of beech sawdust to OFMSW allowed to operate co-digestion reactors with an average OLR of 8.3 g VS/kg·d, and reach a maximum 29.0 % TS. Co-digestion lowered by 22 % the TAN content, though an average 186 L/kg VS added of biogas was obtained. Therefore, the addition of sawdust, as an example of lignocellulosic substrate, to OFMSW (i.e. 1-2 g VS-Sawdust/g VS-OFMSW) is an adequate strategy to stabilize HS-AD at very high TS contents (i.e. 20-30 %). Nonetheless, a compromise must be found between increasing the TS content and reducing the specific biogas production by co-digestion, since both aspects strongly determine the HS-AD economy for OFMSW treatment.
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## Chapter 5

| <b>High-Solids</b> | Anaerobic | Digestion | Model | for |
|--------------------|-----------|-----------|-------|-----|
| Homogenized        | Reactors  |           |       |     |

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#### Abstract

During high-solids anaerobic digestion (HS-AD) of the organic fraction of municipal solid waste (OFMSW), an important total solid (TS) removal occurs, leading to the modification of the reactor content mass/volume, in contrast to 'wet' anaerobic digestion (AD). Therefore, HS-AD mathematical simulations need to be approached differently than 'wet' AD simulations. This study aimed to develop a modelling tool based on the anaerobic digestion model No.1 (ADM1) capable of simulating the TS and the reactor mass/volume dynamics in the HS-AD of OFMSW. Four hypotheses were used, including the effects of apparent concentrations at high TS. The model simulated adequately HS-AD of OFMSW in batch and continuous mode, particularly the evolution of TS, reactor mass, ammonia and volatile fatty acids. By adequately simulating the reactor content mass/volume and the TS, this model might bring further insight about potentially inhibitory mechanisms (i.e. NH<sub>3</sub> buildup and/or acidification) occurring in HS-AD of OFMSW.

**Keywords**: High-Solids Anaerobic Digestion; ADM1; Reactor Mass Simulation; Total Solids; Apparent Concentrations.



#### **Graphical Abstract**

## 5.1 Introduction

Anaerobic digestion (AD) is a biochemical treatment technology for organic waste valorization yielding a high-methane-content biogas and a partially stabilized organic material with potential applications as soil amendment (Mata-Álvarez, 2003). High-solids anaerobic digestion (HS-AD) is a particular case of AD operated at a total solid (TS) content  $\geq 10$  %, in contrast to 'wet' AD applications (i.e. TS < 10 %) (Abbassi-Guendouz et al., 2012). Thus, HS-AD has the advantage of minimizing the reactor volume, as well as the need for water addition. On the other hand, HS-AD is normally associated with an important reduction of the total (TS) and volatile (VS) solid content, during the biological degradation of the organic matter. For example, HS-AD of the organic fraction of municipal solid waste (OFMSW) might lead to a TS removal of 30 - 80 % (Cecchi et al., 2002; Mata-Álvarez, 2003; Pavan et al., 2000). However, some drawbacks limit the applicability of HS-AD as, for example, the reduced kinetics expected as a consequence of the hampered mass transfer, and the high risk of acidification due to organic overloading (Benbelkacem et al., 2015; De Baere, 2000).

Among the solid wastes used in HS-AD, the OFMSW is particularly suited for anaerobic treatment due to its elevated TS content (i.e. 25 - 30 %), biodegradation potential and possibility to recover nutrients (i.e. nitrogen and phosphorous) from its composition (De Baere et al., 2013; Mata-Álvarez, 2003). However, HS-AD of OFMSW is normally associated with a high risk of inhibition due to the high protein content, leading to free ammonia nitrogen (NH<sub>3</sub>), as one of the most important inhibitors (Chen et al., 2008; Kayhanian, 1999; Rajagopal et al., 2013).

Understanding the biochemical and physical-chemical dynamics in HS-AD is crucial to ease the design and operation of HS-AD reactors, minimizing the risk of acidification/inhibition. Particularly important is the knowledge about the interactions between the main four phases – microorganisms, solids, liquids and gases – in HS-AD, since it might allow to increase the waste treatment capabilities and methane yield (Mata-Álvarez, 2003; Vavilin et al., 2004; Xu et al., 2015). In this line, an adapted mathematical model is required for the operational analysis and technology development of HS-AD, as some of the main applications for 'wet' AD of the anaerobic digestion model No.1 (ADM1) (Batstone, 2006; Batstone et al., 2002; Batstone et al., 2015; Xu et al., 2015).

ADM1 is a structured model gathering together the main biochemical and physicalchemical processes of AD (Batstone et al., 2002; Batstone et al., 2015). Biochemical processes include the disintegration, hydrolysis, acidogenesis, acetogenesis and methanogenesis of complex substrates composed of carbohydrates, proteins and lipids in chemical oxygen demand (COD) units. Physical-chemical processes include the gas transfer AD applications (i.e. TS << 10 %), while a more complex hydraulic and particulate component modeling is required for HS-AD (Batstone et al., 2002; Batstone et al., 2015; Xu et al., 2015). Thus, modelling HS-AD might be particularly challenging due to the intrinsic complexity of the process (Batstone et al., 2015; Mata-Alvarez et al., 2000; Vavilin et al., 2004; Xu et al., 2015). For example, the (semi-)solid matrix might define the soluble/gaseous transport processes, as well as the capabilities of anaerobic biomass to access the substrates (Bollon et al., 2013; Vavilin et al., 2005).

The mass balance modification, regarding the continuously stirred tank reactor (CSTR) implementation of ADM1 (Batstone et al., 2002), is required to account for the reactor content mass (M<sub>Global</sub>) removal and the specific weight (p<sub>Global</sub>) dynamics in HS-AD (Batstone et al., 2015; Kayhanian et al., 1994; Richards et al., 1991; Vavilin et al., 2004). Noteworthy, the reactor content volume ( $V_{Global}$ ) might describe important fluctuations during HS-AD, depending mainly on the substrate TS and biodegradability, in contrast to 'wet' AD. Furthermore, a given degree of gaseous porosity ( $\epsilon$ ) might be present in the HS-AD matrix, particularly at TS contents  $\geq 25$  % (Batstone et al., 2015; Benbelkacem et al., 2013; Bollon et al., 2013; Vavilin et al., 2003). ADM1 was originally expressed in volumetric units (i.e. kg COD/m<sup>3</sup>). Meanwhile, the most common measurements in HS-AD are normally expressed in mass units (i.e. kg COD/kg), since accounting for the specific weight of (semi-)solid samples – but also the specific weight dynamics in HS-AD – involves the complexity of the analytical techniques (Benbelkacem et al., 2013; Bollon et al., 2013; Kayhanian et al., 1996). For example, the specific weight of a (semi-)solid sample can be approximated by the use of a water pycnometer, where the sample must be appropriately pretreated (i.e. dried/ground), the distilled water fully degassed and analyses performed under temperature-controlled conditions (ASTM, 2002). With all the above, HS-AD simulations need to be approached differently than in 'wet' AD, where  $\rho_{Global}$  and  $V_{Global}$  are often assumed constant, as summarized in Figure 5.1.



Figure 5.1 High-solids vs. 'wet' anaerobic digestion

This study aimed at developing a mathematical tool based on the ADM1 biochemical framework, capable of simulating the solids and reactor content mass/volume dynamics in HS-AD of OFMSW, including the interrelationship between TS (and VS) removal and biogas production. By simulating adequately the global mass/volume and TS dynamics, the presented model might serve as a link between 'wet' AD and HS-AD, while it might help to explore potential inhibitory/acidification mechanisms occurring during HS-AD of OFMSW. Meanwhile, the proposed model was aimed to be as general as possible, since different HS-AD applications (i.e. organic substrate and/or reactor configuration) could be simulated, provided that the main hypotheses presented in the methodology section are fulfilled. Furthermore, the eventual model user is encouraged to further calibrate the model parameters and/or modify the model structure, in order to adapt the HS-AD model for any specific need.

## 5.2 Materials and Methods

## 5.2.1 High-Solids Model Implementation

The main basis for the dynamic model presented in this study was ADM1 (Batstone et al., 2002), including the modifications suggested by Blumensaat et al. (2005) for closing nitrogen and carbon balances. The simulation of the HS-AD of OFMSW required four preliminary hypotheses in order to reduce the complexity of the model. Firstly, HS-AD was assumed to take place in a homogenized (i.e. completely mixed) reactor [Hypothesis 1]. Secondly, the effect of porosity and transport processes was assumed to be negligible [Hypothesis 2]. Then, the specific weight of solids and solvent was considered constant [Hypothesis 3]. Finally, the biochemical reactions were assumed to occur predominantly in water [Hypothesis 4].

With these hypotheses, ADM1 required some particular modifications in order to simulate the TS and mass/volume dynamics in HS-AD, while allowing the calibration of the proposed model. The main modifications implemented in ADM1 in order to simulate HS-AD were the inclusion of mass balances modifying the reactor mass and volume (needed to account for the organic solid removal in HS-AD) and the inclusion of apparent concentrations (as a link between 'wet' and high-solids applications).

#### 5.2.1.1 Mass Balances in High-Solid Anaerobic Digestion Reactors

1 . .

The simulation of the reactor mass and TS/VS content of homogenized HS-AD reactors required the implementation of the global ( $M_{Global}$ ) [Equation 5.1], solid material ( $M_{Solids}$ ) [Equation 5.2], liquid-solvent content ( $M_{Solvent}$ ) [Equation 5.3] and inert material ( $M_{Inerts}$ ) [Equation 5.4] mass balances. In this study, the solvent was considered as only water, while the solid material included all the organic and inorganic compounds (i.e. particulates and soluble compounds, VFA, microorganisms) inside the reactor, except water. In mass balances, the mass content ( $M_i$ ) – global or partial – dynamics were related to the corresponding mass fluxes ( $m_i$ ), particularly the gases flowing out of the reactor as a consequence of methanogenesis. The implementation of reactor mass balances is crucial in HS-AD, since it accounts for the importance of mass and water removal due to biogas production, in contrast to 'wet' AD (Henze et al., 1997; Kayhanian et al., 1996; Richards et al., 1991).

$$\frac{dM_{Global}}{dt} = m_{Influent,Global} - m_{Effluent,Global} - m_{Biogas}$$
(5.1)

$$\frac{dM_{Solids}}{dt} = m_{Influent,Solids} - m_{Effluent,Solids} - (m_{Biogas} - m_{Vapor})$$
(5.2)

$$\frac{dM_{Solvent}}{dt} = m_{Influent,Solvent} - m_{Effluent,Solvent} - m_{Vapor}$$
(5.3)

$$\frac{dM_{Inerts}}{dt} = m_{Influent,Inerts} - m_{Effluent,Inerts}$$
(5.4)

The biogas (m<sub>Biogas</sub>) [Equation 5.5] and vapor (m<sub>Vapor</sub>) [Equation 5.6] outflows in the mass balances were calculated from the volumetric biogas flow (Q<sub>g</sub>), obtained as shown in the CSTR implementation of ADM1 (Batstone et al., 2002), by using the molar gas composition ( $x_i$ ) and the molecular weight ( $Mr_i$ ) of each gaseous compound in the gas phase. The biogas was assumed to be composed of CH<sub>4</sub>, CO<sub>2</sub>, H<sub>2</sub>, H<sub>2</sub>O and NH<sub>3</sub>. The reactor headspace was assumed to be vapor saturated, being vapor pressure ( $P_v$ ) expressed as a function of temperature (T). On the other hand, an inert gas was added to account for the initial flushing in AD experiments (i.e. by N<sub>2</sub>), assuming for it a negligible liquid solubility. Importantly, the inert gas was not included in m<sub>Biogas</sub> calculations. Once knowing the M<sub>Global</sub>, M<sub>Solids</sub> and M<sub>Inerts</sub>, the TS and VS contents were approximated in dynamic mode by using the corresponding definition (EPA, 2001) [Equations 5.7 & 5.8]. Noteworthy, TS and VS in the proposed model were dimensionless (i.e. kg Solids/kg Total), varying from 0 to 1.

$$m_{Biogas} = \frac{P_T Q_g}{RT} \sum x_i M r_i \tag{5.5}$$

$$m_{Vapor} = \frac{P_v Q_g}{RT} M r_{H2O}$$
(5.6)

$$TS = \frac{M_{Solids}}{M_{Global}}$$
(5.7)

$$VS = \frac{M_{Solids} - M_{Inerts}}{M_{Global}}$$
(5.8)

The liquid-gas transfer of gaseous species in the CSTR implementation of ADM1 depends on the ratio between the reactor content volume ( $V_{Global}$ ; ' $V_{Iiq}$ ' in ADM1) and the gas volume ( $V_g$ ), while their sum yields the design/overall reactor volume ( $V_{Reactor}$ ) (Batstone et al., 2002). Thus, since a considerable reduction of  $V_{Global}$  – alongside  $M_{Global}$  removal – can occur in HS-AD associated with methanogenesis, the reactor volume was approximated by the specific weigh of the reactor content ( $\rho_{Global}$ ). Importantly,  $\rho_{Global}$  varies also in HS-AD, as it gathers together the individual dynamics of all the mass compounds in the system (Kayhanian et al., 1996). Therefore, to simulate  $\rho_{Global}$ , it is necessary to know the specific weight of all the materials within HS-AD ( $\rho_i$ ), but also their corresponding mass fraction ( $m_i$ ) [Equation 5.9]. For simplicity, the simulations in this study used a common specific weight for all the solid compounds ( $\rho_{Solids}$ ) and a solvent specific weight ( $\rho_{Solvent}$ ). With these simplifications, the  $V_{Global}$  dynamics could be approximated with Equation 5.10.

$$\frac{1}{\rho_{Global}} = \sum_{i} \frac{m_i}{\rho_i} \tag{5.9}$$

$$\frac{dV_{Global}}{dt} = \frac{1}{\rho_{Solids}} \cdot \frac{dM_{Solids}}{dt} + \frac{1}{\rho_{Solvent}} \cdot \frac{dM_{Solvent}}{dt}$$
(5.10)

The distinction between mass and volume in the proposed model for homogenized HS-AD reactors permitted the use of ADM1 volumetric units (i.e.  $\text{kmol/m}^3$ ), while implementing the different influent and effluent mass and/or volumetric flows when operating HS-AD in (semi-)continuous mode. Finally, for illustrative purposes only, an adaptive volumetric effluent (Q<sub>Effluent</sub>) was added to the model – in terms of a proportional controller – to maintain V<sub>Global</sub> if required. This strategy permitted to compensate for the potential organic mass removal in HS-AD and, therefore, to stabilize the HS-AD system, as further discussed





Figure 5.2 Schematic representation of the high-solids anaerobic digestion model implementation

## 5.2.1.2 Apparent Concentrations – Soluble Species Recalculation

The (soluble) apparent concentrations  $(S_{T,i,App})$  were used in the HS-AD model biochemistry and physical-chemistry to reproduce the effect of high TS in HS-AD, in contrast to 'wet' AD. This modification was related to the assumption that the main biochemical reactions might occur predominantly in the presence of water (Hypothesis 4). Similarly, the apparent concentrations served to link the global (i.e. kmol/kg Total) and liquid fraction (i.e. kmol/kg Solvent) measurements in HS-AD. The apparent concentrations were calculated for all the soluble species of ADM1 using TS,  $\rho_{Global}$  and  $\rho_{Solvent}$  [Equation 5.11]. Importantly, the long chain fatty acids (LCFA,  $S_{fa}$ ) were not considered as soluble in HS-AD, due to their highly non-polar nature and reduced solubility in water (i.e. palmitic acid solubility = 1.2 mg/L at 60 °C). With this approach, the proposed model simulates the mass balance of dynamic variables  $(C_{T,i})$  – either particulate  $(X_{T,i})$  or soluble  $(S_{T,i})$  – as a function of V<sub>Global</sub> (i.e. kmol/m<sup>3</sup> Total) [Equation 5.12], while the apparent concentrations (S<sub>T,i,App</sub>) (i.e. kmol/m<sup>3</sup> Solvent) were used only for the soluble species included in the biochemical and physical-chemical rates of ADM1 ( $r_{i,ADM1}$ ) (i.e. uptake of acetate). It is important to mention that Equation 5.12 is the mass balance of an individual component in AD and, therefore, should be based in the chain rule in order to account for the V<sub>Global</sub> dynamics, in contrast to the CSTR implementation of ADM1 (Batstone et al., 2002). On the other hand, it should be noted that the effect of apparent concentrations becomes negligible at low TS contents (i.e. TS < 5 %) with  $\rho_{Global}$  tending to  $\rho_{Solvent}$ , as  $S_{T,i,App}$  progressively approaches to  $S_{T,i}$  in these conditions. With all the above, the sole implementation of the HS-AD mass balances and the use of apparent concentrations in this study might allow to simulate indistinctly 'wet' AD and HS-AD conditions, and/or the transition between these two AD regimes, for example, during a prolonged HS-AD operation.

$$S_{T,i,App}\left(\frac{kg \text{ or } kmol}{m^3 \text{ Solvent}}\right) = \frac{S_{T,i}\left(\frac{kg \text{ or } kmol}{m^3 \text{ Total}}\right)}{(1 - TS)\left(\frac{kg \text{ Solvent}}{kg \text{ Total}}\right)} \cdot \frac{\rho_{Solvent}\left(\frac{kg \text{ Solvent}}{m^3 \text{ Solvent}}\right)}{\rho_{Global}\left(\frac{kg \text{ Total}}{m^3 \text{ Total}}\right)}$$
(5.11)

$$\frac{dC_{T,i}}{dt} = \frac{1}{V_{Global}} \cdot \left( Q_{Influent} \cdot C_{T,0} - \frac{m_{Effluent}}{\rho_{Global}} \cdot C_{T,i} \right) + \sum r_{i,ADM1} - \frac{C_{T,i}}{V_{Global}} \cdot \frac{dV_{Global}}{dt} \quad (5.12)$$

### 5.2.1.3 Kinetic Rates

The ADM1 biochemical rates and inhibitions were used for the verification of the model implementation according to the protocol proposed by Rosén et al. (2006). The model verification aimed to test/assess the ADM1 implementation (code) alongside the adequate mathematical solution of the mass balances, determining the TS and organic removal both in 'wet' and high-solids AD applications. On the other hand, a slightly different set of biochemical rates was used for HS-AD model calibration. Thus, calibration aimed to test/assess the HS-AD model performance under real experimental conditions. The biochemical kinetics used in this study are shown in Table 5.1.

The biochemical rates used in the HS-AD model were associated with the inhibitory functions as originally proposed in ADM1 (Batstone et al., 2002; Rosén et al., 2006) [Equations 5.13 to 5.16]. However, all the soluble species terms included in the HS-AD biochemical rates – excluding  $S_{fa}$  – were expressed in terms of apparent concentrations, as mentioned in section 5.2.1.2.

| D                           | Rate (p <sub>j</sub> , kg COD m <sup>-3</sup> d <sup>-1</sup> )                                                                                                                                                                                                          |                                                                                                                                                                                                            |  |  |  |
|-----------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|--|--|
| Process -                   | Model Verification                                                                                                                                                                                                                                                       | Model Calibration                                                                                                                                                                                          |  |  |  |
| Disintegration              | $k_{dis} \cdot X_c$                                                                                                                                                                                                                                                      | -                                                                                                                                                                                                          |  |  |  |
| Hydrolysis of Carbohydrates | $k_{h,ch}{\boldsymbol{\cdot}} \mathbf{X}_{ch}$                                                                                                                                                                                                                           | $k_{ m h,ch} \cdot X_{ m ch}$                                                                                                                                                                              |  |  |  |
| Hydrolysis of Proteins      | $k_{h,pr} {\boldsymbol \cdot} {\mathbf X}_{pr}$                                                                                                                                                                                                                          | $k_{h,pr} \cdot X_{pr}$                                                                                                                                                                                    |  |  |  |
| Hydrolysis of Lipids        | $\mathbf{k}_{\mathrm{h,li}} \cdot \mathbf{X}_{\mathrm{li}}$                                                                                                                                                                                                              | $k_{\rm h,li}{\cdot}X_{\rm li}$                                                                                                                                                                            |  |  |  |
| Sugars Uptake               | $k_{m,su} {\cdot} S_{su,App} / (K_{S,Xsu} {+} S_{su,App}) {\cdot} X_{su} {\cdot} I_{pH} {\cdot} I_{in}$                                                                                                                                                                  | $k_{m,su} \cdot S_{su,App} / (K_{S,Xsu} + S_{su,App}) \cdot X_{su} \cdot I_{pH} \cdot I_{in}$                                                                                                              |  |  |  |
| Aminoacids Uptake           | $k_{m,aa} {\cdot} S_{aa,App} / (K_{S,Xaa} {+} S_{aa,App}) {\cdot} X_{aa} {\cdot} I_{pH} {\cdot} I_{in}$                                                                                                                                                                  | $k_{m,aa} \cdot S_{aa,App} / (K_{S,Xaa} + S_{aa,App}) \cdot X_{aa} \cdot I_{pH} \cdot I_{in}$                                                                                                              |  |  |  |
| LCFA Uptake                 | $k_{m,fa} \cdot S_{fa}/(K_{S,Xfa} + S_{fa}) \cdot X_{fa} \cdot I_{pH} \cdot I_{in} \cdot I_{h2}$                                                                                                                                                                         | $k_{m,fa} \cdot S_{fa} / (K_{S,Xfa} + S_{fa}) \cdot X_{fa} \cdot I_{pH} \cdot I_{in} \cdot I_{h2}$                                                                                                         |  |  |  |
| Valerate Uptake             | $\begin{split} &k_{m,c4} \cdot \mathbf{S}_{va,App} / (\mathbf{K}_{S,Xc4} + \mathbf{S}_{va,App}) \cdot \mathbf{X}_{c4} \\ &\cdot \mathbf{S}_{va,App} / (1 + \mathbf{S}_{bu,App} + 10^{-6}) \cdot \mathbf{I}_{pH} \cdot \mathbf{I}_{in} \cdot \mathbf{I}_{h2} \end{split}$ | $\begin{array}{l} k_{m,c5} \cdot S_{va,App} / (K_{S,Xc5} + S_{va,App}) \cdot X_{c5} \cdot I_{pH} \\ \cdot I_{in} \cdot I_{h2} \end{array}$                                                                 |  |  |  |
| Butyrate Uptake             | $\begin{array}{l} k_{m,c4} \cdot S_{bu,App}/(K_{S,Xc4} + S_{bu,App}) \cdot X_{c4} \\ \cdot S_{bu,App}/(1 + S_{bu,App} + 10^{-6}) \cdot I_{pH} \cdot I_{in} \cdot I_{h2} \end{array}$                                                                                     | $\begin{array}{l} k_{m,c4} \cdot S_{bu,App} / (K_{S,Xc4} + S_{bu,App}) \cdot X_{c4} \cdot I_{pH} \\ \cdot I_{in} \cdot I_{h2} \end{array}$                                                                 |  |  |  |
| Propionate Uptake           | $\begin{array}{l} k_{m, pro} \cdot \mathbf{S}_{pro, App} / (\mathbf{K}_{S, Xpro} + \mathbf{S}_{pro, App}) \cdot \mathbf{X}_{pro} \cdot \mathbf{I}_{pH} \\ \cdot \mathbf{I}_{in} \cdot \mathbf{I}_{h2} \end{array}$                                                       | $\begin{array}{l} k_{m, pro} \cdot S_{pro, App} / (K_{S, Xpro} + S_{pro, App}) \cdot X_{pro} \cdot I_{pH} \\ \cdot I_{in} \cdot I_{h2} \end{array}$                                                        |  |  |  |
| Acetate Uptake              | $\begin{array}{l} k_{m,ac} \! \cdot \! S_{acApp} \! / \! (K_{S,Xac} \! + \! S_{ac,App}) \! \cdot \! X_{ac} \! \cdot \! I_{pH} \! \cdot \! I_{in} \\ \cdot \! I_{nh3} \end{array}$                                                                                        | $\begin{array}{l} k_{m,ac} \cdot \mathbf{S}_{ac,App} / (\mathbf{K}_{S,Xac} + \mathbf{S}_{ac,App}) \cdot \mathbf{X}_{ac} \cdot \mathbf{I}_{pH} \\ \cdot \mathbf{I}_{in} \cdot \mathbf{I}_{nh3} \end{array}$ |  |  |  |
| Hydrogen Uptake             | $k_{m,h2} \cdot S_{h2,App} / (K_{S,Xh2} + S_{h2,App}) \cdot X_{h2} \cdot I_{pH} \cdot I_{in}$                                                                                                                                                                            | $k_{m,h2} \boldsymbol{\cdot} S_{h2,App} / (K_{S,Xh2} \boldsymbol{+} S_{h2,App}) \boldsymbol{\cdot} X_{h2} \boldsymbol{\cdot} I_{pH} \boldsymbol{\cdot} I_{in}$                                             |  |  |  |
| Sugar Degraders Decay       | $k_d \cdot X_{su}$                                                                                                                                                                                                                                                       | $k_{d} \cdot X_{su}$                                                                                                                                                                                       |  |  |  |
| Aminoacids Degraders Decay  | $k_d \cdot X_{aa}$                                                                                                                                                                                                                                                       | $k_{d}$ · $X_{aa}$                                                                                                                                                                                         |  |  |  |
| LCFA Degraders Decay        | $k_d \cdot X_{fa}$                                                                                                                                                                                                                                                       | $k_{d}$ · $X_{fa}$                                                                                                                                                                                         |  |  |  |
| Valerate Degraders Decay    | -                                                                                                                                                                                                                                                                        | $k_d \cdot X_{c5}$                                                                                                                                                                                         |  |  |  |
| Butyrate Degraders Decay    | $k_d \cdot X_{c4}$                                                                                                                                                                                                                                                       | $k_d \cdot X_{c4}$                                                                                                                                                                                         |  |  |  |
| Propionate Degraders Decay  | $k_d \cdot X_{pro}$                                                                                                                                                                                                                                                      | $k_d$ · $X_{pro}$                                                                                                                                                                                          |  |  |  |
| Acetate Degraders Decay     | $k_d \cdot X_{ac}$                                                                                                                                                                                                                                                       | $k_{d} {\boldsymbol \cdot} X_{ac}$                                                                                                                                                                         |  |  |  |
| Hydrogen Degraders Decay    | $k_d \cdot X_{h2}$                                                                                                                                                                                                                                                       | $k_d \cdot X_{h2}$                                                                                                                                                                                         |  |  |  |

Table 5.1 Biochemical kinetics used for model implementation verification and calibration

$$I_{in} = \frac{S_{in,App}}{K_{S,Sin} + S_{in,App}}$$
(5.13)

$$I_{h2} = \frac{K_{i,Sh2}}{K_{i,Sh2} + S_{h2,App}}$$
(5.14)

$$I_{pH} = \frac{K_{pH}^{N_{pH}}}{K_{pH}^{N_{pH}} + S_{proton}^{N_{pH}}}$$
(5.15)

$$I_{nh3} = \frac{K_{i,Snh3}}{K_{i,Snh3} + S_{nh3,App}}$$
(5.16)

Regarding the HS-AD model implementation used for calibration [Table 5.1], the valerate uptake was assumed to be carried out by valerate degraders ( $X_{c5}$ ), instead of butyrate and valerate being both degraded by butyrate degraders ( $X_{c4}$ ), as proposed in ADM1 (Batstone et al., 2002). This last modification was used to account for the different dynamics observed for butyrate and valerate uptake in the experimental data. The valerate parameters and rates were maintained as in the original thermophilic (55 °C) implementation of ADM1, though the  $X_{c5}$  decay was included in the biochemical matrix. On the other hand, the microbial decay was assumed to yield particulate substances (i.e. carbohydrates and proteins) directly, avoiding the use of a composite material ( $X_c$ ) and the associated disintegration kinetics (Batstone et al., 2015). The biomass decay COD fractioning (i.e.  $f_{ch,xc}$ ) was maintained as proposed by Rosén et al. (2006). However, the inert materials (i.e.  $S_i$  and  $X_i$ ) carbon content ( $C_i$ ) was modified to 0.0405 kmol C/kg COD in order to close the biomass carbon balance, while the inert nitrogen content ( $N_i$ ) was modified to 0.0144 kmol N/kg COD to close the biomass nitrogen balance. This last modification permitted to reduce the stiffness and speed up the model simulations in this study.

The degradation of the protein content of an organic waste determines the total ammonia nitrogen (TAN, S<sub>in</sub>) in HS-AD (Kayhanian, 1999). In this line, the nitrogen balance has to be closed for the microorganisms in ADM1, while adding complex substrates implies the fulfilment of the corresponding nitrogen balances. For this study, two nitrogen balances were used for the biomass and substrate as shown in Equations 5.17 and 5.18, respectively, assuming a common nitrogen content for proteins/amino acids ( $N_{aa}$ ). With this approach, two new inert variables ( $S_{i,subs}$  and  $X_{i,subs}$ ) were added to ADM1 in order to calibrate the initial protein content (X<sub>pr</sub>) and/or the experimental TAN dynamics. The nitrogen balance for biomass [Equation 5.17] remained closed as mentioned before, while the protein fraction of the substrate-inoculum mixture  $(f_{pr,subs})$  could be adjusted by calibrating the inert nitrogen content of the substrate-inoculum mixture (Ni,subs), since all the remaining variables in the nitrogen balance ( $N_{subs}$ ,  $f_{si,subs}$  and  $f_{xi,subs}$ ) [Equation 5.18] could be obtained experimentally. For example, the anaerobic biodegradability (i.e. COD<sub>removed</sub>/COD<sub>substrate</sub>) of an organic substrate is equivalent to 1 -  $(f_{si,subs} + f_{xi,subs})$ , while the global nitrogen content of the substrate-inoculum mixture (N<sub>subs</sub>) is the quotient between the total Kjeldahl nitrogen (TKN) and COD (i.e. TKNsubstrate/CODsubstrate).

$$N_{bac} = f_{pr,xc} \cdot N_{aa} + (f_{si,xc} + f_{xi,xc}) \cdot N_i$$
(5.17)

$$N_{subs} = f_{pr,subs} \cdot N_{aa} + \left(f_{si,subs} + f_{xi,subs}\right) \cdot N_{i,subs}$$
(5.18)

## 5.2.2 Verification of the Model Implementation

The proposed model implementation was verified for 'wet' AD according to Rosén et al. (2006). Similarly, the model was further tested for HS-AD conditions. In total, four different verification scenarios were simulated: A) 'wet' AD using the ADM1 implementation of Rosén et al. (2006); B) 'wet' AD using the HS-AD model implementation with a constant  $Q_{Effluent}$ ; C) HS-AD using the HS-AD model and constant  $Q_{Effluent}$ ; and D) HS-AD considering the HS-AD model with an adaptive  $Q_{Effluent}$ . The HS-AD model was coded in MATLAB<sup>®</sup> R2017a. The equation resolution was the ode15s; a variable-step, variable-order solver based on the numerical differentiation formulas of orders 1 to 5. The influent conditions used for model verification are shown in Table 5.2.

Noteworthy, the only difference between the influent conditions during simulations A and B was the introduction of the TS, VS and  $\rho_{Global}$  of the substrate in the last case [Table 5.2], permitting to excite the high-solids module of the proposed HS-AD model, in contrast to the CSTR implementation of ADM1. On the other hand, for illustrative purposes only, a high-solids substrate was included using a different carbohydrate (X<sub>ch</sub>) and particulate inert (X<sub>i</sub>) content, but also TS, VS and  $\rho_{Global}$ , for simulations C and D [Table 5.2]. Thus, the high TS content of the influent conditions (i.e. 25 %), associated predominantly with X<sub>ch</sub> and X<sub>i</sub>, permitted to test the model under HS-AD operation, while avoiding potential inhibitory states due to NH<sub>3</sub> accumulation.

During the verification of the model implementation, all the ADM1 parameters were used as proposed by Rosén et al. (2006) for mesophilic (35 °C) AD operation, though the original hydrolysis constant for carbohydrates ( $k_{h,ch}$ ) had to be reduced to 0.10 days in the HS-AD verification only (simulations C and D), in order to avoid reactor overloading and acidification (i.e. pH  $\leq$  6.0) during the initial days of simulation. 200 days of 'wet' AD or HS-AD operation were simulated for each verification scenario. The organic loading rate (OLR) was evaluated as the daily substrate addition in COD units divided by V<sub>Global</sub>, while the hydraulic retention time (HRT) was evaluated as the quotient between V<sub>Global</sub> and Q<sub>Effluent</sub>.

|                             | Μ            | lodel Verification        |                   | Units     |                        |
|-----------------------------|--------------|---------------------------|-------------------|-----------|------------------------|
| Name                        | Simulation A | Simulation A Simulation B |                   |           | - Model<br>Calibration |
| $S_{su}$                    | 0.010        | 0.010 0.010               |                   | 13.557    | kg COD m <sup>-3</sup> |
| $\mathbf{S}_{\mathrm{aa}}$  | 0.001        | 0.001                     | 0.001             | 2.207     | kg COD m <sup>-3</sup> |
| $\mathbf{S}_{\mathrm{fa}}$  | 0.001        | 0.001                     | 0.001 0.001 1.393 |           | kg COD m <sup>-3</sup> |
| $\mathbf{S}_{va}$           | 0.001        | 0.001                     | 0.001             | 0.734     | kg COD m <sup>-3</sup> |
| $\mathbf{S}_{\mathrm{bu}}$  | 0.001        | 0.001                     | 0.001             | 0.500     | kg COD m <sup>-3</sup> |
| $\mathbf{S}_{\mathrm{pro}}$ | 0.001        | 0.001                     | 0.001             | 2.059     | kg COD m <sup>-3</sup> |
| $\mathbf{S}_{\mathrm{ac}}$  | 0.001        | 0.001                     | 0.001             | 0.103     | kg COD m <sup>-3</sup> |
| Sh2                         | 1.000E-08    | 1.000E-08                 | 1.000E-08         | 1.000E-08 | kg COD m <sup>-3</sup> |
| $\mathbf{S}_{\mathrm{ch4}}$ | 1.000E-08    | 1.000E-08                 | 1.000E-08         | 1.000E-08 | kg COD m <sup>-3</sup> |
| $\mathbf{S}_{\mathrm{ic}}$  | 0.040        | 0.040                     | 0.040             | 0.029     | kmol C m-3             |
| $\mathbf{S}_{\mathrm{in}}$  | 0.010        | 0.010                     | 0.010             | 0.186     | kmol N m <sup>-3</sup> |
| $\mathbf{S}_{i}$            | 0.020        | 0.020                     | 0.020             | 0.000     | kg COD m <sup>-3</sup> |
| $\mathbf{S}_{i,subs}$       | -            | -                         | -                 | 32.227    | kgCOD m <sup>-3</sup>  |
| $X_{c}$                     | 2.000        | 2.000                     | 2.000             | -         | kg COD m <sup>-3</sup> |
| $X_{ch}$                    | 5.000        | 5.000                     | 120.000           | 40.671    | kg COD m <sup>-3</sup> |
| $X_{pr}$                    | 20.000       | 20.000                    | 20.000            | 30.902    | kg COD m <sup>-3</sup> |
| $X_{g}$                     | 5.000        | 5.000                     | 5.000             | 12.534    | kg COD m <sup>-3</sup> |
| $X_{su}$                    | 0.010        | 0.010                     | 0.010             | 0.050     | kg COD m <sup>-3</sup> |
| $X_{aa}$                    | 0.010        | 0.010                     | 0.010             | 0.050     | kg COD m <sup>-3</sup> |
| $\mathbf{X}_{\mathrm{fa}}$  | 0.010        | 0.010                     | 0.010             | 0.001     | kg COD m <sup>-3</sup> |
| $X_{c5}$                    | -            | -                         | -                 | 0.010     | kgCOD m <sup>-3</sup>  |
| $X_{c4}$                    | 0.010        | 0.010                     | 0.010             | 0.002     | kg COD m <sup>-3</sup> |
| $X_{pro}$                   | 0.010        | 0.010                     | 0.010             | 0.005     | kg COD m <sup>-3</sup> |
| $X_{ac}$                    | 0.010        | 0.010                     | 0.010             | 0.003     | kg COD m <sup>-3</sup> |
| $X_{h2}$                    | 0.010        | 0.010                     | 0.010             | 0.070     | kg COD m <sup>-3</sup> |
| $\mathbf{X}_{\mathbf{i}}$   | 25.000       | 25.000                    | 250.000           | 0.000     | kg COD m <sup>-3</sup> |
| $\mathbf{X}_{i,subs}$       | -            | -                         | -                 | 80.567    | kgCOD m <sup>-3</sup>  |
| $\mathbf{S}_{cat}$          | 0.040        | 0.040                     | 0.040             | 0.100     | kmoleq m-3             |
| $\mathbf{S}_{\mathrm{an}}$  | 0.020        | 0.020                     | 0.020             | 0.051     | kmoleq m-3             |
| $ ho_{Global}$              | -            | 1000.000                  | 1100.000          | 1077.633  | kg m <sup>-3</sup>     |
| TS                          | -            | 4.500                     | 25.000            | 15.502    | %                      |
| VS                          | -            | 3.500                     | 23.000            | 12.942    | %                      |

 

 Table 5.2 Influent and initial conditions used for model implementation verification and model calibration

## 5.2.3 Experimental Data and Data Recalculation

The experimental data used to calibrate the HS-AD model consisted in a batch-sacrifice test fed with dried OFMSW and centrifuged inoculum at TS = 15 % operated under thermophilic (55 °C) conditions. In the sacrifice test, 15 replicates were implemented in 250

mL serum bottles. Thus, after measuring the biogas volume and composition, a single replicate was opened, and the HS-AD content thoroughly analyzed for the main physicalchemical variables. The experimental results included the TS, VS,  $\rho_{Global}$ , COD, TKN, TAN, pH, volatile fatty acids (VFA; valeric, butyric, propionic and acetic acids), mono-valent ions (Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup>), biogas composition (CH<sub>4</sub>, CO<sub>2</sub> and H<sub>2</sub>) and methane yield. The serum bottles were agitated only on those days when the biogas production was measured. Further information about the experimental setup, substrate, inoculum and physical-chemical analyses is presented as Supplementary Information.

Importantly, an experimental bias might exist on TS measurements whether volatile compounds (i.e. NH<sub>3</sub>, CO<sub>2</sub> and VFA) are lost when drying at 105 °C (Angelidaki et al., 2009; EPA, 2001). For this study, the mass of volatile substances at 105 °C (M<sub>volatiles</sub>) was assumed to be equivalent to the total mass of VFA (S<sub>ac</sub>, S<sub>pro</sub>, S<sub>bu</sub> and S<sub>va</sub>), TAN (S<sub>in</sub>) and inorganic carbon (S<sub>ic</sub>) [Equation 5.19]. Thus, the simulated TS and VS were recalculated *a posteriori* (TS<sub>Recalc</sub> and VS<sub>Recalc</sub>) [Equation 5.20 and 5.21] in order to compare them with the experimental values.

$$M_{Volatiles} = \left(S_{ac} \cdot \frac{60}{64} + S_{pro} \cdot \frac{74}{112} + S_{bu} \cdot \frac{88}{160} + S_{va} \cdot \frac{102}{208} + S_{in} \cdot 17 + S_{ic} \cdot 44\right) \cdot V_{Global}$$
(5.19)

$$TS_{Recalc} = \frac{M_{Solids} - M_{Volatiles}}{M_{Global}}$$
(5.20)

$$VS_{Recalc} = \frac{M_{Solids} - M_{Inerts} - M_{Volatiles}}{M_{Global}}$$
(5.21)

#### 5.2.4 Model Calibration

The calibration of some of the main biochemical parameters in this study aimed to obtain the best fitting with the experimental data for a homogenized HS-AD laboratory-scale reactor, in order to assess the correct simulations of the TS and reactor content dynamics. The model calibration was carried out by trial and error, mainly for the hydrolysis (i.e.  $k_{h,ch}$ ) and maximum growth rate (i.e.  $k_{m,su}$ ) constants, aiming to maintain as close as possible the parameters proposed for thermophilic (55 °C) AD in ADM1 (Batstone et al., 2002). Noteworthy, the initial composition (i.e.  $S_{ac}$ ,  $S_{in}$ ) was chosen based on the evaluation of the experimental data available (i.e. VFA, TAN), while all the initial microorganisms concentrations (i.e.  $X_{ac}$ ,  $X_{su}$ ) were calibrated also by trial and error, alongside the main biochemical parameters, as further discussed in section 5.3.2.1.

## 5.3 Results and Discussion

## 5.3.1 Model Implementation Verification

### 5.3.1.1 'Wet' AD Verification

The model verification for 'wet' AD operating in a CSTR (simulation A) showed minimal differences (i.e.  $4^{th}-5^{th}$  significant digit) compared to the results suggested by Rosén et al. (2006) [Table 5.3], being these differences likely associated with the slightly different equation resolution method used [U. Jeppsson, Personal Communication]. Importantly, when using the HS-AD model implementation for 'wet' AD (simulation B), the results were again very close to the original 'wet' ADM1 verification, though some differences could be observed for all the dynamic variables [Table 5.3]. For example, the acetic acid (S<sub>ac</sub>) predicted with the HS-AD model implementation (simulation B) was around 39 % higher than that in the original ADM1 (simulation A). The TS concentration effect of apparent concentrations might define some differences among all the soluble species during 'wet' AD (i.e. S<sub>ac</sub>, S<sub>h2</sub>, S<sub>nh3</sub>), though the apparent concentrations effect in 'wet' applications was relatively small in simulation B due to the low TS content (i.e. < 5 %) [Equation 5.11].

It is important to mention that the differences between simulations A and B were related to the fact that the 'wet' AD simulation using the HS-AD model (simulation B) did not reach steady-state. Thus, a steady-state operation in simulation B was not reached even after 200 days, particularly due to the implementation of a common volumetric influent/effluent (i.e.  $Q_{Influent} = Q_{Effluent}$ ). In this line, simulation B showed an overall 37 % reduction in the TS content after 200 days, as well as a 13 % reduction in the V<sub>Global</sub> (but also HRT), and a 0.5 % reduction in  $\rho_{Global}$  [Table 5.3]. Therefore, a daily-averaged 0.06 % V<sub>Global</sub> modification occurred in 'wet' AD using the HS-AD model, which might be considered negligible for short operation periods, but increasingly important for longer operation (Henze et al., 1997; Richards et al., 1991). The progressive reduction of the HRT during simulation B led to a proportional increase in the OLR from 2.85 to 3.27 kg COD/m<sup>3</sup>·d [Figure 5.3a], explaining the differences between simulations A and B (i.e. S<sub>ac</sub>) mentioned before. Interestingly, the reduction in  $\rho_{Global}$  (i.e. 0.994 kg/L) below  $\rho_{Solvent}$  (i.e. 1.000 kg/L) suggests that the influent conditions (i.e.  $\rho_{Global} = \rho_{Solvent}$ ) and/or the model simplifications (i.e.  $\rho_{Solids} = const.$ ) required further testing.

|                            | ADM1 Implementation        |          | HS-AD Model Implementation |                             |                               |                                        |
|----------------------------|----------------------------|----------|----------------------------|-----------------------------|-------------------------------|----------------------------------------|
| Variable                   | Rosen & Jeppsson<br>(2006) | 'Wet' AD | 'Wet' AD<br>Const.         | HS-AD Const.<br>Effluent ** | HS-AD<br>Variable<br>Effluent | Units                                  |
| S                          | 0.01105                    | 0.01105  | 0.01260                    | 0.01602                     | 0.01000                       | ka COD m <sup>-3</sup>                 |
| Ssu                        | 0.10762                    | 0.01195  | 0.01209                    | 0.01092                     | 0.01000                       | kg COD III                             |
| S <sub>ac</sub>            | 0.15763                    | 0.15721  | 0.15222                    | 0.10339                     | 0.11028                       | kg COD III                             |
| S <sub>1C</sub>            | 0.13208                    | 0.13270  | 0.13232                    | 0.08451                     | 0.11028                       | kmole N m <sup>-3</sup>                |
| Sin<br>V.                  | 0.13023                    | 0.13023  | 0.13129                    | 60 72602                    | 0.07803                       | $ka COD m^{-3}$                        |
| Ach<br>V                   | 0.02793                    | 0.02793  | 0.03183                    | 5 29796                     | 6 15909                       | kg COD III                             |
| $\Lambda_{su}$             | 0.42017                    | 0.42017  | 0.43028                    | 2 25004                     | 0.13898                       | kg COD III                             |
| $\Lambda_{ac}$             | 0.70050                    | 170      | 170                        | 2.33994                     | 2.32894                       | kg COD III                             |
| QEffluent                  | 170                        | 170      | 170                        | 170                         | 7.16                          | $m^3 d^{-1}$                           |
| рп                         | /.4/                       | 7.40     | /.40                       | 7.20                        | 7.10                          | III' u<br>Irmal C m <sup>-3</sup>      |
| Sco2                       | 0.0099                     | 0.0099   | 0.0096                     | 0.0128                      | 0.0134                        | kmol C m <sup>2</sup>                  |
| S <sub>nh3</sub>           | 0.0041                     | 0.0041   | 0.0042                     | 0.0015                      | 0.0012                        | kmol N m                               |
| Рт                         | 1.069                      | 1.069    | 1.069                      | 1.180                       | 1.220                         | bar                                    |
| $Q_{g}$                    | 2956                       | 2956     | 2939                       | 9752                        | 12472                         | Nm <sup>3</sup> d <sup>-1</sup>        |
| $%CH_4$                    | 61*                        | 60.9     | 60.8                       | 50.6                        | 49.9                          | %                                      |
| $%CO_2$                    | 34*                        | 33.9     | 34.0                       | 44.7                        | 45.5                          | %                                      |
| $V_{Global}$               | 3400                       | 3400     | 2967                       | 1717                        | 3400                          | m <sup>3</sup>                         |
| $ ho_{Global0}$            | -                          | 1000     | 1000                       | 1100                        | 1100                          | kg m <sup>-3</sup>                     |
| ρ <sub>Global</sub>        | -                          | 1000     | 995                        | 1082                        | 1077                          | kg m <sup>-3</sup>                     |
| HRT                        | 20*                        | 20       | 20                         | 20                          | 20                            | d                                      |
| HRT <sub>real</sub>        | -                          | 20       | 17                         | 10                          | 20                            | d                                      |
| OLR                        | -                          | 2.85     | 2.85                       | 19.85                       | 19.85                         | kg COD m <sup>-3</sup> d <sup>-1</sup> |
| <b>OLR</b> <sub>real</sub> | -                          | 2.85     | 3.27                       | 39.32                       | 19.86                         | kg COD m <sup>-3</sup> d <sup>-1</sup> |
| $TS_0$                     | 4.5 *                      | -        | 4.5                        | 25.0                        | 25.0                          | %                                      |
| TS                         | -                          | -        | 2.9                        | 20.4                        | 19.0                          | %                                      |
| TS <sub>Recalc</sub>       | -                          | -        | 1.9                        | 19.8                        | 18.5                          | %                                      |
| $VS_0$                     | -                          | -        | 3.5                        | 23.0                        | 23.0                          | %                                      |
| VS                         | -                          | -        | 1.8                        | 18.2                        | 16.9                          | %                                      |
| $VS_{\text{Recalc}}$       | -                          | -        | 0.9                        | 17.6                        | 16.3                          | %                                      |

Table 5.3 Summary of steady-state results for model implementation verification

\*Mentioned Only; \*\*No Steady-State Reached.

The specific weight of a complex sample ( $\rho_{Global}$ ) depends on all the compounds involved [Equation 5.9]. Since the measurement of all the variables  $\rho_i$  in an AD sample is rarely available, the  $\rho_i$  of each compound needs to be known/assumed for simulations. In this line, the specific weight of a sample solid fraction ( $\rho_{Solids}$ ) can be approximated by knowing the specific weight of the solvent ( $\rho_{Solvent}$ ), though  $\rho_{Solvent}$  is again function of all the different compounds in solution, as well as a function of temperature and pressure (Lide, 2004). As a preliminary approach,  $\rho_{Solvent}$  was assumed to be close to the specific weight (density) of water at 0 °C and 1 bar (i.e.  $\rho_{Solvent} = 1 \text{ kg/L}$ ), since the density of water is 999.84 kg/m<sup>3</sup> at 0 °C, 993.64 kg/m<sup>3</sup> (0.63 % error) at 35 °C, and 985.19 kg/m<sup>3</sup> (1.48 % error) at 55 °C (Kell, 1975; Lide, 2004), thus being approximately constant at any of these temperatures. With this strategy, the specific weights obtained for the overall sample ( $\rho_{Global}$ ) and/or the solid fraction ( $\rho_{Solids}$ ) were considered relative regarding the specific weight of solvent ( $\rho_{Solvent}$ ). Meanwhile,  $\rho_{Solvent}$  (but also  $\rho_{Solids}$ ) could be set to any value, or modified by any expression (i.e. as a function of temperature), without modifying the structure of the model. Thus, once knowing the  $\rho_{Solvent}$ , the  $\rho_{Global}$  and TS of a (semi-)solid sample,  $\rho_{Solids}$  could be approximated by using the mass balance [Equation 5.9].



*Figure 5.3* Hydraulic retention time and organic loading rate in model implementation verification: a) 'wet' anaerobic digestion (simulations A and B); and b) high-solids anaerobic digestion (simulations C and D)

Previous research indicated that  $\rho_{Solids}$  ranges from 1.3 kg/L in lignocellulosic materials to 1.5 kg/L in OFMSW and 2.5 kg/L for inorganic inert solids (i.e. sand). On the other hand,

the specific weight of microorganisms is reported between 0.8 and 1.4 kg/L (van Veen et al., 1979), though this fraction might be a negligible part (i.e. 5 %) of the whole reactor mass content. Therefore, a compromise value of  $\rho_{Solids} = 1.5$  kg/L was chosen for the preliminary model verification/calibration, though further testing must be devoted to this particular variable, since it could influence other aspects of the HS-AD simulations (i.e. V<sub>Global</sub>), as mentioned before.

#### 5.3.1.2 HS-AD Verification

Regarding the HS-AD model verification with constant  $Q_{Effluent}$  (simulation C), the HS-AD simulation did not reach the steady state after 200 days, while longer simulations (i.e. 365 days) yielded reactor acidification (i.e.  $pH \le 6.0$ ) – data not shown. This is due to a progressive reduction of  $V_{Global}$  in HS-AD when maintaining a volumetric outflow equal to the volumetric inflow (i.e.  $Q_{Influent} = Q_{Effluent}$ ) (Kayhanian et al., 1996; Richards et al., 1991). Thus, the HRT decreases – and the OLR increases – proportionally to the  $V_{Global}$  reduction in HS-AD until the 'washout' of methanogens occurs and the reactor acidifies. For example, a 50 % reduction in HRT was observed with the influent conditions tested in simulation C [Figure 5.3b], with an approximately daily-averaged  $V_{Global}$  reduction of 0.25 %.

Meanwhile, a rapid stabilization of the HS-AD process was obtained when choosing a constant reactor volume as a set point (i.e.  $V_{Setpoint} = V_{Global0}$ ) and recalculating  $Q_{Effluent}$  [Table 5.3 and Figure 5.3b]. Noteworthy, the  $Q_{Effluent}$  recalculation operation yielded a reduction of around 5.6 % of the steady-state value regarding  $Q_{Influent}$ , and a 24 % TS removal compared to the substrate TS (i.e. from 25 to 19 %). These results condense the importance of reducing the effluent compared to the influent (i.e.  $Q_{Influent} > Q_{Effluent}$ ) to reach steady-state HS-AD, in order to compensate the organic removal by methanogenesis (Kayhanian et al., 1994; Kayhanian et al., 1996; Richards et al., 1991). Furthermore, the use of apparent concentrations might be also crucial for HS-AD simulations, since practically all the biochemical rates were affected (i.e. speeded-up/slowed-down) by the TS concentration effect on soluble substrates (i.e.  $S_{ac}$ ) and/or inhibitors (i.e.  $S_{nb3}$ ) [Table 5.1]. For example, a 26 % increase in all the soluble concentrations (i.e.  $S_{su}$  and  $S_{h2}$ ) was obtained by the tested HS-AD conditions in steady-state operation – data not shown.

The water/solvent in this study was assumed to be conservative, since the same water entering leaves the system as a liquid effluent (m<sub>Effluent,Solvent</sub>) or vapor (m<sub>Vapor</sub>), but is not produced/consumed. Importantly, production/consumption of water in the biochemical processes (i.e. hydrolysis, methanogenesis) might occur, linking Equations 5.2 and 5.3. However, the production/consumption of water is tightly linked to the stoichiometry of all the reactions occurring in HS-AD, while the stoichiometry of all the biochemical reactions in

ADM1 requires further development (De Gracia et al., 2006; Kleerebezem et al., 2006; Rodríguez et al., 2006). Therefore, using Equations 5.1 to 5.4 is a reasonable hypothesis that can be modified, once the global stoichiometry of HS-AD is well-defined. In this last case, the Petersen matrix originally proposed for ADM1 would need to account for water as another dynamic variable. For example, De Gracia et al. (2006) included water (i.e.  $S_{h2o}$ ) in the Petersen matrix of ADM1, though the AD stoichiometry was partially assumed (i.e. elemental composition). Furthermore, in order to use Equations 5.1 to 5.4 in this study, it was also assumed that the organic solid destruction only proceeds when biogas production occurs. In other words, whether hydrolysis, acidogenesis and/or acetogenesis occur, but not biogas production (i.e. CH4, CO<sub>2</sub> and/or H<sub>2</sub>), complex substrates (i.e. carbohydrates) are just transformed into more simple substrates (i.e. sugars, VFA), being both of them jointly included in the term m<sub>Effluent,Solids</sub>. With these two last assumptions, the hydrolysis to acidogenesis steps were not included in Equations 5.1 to 5.4. However, the mass volatile compounds at 105 °C (M<sub>Volatiles</sub>) needed to be accounted in the TS and VS calculations, as shown in Equations 5.19 to 5.21.

Due to the considerably higher COD of the influent conditions [Table 5.2], the OLR was around 7 times higher for HS-AD than for 'wet' AD simulations [Table 5.3], which directly relates to the higher chances of HS-AD acidification, and the necessity to reduce considerably the  $k_{h,ch}$  for HS-AD simulations. In either case, HS-AD experimental data are required to calibrate biochemical parameters (i.e.  $k_{h,ch}$ ).

## 5.3.2 Model Calibration

#### 5.3.2.1 Comparison Between Simulated and Experimental Values

The HS-AD simulation of OFMSW in batch conditions at 15 % TS closely matched all the experimental variables [Figure 5.4], though slight disagreements were also observed between the experimental data and the simulated values. The initial conditions and modified parameters used are shown in Tables 5.2 and 5.4, respectively. Firstly, the cumulative methane production was 830 NmL CH<sub>4</sub> [Figure 5.4a], coinciding to that obtained experimentally, while the biogas composition was also well simulated – data not shown. Importantly, the overall biogas production was associated with 1.7 g M<sub>Global</sub> removal (i.e. 4.6 %), in agreement with the 1.5 - 2.0 g that could have been removed according to the experimental biogas flow/composition. Noteworthy, the simulation suggested that  $\rho_{Global}$  was reduced from 1078 to 1064 kg/m<sup>3</sup> (i.e. 1.2 % reduction) along the whole experimental period (data not shown), though the  $\rho_{Global}$  modification should be further validated with



experimental data, as discussed before. The  $M_{Global}$  and  $\rho_{Global}$  modification yielded a  $V_{Global}$  reduction of 3.5 % – data not shown.

*Figure 5.4* Batch mono-digestion of OFMSW at 15 % total solids: a) accumulated methane production and reactor mass content; b) volatile fatty acids; c) total and free ammonia nitrogen; and d) total and volatile solids

The initial composition in the batch experiment [Table 5.2] was based on the availability of experimental data (i.e. COD, TS and CH<sub>4</sub> yield), but also on a reasoned assessment of the substrate and/or inoculum composition. For example, the protein content of the substrate/inoculum mixture (i.e.  $X_{pr} + S_{aa}$ ) was adjusted according to the nitrogen content of proteins and amino acids (N<sub>aa</sub>) [Table 5.4] and the inert materials (i.e.  $X_i + S_i$ ) to simulate the TAN (S<sub>in</sub>) dynamics, as mentioned in section 5.2.1.3. Unfortunately, apart from

the CH<sub>4</sub> yield and COD of the initial mixture, no data were available regarding the remaining complex substances (i.e. particulates) involved in the biochemical framework of the model. Therefore, the distinction between the initial carbohydrate/sugars ( $X_{ch}/S_{su}$ ) and lipids/LCFA ( $X_g/S_{fa}$ ) had to be tuned alongside the biochemical parameters to simulate the initial days of the batch setup.

| Parameter                     | ADM1 | This Study | Units                  |
|-------------------------------|------|------------|------------------------|
| k <sub>h,ch</sub>             | 10   | 0.05       | d-1                    |
| $\mathbf{k}_{\mathrm{h,pr}}$  | 10   | 0.05       | d-1                    |
| k <sub>h,li</sub>             | 10   | 0.07       | d-1                    |
| k <sub>m,su</sub>             | 70   | 35         | d-1                    |
| k <sub>m,fa</sub>             | 10   | 4          | d-1                    |
| k <sub>m,c5</sub>             | 30   | 1          | d-1                    |
| k <sub>m,c4</sub>             | 30   | 6          | d-1                    |
| k <sub>m,pro</sub>            | 20   | 1          | d-1                    |
| $pH_{\rm LL,ac}$              | 6    | 5.8        |                        |
| $pH_{\text{UL},\text{ac}}$    | 7    | 6.8        |                        |
| $\mathbf{f}_{bu,su}$          | 0.13 | 0.37       |                        |
| $\mathbf{f}_{\text{pro,su}}$  | 0.27 | 0.11       |                        |
| $\mathbf{f}_{\mathrm{ac,su}}$ | 0.41 | 0.40       |                        |
| $\mathbf{f}_{\mathrm{h2,su}}$ | 0.19 | 0.12       |                        |
| $N_{i,subs}$                  | -    | 0.001      | kmol N m <sup>-3</sup> |

Table 5.4 Main parameters modified for model calibration

During the initial 20 days of experiment, pH was observed to drop from 7.3 to 6.3 - data not shown – due to VFA accumulation [Figure 5.4b]. Thus, the initial VFA and pH dynamics were simulated by a plausible set of microorganism concentrations, hydrolysis constants and initial substrate/inoculum fractionation [Tables 5.2 and 5.4]. The initial microbial concentrations are crucial in the simulation of AD batch experiments, though they are normally unknown due to the difficulties for measuring the populations involved (Donoso-Bravo et al., 2011; Flotats et al., 2010). Importantly, the hydrolysis constants (k<sub>h</sub>) were considerably reduced compared to the original values proposed in ADM1 for thermophilic (55 °C) operation (i.e.  $k_{h,ch} = 0.05 d^{-1} vs$ . 10 d<sup>-1</sup>, respectively), though the calibrated values were in accordance with reported hydrolysis rates for simulation of OFMSW (Batstone et al., 2002; Kayhanian, 1995; Mata-Álvarez, 2003; Vavilin et al., 2005).

In order to obtain the best fitting between the simulated and experimental VFA dynamics from day 20, the maximum growth rate (km) of some microbial populations was also considerably reduced. For example, the maximum growth rate of propionate degraders  $(k_{m,pro})$  was reduced to 1 d<sup>-1</sup>, in contrast to the 20 d<sup>-1</sup> proposed by ADM1 for thermophilic (55 °C) operation [Table 5.4]. Noteworthy, the extremely low k<sub>m</sub> used for model calibration, in contrast to the original values of ADM1, might be suggesting that some inhibition in the VFA uptake was occurring in the experiment. Thus, NH<sub>3</sub> reached particularly high contents in the reactor (i.e. 0.16 mol N/kg) [Figure 5.4c] mainly due to the high pH observed (i.e.  $\geq$ 8.0), while  $NH_3$  is a well-known inhibitor of acetoclastic and hydrogenotrophic methanogens (Angelidaki et al., 1993; Gallert et al., 1997; Jokela et al., 2003). In this line, the implementation of reversible NH<sub>3</sub> inhibition [Equation 5.16] in hydrogen uptake could match adequately all the VFA, since valerate and propionate degraders are inhibited by H<sub>2</sub> buildup in ADM1 (Batstone et al., 2002). However, this last strategy led to  $H_2$  accumulation in the gas phase (i.e. 2 - 5 %, data not shown), though no H<sub>2</sub> was detected experimentally. Therefore, all the VFA-degrading populations might be affected in some degree by NH<sub>3</sub> accumulation, as suggested by Poggi-Varaldo et al. (1997).

The model suggested a 5 - 15 % difference between the simulated and experimental TS and VS contents, despite the experimental trends were well approximated in both cases [Figure 5.4d]. Therefore, since the simulated M<sub>Global</sub>, CH<sub>4</sub> yield and COD showed good simulations, an experimental bias was suspected in the experimental TS/VS measurement. Noteworthy, the recalculated TS and VS [Equations 5.19 to 5.21] improved considerably the matching of the TS and VS simulations with the values observed experimentally, though some differences were also observed from day 20 onwards. Meanwhile, the TS and VS recalculation is supported by the fact that some organic material (i.e. VFA), ammonia nitrogen (i.e. NH<sub>3</sub>) and/or inorganic carbon (i.e. CO<sub>2</sub>) might volatilize when drying the samples at 105 °C for prolonged periods of time (i.e. 24 h) (Angelidaki et al., 2009; EPA, 2001). With all the above, the observed differences between the TS and VS recalculated and experimental values [Figure 5.4d] were likely related to the differences in the propionate and valerate simulations [Figure 5.4b] during the same period. Therefore, the model calibration might require further improvement as also discussed in next section.

#### 5.3.2.2 Need for Further Calibration

The model calibration in this study was aimed to be minimal because of: 1) the complexity of HS-AD vs. the assumptions taken (i.e. homogenized reactor); 2) the little data available regarding solids mass dynamics (i.e. TS/VS); 3) the high number of biochemical parameters involved (i.e. > 10); and 4) the 'strong' interrelationship between parameters and

the initial conditions in structured AD models (Batstone et al., 2015; Donoso-Bravo et al., 2011; Flotats et al., 2010; Vanrolleghem et al., 1995). Thus, the calibration in this study was mainly addressed to the simultaneous fitting of the overall dynamics of TS/VS removal, reactor mass, biogas production, VFA and pH, in order to assess the potentiality of the proposed model to simulate a homogenized HS-AD matrix.

The parameter modification compared to ADM1 values [Table 5.4] was needed to obtain an adequate fitting of the overall set of experimental data for the sacrifice test in this study. Importantly, most of the biochemical parameters modified were within the recommended range suggested in ADM1, with the exception of the maximum propionate and valerate growth rates (i.e. k<sub>m,pro</sub> and k<sub>m,va</sub>) that could be associated to NH<sub>3</sub> inhibition, as mentioned in section 5.3.2.1. For example, the lower and upper pH levels for acetate uptake (pH<sub>LL,ac</sub> and pH<sub>UL,ac</sub>, respectively) might vary around 30 % from the values proposed in ADM1 (i.e.  $pH_{LL,ac} = 6.0$  and  $pH_{UL,ac} = 7.0$ ) (Batstone et al., 2002). However, it must be highlighted that the implementation of a single experimental dataset was not enough to calibrate a large number of parameters since, for example, different combinations of biochemical parameters and/or initial conditions (i.e. microorganisms) could yield practically the same agreement between experimental and simulated results (Girault et al., 2011; Jabłonski et al., 2014; Vanrolleghem et al., 1995; Vavilin et al., 2008). Therefore, more experimental datasets (i.e. laboratory and/or large scale applications) are needed to refine the calibration of the proposed parameters for HS-AD of OFMSW. Meanwhile, a sensitivity analysis and an adequate parameter optimization strategy might reveal important aspects about the main biochemical and physical-chemical processes occurring in HS-AD of OFMSW.

With all the above, the minimal model calibration showed the potentiality of using adequately the mass balances alongside the biochemical framework of ADM1 to simulate HS-AD of OFMSW. Thus, the HS-AD model simulates particularly well the TS, VS, and M<sub>Global</sub> dynamics of HS-AD, provided the four preliminary hypotheses proposed are fulfilled. Meanwhile, further studies are needed in order to improve the biochemical calibration of the HS-AD model, with the aim to explore the different acidification/inhibitory mechanisms of HS-AD fed with OFMSW. Further calibration will be also helpful to double check the hypotheses used, assess the HS-AD model performance and/or highlight potential areas requiring further model development. Summarizing, the user could calibrate the model parameters and/or readapt the HS-AD model structure as required for any particular HS-AD application.

### 5.4 Conclusions

In this study, a novel ADM1-based model was developed to simulate the solids and reactor mass/volume dynamics of homogenized HS-AD reactors. An adequate mass balance implementation condensed the effects of biogas production on HS-AD mass/volume, being critical to simulate relatively long operations. Apparent concentrations accounted for the TS concentration effect on soluble species. The model was verified for 'wet' AD and HS-AD, serving as a link between both operational regimes. The model simulated particularly well HS-AD of OFMSW in batch, including the TS and reactor mass, while further model calibration might serve to assess inhibitory mechanisms in HS-AD of OFMSW.

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## Chapter 6

# Coupling a Non-Ideal Bio-Physical-Chemical Module to the High-Solids Anaerobic Digestion Model

This chapter has been submitted as:

Pastor-Poquet, V., Papirio, S., Steyer, J.-P., Trably, E., Escudié, R., & Esposito, G. (*submitted*). Non-Ideal Bio-Physical-Chemical Effects on High-Solids Anaerobic Digestion of the Organic Fraction of Municipal Solid Waste.

#### Abstract

This study evaluates the main effects of including 'non-ideal' bio-physical-chemical corrections in high-solids anaerobic digestion (HS-AD) simulations, using the organic fraction of municipal solid waste (OFMSW) as substrate, at total solid (TS) contents between 10 and 40 %. A relatively simple 'non-ideal' module was developed to account for the effects of a high ionic strength (I, e.g. > 0.2 M) on the main ionic equilibriums of HS-AD. As a novel approach, the 'non-ideal' module was coupled to the HS-AD model for homogenized reactors, to jointly evaluate the effects of 'non-ideality' and the TS content dynamics on the HS-AD bio-physical-chemistry. 'Non-ideality' influenced the pH calculations, soluble concentration of inhibitory compounds (i.e. NH<sub>3</sub>), volatile fatty acid accumulation, and liquid-gas transfer (i.e. CO<sub>2</sub>), particularly at higher TS (i.e.  $\geq 20$  %). Meanwhile, the HS-AD mass/volume dynamics and the apparent concentrations for soluble compounds (i.e. kmol/m<sup>3</sup> Solvent) were crucial to assess the influence of 'non-ideality'. Fitting the experimental data for batch assays at 15 % TS showed that HS-AD of OFMSW might be operated at  $I \ge 0.5$  M. Therefore, all HS-AD simulations should account for 'non-ideal' corrections, particularly when assessing the main inhibitory mechanisms (i.e. NH<sub>3</sub> buildup and acidification) potentially occurring in HS-AD of OFMSW. In this line, further bio-physical-chemical mechanisms (e.g. precipitation) should be also evaluated in future model implementations, to enhance HS-AD simulations using OFMSW as substrate.

**Keywords**: High-Solids Anaerobic Digestion Model; Non-Ideal Bio-Physical-Chemical Corrections; Ionic Strength; Total Solids Dynamics; Apparent Concentrations; Ammonia Inhibition.



## **Graphical Abstract**

## 6.1 Introduction

Anaerobic digestion (AD) models enhance our current understanding about the biogas production dynamics and/or inhibitory mechanisms, while revealing potential opportunities for bioprocess optimization (Lauwers et al., 2013; Steyer et al., 2006). The Anaerobic Digestion Model No.1 (ADM1) is a structured model gathering together the main biophysical-chemical processes occurring in AD (Batstone et al., 2002; Batstone et al., 2015). Among the biochemical processes, ADM1 includes the disintegration, hydrolysis, acidogenesis, acetogenesis and methanogenesis of organic substrates, composed of carbohydrates, proteins and lipids, expressed in chemical oxygen demand (COD) units. Physical-chemical processes include the liquid-gas transfer of CH4, CO<sub>2</sub>, and H<sub>2</sub>, and the ionic equilibriums of volatile fatty acids (VFA; acetic, propionic, butyric and valeric), inorganic nitrogen (i.e. NH<sub>3</sub>), and inorganic carbon (i.e. CO<sub>2</sub>).

High-solids anaerobic digestion (HS-AD) is a particular AD operation at total solid (TS) content  $\geq 10$  % (Abbassi-Guendouz et al., 2012; Karthikeyan et al., 2013). In HS-AD of the organic fraction of municipal solid waste (OFMSW), a 30 - 80 % volatile solid (VS) removal generally occurs as a consequence of biogas production (Mata-Álvarez, 2003). Thus, the biogas production in HS-AD leads to the reduction of the reactor content mass (M<sub>Global</sub>) and/or volume (V<sub>Global</sub>), but also the reactor content specific weight ( $\rho_{Global}$ ), in contrast to 'wet' AD (i.e. TS < 10 %) (Kayhanian et al., 1996; Pastor-Poquet et al., 2018).

Aiming to account for the importance of mass removal in HS-AD simulations, a HS-AD model based on the ADM1 biochemistry was developed (Pastor-Poquet et al., 2018). The main difference between the HS-AD model and the CSTR implementation of ADM1 (Batstone et al., 2002) lies on the simulation of  $M_{Global}$ ,  $V_{Global}$ , TS, VS, and  $\rho_{Global}$  dynamics by using a more extended set of mass balances for homogenized HS-AD reactors. For example, apart from the mass balances for soluble and particulate substances in ADM1, the HS-AD model includes the mass balance of reactor mass ( $M_{Global}$ ), solvent ( $M_{Solvent}$ ), and inert ( $M_{Inerts}$ ) contents, allowing the dynamic calculation of TS and VS. On the other hand, apparent concentrations (i.e. kg COD/m<sup>3</sup> Solvent) were used in the bio-physical-chemical framework of the HS-AD model, in order to account for the TS concentration effect on HS-AD solutes (i.e. VFA), and in contrast to ADM1 that uses global concentrations (i.e. kg COD/m<sup>3</sup> Total).

An important limitation of the physical-chemical framework of ADM1 is the absence of corrections for the 'non-ideal' solution effects on AD (Batstone et al., 2012; Solon et al., 2015; Tait et al., 2012). In solution, a global species concentration  $(S_{T,i})$  includes the corresponding dissociated  $(S_i^{Zi})$  and un-dissociated  $(S_i^{Zi=0})$  species concentrations, with their associated ion charge  $(Z_i)$ . Thus, the 'ideal' dissociated/un-dissociated species can be

obtained from  $S_{T,i}$  once knowing the mass balance, the 'ideal' equilibrium constant (K<sub>a,i</sub>), and the solution pH. For example, the total ammonia/inorganic nitrogen (TAN, S<sub>in</sub>) in AD is mainly dissociated into ammonium ion (NH<sub>4</sub><sup>+</sup>, S<sub>nh4+</sub>) and free ammonia (NH<sub>3</sub>, S<sub>nh3</sub>), as a function of the equilibrium constant for inorganic nitrogen (K<sub>a,in</sub>), and the proton concentration (H<sup>+</sup>, S<sub>h+</sub>) [Equation 6.1]. Using the inorganic nitrogen mass balance [Equation 6.2] and the 'ideal' ammonia equilibrium [Equation 6.3], S<sub>nh4+</sub> and S<sub>nh3</sub> can be approximated for a given pH – S<sub>h+</sub> concentration.

$$NH_4^+ \stackrel{K_{a,in}}{\longleftrightarrow} NH_3 + H^+$$
 (6.1)

$$S_{in} = S_{nh_4^+} + S_{nh_3} \tag{6.2}$$

$$K_{a,in} = \frac{S_{nh_3} \cdot S_{h^+}}{S_{nh_4^+}}$$
(6.3)

Ionic strength (*I*) estimates the level of ionic interactions of an aqueous solution, and can be approximated from  $S_i^{Zi}$  and  $Z_i$  [Equation 6.4] (Parkhurst et al., 1999; Solon et al., 2015). Whether a solution is not infinitely diluted (i.e.  $\Sigma S_i^{Zi} \neq 0$ ), the hypothesis of 'ideality' (i.e.  $I \sim 0$ ) is not further valid, and all the 'non-ideal' equilibriums involved in the solution must be expressed in terms of activities, instead of molal concentrations (Batstone et al., 2012; Tait et al., 2012). The activity of a solute (a<sub>i</sub>) is the product of the molal concentration ( $S_i^{Zi}$ , kmol/kg Solvent) by the coefficient of activity ( $\gamma_i$ ) [Equation 6.5]. 'Non-ideality' corrections are required for AD solutions when  $I \ge 0.2$  M, being potentially important in HS-AD due to the high organic concentration used (Batstone et al., 2015; Solon et al., 2015; Tait et al., 2012).

$$I = \frac{1}{2} \sum S_i^{Z_i} \cdot Z_i^{2}$$
(6.4)

$$a_i = \gamma_i \cdot S_i^{\ Z_i} \tag{6.5}$$

For an 'ideal' solution  $\gamma_i = 1$ , whereas for a 'non-ideal' solution  $\gamma_i < 1$  for dissociate species (i.e.  $Z_i \neq 0$ ) and  $\gamma_i > 1$  for un-dissociated species (i.e.  $Z_i = 0$ ). Thus,  $\gamma_i$  is mainly a function of *I* and, for a moderately concentrated solution (i.e.  $I \le 0.2$  M), the Davies equation [Equation 6.6] is commonly used for assessing the activity of ionic species (Allison et al., 1991; Parkhurst et al., 1999). However, when I > 0.2 M,  $\gamma_i$  tends to unity with increasing *I* by using the Davies equation (Solon, 2016; Tait et al., 2012). Therefore, the WATEQ Debye-
Hückel equation [Equation 6.7] is recommended for  $0.2 \le I \le 1.0$  M, as  $\gamma_i$  progressively tends to zero with increasing *I* (Parkhurst et al., 1999; Solon et al., 2015).

$$\log_{10}(\gamma_i) = -A \cdot Z_i^{\ 2} \cdot \left(\frac{\sqrt{I}}{1 + \sqrt{I}} - 0.3 \cdot I\right)$$
(6.6)

$$log_{10}(\gamma_i) = -\frac{A \cdot Z_i^2 \cdot \sqrt{I}}{1 + B \cdot a_i^0 \cdot \sqrt{I}} + b_i \cdot I$$
(6.7)

Liquid-gas transfer, ionic speciation, ion pairing and precipitation are among the most important physical-chemical processes affecting and being affected by 'non-ideality' in AD. In particular, the ionic speciation determines the medium pH, as well as the concentration of soluble inhibitory substances (i.e. NH<sub>3</sub>), being two of the most important parameters regulating the biogas production in ADM1 (Batstone et al., 2002; Rosén et al., 2006; Xu et al., 2015). Therefore, failing to include 'non-ideal' corrections in ADM1-based models might result in an artificially high NH<sub>3</sub> concentration, subsequently influencing the parameter calibration related to NH<sub>3</sub> inhibition (Hafner et al., 2009; Nielsen et al., 2008; Patón et al., 2018).

With all the above, the 'non-ideal' approach may be particularly important to assess the main inhibitory mechanisms in HS-AD of OFMSW, since HS-AD is easily subjected to reactor inhibition by high levels of NH<sub>3</sub>, as a consequence of the high protein content of OFMSW and the reduced free water available in the process (García-Bernet et al., 2011; Kayhanian, 1999). The NH<sub>3</sub> build-up in HS-AD may lead to VFA accumulation and eventual reactor failure by acidification (i.e.  $pH \le 6.0$ ). On the other hand, acidification might be also the result of substrate overload due to the imbalance between acidogenic-methanogenic growth and/or the elevated organic content of HS-AD (Pastor-Poquet et al., 2018; Staley et al., 2011). Noteworthy, the release of inorganic carbon (i.e.  $CO_2/HCO_3^-$ ) by acetoclastic methanogens is one of the main pH buffering agents in AD, potentially counteracting reactor acidification (Steyer et al., 2006). Therefore, the risk of acidification might be also affected by the 'non-ideal' effect on the CO<sub>2</sub> liquid-gas transfer (Patón et al., 2018).

This study evaluates for the first time the main effects of including 'non-ideal' biophysical-chemical corrections in HS-AD simulations using OFMSW as substrate, at TS contents from 10 to 40 %. With this aim, a relatively simple 'non-ideal' calculation module, based on the Visual MINTEQ (Allison et al., 1991) and Phreeqc (Parkhurst et al., 1999) physical-chemical engines, was developed to assess the potential effects of a high I (e.g. > 0.2 M) upon the main ionic equilibriums of HS-AD, while speeding-up model simulations. Coupling the proposed 'non-ideal' module with the HS-AD model (Pastor-Poquet et al., 2018) permitted to explore some of the main inhibitory mechanisms (i.e. NH<sub>3</sub> buildup and acidification) in HS-AD of OFMSW, particularly at relatively high TS contents (i.e.  $\geq 20$  %). Moreover, the 'non-ideal' HS-AD model configuration could be easily adapted to any other organic substrate (i.e. manure, agricultural waste) or HS-AD reactor configuration (i.e. laboratory or industrial scale).

## 6.2 Methodology

## 6.2.1 Activity Coefficients and Modified Equilibrium Constants

In this study, the Extended Debye-Hückel (EDH) equation [Equation 6.8] was used to approximate the activity coefficients ( $\gamma_i$ ) in HS-AD. EDH is a particular case of the WATEQ Debye-Hückel equation [Equation 6.7], whose parameters (A, B and  $a_i^0$ ) are known for the main ionic species normally measured in AD (e.g. CH<sub>3</sub>COO<sup>-</sup>, CH<sub>3</sub>CH<sub>2</sub>COO<sup>-</sup>, NH<sub>4</sub><sup>+</sup> and Na<sup>+</sup>) (Ball et al., 1991; Stumm et al., 1996). Importantly, the activity coefficients for non-charged species ( $\gamma_0$ ) in solution (i.e. NH<sub>3</sub>, CO<sub>2</sub>) were also calculated as a function of *I* [Equation 6.9], using  $b_i = 0.1$  (Parkhurst et al., 1999).

$$log_{10}(\gamma_i) = -\frac{A \cdot Z_i^2 \cdot \sqrt{I}}{1 + B \cdot a_i^0 \cdot \sqrt{I}}$$
(6.8)

$$\log_{10}(\gamma_0) = -b_i \cdot I \tag{6.9}$$

To include 'non-ideal' effects in AD, the 'ideal' dissociation/equilibrium constants ( $K_{a,i}$ ) were corrected in terms of activities ( $a_i$ ) to obtain the modified equilibrium constants ( $K_{a,i}$ ) (Nielsen et al., 2008; Tait et al., 2012). For example,  $K_{a,in}$  expressed in activity terms [Equation 6.10] can be reorganized to obtain the modified equilibrium constant for inorganic nitrogen ( $K_{a,in}$ ) [Equation 6.11]. Importantly, the proton activity ( $a_{h+}$ ) must be used for pH calculations [Equation 6.12] under 'non-ideal' conditions (Allison et al., 1991; Parkhurst et al., 1999). Therefore, since the 'non-ideal' set of equations (i.e. Equations 6.2, 6.8, 6.9, 6.11 and 6.12) is implicit in  $S_{h+}$ , the calculation of pH, *I*, and  $K_{a,i}$ ' must be solved iteratively, fulfilling both equilibriums and mass balances in an ionic solution.

$$K_{a,in} = \frac{a_{nh_3} \cdot a_{h^+}}{a_{nh_4}^+} = \frac{\gamma_{nh_3} \cdot S_{nh_3} \cdot \gamma_{h^+} \cdot S_{h^+}}{\gamma_{nh_4}^+ \cdot S_{nh_4}^+} = \frac{\gamma_{nh_3} \cdot \gamma_{h^+}}{\gamma_{nh_4}^+} \cdot \frac{S_{nh_3} \cdot S_{h^+}}{S_{nh_4}^+}$$
(6.10)

$$K_{a,in}' = K_{a,in} \cdot \frac{\gamma_{nh4^+}}{\gamma_{nh_3} \cdot \gamma_{h^+}} = \frac{S_{nh_3} \cdot S_{h^+}}{S_{nh_4^+}}$$
(6.11)

$$pH = -log_{10}(a_{h^+}) = -log_{10}(\gamma_{h^+} \cdot S_{h^+})$$
(6.12)

For the objective of this study, the main global species used were acetate ( $S_{ac}$ ), propionate ( $S_{pro}$ ), butyrate ( $S_{bu}$ ), valerate ( $S_{va}$ ), inorganic carbon ( $S_{ic}$ ), inorganic nitrogen ( $S_{in}$ ), and mono-valent inorganic cations ( $S_{cat}$ ) and anions ( $S_{an}$ ), as originally proposed in ADM1 (Batstone et al., 2002). The schematic representation of the iterative module for including the 'non-ideality' of an AD solution is shown in Figure 6.1. All the required equilibrium constants for an 'ideal' solution ( $K_{a,i}$ ), and their temperature dependence using the van't Hoff equation, were extracted from Batstone et al. (2002) and Lide (2004).

In order to keep the physical-chemical module as simple as possible, the proposed calculation procedure did not consider ion-pairing or precipitation. Noteworthy, ion-pairing and precipitation are based on further ionic equilibriums, whereas the due kinetic rates of nucleation and crystal growth phenomena must be adequately accounted also for precipitation (Huber et al., 2017; Vaneeckhaute et al., 2018). Further information about those mechanisms and some potential strategies for their implementation in ADM1-based models can be found elsewhere (Flores-Alsina et al., 2015; Lizarralde et al., 2015; Mbamba et al., 2015; Parkhurst et al., 1999; Vaneeckhaute et al., 2018), as also mentioned in section 6.3.1.3.

The gaseous species used in this study were CH<sub>4</sub>, H<sub>2</sub>, CO<sub>2</sub>, and NH<sub>3</sub>. The addition of the NH<sub>3</sub> liquid-gas transfer in the HS-AD model was shown elsewhere (Pastor-Poquet et al., 2018). The Henry's constant (K<sub>H,i</sub>) of each gaseous species was modified by the introduction of  $\gamma_0$ , obtaining the modified Henry's constant (K<sub>H,i</sub>) [Equation 6.13]. The K<sub>H,i</sub> reference values and their dependence with temperature via the van't Hoff equation were taken from Batstone et al. (2002) and Lide (2004).

$$K_{H,i}'\left(\frac{kmol}{m^3 \cdot bar}\right) = \frac{K_{H,i}\left(\frac{kmol}{m^3 \cdot bar}\right)}{\gamma_0} = \frac{S_{g,i}\left(\frac{kmol}{m^3}\right)}{P_i\left(bar\right)}$$
(6.13)



**Figure 6.1** Schematic representation of the 'ideal' or 'non-ideal' physical-chemical implementation used for all ADM1-based models in this study. t refers to the simulation time-step. Tol refers to tolerance (in this study  $Tol = 10^{-6}$ ). I is the ionic strength; while  $S_{T,i}$  is the global concentration;  $K_{a,i}$  is the dissociation equilibrium constant; and  $\gamma_i$  is the activity coefficient of soluble species

## 6.2.2 Model Implementation Verification

#### 6.2.2.1 Model Comparison

The 'non-ideal' calculation module [Figure 6.1] was used to upgrade the CSTR implementation of ADM1 as suggested by Rosén et al. (2006), and the HS-AD model proposed by Pastor-Poquet et al. (2018). Four different models were compared: standard ADM1 (ADM1); ADM1 using 'non-ideal' conditions (ADM1 Non-Ideal); the HS-AD model (HS-AD Model); and the HS-AD model using 'non-ideal' conditions (HS-AD Model Non-Ideal). 365 days of continuous HS-AD operation were used in each simulation. Apparent (i.e. kmol/m<sup>3</sup> Solvent) and global (i.e. kmol/m<sup>3</sup> Total) concentrations were used to express exactly the same HS-AD results, since both concentrations are related to each other by the TS, as well as global ( $\rho_{Global}$ ) and solvent ( $\rho_{Solvent}$ ) specific weights (Pastor-Poquet et al., 2018). Particularly, apparent concentrations were used in the HS-AD model to account for the TS concentration effect on all the soluble species in a low water environment as HS-AD.

Importantly, simulation of a continuous HS-AD reactor using the HS-AD model required the reduction of the volumetric effluent ( $Q_{Effluent}$ ) compared to the influent ( $Q_{Influent}$ ) to maintain  $V_{Global}$  constant. With this aim, a proportional controller for  $Q_{Effluent}$  was used as described by Pastor-Poquet et al. (2018), allowing also the comparison between the steady-state results obtained with the CSTR implementation of ADM1 and the HS-AD model. On the other hand, all the simulated TS and VS were recalculated (i.e. TS<sub>Recalc</sub> and VS<sub>Recalc</sub>, respectively) as shown by Pastor-Poquet et al. (2018), to include the potential losses of volatile materials (i.e. CO<sub>2</sub>, NH<sub>3</sub> and VFA) when drying a sample at 105°C (EPA, 2001). The organic loading rate (OLR) was approximated as the daily influent COD per unit of V<sub>Global</sub> (i.e. kg COD/m<sup>3</sup>·d), while the hydraulic retention time (HRT) was evaluated as the quotient between V<sub>Global</sub> and Q<sub>Effluent</sub> (i.e. days). The overall biomass content (X<sub>biomass</sub>) was the sum of all microbial concentrations in ADM1: X<sub>biomass</sub> = X<sub>su</sub> + X<sub>aa</sub> + X<sub>fa</sub> + X<sub>c4</sub> + X<sub>pro</sub> + X<sub>ac</sub> + X<sub>h</sub>2.

As a novel approach, the four model configurations presented above were used to assess simultaneously the influence of the varying reactor content mass/volume, the effect of the apparent concentrations, and the solution 'non-ideality' in HS-AD simulations. The biochemical rates used for model verification are reported in Table 6.1. All the model parameters were as in Rosén et al. (2006) for mesophilic (35°C) AD. Continuous influent conditions were used at 10, 20 and 30 % TS [Table 6.2], together with a Q<sub>Influent</sub> of 170 m<sup>3</sup>/d, a V<sub>Global</sub> of 3400 m<sup>3</sup>, and a reactor design volume (V<sub>Reactor</sub>) of 3700 m<sup>3</sup>. With these specifications, all the simulations were performed at an HRT of 20 d, while the OLR was proportionally increased for higher TS influents. All the influent conditions simulated an OFMSW inflow with a relatively high content of proteins (X<sub>pr</sub>) at different dilutions [Table 6.2], permitting to assess differently the NH<sub>3</sub> inhibition on acetate uptake, particularly when reaching steady-state HS-AD.

| D                              | Rate (r <sub>j</sub> , kg                                                                                                                                                                    | COD m <sup>-3</sup> d <sup>-1</sup> )                                                                                                                                          |
|--------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Process —                      | Verification                                                                                                                                                                                 | Calibration                                                                                                                                                                    |
| Disintegration                 | $k_{dis} \cdot X_c$                                                                                                                                                                          | -                                                                                                                                                                              |
| Hydrolysis of<br>Carbohydrates | $k_{h,ch}\!\cdot\!X_{ch}$                                                                                                                                                                    | $k_{h,ch} {\boldsymbol \cdot} {\mathbf X}_{ch}$                                                                                                                                |
| Hydrolysis of Proteins         | $k_{ m h,pr}$ · $X_{ m pr}$                                                                                                                                                                  | $k_{h,pr}$ · $X_{pr}$                                                                                                                                                          |
| Hydrolysis of Lipids           | $k_{h,li}$ · $X_{li}$                                                                                                                                                                        | $k_{\rm h,li}{\scriptstyle\star} X_{\rm li}$                                                                                                                                   |
| Sugars Uptake                  | $k_{m,su} {\cdot} S_{su,App} / (K_{S,Xsu} {+} S_{su,App}) {\cdot} X_{su} {\cdot} I_{pH} {\cdot} I_{in}$                                                                                      | $k_{m,su} \cdot S_{su,App} / (K_{S,Xsu} + S_{su,App}) \cdot X_{su} \cdot I_{pH} \cdot I_{in}$                                                                                  |
| Aminoacids Uptake              | $k_{m,aa} \cdot S_{aa,App} / (K_{S,Xaa} + S_{aa,App}) \cdot X_{aa} \cdot I_{pH} \cdot I_{in}$                                                                                                | $k_{m,aa} \cdot S_{aa,App} / (K_{S,Xaa} + S_{aa,App}) \cdot X_{aa} \cdot I_{pH} \cdot I_{in}$                                                                                  |
| LCFA Uptake                    | $k_{m,fa} \cdot S_{fa} / (K_{S,Xfa} + S_{fa}) \cdot X_{fa} \cdot I_{pH} \cdot I_{in} \cdot I_{h2}$                                                                                           | $k_{m,fa} \cdot S_{fa} / (K_{S,Xfa} + S_{fa}) \cdot X_{fa} \cdot I_{pH} \cdot I_{in} \cdot I_{h2}$                                                                             |
| Valerate Uptake                | $\begin{array}{l} k_{m,c4} \cdot S_{va,App} / (K_{S,Xc4} + S_{va,App}) \cdot X_{c4} \cdot \\ S_{va,App} / (1 + S_{bu,App} + 10^{-6}) \cdot I_{pH} \cdot I_{in} \cdot I_{h2} \end{array}$     | $\begin{array}{l} k_{m,c5} \cdot S_{va,App} / (K_{S,Xc5} + S_{va,App}) \cdot X_{c5} \cdot I_{pH} \cdot I_{in} \cdot \\ I_{h2} \cdot I_{nh3} \end{array}$                       |
| Butyrate Uptake                | $\begin{array}{l} k_{m,c4} \cdot S_{bu,App} / (K_{S,Xc4} + S_{bu,App}) \cdot X_{c4} \cdot \\ S_{bu,App} / (1 + S_{bu,App} + 10^{-6}) \cdot I_{pH} \cdot I_{in} \cdot I_{h2} \end{array}$     | $\frac{k_{m,c4}\cdot S_{bu,App}}{I_{in}\cdot I_{h2}} \cdot X_{c4} \cdot I_{pH} \cdot I_{in} \cdot I_{h2}$                                                                      |
| Propionate Uptake              | $\begin{array}{c} k_{m,pro} \cdot \mathbf{S}_{pro,App} / (\mathbf{K}_{S,Xpro} + \mathbf{S}_{pro,App}) \cdot \mathbf{X}_{pro} \cdot \mathbf{I}_{pH} \cdot \\ I_{in} \cdot I_{h2} \end{array}$ | $\begin{array}{l} k_{m,pro} \cdot S_{pro,App} / (K_{S,Xpro} + S_{pro,App}) \cdot X_{pro} \cdot I_{pH} \cdot \\ I_{in} \cdot I_{h2} \cdot I_{nh3} \end{array}$                  |
| Acetate Uptake                 | $\begin{array}{c} k_{m,ac} {\cdot} S_{acApp} / (K_{S,Xac} {+} S_{ac,App}) {\cdot} X_{ac} {\cdot} I_{pH} {\cdot} \\ I_{in} {\cdot} I_{nh3} \end{array}$                                       | $\begin{array}{c} k_{m,ac} \!\cdot\! S_{ac,App} \! / \! (K_{S,Xac} \!\!+ \!\! S_{ac,App}) \!\cdot\! X_{ac} \!\cdot\! I_{pH} \!\cdot\! \\ I_{in} \!\cdot\! I_{nh3} \end{array}$ |
| Hydrogen Uptake                | $\begin{array}{l} k_{m,h2} \cdot S_{h2,App} / (K_{S,Xh2} + S_{h2,App}) \cdot X_{h2} \cdot \\ I_{pH} \cdot I_{in} \end{array}$                                                                | $k_{m,h2} \cdot S_{h2,App} / (K_{S,Xh2} + S_{h2,App}) \cdot X_{h2} \cdot I_{pH} \cdot I_{in}$                                                                                  |
| Sugar Degraders<br>Decay       | $k_d {\cdot} X_{su}$                                                                                                                                                                         | $k_{d} \cdot X_{su}$                                                                                                                                                           |
| Aminoacids<br>Degraders Decay  | $k_d \cdot X_{aa}$                                                                                                                                                                           | $k_{d} \cdot X_{aa}$                                                                                                                                                           |
| LCFA Degraders<br>Decay        | $k_d {\cdot} X_{\mathrm{fa}}$                                                                                                                                                                | $k_d \cdot X_{fa}$                                                                                                                                                             |
| Valerate Degraders<br>Decay    | -                                                                                                                                                                                            | $k_{d} \cdot X_{c5}$                                                                                                                                                           |
| Butyrate Degraders<br>Decay    | $k_d \cdot X_{c4}$                                                                                                                                                                           | $k_d \cdot X_{c4}$                                                                                                                                                             |
| Propionate Degraders<br>Decay  | $k_d {\cdot}  X_{pro}$                                                                                                                                                                       | $k_d \cdot X_{pro}$                                                                                                                                                            |
| Acetate Degraders<br>Decay     | $k_d \cdot X_{ac}$                                                                                                                                                                           | $k_{d} \cdot X_{ac}$                                                                                                                                                           |
| Hydrogen Degraders<br>Decay    | $k_d {\cdot}  X_{h2}$                                                                                                                                                                        | $k_{d} \cdot X_{h2}$                                                                                                                                                           |

 Table 6.1 Biochemical rates used for model implementation verification and model calibration

with

 $I_{in} = S_{in,App} / (K_{i,Sin} + S_{in,App})$ 

 $I_{h2} = K_{i,Sh2}/(K_{i,Sh2} + S_{h2,App})$ 

 $I_{pH} = K_{pH} ^N_{pH} / (K_{pH} ^N_{pH} + S_{h+} ^N_{pH})$ 

 $I_{nh3}=K_{i,Snh3}/(K_{i,Snh3}+S_{nh3,App})$ 

|                             |           | Verification |           |             | <b>TT 1</b> .          |
|-----------------------------|-----------|--------------|-----------|-------------|------------------------|
| Name                        | TS = 10 % | TS = 20 %    | TS = 30%  | Calibration | Units                  |
| Ssu                         | 0.010     | 0.010        | 0.010     | 10.846      | kg COD m <sup>-3</sup> |
| $\mathbf{S}_{\mathrm{aa}}$  | 0.001     | 0.001        | 0.001     | 2.125       | kg COD m <sup>-3</sup> |
| $\mathbf{S}_{\mathrm{fa}}$  | 0.001     | 0.001        | 0.001     | 0.649       | kg COD m <sup>-3</sup> |
| $\mathbf{S}_{\mathrm{va}}$  | 0.001     | 0.001        | 0.001     | 0.000       | kg COD m <sup>-3</sup> |
| $\mathbf{S}_{\mathrm{bu}}$  | 0.001     | 0.001        | 0.001     | 0.500       | kg COD m <sup>-3</sup> |
| $\mathbf{S}_{\mathrm{pro}}$ | 0.001     | 0.001        | 0.001     | 2.059       | kg COD m <sup>-3</sup> |
| $\mathbf{S}_{\mathrm{ac}}$  | 0.001     | 0.001        | 0.001     | 0.103       | kg COD m <sup>-3</sup> |
| $\mathbf{S}_{h2}$           | 1.000E-08 | 1.000E-08    | 1.000E-08 | 1.000E-08   | kg COD m <sup>-3</sup> |
| $\mathbf{S}_{ch4}$          | 1.000E-05 | 1.000E-05    | 1.000E-05 | 1.000E-05   | kg COD m <sup>-3</sup> |
| $\mathbf{S}_{\mathrm{ic}}$  | 0.040     | 0.040        | 0.040     | 0.029       | kmol C m <sup>-3</sup> |
| $\mathbf{S}_{\mathrm{in}}$  | 0.010     | 0.010        | 0.010     | 0.186       | kmol N m <sup>-3</sup> |
| $\mathbf{S}_{i}$            | 0.020     | 0.020        | 0.020     | 0.000       | kg COD m <sup>-3</sup> |
| $\mathbf{S}_{i,subs}$       | -         | -            | -         | 34.706      | kg COD m <sup>-3</sup> |
| $X_{c}$                     | 2.000     | 2.000        | 2.000     | -           | kg COD m <sup>-3</sup> |
| $X_{ch}$                    | 10.000    | 20.000       | 30.000    | 43.382      | kg COD m <sup>-3</sup> |
| $\mathbf{X}_{\mathrm{pr}}$  | 20.000    | 40.000       | 60.000    | 29.756      | kg COD m <sup>-3</sup> |
| $\mathbf{X}_{\mathrm{g}}$   | 1.000     | 2.000        | 3.000     | 5.843       | kg COD m <sup>-3</sup> |
| $\mathbf{X}_{\mathrm{su}}$  | 0.010     | 0.010        | 0.010     | 0.050       | kg COD m <sup>-3</sup> |
| $\mathbf{X}_{aa}$           | 0.010     | 0.010        | 0.010     | 0.050       | kg COD m <sup>-3</sup> |
| $\mathbf{X}_{\mathrm{fa}}$  | 0.010     | 0.010        | 0.010     | 0.001       | kg COD m <sup>-3</sup> |
| $X_{c5}$                    | -         | -            | -         | 0.002       | kg COD m <sup>-3</sup> |
| $X_{c4}$                    | 0.010     | 0.010        | 0.010     | 0.001       | kg COD m <sup>-3</sup> |
| $\mathbf{X}_{\mathrm{pro}}$ | 0.010     | 0.010        | 0.010     | 0.002       | kg COD m <sup>-3</sup> |
| $X_{ac}$                    | 0.010     | 0.010        | 0.010     | 0.002       | kg COD m <sup>-3</sup> |
| $X_{h2}$                    | 0.010     | 0.010        | 0.010     | 0.070       | kg COD m <sup>-3</sup> |
| $\mathbf{X}_{i}$            | 60.000    | 120.000      | 180.000   | 0.000       | kg COD m <sup>-3</sup> |
| $X_{i,subs}$                | -         | -            | -         | 86.765      | kg COD m <sup>-3</sup> |
| $\mathbf{S}_{cat}$          | 0.040     | 0.040        | 0.040     | 0.100       | kmoleq m-3             |
| $\mathbf{S}_{an}$           | 0.020     | 0.020        | 0.020     | 0.051       | kmoleq m-3             |
| $ ho_{Global}$              | 1050.000  | 1080.000     | 1100.000  | 1077.633    | kg m <sup>-3</sup>     |
| TS                          | 10.000    | 20.000       | 30.000    | 15.502      | %                      |
| VS                          | 8.000     | 18.000       | 28.000    | 12.942      | %                      |

**Table 6.2** Influent conditions used for model implementation verification at different total solid (TS) contents and initial conditions used for model calibration

#### 6.2.2.2 'Non-Ideal' Calculations

pH calculations were performed as shown in Rosén et al. (2006) and Volcke et al. (2005). In order to implement 'non-ideal' conditions, the  $K_{a,i}$  of all the ionic species in ADM1 (i.e.  $S_{in}$ ,  $S_{ic}$ ,  $S_{ac}$ ) were modified at each time-step, as shown in section 6.2.1. For 'non-ideal' simulations,  $S_{cat}$  and  $S_{an}$  were entirely associated to Na<sup>+</sup> and Cl<sup>-</sup>, respectively. Importantly, apparent concentrations (i.e. kmol/m<sup>3</sup> Solvent) were used in the pH calculations – as well as in all the bio-physical-chemical dynamics – of the HS-AD model, in contrast to the CSTR implementation of ADM1 that used global concentrations (i.e. kmol/m<sup>3</sup> Total).

In some HS-AD model simulations, the Phreeqc engine (Charlton et al., 2011; Parkhurst et al., 1999) was used for pH, *I* and  $\gamma_i$  calculations, as an alternative to the proposed 'non-ideal' module [Figure 6.1]. In these cases, precipitation was not used, though ion pairing is one of the main features of Phreeqc. It must be mentioned that the proposed module for assessing 'non-ideality' in HS-AD simulations [Figure 6.1] is a simplification of more complex physical-chemical engines (i.e. Visual MINTEQ and Phreeqc). Nonetheless, the proposed 'non-ideal' module – instead of Phreeqc – served to compare 'ideal' and 'non-ideal' HS-AD simulations, using the same pH calculation routine in both cases, by only modifying the equilibrium constants (K<sub>a,i</sub>) at each simulation time-step in the 'non-ideal' implementation.

To illustrate the existing link between 'non-ideality' and the main NH<sub>3</sub> inhibition parameters in structured HS-AD models, the NH<sub>3</sub> half-inhibition constant for acetoclastic methanogens ( $K_{i,Snh3,Xac}$ ) was slightly modified in some cases. Thus, simulations using the original  $K_{i,Snh3,Xac}$  for mesophilic (35°C) conditions (i.e. 0.0018 kmol N/m<sup>3</sup>) (Batstone et al., 2002) were compared with simulations using slightly different  $K_{i,Snh3,Xac}$  (i.e. 0.0008 and 0.0028 kmol N/m<sup>3</sup>). To compare the different values for the soluble acetate concentration ( $S_{ac}$ ) under 'ideal' ( $S_{ac,Ideal}$ ) and 'non-ideal' ( $S_{ac,Non-Ideal}$ ) conditions at the same influent TS, the relative acetate difference was used [Equation 6.14]. To compare the different values for the NH<sub>3</sub> concentration ( $S_{nh3}$ ) under 'ideal' ( $S_{nh3,Ideal}$ ) and 'non-ideal' ( $S_{nh3,Non-Ideal}$ ) conditions, the relative NH<sub>3</sub> difference was used [Equation 6.15]. The Henry's constant for CO<sub>2</sub> ( $K_{H,co2}$ ) reduction between 'ideal' ( $K_{H,co2,Ideal}$ ) and 'non-ideal' ( $K_{H,co2,Non-Ideal}$ ) conditions was also expressed as relative difference [Equation 6.16].

Acetate Difference (%) = 
$$\frac{(S_{ac,Non-Ideal} - S_{ac,Ideal})}{S_{ac,Ideal}} \cdot 100$$
 (6.14)

$$NH_3 Difference (\%) = \frac{(S_{nh3,Non-Ideal} - S_{nh3,Ideal})}{S_{nh3,Ideal}} \cdot 100$$
(6.15)

$$K_{H,co2} Difference (\%) = \frac{(K_{H,co2,Non-Ideal} - K_{H,co2,Ideal})}{K_{H,co2,Ideal}} \cdot 100$$
(6.16)

## 6.2.3 Experimental Data and Model Calibration

A HS-AD batch experiment fed with OFMSW under thermophilic (55°C) conditions was used for model calibration. The batch experiment consisted of a sacrifice test starting at 15 % TS, where one replicate was opened – 'sacrificed' – periodically, and the main physical-chemical analyses (e.g. TS, VFA) were performed. Experimental data included the cumulative methane production, gas composition (i.e. CH<sub>4</sub> and CO<sub>2</sub>), TS and VS, TAN, VFA, pH, and mono-valent ions (i.e. Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup>). Further information about the experimental setup and physical-chemical analyses used can be found in Pastor-Poquet et al. (2018).

For model calibration, the 'non-ideal' CSTR implementation of ADM1 (ADM1 Non-Ideal) and the HS-AD model (HS-AD Model Non-Ideal) were compared, using the biochemical rates reported in Table 6.1. Noteworthy, these rates were slightly different than those used in the original ADM1 implementation (Batstone et al., 2002), since a new population for valerate degraders ( $X_{c5}$ ) was included, while the composite ( $X_c$ ) disintegration was disregarded, as shown by Pastor-Poquet et al. (2018). As an example, a reversible (noncompetitive) NH<sub>3</sub> inhibition function [Equation 6.17] was also used for propionate and valerate uptakes in model calibration [Table 6.1], to account for the potential methanogenic and/or acetogenic NH<sub>3</sub> inhibition observed in the experimental dataset (Pastor-Poquet et al., 2018). The initial conditions [Table 6.2] were recalculated based on the experimental data available. The biochemical parameters for thermophilic (55°C) conditions were extracted from Batstone et al. (2002). Meanwhile, some parameters were also modified aiming to fit adequately the experimental data [Table 6.3]. Parameter calibration and all the initial biomass concentrations (e.g. X<sub>ac</sub>) were approximated by trial-and-error. The detailed methodology used for obtaining the initial conditions and for model calibration were described elsewhere (Pastor-Poquet et al., 2018).

$$I_{nh3} = \frac{K_{i,Snh3}}{K_{i,Snh3} + S_{nh3,App}}$$
(6.17)

| Parameter                      | ADM1 | This Study | Units                  |
|--------------------------------|------|------------|------------------------|
| k <sub>h,ch</sub>              | 10   | 0.05       | d-1                    |
| k <sub>h,pr</sub>              | 10   | 0.05       | d <sup>-1</sup>        |
| k <sub>h,li</sub>              | 10   | 0.07       | d <sup>-1</sup>        |
| k <sub>m,su</sub>              | 70   | 35         | d <sup>-1</sup>        |
| k <sub>m,fa</sub>              | 10   | 4          | d <sup>-1</sup>        |
| k <sub>m,c5</sub>              | 30   | 8          | d-1                    |
| k <sub>m,c4</sub>              | 30   | 8          | d-1                    |
| k <sub>m,pro</sub>             | 20   | 10         | d-1                    |
| K <sub>i,Snh3,Xc5</sub>        | -    | 0.006      | kmol N m <sup>-3</sup> |
| Ki,Snh3,Xpro                   | -    | 0.006      | kmol N m <sup>-3</sup> |
| $pH_{LL,ac}$                   | 6    | 5.6        |                        |
| $pH_{\text{UL,ac}}$            | 7    | 6.6        |                        |
| $\mathbf{f}_{\mathrm{bu,su}}$  | 0.13 | 0.37       |                        |
| $\mathbf{f}_{\mathrm{pro,su}}$ | 0.27 | 0.11       |                        |
| $\mathbf{f}_{\mathrm{ac,su}}$  | 0.41 | 0.40       |                        |
| $f_{h2,su}$                    | 0.19 | 0.12       |                        |
| $\mathbf{N}_{i,subs}$          | -    | 0.001      | kmol N m <sup>-3</sup> |

 Table 6.3 Biochemical parameters modified for model calibration at thermophilic (55°C)

 conditions

It must be stated that both the initial conditions and/or the biochemical model parameterization are tightly related to the model structure (Dochain et al., 2001; Donoso-Bravo et al., 2011; Poggio et al., 2016). Thus, in order to minimize the differences between the CSTR implementation of ADM1 and the HS-AD model, the same set of initial conditions [Table 6.2] and thermophilic (55°C) parameters [Table 6.3] were used in both cases. The adjustment/fitting of the model implementations regarding the experimental data was evaluated by the weighted sum of squares, calculated as shown by Flotats et al. (2003). The weighted sum of squares included the cumulative methane production ( $V_{ch4}$  Cum.), gas composition (CH<sub>4</sub> + CO<sub>2</sub>), pH, TAN (S<sub>in</sub>), and VFA (S<sub>ac</sub>, S<sub>pro</sub>, S<sub>bu</sub> & S<sub>va</sub>).

## 6.3 Results and Discussion

## 6.3.1 Verification of the 'Non-Ideal' Model Implementation

#### 6.3.1.1 Effects of 'Non-Ideality' on Standard ADM1

The main difference between the 'ideal' ADM1 simulations using different influent TS was the  $S_{in}$  and  $S_{ac}$  accumulation, but also the reduction of the acetoclastic methanogens concentration ( $X_{ac}$ ) along higher operating TS [Table 6.4]. These results are related to the higher OLR used at higher influent TS, since the protein content (i.e. 0.22 kg COD/kg COD), as well as the anaerobic biodegradability (i.e. 0.35 kg COD/kg COD) were set equal for all the influent conditions [Table 6.2]. Meanwhile, the  $S_{ac}$  accumulation at higher influent TS [Figure 6.2a] was also related to the NH<sub>3</sub> half-inhibition constant for acetoclastic methanogens used in all simulations (i.e.  $K_{i,Snh3,Xac} = 0.0018$  kmol N/m<sup>3</sup>), since an increasing  $S_{nh3}$  exacerbates inhibition [Table 6.1]. Thus, the  $X_{ac}/X_{biomass}$  ratio was observed to decrease from 20.6 to 16.6 % at 10 and 30 % influent TS, respectively [Figure 6.2b]. Importantly, this last phenomenon might imply a greater risk of methanogenic overloading at increasing OLR in HS-AD simulations under 'ideal' conditions, since a proportionally lower  $X_{ac}$  is available to counteract the  $S_{ac}$  buildup.

The CSTR implementation of ADM1 using 'non-ideal' conditions (ADM1 Non-Ideal) showed an increasing *I* alongside the higher influent TS used, from 0.166 M at 10 % TS up to 0.390 M at 30 % TS [Table 6.4]. These results suggest that the bio-physical-chemistry in HS-AD of OFMSW might be considerably 'non-ideal' (i.e.  $I \ge 0.2$  M), being the solution 'non-ideality' exacerbated at higher operating TS contents and/or by the occurrence of inhibitory mechanisms (i.e. NH<sub>3</sub> build-up). Therefore, an adequate 'non-ideal' methodology seems to be required to account for ionic speciation in HS-AD simulations (Batstone et al., 2015; Tait et al., 2012), though the *I* range for HS-AD of OFMSW should be better assessed by experimental data, as shown in section 6.3.3.

|                            |         |         |                                                                     |                                                                        | ,                                                                                |                                                                                           |                                                               |                                                           |                                                    |                                                           |                                                     |                                                                          |                                                              |                                                                            |                                                                           |                                                                           |                                                                         | Ì                                                                         | 1                                                      |
|----------------------------|---------|---------|---------------------------------------------------------------------|------------------------------------------------------------------------|----------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|---------------------------------------------------------------|-----------------------------------------------------------|----------------------------------------------------|-----------------------------------------------------------|-----------------------------------------------------|--------------------------------------------------------------------------|--------------------------------------------------------------|----------------------------------------------------------------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------|-------------------------------------------------------------------------|---------------------------------------------------------------------------|--------------------------------------------------------|
| Variable                   | 10%TS   | 20%TS   | 30%TS                                                               | 10%TS                                                                  | 20%TS                                                                            | 30%TS                                                                                     | 105                                                           | %TS                                                       | 20                                                 | %TS                                                       | 30                                                  | %TS                                                                      | 10                                                           | 6TS                                                                        | 20%                                                                       | 6TS                                                                       | 30%                                                                     | %TS                                                                       | Unit                                                   |
|                            | 10/013  | C10/01  | 20/013                                                              | 10/013                                                                 | C10/02                                                                           | 010/010                                                                                   | Global                                                        | Apparent                                                  | Global                                             | Apparent                                                  | Global                                              | Apparent                                                                 | Global                                                       | Apparent                                                                   | Global                                                                    | Apparent                                                                  | Global                                                                  | Apparent                                                                  |                                                        |
| TS                         | 10.0    | 20.0    | 30.0                                                                | 10.0                                                                   | 20.0                                                                             | 30.0                                                                                      | ~                                                             | 3.3                                                       | _                                                  | 7.2                                                       | 2                                                   | 6.5                                                                      | ~                                                            | 3                                                                          | 1                                                                         | 7.1                                                                       | 20                                                                      | 5.4                                                                       | %                                                      |
| $TS_{Recalc}$              | 9.1     | 18.4    | 27.5                                                                | 9.1                                                                    | 18.5                                                                             | 27.8                                                                                      | ~1                                                            | 7.4                                                       | 1                                                  | 5.5                                                       | 2                                                   | 3.8                                                                      |                                                              | .4                                                                         | 1                                                                         | 5.6                                                                       | 23                                                                      | 3.9                                                                       | %                                                      |
| QEffluent                  | 170     | 170     | 170                                                                 | 170                                                                    | 170                                                                              | 170                                                                                       | _                                                             | 89                                                        | _                                                  | 166                                                       | _                                                   | 64                                                                       | _                                                            | 89                                                                         | 1                                                                         | 56                                                                        | 1                                                                       | 63                                                                        | m³ d                                                   |
| $V_{Global}$               | 3400    | 3400    | 3400                                                                | 3400                                                                   | 3400                                                                             | 3400                                                                                      | 5                                                             | 400                                                       | ىپ                                                 | 400                                                       | 3                                                   | 399                                                                      | دي                                                           | 100                                                                        | 34                                                                        | 00                                                                        | 33                                                                      | 661                                                                       | m³                                                     |
| PGlobal                    | 1050    | 1080    | 1100                                                                | 1050                                                                   | 1080                                                                             | 1100                                                                                      | 10                                                            | 044                                                       | Ŀ                                                  | 690                                                       | 1                                                   | 086                                                                      | -                                                            | )44                                                                        | 10                                                                        | 69                                                                        | 10                                                                      | )86                                                                       | kg n                                                   |
| OLR                        | 4.7     | 9.2     | 13.8                                                                | 4.7                                                                    | 9.2                                                                              | 13.8                                                                                      | 4                                                             | 1.7                                                       |                                                    | 9.2                                                       | _                                                   | 3.8                                                                      | 4                                                            | .7                                                                         | 9                                                                         | .2                                                                        | 15                                                                      | 3.8                                                                       | kg COD                                                 |
| HRT                        | 20.0    | 20.0    | 20.0                                                                | 20.0                                                                   | 20.0                                                                             | 20.0                                                                                      | 2                                                             | 0.0                                                       | 2                                                  | 0.0                                                       | 2                                                   | 0.0                                                                      | 2                                                            | 0.0                                                                        | 21                                                                        | 0.0                                                                       | 2(                                                                      | 0.0                                                                       | b                                                      |
| $Q_{g}$                    | 3224    | 6043    | 8426                                                                | 3212                                                                   | 6307                                                                             | 9189                                                                                      | 3,                                                            | 229                                                       | 5                                                  | 879                                                       | 8                                                   | 203                                                                      | يب                                                           | 218                                                                        | 63                                                                        | 14                                                                        | 88                                                                      | 316                                                                       | Nm <sup>3</sup>                                        |
| %CH4                       | 57.3    | 55.6    | 53.1                                                                | 57.7                                                                   | 57.6                                                                             | 56.7                                                                                      | S1                                                            | 7.3                                                       | 5                                                  | 4.3                                                       | 5                                                   | 1.8                                                                      | 5                                                            | 7.6                                                                        | S                                                                         | 1.4                                                                       | 54                                                                      | 4.9                                                                       | %                                                      |
| %CO2                       | 37.5    | 39.5    | 42.1                                                                |                                                                        |                                                                                  |                                                                                           |                                                               | 1                                                         |                                                    | 1                                                         |                                                     |                                                                          |                                                              |                                                                            |                                                                           |                                                                           |                                                                         |                                                                           |                                                        |
| pН                         | 7.42    | 7.49    |                                                                     | 37.1                                                                   | 37.5                                                                             | 38.5                                                                                      | يى                                                            | /.6                                                       | 4                                                  | 0.7                                                       | 4                                                   | 3.4                                                                      | 3                                                            | 7.2                                                                        | ų                                                                         | 1.7                                                                       | 4(                                                                      | 0.4                                                                       | %                                                      |
| Ι                          | ,       |         | 7.33                                                                | 37.1<br>7.31                                                           | 37.5<br>7.50                                                                     | 38.5<br>7.55                                                                              | 3.                                                            | .44                                                       | 74                                                 | .44                                                       | 7 4                                                 | 3.4<br>.22                                                               | 3                                                            | 7.2<br>33                                                                  | 7. 3                                                                      | 1.7<br>55                                                                 | 4(<br>7.                                                                | 0.4<br>.49                                                                | %                                                      |
| $\mathbf{S}_{\mathrm{ac}}$ | 0.165   | 4.570   | 7.33                                                                | 37.1<br>7.31<br>0.166                                                  | 37.5<br>7.50<br>0.278                                                            | 38.5<br>7.55<br>0.390                                                                     | 7                                                             | - 44 .6                                                   | 7 4                                                | .44                                                       | 4 17                                                | 3.4<br>.22                                                               | 3<br>0.                                                      | 7.2<br>33<br>176                                                           | 3<br>7.                                                                   | 55<br>521                                                                 | 40<br>7.                                                                | ).4<br>.49<br>512                                                         | % kmol                                                 |
| $\mathbf{S}_{\text{in}}$   | 0.129   | 0.246   | 7.33<br>-<br>15.128                                                 | 37.1<br>7.31<br>0.166<br>0.086                                         | 37.5<br>7.50<br>0.278<br>0.327                                                   | 38.5<br>7.55<br>0.390<br>3.618                                                            | 3<br>7<br>0.182                                               | .44<br>-<br>0.190                                         | 4<br>7.371                                         | -<br>.44<br>8.328                                         | 4<br>7<br>19.490                                    | 3.4<br>.22<br>-<br>24.426                                                | 3<br>7<br>0.088                                              | 7.2<br>33<br>1176<br>0.092                                                 | 3<br>7<br>0.5<br>37                                                       | 7.7<br>55<br>921<br>0.606                                                 | 40<br>7.<br>0.5<br>10.002                                               | ).4<br>.49<br>512<br>12.516                                               | %<br>kmol<br>kg COI                                    |
| $S_{nh3}$                  | 0.00362 | 0.00820 | 7.33<br>-<br>15.128<br>0.365                                        | 37.1<br>7.31<br>0.166<br>0.086<br>0.129                                | 37.5<br>7.50<br>0.278<br>0.327<br>0.245                                          | 38.5<br>7.55<br>0.390<br>3.618<br>0.363                                                   | 3<br>0.182<br>0.130                                           | 7.6<br>- 44<br>0.190<br>0.136                             | 4<br>7.371<br>0.253                                | .44<br>-<br>8.328<br>0.286                                | 4<br>19.490<br>0.381                                | 3.4<br>.22<br>-<br>24.426<br>0.477                                       | 3<br>7<br>0.088<br>0.130                                     | 7.2<br>.33<br>0.092<br>0.136                                               | 3:<br>7.<br>0.537<br>0.252                                                | 7.7<br>55<br>0.606<br>0.285                                               | 40<br>7.<br>0.5<br>10.002<br>0.379                                      | ).4<br>,49<br>512<br>12.516<br>0.475                                      | %<br>kmol<br>kg COI                                    |
| $\mathbf{S}_{ic}$          | 0.153   | 0.199   | 7.33<br>-<br>15.128<br>0.365<br>0.00839                             | 37.1<br>7.31<br>0.166<br>0.086<br>0.129<br>0.00196                     | 37.5<br>7.50<br>0.278<br>0.327<br>0.245<br>0.00527                               | 38.5<br>7.55<br>0.390<br>3.618<br>0.363<br>0.00810                                        | 3<br>0.182<br>0.130<br>0.00387                                |                                                           | 4<br>7.371<br>0.253<br>0.00755                     |                                                           | 4<br>19,490<br>0.381<br>0.00692                     | 3.4<br>.22<br>-<br>24.426<br>0.477<br>0.00867                            | 3<br>7<br>0.088<br>0.130<br>0.00207                          | 7.2<br>33<br>176<br>0.092<br>0.136<br>0.00216                              | 3<br>7<br>0.537<br>0.252<br>0.00588                                       | 7.7<br>55<br>0.606<br>0.285<br>0.00663                                    | 40<br>7.<br>0.5<br>10.002<br>0.379<br>0.00693                           | ),4<br>,49<br>512<br>12.516<br>0.475<br>0.00868                           | %<br>kmol 1<br>kg COE<br>kmole N                       |
| $S_{co2}$                  | 0.01100 | 0.01216 | 7.33<br>-<br>15.128<br>0.365<br>0.00839<br>0.154                    | 37.1<br>7.31<br>0.166<br>0.086<br>0.129<br>0.00196<br>0.158            | 37.5<br>7.50<br>0.278<br>0.327<br>0.245<br>0.00527<br>0.267                      | 38.5<br>7.55<br>0.390<br>3.618<br>0.363<br>0.00810<br>0.00810                             | 3<br>7<br>0.182<br>0.130<br>0.154                             | 7.6<br>-<br>0.190<br>0.136<br>0.00404<br>0.161            | 4<br>7.371<br>0.253<br>0.00755<br>0.162            | .44<br>-<br>8.328<br>0.286<br>0.00853<br>0.183            | 4<br>19.490<br>0.381<br>0.00692<br>0.102            | 3.4<br>.22<br>-<br>24.426<br>0.477<br>0.00867<br>0.128                   | 3<br>7<br>0.088<br>0.130<br>0.00207<br>0.160                 | 7.2<br>.33<br>0.092<br>0.136<br>0.00216<br>0.167                           | 3<br>7,<br>0.537<br>0.252<br>0.00588<br>0.269                             | 55<br>52<br>0.606<br>0.285<br>0.00663<br>0.304                            | 40<br>7.<br>0.5<br>10.002<br>0.379<br>0.00693<br>0.246                  | ).4<br>,49<br>512<br>12.516<br>0.475<br>0.00868<br>0.308                  | %<br>kmol<br>kg COI<br>kmole N<br>kmole N              |
| $\mathbf{X}_{\mathrm{ac}}$ | 0.78    | 1 76    | 7.33<br>-<br>15.128<br>0.365<br>0.00839<br>0.154<br>0.01347         | 37.1<br>7.31<br>0.166<br>0.086<br>0.129<br>0.00196<br>0.158<br>0.01048 | 37.5<br>7.50<br>0.278<br>0.327<br>0.245<br>0.00527<br>0.267<br>0.267             | 38.5<br>7.55<br>0.390<br>3.618<br>0.363<br>0.00810<br>0.330<br>0.330                      | 3<br>0.182<br>0.130<br>0.130<br>0.154<br>0.01055              | 7.6<br>-<br>0.190<br>0.136<br>0.161<br>0.01102            | 4<br>7.371<br>0.253<br>0.00755<br>0.162<br>0.01107 | .44<br>-<br>8.328<br>0.286<br>0.00853<br>0.183<br>0.01250 | 4<br>19,490<br>0.381<br>0.00692<br>0.102<br>0.01104 | 3.4<br>.22<br>-<br>24.426<br>0.477<br>0.00867<br>0.128<br>0.01384        | 3<br>7<br>0.088<br>0.130<br>0.160<br>0.160                   | 7.2<br>.33<br>1176<br>0.092<br>0.136<br>0.00216<br>0.167<br>0.1047         | 3<br>0.537<br>0.252<br>0.00588<br>0.269<br>0.00960                        | 55<br>55<br>0.606<br>0.285<br>0.00663<br>0.304<br>0.01084                 | 40<br>7.<br>0.5<br>10.002<br>0.379<br>0.00693<br>0.246<br>0.00924       | 0.4<br>.49<br>512<br>12.516<br>0.475<br>0.00868<br>0.308<br>0.01156       | %<br>kmol<br>kg COI<br>kmole ?<br>kmole ?<br>kmole (   |
| Xbiomass                   | 3.77    | 00.1    | 7.33<br>-<br>15.128<br>0.365<br>0.00839<br>0.154<br>0.01347<br>1.73 | 37.1<br>7.31<br>0.166<br>0.086<br>0.129<br>0.00196<br>0.158<br>0.01048 | 37.5<br>7.50<br>0.278<br>0.327<br>0.0245<br>0.00527<br>0.00527<br>0.267<br>0.267 | 38.5<br>7.55<br>0.390<br>3.618<br>0.363<br>0.00810<br>0.00810<br>0.330<br>0.01140<br>2.14 | 3:<br>7<br>0.182<br>0.130<br>0.00387<br>0.154<br>0.01055<br>0 | 7.6<br>-<br>0.190<br>0.136<br>0.00404<br>0.161<br>0.01102 | 4<br>7.371<br>0.253<br>0.162<br>0.01107<br>1       |                                                           | 4<br>19,490<br>0.381<br>0.102<br>0.102<br>1         | 3.4<br>.22<br>-<br>24.426<br>0.477<br>0.00867<br>0.128<br>0.01384<br>.65 | 3<br>7<br>0.088<br>0.130<br>0.00207<br>0.160<br>0.01002<br>0 | 7.2<br>.33<br>0.092<br>0.136<br>0.00216<br>0.167<br>0.167<br>0.01047<br>79 | 3<br>7,<br>0,537<br>0,252<br>0,00588<br>0,00588<br>0,269<br>0,00960<br>1. | 7.7<br>55<br>0.606<br>0.285<br>0.00663<br>0.304<br>0.304<br>0.01084<br>54 | 40<br>7.<br>0.5<br>10.002<br>0.379<br>0.00693<br>0.246<br>0.00924<br>1. | ),4<br>49<br>512<br>12,516<br>0,475<br>0,00868<br>0,308<br>0,01156<br>.99 | %<br>kmol<br>kg COE<br>kmole ?<br>kmole ?<br>kmole COE |



**Figure 6.2** Summary of results for model implementation verification as a function of influent total solids (TS). Comparison between standard ADM1, ADM1 Non-Ideal, HS-AD Model and HS-AD Model Non-Ideal outputs: a) Total acetate concentration ( $S_{ac}$ ) vs. initial TS; b) total acetoclastic methanogens to biomass ratio ( $X_{ac}/X_{biomass}$ ) vs. initial TS; c) total acetate concentration ( $S_{ac}$ ) vs. total NH3 concentration ( $S_{nh3}$ ); and d) Henry's constant difference for CO<sub>2</sub> ( $K_{H,co2}$ ) vs. ionic strength. The global and apparent concentrations are interrelated by TS, and the specific weight of reactor content ( $\rho_{Global}$ ) and aqueous solvent ( $\rho_{Solvent} = 1000 \text{ kg/m}^3$ ). The NH3 half-inhibition constant for acetoclastic methanogens ( $K_{i,Snh3,Xac}$ ) was 0.0018 kmol N/m<sup>3</sup>

The 'non-ideal' ADM1 implementation affected practically all the simulated dynamics (e.g.  $S_{ic}$ ,  $S_{ac}$  and  $X_{ac}$ ), in comparison to the 'ideal' ADM1 implementation [Table 6.4]. Particularly,  $S_{nh3}$  decreased by 3 - 45 % when using the 'non-ideal' in contrast to the 'ideal' methodology at each operating TS (i.e. 10 - 30 %), substantially mitigating the acetoclastic inhibition and  $S_{ac}$  accumulation [Figure 6.2a]. The potential alleviation of NH<sub>3</sub> inhibition by using 'non-ideal' conditions was also suggested by Hafner et al. (2009) for AD digesters using cow/swine manure as substrate. In this study, the implementation of 'non-ideal' ADM1 calculations also showed an 8 to 20 % increase in the  $X_{ac}/X_{biomass}$  ratio at higher TS (i.e. 20 - 30 %) compared to the 'ideal' implementation [Figure 6.2b]. Thus, 'non-ideal' conditions potentially allow a higher operating OLR when simulating HS-AD of OFMSW, since the reduced  $S_{nh3}$  leads to a relatively higher  $X_{ac}$  to counteract substrate overloading and  $S_{ac}$  accumulation.

It must be noted that, due to the inherent structure of both the biochemical (i.e. Monod equation) and physical-chemical (i.e. charge balance) framework in ADM1, AD simulations are highly non-linear (Donoso-Bravo et al., 2011; Solon, 2016; Volcke et al., 2005). In other words, an increase in the influent conditions (i.e. OLR) of an ADM1-based model might not lead to a proportional increase in the output dynamics (e.g.  $S_{ac}$  and  $S_{nh3}$ ) at steady-state. For example, the  $S_{ac}$  accumulation was observed to increase exponentially alongside the  $S_{nh3}$  build-up both with the 'ideal' and 'non-ideal' implementations of ADM1 [Figure 6.2c]. This last effect is related to the Monod kinetics, as well as the reversible inhibition function used for acetoclastic methanogenesis in ADM1 [Table 6.1]. Therefore, the implementation of 'non-ideal' conditions may be crucial in HS-AD simulations, since minimal changes in  $S_{nh3}$  – associated to the 'non-ideal' physical-chemistry – might lead to considerable differences in the anaerobic kinetic rates and/or inhibition potential using structured HS-AD models.

Finally, K<sub>H,i</sub> for gaseous species (i.e. CH<sub>4</sub> and CO<sub>2</sub>) decreased linearly alongside increasing *I* by using 'non-ideal' conditions in HS-AD. For example, K<sub>H,co2</sub> showed a 8.6 % reduction at an *I* of 0.39 M using ADM1 Non-Ideal [Equation 6.16], corresponding to a 30 % influent TS [Table 6.4 and Figure 6.2d]. Similarly, a linear relationship was also obtained for the K<sub>H,co2</sub> reduction at increasing TS contents from 10 to 40 %: *K<sub>H,co2</sub> Difference* (%) = -0.242 · TS (%) - 1.343,  $r^2 = 1.000$  – data not shown. The K<sub>H,i</sub> reduction with increasing TS strongly influences the liquid-gas transfer in HS-AD simulations. For example, the K<sub>H,co2</sub> reduction exacerbates the CO<sub>2</sub> volatilization in HS-AD, potentially reducing the available inorganic carbon content (S<sub>ic</sub>, HCO<sub>3</sub><sup>-</sup>), as an important source of buffering capacity and resistance against organic overloading (Patón et al., 2018; Poggio et al., 2016; Steyer et al., 2006). Therefore, 'non-ideal' conditions are also needed to evaluate the liquid-gas transfer (i.e. CO<sub>2</sub>) in HS-AD simulations, as a potential trigger for reactor acidification.

## 6.3.1.2. 'Non-Ideal' Implementation of the HS-AD Model

The main difference between the CSTR implementation of ADM1 and the HS-AD model lies on the simulation of  $M_{Global}$ ,  $V_{Global}$ , TS, VS, and  $\rho_{Global}$  dynamics by the HS-AD model (Pastor-Poquet et al., 2018). Moreover,  $Q_{Effluent}$  had to be reduced compared to  $Q_{Influent}$  when using the HS-AD model, as mentioned in section 6.2.2.1. Therefore, all simulations using the HS-AD model resulted in noticeable differences in the values of these operational variables (i.e. TS, VS and  $Q_{Effluent}$ ) at steady-state [Table 6.4], in comparison to the corresponding influent conditions [Table 6.2]. On the other hand, the use of apparent concentrations (i.e.  $S_{ac,App}$ , kg COD/m<sup>3</sup> Solvent) increased relatively the soluble global species concentrations (i.e.  $S_{ac}$ , kg COD/m<sup>3</sup> Total) at higher operating TS [Table 6.4], due to the relatively lower amount of free water in HS-AD (Pastor-Poquet et al., 2018).

The previous conclusions about the NH<sub>3</sub> inhibition alleviation and the increasing liquidgas transfer (i.e. CO<sub>2</sub>) using ADM1 Non-Ideal – section 6.3.1.1 – are also valid for HS-AD Model Non-Ideal. In particular, S<sub>ac</sub> was from 48 to 93 % lower for 'non-ideal' than 'ideal' HS-AD model simulations [Table 6.4 and Figure 6.2a]. However, it must be highlighted that 'non-ideal' conditions were further exacerbated using the HS-AD model, likely due to the inclusion of apparent concentrations in the bio-physical-chemical framework. Thus, HS-AD Model Non-Ideal showed a 5 - 32 % increase on *I* compared to ADM1 Non-Ideal [Table 6.4]. Meanwhile, the K<sub>H,co2</sub> reduction [Equation 6.16] at influent TS contents from 10 to 40 % showed a more pronounced slope than that obtained with ADM1:  $K_{H,co2}$  Difference (%) = - 0.400 · TS (%) + 0.565,  $r^2 = 0.991$  – data not shown.

Interestingly, when using HS-AD Model Non-Ideal, some seemingly contradictory results were observed regarding the NH<sub>3</sub> inhibition between the 'ideal' and 'non-ideal' simulations at steady-state: At 30 % influent TS, the apparent NH<sub>3</sub> concentration ( $S_{nh3,App}$ ) was 0.00867 and 0.00868 kmol N/m<sup>3</sup> Solvent (i.e. 0.12 % difference), while  $S_{ac}$  was 19.5 and 10.0 kg COD/m<sup>3</sup> Total, for the 'ideal' and 'non-ideal' HS-AD model implementations, respectively [Table 6.4]. In other words, the steady-state  $S_{ac}$  was substantially lower at an equivalent  $S_{nh3,App}$ . Meanwhile, the steady-state  $S_{ac}$  vs.  $S_{nh3}$  still fulfilled the Monod inhibition framework [Figure 6.2c].

To emphasize these last results, the relative differences in the acetate [Equation 6.14] and NH<sub>3</sub> [Equation 6.15] concentrations were used. Thus,  $S_{ac,Non-Ideal}$  was lower than  $S_{ac,Ideal}$  – the acetate difference was negative – at any influent TS [Table 6.4 and Figure 6.3a]. Nevertheless, the NH<sub>3</sub> difference between  $S_{nh3,Non-Ideal}$  and  $S_{nh3,Ideal}$  at 30 % TS was positive, in contrast to 10 and 20 % TS influent conditions [Table 6.4 and Figure 6.3b]. Similar 'contradictory' results were also observed at higher influent TS contents (i.e. 35 - 40 % TS), where  $S_{ac}$  was lower (i.e. 26 - 35 %), while  $S_{nh3}$  was higher (i.e. 1 - 3 %), for the 'non-ideal' in contrast to the 'ideal' HS-AD model implementation [Figure 6.3].



**Figure 6.3** Contour plots for the relative difference between the 'ideal' and 'non-ideal' implementations of both ADM1 and the HS-AD model at different influent total solid (TS) contents: a) Acetate (S<sub>ac</sub>) difference [Equation 6.14]; and b) NH<sub>3</sub> (S<sub>nh3</sub>) difference [Equation 6.15]. Values in parentheses show the NH<sub>3</sub> half-inhibition constants used for acetoclastic methanogens (K<sub>i,Snh3,Xac</sub>, kmol N/m<sup>3</sup>). Positive values over the 'inversion' threshold in panel b represent the influent TS at which the steady-state NH<sub>3</sub> concentration is higher for the 'nonideal' than for the 'ideal' model implementation

Summarizing, results above seemed to contradict the expected trend for acetoclastic inhibition in HS-AD simulations at steady-state: a higher  $S_{nh3}$  concentration should lead to a higher  $S_{ac}$  accumulation. However, these seemingly contradictory results on NH<sub>3</sub> inhibition were only related to the direct comparison of two strongly non-linear model implementations (i.e. 'ideal' *vs.* 'non-ideal'). More in particular, during the initial 40 days of HS-AD model

simulations using a 30 % influent TS, the  $X_{ac}$  growth was promoted by the 'non-ideal' in contrast to the 'ideal' model implementation, due to a lower operating  $S_{nh3,App}$ , as further discussed in section 6.3.1.3.

All the above simulations were performed using  $K_{i,Snh3,Xac} = 0.0018$  kmol N/m<sup>3</sup>. Importantly, when shifting  $K_{i,Snh3,Xac}$  towards lower/higher values in HS-AD Model Non-Ideal, the TS threshold where  $S_{ac,Ideal} > S_{ac,Non-Ideal}$  for  $S_{nh3,Ideal} < S_{nh3,Non-Ideal}$  ('inversion' threshold) also shifted [Figure 6.3]. For example, using  $K_{i,Snh3,Xac} = 0.0008$  kmol N/m<sup>3</sup>, the 'inversion' threshold occurred at around 20 % influent TS, while using  $K_{i,Snh3,Xac} = 0.0028$  kmol N/m<sup>3</sup>, the 'inversion' threshold occurred between 35 and 40 % TS. Similar acetoclastic inhibition results were also obtained between the 'ideal' and 'non-ideal' ADM1 implementations, though the 'inversion' thresholds shifted towards slightly higher operating TS regarding the HS-AD model [Figure 6.3]. For example, using  $K_{i,Snh3,Xac} = 0.0018$  kmol N/m<sup>3</sup>, the inversion threshold using ADM1 was 40 % influent TS, instead of 30 % influent TS. All these results indicate that 'non-ideality' is tightly interrelated to the NH<sub>3</sub> inhibition parameters, but also to the overall HS-AD model structure.

### 6.3.1.3 The Effects of 'Non-Ideality' during the Initial Days of HS-AD Simulations

During the initial 20 days of HS-AD simulations using 30 % influent TS,  $X_{ac}$  was observed to increase considerably faster under 'non-ideal' than 'ideal' conditions [Figure 6.4a], explaining the lower  $S_{ac}$  buildup under 'non-ideal' conditions [Figure 6.4b]. pH was equivalent during the initial 10 days of 'ideal' and 'non-ideal' simulations, though pH for 'non-ideal' simulations was up to 0.27 units higher from day 10 [Figure 6.4c and Table 6.4]. Meanwhile, a lower  $S_{nh3,App}$  was observed along the initial 40 days of 'non-ideal' simulations [Figure 6.4d], despite the apparent TAN ( $S_{in,App}$ ) was equivalent in both the 'ideal' and 'non-ideal' model implementations [Figure 6.4e]. Therefore, the 'non-ideal' bio-physical-chemistry of HS-AD at 30 % influent TS led to a lower  $S_{nh3,App}$ , mitigating the NH<sub>3</sub> inhibition and promoting the  $X_{ac}$  growth, as previously observed for 10 and 20 % influent TS. Nonetheless, the steady-state results [Table 6.4] prevented observing the real effect of 'non-ideality' in HS-AD simulations.



Figure 6.4 Effect of 'non-ideality' during the initial 40 days of HS-AD model simulations at 30 % influent TS. Comparison between 'ideal' and 'non-ideal' conditions, including the Phreeqc engine: a) Acetoclastic methanogens concentration (X<sub>ac</sub>); b) total acetate concentration (S<sub>ac</sub>); c) pH; d) apparent NH<sub>3</sub> concentration (S<sub>nh3,App</sub>); e) total ammonia nitrogen concentration (S<sub>in,App</sub>); and f) ionic strength (I). The NH<sub>3</sub> half-inhibition constant for acetoclastic methanogens (K<sub>i,Snh3,Xac</sub>) was 0.0018 kmol N/m<sup>3</sup>

With all the above, the 'inversion' threshold on the NH<sub>3</sub> concentration at steady-state [Figure 6.3b] is the consequence of comparing two strongly non-linear model implementations (i.e. 'ideal' *vs.* 'non-ideal') at steady-state, being non-linearity associated to the complexity of the biochemical and physical-chemical framework of ADM1-based models, as mentioned before. Importantly, the occurrence of the NH<sub>3</sub> 'inversion' threshold further stresses the fact that 'ideal' ADM1-based models should not be applied to HS-AD (i.e. TS  $\geq 10$  %), since the equation non-linearities might lead to important differences in both the dynamics and the steady state results (i.e. pH, X<sub>ac</sub>, S<sub>nh3</sub>, S<sub>ac</sub>) of HS-AD simulations. The 'inversion' threshold on the NH<sub>3</sub> inhibition at steady-state was also observed when using slightly different initial conditions (i.e. X<sub>pr.0</sub>, S<sub>in.0</sub>, S<sub>ac.0</sub>, S<sub>cat.0</sub>, X<sub>su.0</sub> and/or X<sub>aa.0</sub> – data not shown), since steady-state AD simulations should not depend on the initial conditions used (Donoso-Bravo et al., 2011). Thus, all the above results indicate that a high *I* (i.e.  $\geq 0.2$  M) strongly influenced the bio-physical-chemistry of HS-AD simulations, particularly the NH<sub>3</sub> inhibition dynamics during the initial days of reactor operation at high TS contents (i.e.  $\geq 20$  - 30 %).

To assess 'non-ideal' effects on AD, some of the most complete physical-chemical engines for 'non-ideal' characterizations are Visual MINTEQ (Allison et al., 1991) and Phreeqc (Parkhurst et al., 1999) software, including the direct ADM1 implementation in Phreeqc (C code) described by Huber et al. (2017), the generic nutrient recovery model of Vaneeckhaute et al. (2018), but also the physical-chemical module developed by Flores-Alsina et al. (2015) and Solon et al. (2015) for plant-wide wastewater treatment. Indeed, the high organic content in HS-AD might strongly determine the precipitation, ion-pairing and ion-surface interactions (Batstone et al., 2012; Huber et al., 2017), requiring even further complexity of the HS-AD bio-physical-chemical framework than for 'wet' AD applications (i.e. TS < 10 %). On the other hand, more simple 'non-ideal' modules for AD solutions have been also used by González-Cabaleiro (2015) and Nielsen et al. (2008). In this line, the model complexity depends on the model objectives and experimental data available, being always recommended to keep the model as simple as possible, though well suited for addressing the envisaged objectives (Eberl et al., 2006).

To validate the 'non-ideal' module proposed in this study [Figure 6.1], 'non-ideal' simulations of the HS-AD model were also performed coupling the Phreeqc engine (Charlton et al., 2011). In spite of the higher complexity of Phreeqc, both 'non-ideal' modules yielded practically the same HS-AD dynamics (i.e.  $S_{ac}$ ,  $S_{in}$ ,  $X_{ac}$ ) using 30 % influent TS [Figure 6.4], being the 2 - 6 % higher *I* the most noticeable difference when Phreeqc was used as 'non-ideal' module [Figure 6.4f]. The Phreeqc engine coupling to the HS-AD model also yielded closely-matching results to the proposed 'non-ideal' module under all the HS-AD simulations presented in section 6.3.1.2 – data not shown. Importantly, due to the reduced complexity of the proposed 'non-ideal' module [Figure 6.1] and/or the coupling of an

'external' software, the simulation speed increased considerably (i.e. 7 - 8 times faster) compared to when using the Phreeqc engine as 'non-ideal' module.

### 6.3.2 HS-AD Calibration under 'Non-Ideal' Conditions

The calibration in this study was not aimed to be exhaustive due to the great number of parameters (i.e. > 15) and/or initial conditions (i.e. > 10) involved in an ADM1-based model, as well as the reduced number of experimental data available (Dochain et al., 2001; Donoso-Bravo et al., 2011; Poggio et al., 2016). Instead, the model calibration aimed to assess the operative levels of *I* in HS-AD fed with OFMSW. Moreover, real data calibration could also serve to evaluate the influence of the model complexity (i.e. mass balances) regarding the need for 'non-ideal' calculations in HS-AD.

For the calibration of ADM1 Non-Ideal and HS-AD Model Non-Ideal, the same initial conditions [Table 6.2] and biochemical parameters [Table 6.3] were used in both models, yielding a similar degree of adjustment regarding the experimental data (i.e. weighted sum of squares = 2.2 - 2.5) [Table 6.5]. Nonetheless, HS-AD Model Non-Ideal outperformed ADM1 Non-Ideal in terms of simulating the TS, VS, and M<sub>Global</sub> dynamics due to the use of a more extended set of mass balances. Moreover, HS-AD Model Non-Ideal adjustment improved considerably towards the end of the experiment, in contrast to the ADM1 Non-Ideal simulations [Figure 6.5]. For example, the experimental matching in S<sub>in</sub>, S<sub>pro</sub>, S<sub>va</sub>, and gas composition improved from day 15 - 20 onwards, as M<sub>Global</sub> and/or V<sub>Global</sub> reduction by methanogenesis occurred in the system. In this line, HS-AD Model Non-Ideal predicted 1.6 g of M<sub>Global</sub> were removed, equivalent to a 4.4 % of the initial reactor content, during 92 days of batch operation [Table 6.5].

Both ADM1 Non-Ideal and HS-AD Model Non-Ideal simulations showed  $I \ge 0.5$  M from day 50 [Figure 6.5d], associated to the accumulation of S<sub>in</sub> and VFA, with *I* being around 5 - 10 % higher in HS-AD Model, due to the use of apparent concentrations. These results confirm that *I* might be considerably higher than 0.2 M in HS-AD of OFMSW, strongly requiring the implementation of 'non-ideal' conditions at high TS contents (i.e.  $\ge 10$  %) to improve the simulations of pH, biochemical inhibition (i.e. NH<sub>3</sub>), VFA accumulation (i.e. acetate), and liquid-gas transfer (i.e. CO<sub>2</sub>). Furthermore, taking into account the high *I* observed (i.e.  $\ge 0.5$  M), the Davies equation [Equation 6.6] might not be appropriated for HS-AD simulations due to the increasing errors in  $\gamma_i$  at  $I \ge 0.2$  M (Solon, 2016; Tait et al., 2012). For example, a 20 to 25 % higher  $\gamma_{NH4+}$  is obtained at *I* of 0.5 and 0.6 M, respectively, by using the Davies instead of the EDH equation [Equation 6.8].

|                                |              | ADM1 N          | HS-A      | AD Model |                                 |
|--------------------------------|--------------|-----------------|-----------|----------|---------------------------------|
| Variable                       | Experimental | ADMI Non-       | No        | n-Ideal  | Units                           |
|                                |              | Ideal -         | Global    | Apparent | -                               |
| V <sub>CH4</sub> Cum.          | 0.835        | 0.825           | 0.820     | -        | Nm <sup>3</sup> CH <sub>4</sub> |
| $\mathbf{M}_{\mathrm{Global}}$ | -            | 37.12           | 35.50     | -        | g                               |
| TS                             | -            | 15.50           | 12.23     | -        | %                               |
| $TS_{Recalc}$                  | 9.82         | 12.11           | 8.69      | -        | %                               |
| VS                             | -            | 12.94           | 9.55      | -        | %                               |
| $VS_{Recalc}$                  | 6.91         | 9.55            | 6.02      | -        | %                               |
| TAN                            | 0.330        | 0.306           | 0.320     | 0.342    | mol N kg <sup>-1</sup>          |
| NH <sub>3</sub>                | -            | 0.086           | 0.090     | 0.097    | mol N kg <sup>-1</sup>          |
| VFA                            | 14.12        | 9.53            | 10.76     | 11.51    | g COD kg <sup>-1</sup>          |
| pН                             | 8.51         | 8.17            | 8.18      | -        |                                 |
| Ι                              | -            | 0.551           | 0.606     | -        |                                 |
| $%CH_4$                        | 66.0         | 71.5            | 70.3      | -        | %                               |
| $%CO_2$                        | 34.0         | 27.9            | 29.0      | -        | %                               |
|                                |              | Weighted Sum of | Residuals |          |                                 |
| $\mathbf{S}_{\mathrm{va}}$     | -            | 0.284           | 0.302     | -        |                                 |
| $\mathbf{S}_{\mathbf{bu}}$     | -            | 0.570           | 0.584     | -        |                                 |
| $\mathbf{S}_{\mathrm{pro}}$    | -            | 0.431           | 0.238     | -        |                                 |
| $\mathbf{S}_{\mathrm{ac}}$     | -            | 0.160           | 0.174     | -        |                                 |
| $\mathbf{S}_{\mathrm{in}}$     | -            | 0.065           | 0.024     | -        |                                 |
| pH                             | -            | 0.085           | 0.077     | -        |                                 |
| %CH <sub>4</sub>               | -            | 0.264           | 0.241     | -        |                                 |
| $%CO_2$                        | -            | 0.624           | 0.568     | -        |                                 |
| V <sub>CH4</sub> Cum.          | -            | 0.024           | 0.024     | -        |                                 |
| Total                          | -            | 2.507           | 2.231     | -        |                                 |

 Table 6.5 Summary of results for ADM1 and HS-AD model calibration under 'non-ideal' conditions. Batch results correspond to the experimental day 92

With all the above, the influence of 'non-ideality' on the bio-physical-chemistry of HS-AD simulations strongly depends on the model configuration used. Therefore, the HS-AD model (Pastor-Poquet et al., 2018) may be well suited to assess 'non-ideal' effects in HS-AD using OFMSW as a substrate, and particularly the TS concentration effect on the soluble species by using apparent concentrations. Noteworthy, the implementation of apparent concentrations (i.e. kmol/kg Solvent) is in line with the fact that the bio-physical-chemistry of HS-AD occurs predominantly in water. Thus, using apparent concentrations might enhance the predictive capabilities of the 'non-ideal' calculation procedure, while influencing both the kinetic rates and inhibition of anaerobic microorganisms in HS-AD simulations (Pastor-Poquet et al., 2018). On the other hand, an adequate mass balance implementation in HS-AD models is needed when using relatively long simulations (i.e.  $\geq 20$ 



days), as the effect of reactor mass/volume removal by methanogenesis becomes gradually more important to capture all the bio-physical-chemical processes occurring in HS-AD.

**Figure 6.5** Model calibration results. Comparison between ADM1 Non-Ideal and HS-AD Model Non-Ideal: a) Total ammonia nitrogen (TAN); b) total propionate (S<sub>pro</sub>) and valerate (S<sub>va</sub>) concentrations; c) gas composition; and d) ionic strength

To end up, further calibration/optimization alongside a thorough sensitivity analysis is needed for the main biochemical parameters of the HS-AD model, in order to draw adequate conclusions about some of the inhibitory mechanisms (i.e. NH<sub>3</sub> buildup and acidification) potentially occurring in HS-AD of OFMSW. In this line, the faster HS-AD model resolution obtained when coupling the proposed 'non-ideal' module might be particularly suited to speed up the calibration process, where a great number of simulations are usually required to match appropriately the experimental data (Dochain et al., 2001; Donoso-Bravo et al., 2011; Flotats et al., 2006). Alongside, further bio-physical-chemical mechanisms as precipitation, ion pairing and ion-surface interactions should be also evaluated in future model implementations, to adequately address the inherent complexity of HS-AD using OFMSW as substrate.

## 6.4 Conclusions

HS-AD of OFMSW might be operated at  $I \ge 0.5$  M. Therefore, the bio-physicalchemistry of HS-AD simulations needs to account for the 'non-ideal' effects on the pH, soluble inhibitors (i.e. NH<sub>3</sub>), and liquid-gas transfer (i.e. CO<sub>2</sub>), particularly at higher TS contents (i.e.  $\ge 20$  %). In this study, coupling a HS-AD model to a simplified 'non-ideal' module yielded adequate simulations regarding the NH<sub>3</sub> inhibition in HS-AD, both in batch and continuous mode. Using an appropriate set of parameters, the HS-AD model using 'nonideal' conditions might bring further insights about the main inhibitory mechanisms in HS-AD of OFMSW. Similarly, further bio-physical-chemical mechanisms (e.g. precipitation) should be also explored to enhance HS-AD simulations.

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**Chapter 7** 

High-Solids Anaerobic Digestion Model Calibration and Cross-Validation

This chapter presents unpublished results:

Pastor-Poquet, V., Papirio, S., Harmand, J., Steyer, J.-P., Trably, E., Escudié, R., & Esposito,
 G. (*in preparation*). Assessing Practical Identifiability during Calibration and Cross-Validation of a Structured Model for High-Solids Anaerobic Digestion.

#### Abstract

High-solids anaerobic digestion (HS-AD) of the organic fraction of municipal solid waste (OFMSW) is operated a total solid (TS) content  $\geq 10$  % to enhance the waste treatment economy, though it might be associated to free ammonia (NH<sub>3</sub>) inhibition. This study aimed to calibrate and cross-validate a HS-AD model for homogenized reactors, as a main requirement to assess the influence of high NH<sub>3</sub> levels on HS-AD of OFMSW. The practical identifiabilility of structural/biochemical parameters (i.e. 35) and initial conditions (i.e. 32) was evaluated using batch experiments performed at different TS and/or inoculum-tosubstrate ratios. Variance-based global sensitivity analysis and approximate Bayesian computation were used for parameter optimization. The experimental data in this study permitted to correctly estimate up to 8 biochemical parameters. Meanwhile, the rest of parameters and biomass contents were associated to poor practical identifiability. The study also showed the relatively high levels of NH<sub>3</sub> (i.e. up to 2.3 g N/L) and ionic strength (i.e. up to 0.9 M) when increasing the TS content in HS-AD of OFMSW. Therefore, the calibration highlighted the need for target-oriented experimental data to enhance the practical identifiability, but also the need for further testing regarding the NH<sub>3</sub> inhibition mechanisms in HS-AD models.

**Keywords**: HS-AD Model; OFMSW; Ammonia Inhibition; Ionic Strength; Global Sensitivity Analysis; Approximate Bayesian Computation.



#### **Graphical Abstract**

# 7.1 Introduction

High-solids anaerobic digestion (HS-AD) of the organic fraction of municipal solid waste (OFMSW) is operated a total solid (TS) content  $\geq 10$  % to minimize the reactor volume, the need for water addition and the digestate dewatering (Kothari et al., 2014; Pastor-Poquet et al., 2018). HS-AD can also lead up to 80 % TS removal from OFMSW, easing the by-product post-treatment. However, HS-AD of OFMSW is usually associated to free ammonia (NH<sub>3</sub>) inhibition, potentially resulting in volatile fatty acids (VFA) accumulation [Chapter 6 – Non-Ideal Manuscript]. NH<sub>3</sub> inhibition affects both acetoclastic and hydrogenotrophic methanogens but also the rest of VFA degraders in anaerobic digestion (AD), being the degree of inhibition related to the operative parameters (i.e. temperature, pH) and/or the biomass acclimation (Rajagopal et al., 2013).

Adding lignocellulosic waste in OFMSW permits to adjust/increase the carbon-tonitrogen ratio in HS-AD, minimizing the chances of total ammonia nitrogen (TAN) buildup (Pastor-Poquet et al., 2018, In Press). Moreover, lignocellulosic substrates are associated to a reduced hydrolysis rate, permitting to increase the TS content and/or to counteract the VFA accumulation in HS-AD (Capson-Tojo et al., 2017). However, the inclusion of lignocellulosic waste in OFMSW depends on 'external' aspects, as the season or the local waste management strategy. Whether or not a lignocellulosic co-substrate is used, understanding the effects of NH<sub>3</sub> inhibition is crucial to foster the advantages of HS-AD, while ensuring an adequate OFMSW treatment [Chapter 3 – Batch Manuscript].

A HS-AD model was recently developed for homogenized reactors, as an adequate tool to evaluate the effects of NH<sub>3</sub> inhibition potentially occurring in HS-AD of OFMSW (Pastor-Poquet et al., 2018)[ Chapter 6 – Non-Ideal Manuscript]. This structured model, based on the Anaerobic Digestion Model No.1 (ADM1) (Batstone et al., 2002), gathers together the main biochemical and physical-chemical processes in HS-AD. In the HS-AD model, apparent (i.e. kmol/kg H<sub>2</sub>O) instead of global (i.e. kmol/kg) concentrations define the effect of TS upon soluble substances, as a consequence of the low water content within HS-AD. Meanwhile, a more extended set of mass balances allows the simulation of the organic mass removal by biogas production. A solution 'non-ideality' subroutine was subsequently included in the HS-AD model as a function of the ionic strength (I), since 'non-ideality' strongly affects the pH, liquid-gas transfer (i.e. CO<sub>2</sub>) and NH<sub>3</sub> inhibition in HS-AD simulations [Chapter 6 – Non-ideal Manuscript] (Hafner et al., 2009; Solon et al., 2015).

Simulating the effects of NH<sub>3</sub> inhibition at high TS contents with the HS-AD model requires an adequate set of input parameters,  $\theta$ , to be estimated by calibration (Pastor-Poquet et al., 2018)[Chapter 6 – Non-ideal Manuscript] –  $\theta$  include both the structural/biochemical parameters,  $\theta_P$ , and the initial and/or influent conditions,  $\theta_B$ :  $\theta = (\theta_P, \theta_B)$ . Nonetheless,

calibration of structured AD models is far from trivial due to the equation complexity and large number of  $\theta$  involved (Dochain et al., 2001; Donoso-Bravo et al., 2011; Saltelli et al., 2006).

To calibrate a mathematical model, the  $\theta$  must be structurally and practically identifiable, instead of correlated. The  $\theta$  structural identifiability is assessed from a theoretical point of view, assuming noise-free experimental data and error-free model structure. Noteworthy, nearly all  $\theta$  in ADM1 are (locally) structurally identifiable (Nimmegeers et al., 2017). This is a prerequisite to assess the  $\theta$  practical identifiability, and calibrate the HS-AD model, using 'imperfect' experimental data. Unfortunately, the reduced number of experimental data often available and/or the potential presence of experimental errors yield non-identifiable parameters, i.e. parameters that cannot be uniquely estimated with the experimental data available. These are known as practical identifiability issues.

Calibration usually consists of minimizing an objective function,  $J(\theta)$ , that condenses the 'goodness of fitting' between the experimental data, y, and the model outputs,  $y^{sim}(\theta)$ , being these a function of  $N = [1, +\infty)$  input parameters,  $\theta$  (Dochain et al., 2001; Flotats et al., 2010). Several  $J(\theta)$  can be used to calibrate structured models, as the sum of squares, the weighted sum of squares, or any other user-defined alternative (Donoso-Bravo et al., 2011; Ratto et al., 2001). Assuming the existence of a global minimum (optimum) for an objective function,  $J(\theta_{opt})$ , this value is reached using the optimal set of input parameters,  $\theta_{opt}$ .

Practical identifiability issues commonly translate into  $J(\theta)$  showing many local optimums and/or flat valleys, where the precise value of  $\theta$  cannot be easily determined (Guisasola et al., 2006; Rodriguez-Fernandez et al., 2006). Thus, only practical identification of a reduced  $\theta$  subset (i.e. N' < N) is often possible for ADM1-based models (Nimmegeers et al., 2017). This is the case of model over-parameterization, where the modification of two individual  $\theta$ ,  $\theta_i$  (with i = 1, ..., N), can lead to a similar model response. Particularly, when using batch experiments – highly dependent on the initial conditions – to calibrate AD models, different sets of experimental conditions, including different inoculum-to-substrate ratios (ISR), are needed to reduce the  $\theta$  correlation (Donoso-Bravo et al., 2011; Flotats et al., 2010).

Two main approaches can be used to calibrate complex models: The Bayesian and the frequentist. The frequentist approach aims to find optimal parameter values,  $\theta_{opt}$ , while the Bayesian approach considers the optimal parameters as probabilistic distributions conditioned on the experimental data,  $p(\theta_{opt}/y)$ , instead of single values (Dochain et al., 2001; Ratto et al., 2001; Rodriguez-Fernandez et al., 2006; Saltelli et al., 2006). Within both approaches, when facing over-parameterization, it must be decided which  $\theta_i$  influence significantly the model outputs (sensitivity analysis), and hence need to be adequately calibrated.

ADM1-based models contain a particularly high number of  $\theta_P$  (i.e.  $\geq 35$ ) and  $\theta_B$  (i.e.  $\geq 24$ ).  $\theta_P$  might be obtained from literature. Nonetheless, a different model structure – from where literature parameters were obtained – potentially influences the optimal parameter values/distributions [Chapter 6 – Non-ideal manuscript]. On the other hand,  $\theta_B$  might not be easily determined for ADM1-based models, mainly due to the lack of experimental data and/or the difficulties to translate the data into adequate model units (Donoso-Bravo et al., 2011; Flotats et al., 2003). Furthermore, a reliable assessment of the  $\theta$  confidence range is as important as the value of  $\theta_{opt}$  themselves (Guisasola et al., 2006; Rodriguez-Fernandez et al., 2006).

Parameter inference based on the Bayes' theorem [Equation 7.1] is particularly suited to calibrate structured AD models, since it can deal with complex  $J(\theta)$  showing several optima and/or flat geometries, where frequentist inference might not be well suited (Kennedy et al., 2001; Saltelli et al., 2006; Toni et al., 2010). In Bayesian inference, the prior parameter distribution,  $p(\theta)$ , is thoroughly sampled to obtain the posterior parameter distribution,  $p(\theta|y)$ , conditioned in the experimental data, y, and the likelihood function,  $p(y|\theta)$ , while p(y)can be considered as a normalizing constant. Importantly, any user-defined  $J(\theta)$  arising from  $p(y|\theta)$  can be used in 'informal' statistical approaches [Equation 7.2], as variance-based global sensitivity analysis (GSA) and approximate Bayesian computation (ABC) (Donoso-Bravo et al., 2011; Nott et al., 2012; Ratto et al., 2001).

$$p(\theta|y) = \frac{p(y|\theta)p(\theta)}{p(y)}$$
(7.1)

$$J(\theta) = f(p(y|\theta))$$
(7.2)

Variance-based GSA deriving from the Bayes' theorem [Equation 7.1] provides an appropriate assessment about the potentiality of  $\theta_i$  to influence the model outputs, as well as the correlations existing with the rest of  $\theta$ ,  $\theta_j$  (with j = 1, ..., N and  $i \neq j$ ) (Kennedy et al., 2017; Oakley et al., 2004; Saltelli et al., 2006). Similarly, ABC permits also to highlight potential practical identifiability issues, though yielding simultaneously the most likely  $p(\theta|y)$  (Beaumont et al., 2009; Filippi et al., 2013; Pritchard et al., 1999; Toni et al., 2010). As main disadvantage, Bayesian inference is often computational intensive.

The mathematical performance of the HS-AD model for homogenized reactors was previously verified, though the model was only validated for a single HS-AD batch experiment, due to the high number of  $\theta$  requiring calibration (i.e. > 30) (Pastor-Poquet et al., 2018)[Chapter 6 – Non-ideal Manuscript]. Instead, this study aimed to fully calibrate and cross-validate the HS-AD model for homogenized reactors. In particular, this study assessed

the practical identifiability of 35 structural parameters ( $\theta_P$ ) and 8 initial biomass contents ( $\theta_B$ ), by using four HS-AD batch experiments at different TS and/or ISR, as a source of experimental data. Identifiability was assessed by variance-based GSA and ABC, permitting to approximate also  $p(\theta|y)$ . Importantly, the proposed calibration/validation methodology can be easily readapted to account for further HS-AD datasets (e.g. batch, continuous), organic substrates (e.g. manure, agricultural waste) and/or HS-AD model configurations.

### 7.2 Methodology

### 7.2.1 Experimental Data

To calibrate and cross-validate the HS-AD model, while further evaluate the effects of increasing the initial TS content on HS-AD simulations, four different batch experiments were used at thermophilic (55°C) conditions from 10 to 30 % TS [Table 7.1]. The laboratory-scale reactor design volume ( $V_{Reactor}$ ) was either 160 or 280 mL for the different experiments. In all cases, centrifuged inoculum was used to increase simultaneously the initial TS and ISR. The batch experiments are described next, whereas a thorough description of these batch experiments and the bio-physical-chemical analyses performed was reported elsewhere [Chapter 3 – Batch Manuscript].

| Substrate | Exp.<br>No. | Batch<br>No. | ISR (g<br>VS/g VS) | Initial<br>TS (%) | No.<br>Replicates | Design<br>Volume<br>(mL) | Exp. Time<br>(days) | Calibration<br>or Validation |
|-----------|-------------|--------------|--------------------|-------------------|-------------------|--------------------------|---------------------|------------------------------|
|           | 1           | 1            | 1.00               | 15.0              | 15                | 280                      | 92                  | С                            |
|           |             | 2            |                    | 10.8              | 3                 |                          |                     | V                            |
| OEMSW     | 2           | 3            | 1.50               | 13.4              | 3                 | 160                      | 100                 | С                            |
| OF IVIS W | 2           | 4            | 1.50               | 16.4              | 3                 | 100                      | 100                 | V                            |
|           |             | 5            |                    | 19.6              | 3                 |                          |                     | С                            |
|           | 3           | 6            | 0.50               | 28.3              | 2                 | 280                      | 99                  | С                            |
| OFMENU    |             | 7            |                    | 10.0              | 3                 |                          |                     | С                            |
| OFMSW +   | 4           | 8            | 0.16               | 15.0              | 2                 | 280                      | 284                 | V                            |
| Sawdust   |             | 9            |                    | 30.2              | 1                 |                          |                     | С                            |

 Table 7.1 Batch experiments and initial conditions used for HS-AD model calibration and cross-validation

Experiment 1 consisted of a sacrifice test for mono-digestion of OFMSW using ISR = 1.00 g VS/g VS. In this experiment, the main physical-chemical dynamics (i.e. biogas production and composition, TS, VS, VFA, TAN, and mono-valent ions) were measured at different operational times. In Experiments 2 to 4, the biogas production and composition were measured at different experimental times, whereas the rest of physical-chemical analyses (i.e. TS, VS, VFA, TAN, and ions) were only performed before starting and after ending each experiment. Non-sacrifice experiments included mono-digestion of OFMSW using ISR = 1.50 g VS/g VS (Experiment 2) and ISR = 0.50 g VS/g VS (Experiment 3), but also co-digestion of OFMSW and beech sawdust using ISR = 0.16 g VS/g VS (Experiment 4). Within Experiments 2 and 4, different initial TS contents – dilutions – were evaluated, though all the initial batch conditions contained exactly the same amount of substrate and inoculum. In total, nine different HS-AD setups were assessed, subsequently named as "Batch No. 1 to 9" [Table 7.1].

### 7.2.2 HS-AD Model

The HS-AD model included the main biochemical rates of Pastor-Poquet et al. (2018), though some minor modifications were also implemented [Table 7.2]. Firstly, a reversible non-competitive NH<sub>3</sub> inhibition function [Equation 7.3] was included in the valeric (S<sub>va</sub>), butyric (S<sub>bu</sub>), propionic (S<sub>pro</sub>), and hydrogen (S<sub>h2</sub>) uptakes, similarly to the NH<sub>3</sub> (S<sub>nh3</sub>) inhibition on the acetate (S<sub>ac</sub>) uptake, aiming to simulate the VFA accumulation observed in HS-AD experiments, as a likely consequence of the NH<sub>3</sub> buildup [Chapter 3 – Batch Manuscript]. Secondly, carbohydrates (X<sub>ch</sub>) were split into readily-biodegradable (X<sub>ch,fast</sub>) and slowly-biodegradable (X<sub>ch,slow</sub>) to simulate the relatively slower hydrolysis rates of sawdust and the longer methane production observed in co-digestion experiments (i.e.  $\geq$  200 d) [Table 7.1]. Importantly, the hydrolysis of both X<sub>ch,fast</sub> and X<sub>ch,slow</sub> pooled into soluble sugars (S<sub>su</sub>).

$$I_{nh3} = \frac{K_{i,Snh3}}{K_{i,Snh3} + S_{nh3,App}}$$
(7.3)

| Process                                        | Rate (r <sub>j</sub> , kgCOD m <sup>-3</sup> d <sup>-1</sup> )                                                                      |  |  |
|------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------|--|--|
| Hydrolysis of Fast Biodegradable Carbohydrates | $k_{h,ch,fast} {}^{\star} X_{ch,fast}$                                                                                              |  |  |
| Hydrolysis of Slow Biodegradable Carbohydrates | $k_{h,ch,slow}{\boldsymbol{\cdot}} X_{ch,slow}$                                                                                     |  |  |
| Hydrolysis of Proteins                         | $k_{h,pr} \cdot X_{pr}$                                                                                                             |  |  |
| Hydrolysis of Lipids                           | $\mathbf{k}_{\mathrm{h,li}}\mathbf{\cdot}\mathbf{X}_{\mathrm{li}}$                                                                  |  |  |
| Sugars Uptake                                  | $k_{m,su}\textbf{\cdot} S_{su,App}/(K_{S,Xsu}\textbf{+} S_{su,App})\textbf{\cdot} X_{su}\textbf{\cdot} I_{pH}\textbf{\cdot} I_{in}$ |  |  |
| Aminoacids Uptake                              | $k_{m,aa^*}S_{aa,App}/(K_{S,Xaa}{+}S_{aa,App}){\cdot}X_{aa}{\cdot}I_{pH}{\cdot}I_{in}$                                              |  |  |
| LCFA Uptake                                    | $k_{m,fa} \cdot S_{fa} / (K_{S,Xfa} + S_{fa}) \cdot X_{fa} \cdot I_{pH} \cdot I_{in} \cdot I_{h2}$                                  |  |  |
| Valerate Uptake                                | $k_{m,c5} \cdot S_{va,App} / (K_{S,Xc5} + S_{va,App}) \cdot X_{c5} \cdot I_{pH} \cdot I_{in} \cdot I_{h2} \cdot I_{nh3}$            |  |  |
| Butyrate Uptake                                | $k_{m,c4} \cdot S_{bu,App} / (K_{S,Xc4} + S_{bu,App}) \cdot X_{c4} \cdot I_{pH} \cdot I_{in} \cdot I_{h2} \cdot I_{nh3}$            |  |  |
| Propionate Uptake                              | $k_{m,pro} \cdot S_{pro,App} / (K_{S,Xpro} + S_{pro,App}) \cdot X_{pro} \cdot I_{pH} \cdot I_{in} \cdot I_{h2} \cdot I_{nh3}$       |  |  |
| Acetate Uptake                                 | $k_{m,ac} \cdot S_{ac,App} / (K_{S,Xac} + S_{ac,App}) \cdot X_{ac} \cdot I_{pH} \cdot I_{in} \cdot I_{nh3}$                         |  |  |
| Hydrogen Uptake                                | $k_{m,h2} \cdot S_{h2,App} / (K_{S,Xh2} + S_{h2,App}) \cdot X_{h2} \cdot I_{pH} \cdot I_{in} \cdot I_{nh3}$                         |  |  |
| Sugar Degraders Decay                          | $k_d \cdot X_{su}$                                                                                                                  |  |  |
| Aminoacids Degraders Decay                     | $k_d \cdot X_{aa}$                                                                                                                  |  |  |
| LCFA Degraders Decay                           | $k_d {\cdot} X_{fa}$                                                                                                                |  |  |
| Valerate Degraders Decay                       | k <sub>d</sub> . X <sub>e5</sub>                                                                                                    |  |  |
| Butyrate Degraders Decay                       | $k_{d}$ · $X_{c4}$                                                                                                                  |  |  |
| Propionate Degraders Decay                     | $k_{d}$ · $X_{pro}$                                                                                                                 |  |  |
| Acetate Degraders Decay                        | $k_d \cdot X_{ac}$                                                                                                                  |  |  |
| Hydrogen Degraders Decay                       | $k_d \cdot X_{h2}$                                                                                                                  |  |  |

Table 7.2 Biochemical rates used for the HS-AD model in this study

with

$$\begin{split} &I_{in} = S_{in,App}/(K_{i,Sin} + S_{in,App}) \\ &I_{h2} = K_{i,Sh2}/(K_{i,Sh2} + S_{h2,App}) \\ &I_{pH} = K_{pH} ^{A} N_{pH}/(K_{pH} ^{A} N_{pH} + S_{h+} ^{A} N_{pH}) \\ &I_{nh3} = K_{i,Snh3}/(K_{i,Snh3} + S_{nh3,App}) \end{split}$$
# 7.2.3 Model Calibration and Validation

A common set of biochemical parameters was used for all HS-AD simulations at different ISR and/or TS. All biochemical parameters for thermophilic (55°C) AD were extracted from Batstone et al. (2002), though some of these needed to be calibrated (i.e.  $\theta_P$ ) to improve the model fitting. The initial conditions of the batch experiments were predefined according to the experimental data available, as described by Pastor-Poquet et al. (2018), and also mentioned next. Moreover, different ranges of initial biomass concentrations were used (i.e.  $\theta_B$ ), to assess the potential interrelationship of  $\theta_B$  with  $\theta_P$  in batch experiments.

## 7.2.3.1 Initial Conditions

The initial conditions used for each batch simulation are shown in Table 7.3. Noteworthy, due to the fact that the same amount of substrate and inoculum was used along different initial TS contents in Experiment 2, but also in Experiment 4, mass balances were used to reduce the number of 'unknown' initial conditions [Table 7.1]. To use mass balances, the anaerobic biodegradability (BD) of each substrate-inoculum mixture in Experiment 2 and Experiment 4 was assumed constant, whereas the soluble (S) and particulate (X) components were assumed proportional, among the different TS contents. With these assumptions, mass balances permitted to extrapolate the molar and chemical oxygen demand (COD) concentrations, as a function of the initial reactor content mass (M<sub>Global</sub>), reactor content specific weight ( $\rho_{Global}$ ), and reactor content volume (V<sub>Global</sub>). For example, the concentration of acetoclastic methanogens (X<sub>ac</sub>) in Batch No. 3 was approximated from Batch No. 2 as: X<sub>ac,Batch3</sub> = X<sub>ac,Batch2</sub>·V<sub>Global,Batch2</sub>/V<sub>Global,Batch3</sub>.

The nitrogen content of soluble (S<sub>i</sub>) and particulate (X<sub>i</sub>) inert in each substrateinoculum mixture (N<sub>i,subs</sub>) determined the initial protein (X<sub>pr</sub>) + amino-acid (S<sub>aa</sub>) content, according to the nitrogen balance, as shown elsewhere (Pastor-Poquet et al., 2018). Thus, N<sub>i,subs</sub> was different for each batch experiment [Table 7.3]. Noteworthy, despite the initial conditions of batch experiments were maintained for all the simulations, different biomass concentrations (i.e. sugars, X<sub>su</sub>; amino acids, X<sub>aa</sub>; long-chain fatty acids, X<sub>fa</sub>; valerate, X<sub>c5</sub>; butyrate, X<sub>bu</sub>; propionate, X<sub>pro</sub>; acetate, X<sub>ac</sub>; and hydrogen, X<sub>h2</sub>, degraders) were also assessed, as mentioned before. Since mass balances were used among different TS in Experiments 2 and 4, only the initial conditions of Batch No. 1, 2, 6 and 7 [Table 7.1] were evaluated.

|                             |          |          | Mono-    | digestion |          |          |          | Co-digestie | on       |                                |
|-----------------------------|----------|----------|----------|-----------|----------|----------|----------|-------------|----------|--------------------------------|
| Nama                        | ISR =    |          | ISR      | = 1.50    |          | ISR =    |          | ISR = 0.1   | 6        | Units                          |
| Ttame                       | TS=15.0  | TS=9.5   | TS=13.5  | TS=16.5   | TS=19.4  | TS=28.3  | TS=10.0  | TS=15.0     | TS=30.2  |                                |
|                             | %        | %        | %        | %         | %        | %        | %        | %           | %        |                                |
| $\mathbf{S}_{su}$           | 9.761    | 6.920    | 8.776    | 10.861    | 13.245   | 6.201    | 1.800    | 2.779       | 6.496    | kg COD m-3                     |
| $\mathbf{S}_{aa}$           | 3.187    | 5.856    | 7.346    | 9.099     | 11.201   | 7.679    | 0.972    | 1.503       | 3.571    | kg COD m <sup>-3</sup>         |
| $\mathbf{S}_{\mathrm{fa}}$  | 2.610    | 1.656    | 2.186    | 2.702     | 3.184    | 1.467    | 0.377    | 0.579       | 1.300    | kg COD m <sup>-3</sup>         |
| $\mathbf{S}_{va}$           | 0.791    | 1.015    | 1.282    | 1.582     | 1.936    | 1.061    | 1.467    | 2.269       | 5.314    | kg COD m <sup>-3</sup>         |
| $\mathbf{S}_{bu}$           | 0.500    | 0.195    | 0.244    | 0.302     | 0.370    | 1.518    | 0.230    | 0.355       | 0.831    | kg COD m <sup>-3</sup>         |
| $\mathbf{S}_{\text{pro}}$   | 2.059    | 0.877    | 1.109    | 1.368     | 1.674    | 2.565    | 1.367    | 2.115       | 4.952    | kg COD m <sup>-3</sup>         |
| $\mathbf{S}_{ac}$           | 0.103    | 0.035    | 0.044    | 0.054     | 0.066    | 0.871    | 0.058    | 0.090       | 0.210    | kg COD m <sup>-3</sup>         |
| $\mathbf{S}_{\mathrm{h2}}$  | 1.00E-08 | 1.00E-08 | 1.00E-08 | 1.00E-08  | 1.00E-08 | 1.00E-08 | 1.00E-08 | 1.00E-08    | 1.00E-08 | kg COD m <sup>-3</sup>         |
| $\mathbf{S}_{\mathrm{ch4}}$ | 1.00E-08 | 1.00E-08 | 1.00E-08 | 1.00E-08  | 1.00E-08 | 1.00E-08 | 1.00E-08 | 1.00E-08    | 1.00E-08 | kg COD m <sup>-3</sup>         |
| $\mathbf{S}_{\mathrm{ic}}$  | 0.029    | 0.014    | 0.017    | 0.021     | 0.026    | 0.037    | 0.008    | 0.013       | 0.030    | kmol C m-3                     |
| $\mathbf{S}_{in}$           | 0.186    | 0.125    | 0.157    | 0.194     | 0.238    | 0.229    | 0.033    | 0.051       | 0.120    | kmol N m-3                     |
| $\mathbf{S}_{i}$            | 1.00E-08 | 1.00E-08 | 1.00E-08 | 1.00E-08  | 1.00E-08 | 1.00E-08 | 1.00E-08 | 1.00E-08    | 1.00E-08 | kg COD m <sup>-3</sup>         |
| $S_{i,subs}$                | 34.706   | 29.233   | 37.076   | 45.883    | 55.954   | 90.351   | 27.565   | 42.559      | 99.486   | kgCOD m-3                      |
| X <sub>ch,fast</sub>        | 29.283   | 13.840   | 17.553   | 21.722    | 26.490   | 31.003   | 5.400    | 8.337       | 19.489   | kg COD m <sup>-3</sup>         |
| X <sub>ch,slow</sub>        | -        | -        | -        | -         | -        | -        | 27.360   | 42.242      | 98.743   | kg COD m-3                     |
| $\mathbf{X}_{\mathrm{pr}}$  | 28.680   | 11.713   | 14.692   | 18.197    | 22.402   | 38.393   | 5.834    | 9.016       | 21.425   | kg COD m <sup>-3</sup>         |
| $X_g$                       | 18.271   | 3.312    | 4.373    | 5.405     | 6.367    | 5.866    | 2.637    | 4.053       | 9.102    | kg COD m <sup>-3</sup>         |
| Xsu (*)                     | 0.050    | 0.050    | 0.063    | 0.078     | 0.095    | 0.150    | 0.005    | 0.008       | 0.018    | kg COD m <sup>-3</sup>         |
| X <sub>aa</sub> (*)         | 0.050    | 0.050    | 0.063    | 0.078     | 0.095    | 0.060    | 0.005    | 0.008       | 0.018    | kg COD m <sup>-3</sup>         |
| X <sub>fa</sub> (*)         | 0.010    | 0.020    | 0.025    | 0.031     | 0.038    | 0.030    | 0.001    | 0.001       | 0.002    | kg COD m-3                     |
| X <sub>c5</sub>             | 0.005    | 0.010    | 0.013    | 0.016     | 0.019    | 0.030    | 0.001    | 0.001       | 0.002    | kgCOD m <sup>-3</sup>          |
| (*)<br>X <sub>e4</sub> (*)  | 0.001    | 0.050    | 0.063    | 0.078     | 0.095    | 0.030    | 0.001    | 0.001       | 0.002    | kg COD m <sup>-3</sup>         |
| Xpro                        | 0.005    | 0.020    | 0.025    | 0.031     | 0.038    | 0.030    | 0.001    | 0.001       | 0.003    | kg COD m <sup>-3</sup>         |
| (*)                         | 0.005    | 0.020    | 0.025    | 0.051     | 0.050    | 0.050    | 0.001    | 0.001       | 0.005    | kg COD III                     |
| X <sub>ac</sub> (*)         | 0.024    | 0.150    | 0.190    | 0.234     | 0.286    | 0.100    | 0.003 ** | 0.005       | 0.011    | kg COD m <sup>-3</sup>         |
| X <sub>h2</sub> (*)         | 0.050    | 0.200    | 0.253    | 0.312     | 0.382    | 0.090    | 0.003    | 0.005       | 0.011    | kg COD m <sup>-3</sup>         |
| $X_i$                       | 1.00E-08 | 1.00E-08 | 1.00E-08 | 1.00E-08  | 1.00E-08 | 1.00E-08 | 1.00E-08 | 1.00E-08    | 1.00E-08 | kg COD m <sup>-3</sup>         |
| X <sub>i,subs</sub>         | 86.765   | 73.083   | 92.689   | 114.706   | 139.885  | 225.877  | 68.914   | 106.398     | 248.714  | kg COD m <sup>-3</sup>         |
| S <sub>cat</sub>            | 0.100    | 0.060    | 0.075    | 0.091     | 0.109    | 0.166    | 0.040    | 0.059       | 0.120    | kmoleq m <sup>-3</sup>         |
| $S_{an}$                    | 0.051    | 0.040    | 0.050    | 0.060     | 0.073    | 0.069    | 0.020    | 0.030       | 0.060    | kmoleq m-3                     |
| $M_{Global}$                | 37.12    | 29.92    | 24.02    | 19.80     | 16.47    | 23.45    | 138.23   | 93.13       | 46.13    | g                              |
| ρ <sub>Global</sub>         | 1078     | 1059     | 1075     | 1093      | 1113     | 1128     | 1088     | 1134        | 1316     | kg m <sup>-3</sup>             |
| TS                          | 15.0     | 10.8     | 13.4     | 16.4      | 19.6     | 28.3     | 10.0     | 15.0        | 30.2     | %                              |
| VS                          | 12.4     | 9.1      | 11.4     | 13.9      | 16.6     | 24.0     | 9.6      | 14.2        | 28.6     | %                              |
| N <sub>i,subs</sub>         | 0.0010   | 0.0012   | 0.0012   | 0.0012    | 0.0012   | 0.0010   | 0.0004   | 0.0004      | 0.0004   | kmol N kg<br>COD <sup>-1</sup> |
| V <sub>Reactor</sub>        | 280      | 160      | 160      | 160       | 160      | 280      | 160      | 160         | 160      | mL                             |

Table 7.3 Initial conditions used for all batch simulations in this study

\* These values were assessed by Global Sensitivity Analysis (GSA); \*\* This value was also assessed by Approximate Bayesian Computation (ABC); The inoculum-to-substrate ratio (ISR) is expressed in g VS/g VS

#### 7.2.3.2 Biochemical Parameters, Biomass Concentrations and Calibration Ranges

The modified biochemical parameters ( $\theta_P$ ) and modified biomass concentrations ( $\theta_B$ ) in this study, including their initial values and potential variability ranges, are shown in Table 7.4. In total, 35  $\theta_P$  and 32  $\theta_B$  were evaluated.  $\theta_P$  related to the hydrolysis, sugar fractioning (f<sub>su</sub>), maximum growth rate (k<sub>m</sub>) and half-saturation constant (Ks), but also the pH, NH<sub>3</sub> and H<sub>2</sub> inhibition, since all these  $\theta_P$  are simultaneously associated to the substrate under study, are correlated among themselves, and strongly regulate the biogas production/composition from solid substrates (Batstone et al., 2002; Garcia-Gen et al., 2015) [Chapter 6 – Non-Ideal Manuscript]. On the other hand,  $\theta_B$  included all the initial biomass concentrations in the HS-AD simulations (i.e. X<sub>su</sub>, X<sub>aa</sub>, X<sub>fa</sub>, X<sub>c5</sub>, X<sub>bu</sub>, X<sub>pro</sub>, X<sub>ac</sub> and X<sub>h2</sub>), as these concentrations might not only strongly influence the biogas production during the initial days of batch experiments, but might also be potentially interrelated among themselves and/or to the previous  $\theta_P$ .

The sugars uptake yields butyrate  $(f_{bu,su})$ , propionate  $(f_{pro,su})$ , acetate  $(f_{ac,su})$  and hydrogen  $(f_{h2,su})$  as COD fractions in ADM1. Therefore, a maximum of three fractions can be selected simultaneously to fulfill the COD balance:  $f_{bu,su} + f_{pro,su} + f_{ac,su} + f_{h2,su} = 1$ . In this study,  $f_{bu,su}$ ,  $f_{ac,su}$  and  $f_{h2,su}$  were selected, while  $f_{pro,su}$  was recalculated:  $f_{pro,su} = 1 - f_{bu,su} - f_{ac,su} - f_{h2,su}$ . Importantly, further structural parameters and initial conditions need to be induced in the HS-AD model as, for example, the amino-acid (AA, Saa) fractioning and the biomass yield coefficients (Y<sub>b</sub>), though these were not assessed here aiming to reduce the problem under study. In either case, the proposed methodology for calibration/validation can easily include any further  $\theta$ .

Variability ranges for structural parameters are suggested in ADM1 (Batstone et al., 2002). However, considerably wider ranges were assessed in this study to emphasize the absence of prior knowledge about the optimal values. For simplicity, all  $\theta_P$  were allowed to vary 90 % from their initial values,  $\theta_{P,0}$ :  $(1 - 0.9) \cdot \theta_{P,0} \le \theta_P \le (1 + 0.9) \cdot \theta_{P,0}$ ; uniform  $p(\theta_P)$  [Table 7.4]. As the only exception, the lower pH threshold for acetoclastic methanogens (pH<sub>LL,ac</sub>) was bounded between a pH value where methanogenesis potentially collapses (i.e.  $\le 5.0$ ) and the upper pH threshold for acetoclastic methanogens (pH<sub>UL,ac</sub>, i.e. 7.0), to maintain the suitability of the Hill function to simulate the pH inhibition [Table 7.2].

| Model<br>Parameter                     | Units                          | ADM1         | Initial<br>Value | Lower<br>Threshold | Upper<br>Threshold | Initial<br>Concentra<br>tion | Units                  | Initial<br>Value * | Lower<br>Thres<br>hold * | Upper<br>Threshold<br>* |
|----------------------------------------|--------------------------------|--------------|------------------|--------------------|--------------------|------------------------------|------------------------|--------------------|--------------------------|-------------------------|
| K <sub>h,ch,fast</sub>                 | d-1                            | 10           | 0.120            | 0.012              | 0.228              | Xsu,Batch1                   | kg COD m <sup>-3</sup> | -1.301             | -1.952                   | -0.651                  |
| $\mathbf{K}_{h,pr}$                    | d-1                            | 10           | 0.050            | 0.005              | 0.095              | Xsu,Batch2                   | kg COD m-3             | -1.301             | -1.952                   | -0.651                  |
| $\mathbf{K}_{\mathbf{h},\mathbf{li}}$  | d-1                            | 10           | 0.080            | 0.008              | 0.152              | X <sub>su,Batch6</sub>       | kg COD m-3             | -0.824             | -1.236                   | -0.412                  |
| Kh,ch,slow                             | d-1                            | -            | 0.012            | 0.001              | 0.023              | Xsu,Batch7                   | kg COD m-3             | -2.301             | -3.452                   | -1.151                  |
| $\mathbf{k}_{\mathrm{m,su}}$           | d-1                            | 70           | 70               | 7                  | 133                | Xaa,Batch1                   | kg COD m-3             | -1.301             | -1.952                   | -0.651                  |
| $\mathbf{k}_{m,aa}$                    | d-1                            | 70           | 70               | 7                  | 133                | Xaa,Batch2                   | kg COD m <sup>-3</sup> | -1.301             | -1.952                   | -0.651                  |
| $\mathbf{k}_{\mathbf{m},\mathbf{fa}}$  | d-1                            | 10           | 10               | 1                  | 19                 | Xaa,Batch6                   | kg COD m-3             | -1.222             | -1.833                   | -0.611                  |
| k <sub>m,c5</sub>                      | d-1                            | 30           | 8                | 1                  | 15                 | Xaa,Batch7                   | kg COD m <sup>-3</sup> | -2.301             | -3.452                   | -1.151                  |
| $\mathbf{k}_{\mathrm{m,c4}}$           | d-1                            | 30           | 13               | 1                  | 25                 | X <sub>fa,Batch1</sub>       | kg COD m-3             | -2.000             | -3.000                   | -1.000                  |
| km,pro                                 | d-1                            | 20           | 10               | 1                  | 19                 | Xfa,Batch2                   | kg COD m <sup>-3</sup> | -1.699             | -2.548                   | -0.849                  |
| $\mathbf{k}_{m,ac}$                    | d-1                            | 16           | 16               | 2                  | 30                 | X <sub>fa,Batch6</sub>       | kg COD m-3             | -1.523             | -2.284                   | -0.761                  |
| $\mathbf{k}_{\mathrm{m,h2}}$           | d-1                            | 35           | 20               | 2                  | 38                 | Xfa,Batch7                   | kg COD m <sup>-3</sup> | -3.301             | -4.952                   | -1.651                  |
| $\mathbf{k}_{\mathbf{d}}$              | d-1                            | 0.040        | 0.040            | 0.004              | 0.076              | Xc5,Batch1                   | kg COD m-3             | -2.301             | -3.452                   | -1.151                  |
| K <sub>S,Xsu</sub>                     | kg COD m <sup>-3</sup>         | 1.00         | 1.00             | 0.1                | 1.9                | Xc5,Batch2                   | kg COD m-3             | -2.000             | -3.000                   | -1.000                  |
| K <sub>S,Xaa</sub>                     | kg COD m <sup>-3</sup>         | 0.30         | 0.30             | 0.03               | 0.57               | Xc5,Batch6                   | kg COD m-3             | -1.523             | -2.284                   | -0.761                  |
| K <sub>S,Xfa</sub>                     | kg COD m <sup>-3</sup>         | 0.40         | 0.40             | 0.04               | 0.76               | Xc5,Batch7                   | kg COD m-3             | -3.222             | -4.833                   | -1.611                  |
| K <sub>S,Xc5</sub>                     | kg COD m <sup>-3</sup>         | 0.40         | 0.40             | 0.04               | 0.76               | Xc4,Batch1                   | kg COD m <sup>-3</sup> | -3.000             | -4.500                   | -1.500                  |
| K <sub>S,Xc4</sub>                     | kg COD m <sup>-3</sup>         | 0.40         | 0.40             | 0.04               | 0.76               | Xc4,Batch2                   | kg COD m-3             | -1.301             | -1.952                   | -0.651                  |
| K <sub>S,Xpro</sub>                    | kg COD m <sup>-3</sup>         | 0.30         | 0.30             | 0.03               | 0.57               | Xc4,Batch6                   | kg COD m <sup>-3</sup> | -1.523             | -2.284                   | -0.761                  |
| K <sub>S,Xac</sub>                     | kg COD m <sup>-3</sup>         | 0.30         | 0.30             | 0.03               | 0.57               | Xc4,Batch7                   | kg COD m-3             | -3.222             | -4.833                   | -1.611                  |
| K <sub>S,Xh2</sub>                     | kg COD m <sup>-3</sup>         | 5.00E-<br>05 | 5.00E-<br>05     | 0.000005           | 0.000095           | Xpro,Batch1                  | kg COD m <sup>-3</sup> | -2.301             | -3.452                   | -1.151                  |
| $pH_{LL,ac}$                           | -                              | 6.00         | 5.80             | 5.00               | 6.90               | Xpro,Batch2                  | kg COD m-3             | -1.699             | -2.548                   | -0.849                  |
| Ki,Snh3,Xc5                            | kmol N m <sup>-3</sup>         | -            | 0.0070           | 0.0007             | 0.0133             | Xpro,Batch6                  | kg COD m <sup>-3</sup> | -1.523             | -2.284                   | -0.761                  |
| K <sub>i,Snh3,Xc4</sub>                | kmol N m <sup>-3</sup>         | -            | 0.0100           | 0.0010             | 0.0190             | Xpro,Batch7                  | kg COD m-3             | -3.097             | -4.645                   | -1.548                  |
| Ki,Snh3,Xpro                           | kmol N m <sup>-3</sup>         | -            | 0.0100           | 0.0010             | 0.0190             | Xac,Batch1                   | kg COD m <sup>-3</sup> | -1.620             | -2.430                   | -0.810                  |
| K <sub>i,Snh3,Xac</sub>                | kmol N m <sup>-3</sup>         | 0.0110       | 0.0040           | 0.0004             | 0.0076             | Xac,Batch2                   | kg COD m-3             | -0.824             | -1.236                   | -0.412                  |
| Ki,Snh3,Xh2                            | kmol N m <sup>-3</sup>         | -            | 0.0150           | 0.0015             | 0.0285             | Xac,Batch6                   | kg COD m <sup>-3</sup> | -1.000             | -1.500                   | -0.500                  |
| $\mathbf{f}_{bu,su}$                   | kg COD kg<br>COD <sup>-1</sup> | 0.130        | 0.500            | 0.050              | 0.950              | X <sub>ac,Batch7</sub>       | kg COD m <sup>-3</sup> | -2.523             | -3.784                   | -1.261                  |
| $\mathbf{f}_{\mathrm{ac},\mathrm{su}}$ | kg COD kg<br>COD <sup>-1</sup> | 0.270        | 0.290            | 0.029              | 0.551              | Xh2,Batch1                   | kg COD m <sup>-3</sup> | -1.301             | -1.952                   | -0.651                  |
| fh2,su                                 | kg COD kg<br>COD-1             | 0.190        | 0.100            | 0.010              | 0.190              | Xh2,Batch2                   | kg COD m <sup>-3</sup> | -0.699             | -1.048                   | -0.349                  |
| $\mathbf{K}_{i,Sh2,Xfa}$               | kg COD m <sup>-3</sup>         | 3.00E-<br>05 | 05               | 1.00E-06           | 1.90E-05           | Xh2,Batch6                   | kg COD m <sup>-3</sup> | -1.046             | -1.569                   | -0.523                  |
| K <sub>i,Sh2,Xc5</sub>                 | kg COD m <sup>-3</sup>         | 3.00E-<br>05 | 3.00E-<br>05     | 3.00E-06           | 5.70E-05           | Xh2,Batch7                   | kg COD m <sup>-3</sup> | -2.523             | -3.784                   | -1.261                  |
| K <sub>i,Sh2,Xc4</sub>                 | kg COD m <sup>-3</sup>         | 3.00E-<br>05 | 3.00E-<br>05     | 3.00E-06           | 5.70E-05           | -                            | -                      | -                  | -                        |                         |
| K <sub>i,Sh2,Xpro</sub>                | kg COD m <sup>-3</sup>         | 1.00E-<br>05 | 05               | 1.00E-06           | 1.90E-05           |                              | -                      | -                  | -                        |                         |
| k <sub>L</sub> a                       | d-1                            | 200          | 200              | 20                 | 380                | -                            | -                      | -                  | -                        |                         |

**Table 7.4** Main input parameters ( $\theta$ ) used for global sensitivity analysis (GSA), including the<br/>initial values, lower and upper thresholds

\* Logarithm-transformed:  $log_{10}(\theta_B)$ 

Different methods are available to approximate the initial conditions (i.e. biomass concentrations) in batch simulations as, for example, simulating a continuous reactor fed with exactly the same substrate until reaching steady state, and then use these steady conditions to initialize the batch simulations (Dochain et al., 2001; Donoso-Bravo et al., 2011). However, due to the fact that the inoculum was centrifuged right before setting the batch experiments in this study, absence of prior knowledge about the initial biomass concentrations was preferred. To explore different orders of magnitude in the biomass concentrations (often  $0 < \theta_B < 1$ ), the logarithm-transformed  $\theta_B$  were allowed to vary 50 % from their initial values,  $\theta_{B,0}$ :  $(1 + 0.5) \cdot \log_{10} (\theta_{B,0}) \leq \log_{10} (\theta_B) \leq (1 - 0.5) \cdot \log_{10} (\theta_{B,0})$ ; uniform  $p(\log_{10} (\theta_B))$  [Table 7.4].

## 7.2.3.3 Objective Function

The weighted sum of squares between all the available experimental and the corresponding simulated values was used as objective function,  $J(\theta)$  [Equation 7.4].  $J(\theta)$  was adapted from Flotats et al. (2003) to assess the model 'goodness of fitting', being:  $\theta$  the structural parameters and/or initial conditions implemented in the HS-AD model; R the number of batch simulations; D the number of experimental datasets;  $t_{exp}$  the experimental time of each batch experiment;  $y_{i,j,t}$  the experimental measurements;  $y_{i,j,t}^{sim}(\theta)$  the simulated values; and  $w_{i,j}$  the weighting coefficients – calculated as a function of the average experimental data,  $\bar{y}_{i,j}$  [Equation 7.5]. With this approach,  $J(\theta)$  was lower-bounded by the – preliminarily unknown – global minimum:  $J(\theta) \ge J(\theta_{opt})$ .

$$J(\theta) = \sum_{i=1}^{R} \sum_{j=1}^{D} \sum_{t=t_1}^{texp} w_{i,j} \left( y_{i,j,t} - y_{i,j,t}^{sim}(\theta) \right)^2$$
(7.4)  
$$w_{i,j} = \frac{1}{1}$$
(7.5)

$$w_{i,j} = \frac{1}{\sum_{t=t_1}^{t_{exp}} (y_{i,j,t} - \bar{y}_{i,j})^2}$$
(7.5)

Noteworthy, only 6 out of 9 experimental conditions were used to calculate  $J(\theta)$  in this study: Batch No. 1, 3, 5, 6, 7 and 9 [Table 7.1]. Meanwhile, 3 out of 9 experimental conditions were used for cross-validation: Batch No. 2, 4 and 8 [Table 7.1]. Other experimental combinations could be used for calibration/cross-validation. However, the proposed configuration permitted to include the most extreme HS-AD conditions for model calibration (i.e.  $TS \le 10$  %,  $TS \ge 30$  %), while intermediate conditions (i.e.  $10 \le TS \le 30$  %) were used for cross-validation.

### 7.2.3.4 Global Sensitivity Analysis

GSA was aimed to highlight the most influential  $\theta$  to be calibrated with the available set of experimental data. For GSA, multiple  $\theta$  combinations were used to evaluate  $J(\theta)$ [Equation 7.4]. Latin-hypercube sampling (LHS) served to explore the global  $\theta$  space (Solon et al., 2015). Subsequently,  $J(\theta)$  arrays and their corresponding  $\theta$  were assessed by the GSA engine of Kennedy et al. (2001) and Oakley et al. (2004). The GSA engine calculates the individual (IE) and global (GE) effects of  $\theta_i$  upon the global model output (e.g.  $p(y|\theta)$ ,  $J(\theta)$ ) variance. Thus,  $\theta_i$  showing a relatively low IE and GE are associated to poor practical identifiability, since these influence minimally the model output. On the other hand, the GSA engine provides all the correlations between  $\theta_i$  and  $\theta_j$  (i.e.  $\theta_i \cdot \theta_j$ , being  $i \neq j$ ). Similarly, the total correlation of  $\theta_i$  with  $\theta$  can be inferred by the relative difference between IE and GE – though IE and GE are expressed in different units, as interactions can be repeated in the GE of two or more  $\theta_i$  (Oakley et al., 2004; Saltelli et al., 2006).

A maximum  $\theta$  subset of N' = 30 and/or 400 simulations of  $J(\theta)$  can be evaluated simultaneously with the GSA engine (Kennedy et al., 2017). Therefore, to assess  $\theta$ interactions when N > 30, a combination strategy was followed. Firstly,  $30 \theta_i$  were randomly selected and evaluated by GSA (i.e. GSA No. 1). From these  $\theta$ , those showing the smallest IE (e.g. < 1 %) were disregarded as non-identifiable, removed from the initial  $\theta$  subset, and not used for further GSA. Importantly, non-identifiable  $\theta_i$  were fixed at their initial values [Table 7.4] for all subsequent model simulations, since non-identifiability implies that these parameters can be fixed at any value within the prior distribution (Dochain et al., 2001; Guisasola et al., 2006). Then,  $\theta_P$  and/or  $\theta_B$  non-previously-assessed by GSA were combined with the non-removed  $\theta$  subset, and a new GSA was performed (i.e. GSA No. 2). The GSA methodology was repeated until the last remaining  $\theta$  subset was considered as 'potentially identifiable',  $\theta'$ .

In total, seven GSA with different  $\theta$  combinations were progressively performed [Table 7.5]. In this study, the criterion for non-identifiability was assumed as IE  $\leq 0.20$  %, though this threshold could be modified, as mentioned in section 7.3.1.1. To enhance the GSA representativeness in presence of a high number of  $\theta$  (i.e.  $20 \leq N' \leq 30$ ) and/or wide variability ranges (i.e.  $\pm$  50 %), each GSA was conducted in triplicate and the results averaged. Finally, all  $J(\theta)$  arrays used for GSA were searched for the minimum observed value,  $J_{min}(\theta)$  (i.e.  $\geq J(\theta_{opt})$ ), to be subsequently used in ABC.

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| Global sensitivity analysis (GSA) of input parc       |         |
| ? 7.5 Global sensitivity analysis (GSA) of input parc |         |

function,  $J(\theta)$ , variance

| GS/                                  | A No.1 |       | 9                         | SA No.2 |       | 9                       | SA No.3 |       | 6                         | 5SA No.4 |       |                      | SA No.5 |       | 9                            | SA No.6 |       | 6                            | SA No.7 |       |
|--------------------------------------|--------|-------|---------------------------|---------|-------|-------------------------|---------|-------|---------------------------|----------|-------|----------------------|---------|-------|------------------------------|---------|-------|------------------------------|---------|-------|
| θ                                    | IE (%) | GE    | θ                         | IE (%)  | GE    | θ                       | IE (%)  | GE    | θ                         | IE (%)   | GE    | θ                    | IE (%)  | GE    | θ                            | IE (%)  | GE    | θ                            | IE (%)  | GE    |
| K <sub>h,ch,fast</sub>               | 0.08   | 0.63  | $K_{h,p^{\text{tr}}}$     | 1.75    | 5.23  | $K_{h,pr}$              | 2.75    | 8.62  | $K_{\rm h,pr}$            | 2.21     | 7.33  | $K_{h,p\varepsilon}$ | 10.1    | 5.42  | $\mathrm{K}_{\mathrm{h,pr}}$ | 2.00    | 10.44 | $\mathrm{K}_{\mathrm{h,pr}}$ | 1.97    | 7.72  |
| $\mathrm{K}_{\mathrm{h,pr}}$         | 2.01   | 7.56  | $K_{\rm h, li}$           | 0.25    | 0.69  | $K_{h,li}$              | 0.26    | 2.54  | $K_{h,li}$                | 0.11     | 2.85  | Kh,ch,slow           | 4.28    | 14.61 | ${\rm K}_{\rm h,ch,slow}$    | 4.07    | 14.47 | Kh,ch,slow                   | 4.43    | 17.86 |
| $\mathbf{K}_{\mathbf{h},\mathbf{H}}$ | 0.29   | 2.07  | $K_{h,ch,slow}$           | 5.87    | 16.21 | Kh,ch,slow              | 5.83    | 14.32 | Kh,ch,slow                | 5.22     | 16.59 | k <sub>m,fa</sub>    | 0.46    | 4.14  | k <sub>m,fa</sub>            | 1.15    | 5.66  | k <sub>m,in</sub>            | 1.11    | 8.95  |
| ${ m K}_{ m h,ch,slow}$              | 6.45   | 17.27 | $k_{m,aa}$                | 0.07    | 0.43  | k <sub>m,fa</sub>       | 1.21    | 7.25  | k <sub>m,fa</sub>         | 0.56     | 4.81  | k <sub>m,c5</sub>    | 0.22    | 1.63  | k <sub>m,c5</sub>            | 1.59    | 8.34  | k <sub>m,e5</sub>            | 0.67    | 5.72  |
| k <sub>m,su</sub>                    | 0.16   | 3.82  | k <sub>m,få</sub>         | 0.81    | 5.97  | k <sub>m,c5</sub>       | 0.80    | 3.12  | k <sub>m,c5</sub>         | 0.73     | 3.31  | k <sub>m,c4</sub>    | 1.38    | 11.18 | k <sub>m,e4</sub>            | 1.50    | 13.79 | k <sub>m,e4</sub>            | 2.39    | 15.94 |
| $\mathbf{k}_{\mathrm{m,aa}}$         | 0.53   | 2.43  | k <sub>m,c5</sub>         | 0.33    | 1.49  | k <sub>m,c4</sub>       | 2.23    | 17.17 | k <sub>m,c4</sub>         | 2.47     | 19.12 | k <sub>m,pro</sub>   | 1.77    | 8.66  | kmpro                        | 1.23    | 11.37 | $k_{m,pro}$                  | 0.72    | 9.24  |
| $\mathbf{k}_{m,fa}$                  | 0.91   | 4.34  | k <sub>m,e4</sub>         | 2.12    | 12.96 | $k_{m,pro}$             | 0.51    | 8.59  | k <sub>m,pro</sub>        | 0.75     | 10.21 | k <sub>m,ac</sub>    | 3.56    | 12.26 | k <sub>m,ac</sub>            | 5.11    | 17.49 | $\mathbf{k}_{\mathrm{m,ac}}$ | 4.13    | 17.22 |
| k <sub>m,c5</sub>                    | 0.22   | 3.64  | k <sub>m,pro</sub>        | 0.44    | 10.49 | k <sub>m,ac</sub>       | 7.18    | 17.17 | k <sub>m,ac</sub>         | 4.67     | 11.62 | k <sub>m,h2</sub>    | 3.72    | 13.46 | k <sub>m,h2</sub>            | 4.13    | 15.65 | k <sub>m,h2</sub>            | 1.46    | 12.35 |
| k <sub>m,c4</sub>                    | 4.39   | 17.87 | k <sub>m,ac</sub>         | 3.16    | 10.71 | $\mathbf{k}_{m,h2}$     | 4.07    | 10.68 | $\mathbf{k}_{m,h2}$       | 3.57     | 11.84 | kd                   | 5.16    | 15.78 | $\mathbf{k}_{\mathrm{d}}$    | 3.37    | 9.47  | $\mathbf{k}_{\mathrm{d}}$    | 4.43    | 13.37 |
| $k_{m,pro}$                          | 0.62   | 8.62  | $k_{m,h2}$                | 2.60    | 8.32  | kd                      | 3.67    | 9.00  | $\mathbf{k}_{\mathrm{d}}$ | 3.22     | 8.23  | $K_{S,Xh2}$          | 0.13    | 1.09  | pH <sub>LL,ac</sub>          | 10.24   | 24.61 | pH <sub>LL,ac</sub>          | 11.69   | 29.21 |
| k <sub>m,ac</sub>                    | 4.88   | 16.35 | $\mathbf{k}_{\mathrm{d}}$ | 2.86    | 7.83  | K <sub>S,Xh2</sub>      | 0.40    | 1.76  | K <sub>S,Xh2</sub>        | 0.22     | 0.91  | pHLL,ac              | 11.04   | 25.72 | $f_{bu,su}$                  | 3.34    | 26.74 | $f_{bu,su}$                  | 1.89    | 24.59 |
| $k_{m,h2}$                           | 1.60   | 11.78 | K <sub>S,Xh2</sub>        | 0.71    | 2.03  | pH <sub>LL,ac</sub>     | 11.09   | 22.10 | pH <sub>LL,ac</sub>       | 9.69     | 18.95 | Ki,Snh3,Xh2          | 0.21    | 3.16  | f <sub>ac,su</sub>           | 0.96    | 13.49 | $f_{ac,su}$                  | 0.22    | 9.58  |
| kd                                   | 1.65   | 5.26  | pHLL,ac                   | 11.48   | 25.57 | Ki,Snh3,Xh2             | 0.32    | 2.61  | Ki,Snh3,Xh2               | 0.39     | 1.91  | fbu,su               | 4.03    | 30.77 | fh2,su                       | 5.00    | 14.87 | fh2,su                       | 6.37    | 19.92 |
| K <sub>S,Xsu</sub>                   | 0.05   | 0.34  | Ki,Snh3,Xpro              | 0.20    | 1.79  | $f_{bu,su}$             | 2.72    | 20.48 | $f_{bu,su}$               | 2.93     | 24.05 | $f_{ac,su}$          | 0.81    | 15.33 | Xac,Batch7                   | 1.89    | 11.52 | Xac,Batch7                   | 2.73    | 13.98 |
| K <sub>S,Xaa</sub>                   | 0.15   | 1.12  | Ki,Snh3,Xac               | 0.16    | 1.55  | fac,su                  | 0.41    | 10.59 | fac.su                    | 0.48     | 12.46 | $f_{h2,su}$          | 7.05    | 17.03 | Xh2,Batch7                   | 0.00    | 0.00  |                              |         | ,     |
| $K_{S,Xfa}$                          | 0.15   | 3.83  | Ki,Snh3,Xh2               | 0.24    | 1.70  | $\mathbf{f}_{h2,su}$    | 6.02    | 13.39 | $f_{h2,su}$               | 5.83     | 13.35 | Ki,Sh2,Xc4           | 0.03    | 0.40  | ,                            | ,       | ,     | ,                            | ,       | ,     |
| K <sub>S,Xc5</sub>                   | 0.07   | 0.63  | $f_{bu,su}$               | 3.56    | 30.53 | Ki,Sh2,Xc4              | 0.33    | 4.00  | Ki,Sh2,Xc4                | 0.16     | 4.36  | Xac,Batch7           | 1.63    | 7.72  | ,                            | ,       | ,     | ,                            | ,       | ,     |
| K <sub>S,Xc4</sub>                   | 0.00   | 0.00  | $f_{ac,su}$               | 0.67    | 17.63 | Ki,Sh2,Xpro             | 0.17    | 1.55  | Ki,Sh2,Xpro               | 0.13     | 2.88  | Xh2,Batch2           | 0.07    | 1.00  |                              | ,       | ,     |                              | ,       | ,     |
| KS,Xpro                              | 0.00   | 0.00  | fh2,su                    | 7.20    | 16.29 | X <sub>su</sub> ,Batch6 | 0.06    | 0.69  | Xc4,Batch2                | 0.06     | 0.28  | Xh2,Batch6           | 0.00    | 0.00  | ,                            | ,       |       |                              |         | ,     |
| K <sub>S,Xac</sub>                   | 0.13   | 1.13  | Ki,Sh2,Xfa                | 0.00    | 0.00  | Xaa,Batch6              | 0.02    | 0.38  | Xc4,Batch6                | 0.06     | 0.33  | Xh2,Batch7           | 0.40    | 3.15  | ,                            | ,       | ,     |                              | ,       | ,     |
| $K_{S,Xh2}$                          | 0.79   | 4.53  | Ki,Sh2,Xc5                | 0.03    | 0.88  | $X_{aa,Batch7}$         | 0.00    | 0.00  | Xc4,Batch7                | 0.00     | 0.00  | ,                    | ,       | ,     | ,                            | ,       | ,     |                              | ,       | ,     |
| pH <sub>LL,ac</sub>                  | 6.73   | 16.94 | Ki,Sh2,Xc4                | 0.22    | 2.74  | $X_{fa,Batch1}$         | 0.03    | 0.10  | Xpro,Batch1               | 0.05     | 0.44  | ,                    | ,       | ,     | ,                            |         |       | ,                            |         | ,     |
| Ki,Snh3,Xc5                          | 0.02   | 0.29  | Ki,Sh2,Xpro               | 0.25    | 2.26  | Xfa,Batch2              | 0.00    | 0.00  | Xpro,Batch2               | 0.00     | 0.00  | ,                    | ,       | ,     |                              | ,       | ,     | ,                            | ,       | ,     |
| Ki,Snh3,Xc4                          | 0.16   | 2.64  | kla                       | 0.07    | 0.39  | Xfa,Batch6              | 0.00    | 0.00  | Xpro,Batch6               | 0.05     | 0.64  | ,                    | ,       | ,     | ,                            | ,       | ,     |                              | ,       | ,     |
| Ki,Snh3,Xpro                         | 0.33   | 4.71  | X <sub>su,Batch1</sub>    | 0.05    | 0.47  | Xfa,Batch7              | 0.07    | 0.69  | Xpro,Batch7               | 0.20     | 1.12  | ,                    | ,       |       |                              | ,       | ,     |                              | ,       | ,     |
| Ki,Snh3,Xac                          | 0.99   | 6.99  | X <sub>su,Batch2</sub>    | 0.03    | 0.28  | X <sub>c5,Batch1</sub>  | 0.00    | 0.01  | X <sub>ac,Batch1</sub>    | 0.15     | 1.09  | ,                    | ,       | ,     |                              | ,       | ,     |                              | ,       | ,     |
| Ki,Snh3,Xh2                          | 0.23   | 3.55  | X <sub>su,Batch6</sub>    | 0.22    | 3.38  | X <sub>c5,Batch2</sub>  | 0.16    | 0.72  | X <sub>ac,Batch2</sub>    | 0.14     | 0.38  |                      |         | ,     |                              |         | ,     |                              |         | ,     |
| $f_{bu,su}$                          | 1.97   | 24.39 | X <sub>su,Batch7</sub>    | 0.04    | 0.29  | Xc5,Batch6              | 0.03    | 0.14  | Xac,Batch6                | 0.16     | 1.89  | ,                    |         | ,     | ,                            | ,       | ,     |                              | ,       | ,     |
| $f_{ac,su}$                          | 0.56   | 13.69 | X <sub>aa,Batch1</sub>    | 0.05    | 09.0  | Xe5,Batch7              | 0.07    | 0.26  | Xac,Batch7                | 1.99     | 7.48  | ,                    |         |       |                              | ,       | ,     |                              | ,       | ,     |
| fh2,su                               | 4.53   | 12.54 | X <sub>aa,Batch2</sub>    | 0.00    | 0.00  | Xc4,Batch1              | 0.06    | 0.76  | Xh2,Batch1                | 0.18     | 0.74  | ,                    |         |       |                              | ,       | ,     |                              | ,       | ,     |
| $\Sigma \text{ IE } (\%) =$          | 40.67  |       |                           | 45.46   |       |                         | 50.46   |       |                           | 46.38    |       |                      | 46.94   |       |                              | 45.57   |       |                              | 44.21   |       |
| Total (%) =                          | 72.37  | ,     | ,                         | 75.17   | ,     |                         | 78.67   |       |                           | 74.74    | ,     |                      | 72.81   | ,     |                              | 70.49   |       |                              | 68.79   | ,     |
|                                      |        |       |                           |         |       |                         |         |       |                           |          |       |                      |         |       |                              |         |       |                              |         |       |

### 7.2.3.5 Approximate Bayesian Computation

The  $\theta'$  posterior distribution,  $p(\theta'|y)$ , was assessed by ABC (Pritchard et al., 1999; Toni et al., 2010). In short, multiple simulations were carried at different  $\theta'$  combinations sampled by LHS, whereas relatively high  $J(\theta')$  values were discarded by a progressively stringent criterion, based in a tolerance coefficient,  $\varepsilon$  (i.e. > 1.0). In other words, only  $J(\theta') - J_{min}(\theta') \le \varepsilon$  were accepted for posterior evaluation:  $p(\theta'|J(\theta') - J_{min}(\theta') \le \varepsilon)$ . With this approach, identifiability was further assessed by the convergence in the  $\theta'$  confidence range (Toni et al., 2010).

In this study,  $\varepsilon$  was successively reduced from 2.50 to 1.05 (i.e. 2.50, 1.80, 1.30, 1.10 and 1.05). Within each explored  $J(\theta') - J_{min}(\theta') \le \varepsilon$  range, 400 simulations were used.  $\theta'$  were allowed to vary within the same range used for GSA [Table 7.4]. Meanwhile, the 5 to 95 % interquartile range of each  $\theta'$  was used as confidence range, but also as a criterion for identifiability/convergence. The posterior mean, median, mode, Kurtosis, Skewness and correlation matrix were also evaluated, as described in Martin et al. (2010).

### 7.2.3.6 Cross-Validation

Cross-validation assesses the model 'goodness of fitting' in experiments not used for calibration (Bennett et al., 2013). In this study, the  $\theta'$  posterior mean was considered as  $\theta_{opt}$ . Thus,  $\theta_{opt}$  were used to simulate all batch experiments, including the three experimental conditions selected for cross-validation: Batch No. 2, 4 and 8 [Table 7.1].

## 7.3 Results and Discussion

## 7.3.1 GSA – Selecting the Most Influencing Input Parameters for Calibration

### 7.3.1.1 Preliminary Identifiability Assessment

GSA results are summarized in Table 7.5. GSA was started with 30  $\theta_P$  and progressively lead to only 14  $\theta'$ : 13  $\theta_P$  (i.e. K<sub>h,pr</sub>, K<sub>h,ch,slow</sub>, k<sub>m,fa</sub>, k<sub>m,c5</sub>, k<sub>m,c4</sub>, k<sub>m,pro</sub>, k<sub>m,ac</sub>, k<sub>m,h2</sub>, k<sub>d</sub>, pH<sub>LL,ac</sub>, f<sub>bu,su</sub>, f<sub>ac,su</sub>, f<sub>h2,su</sub>) and 1  $\theta_B$  (i.e. X<sub>ac,Batch7</sub>). In this study,  $\theta_i$  showing IE  $\leq 0.20$  % were fixed at their initial values [Table 7.4] to enhance the capabilities of GSA and ABC for calibrating structured AD models. The overall  $J(\theta)$  variance explained by the GSA engine was around 70 % in all cases, confirming the validity of this methodology to assess the most influential  $\theta$  in the HS-AD model (Oakley et al., 2004). IE provides a relative measure of the  $\theta_i$  practical identifiability, while a high  $\theta_i$  correlation – high difference between IE and GE – suggest that  $\theta_i$  cannot be independently calibrated with the available set of experimental data (Saltelli et al., 2006). As an example, GSA No. 1 showed that 40.7 % out of 72.4 % of the global  $J(\theta)$  variance was explained by adding up the IE of 30  $\theta_P$  [Table 7.5], while the remaining 31.7 % variance was explained by correlations among these. For example,  $k_{m,c4}$  explained individually around 4.4 % of the global variance (i.e. IE), though showing GE up to 17.9. This meant that the correlation of  $k_{m,c4}$  with other  $\theta$  was high, and any improvement in  $J(\theta)$  obtained by solely modifying  $k_{m,c4}$  could be partially/totally compensated by the modification of a correlated  $\theta_i$ . In this line, the  $k_{m,c4}$  f<sub>bu,bu</sub> correlation in GSA No. 1 explained up to 2.8 % of the global variance (data not shown), due to the influence of  $f_{bu,su}$  in  $J(\theta)$  (i.e. IE = 2.0 % and GE = 24.4) [Table 7.5].

In this study,  $\theta_i$  were disregarded by a low-demanding criterion (i.e. IE  $\leq 0.20$  %) [Table 7.5], since any chosen criterion for 'potential identifiability' would influence the GSA results when N  $\geq$  30. Meanwhile, GSA also depends on N' (i.e.  $\leq$  30) and/or the particular combination of  $\theta_P$  and  $\theta_B$  used. Thus, using a more demanding identifiability criterion (e.g. IE  $\leq 0.50$  % instead of 0.20 %) might have led to discard  $\theta_i$  during preliminary GSA runs, that would be subsequently characterized as 'potentially identifiable'. For example, GSA No. 1 showed IE = 0.22 % for k<sub>m,c5</sub>, whereas GSA No. 7 eventually showed IE = 0.67 % [Table 7.5]. To enhance identifiability, a strategy to reduce the gap between IE and GE for each  $\theta_i$  is required (Kennedy et al., 2017). However, reducing the IE to GE gap in this study would require to readapt the criterion used for 'potential identifiability'. One strategy might consist on fixing those  $\theta_i$  showing IE  $\leq 0.30$  % in GSA No. 7 (i.e.  $f_{ac,su}$ ), and perform further rounds of GSA (i.e. GSA No. 8), until IE ~ GE for all  $\theta_i$ .

With all the above, a second assessment for identifiability can be useful when using variance-based GSA for structured AD models. ABC is a well-suited tool in this regard, yielding also  $p(\theta|y)$ , in contrast to GSA. Importantly, both methodologies should yield equivalent results regarding the  $\theta_i$  identifiability, though ABC is much computational intensive than GSA, as explained in section 7.3.2.1.

## 7.3.1.2 Importance of the Available Data for Model Calibration

Provided the  $\theta$  are structurally identifiable, practical identifiability relates to the quantity as well as the quality (i.e. experimental errors and/or the sampling frequency) of the experimental data available (Donoso-Bravo et al., 2011; Guisasola et al., 2006; Nimmegeers et al., 2017; Rodriguez-Fernandez et al., 2006). Particularly, a reduced number of experimental data associated to some model dynamics usually prevents practical identifiability of the  $\theta$  involved in these specific dynamics. For example, Y<sub>b</sub> might not be

identifiable in AD models provided the biomass concentration dynamics were measured (Bernard et al., 2001).

In this study, the hydrolysis constant of readily-biodegradable carbohydrates ( $K_{h,ch,fast}$ ) and lipids ( $K_{h,li}$ ), but also the maximum growth rate of sugar ( $k_{m,su}$ ) and amino acid ( $k_{m,aa}$ ) degraders, showed a reduced influence in  $J(\theta)$  by GSA. These results suggest that either insufficient experimental data was available to calibrate  $K_{h,ch,fast}$ ,  $K_{h,li}$ ,  $k_{m,su}$  and  $k_{m,aa}$ , and/or that the biogas production in the HS-AD batch experiments [Table 7.1] was strongly influenced mainly by the VFA uptake – as limiting step. In the same line, due to the Monod properties,  $k_m$  and  $K_s$  might be correlated when using batch experiments for calibration (Dochain et al., 2001; Flotats et al., 2010; Guisasola et al., 2006). Nevertheless, GSA showed negligible influence for all Ks in this study, likely due to using different batch experiments (i.e. ISR and TS) and/or a reduced number of experimental data to obtain  $J(\theta)$  [Equation 7.4].

Importantly, both the NH<sub>3</sub> and H<sub>2</sub> inhibition parameters [Table 7.2] were shown as nonidentifiable in this study, in spite of the strong influence of these parameters to regulate the biogas production in an ADM1-based model, as mentioned in section 7.2.3.2. These results were associated to the reduced TAN and VFA dynamics in the experimental data, since only one single sacrifice experiment was available for calibration/validation. Therefore, despite using different initial conditions (i.e. ISR and/or TS) for model calibration, the NH<sub>3</sub> inhibition in HS-AD of OFMSW cannot be assessed by using traditional batch experiments, where only the biogas production and composition are usually evaluated.

The above results condense the importance of an adequate sampling to enhance identifiability in AD models, but also to test hypotheses regarding the effects of inhibitory substances in HS-AD. Particularly, an extensive VFA, pH and TAN sampling at different operational times during batch experiments is required to identify crucial parameters regarding NH<sub>3</sub> inhibition in HS-AD of OFMSW. Therefore, sacrifice experiments and/or any sampling technique for batch setups – allowing the thorough characterization of the reactor content – should be recommended to calibrate structured HS-AD models using batch experiments.

### 7.3.1.3 The Importance of Initial Conditions for Model Calibration

Interestingly, practically all  $\theta_B$ , with the exception of X<sub>ac,Batch7</sub>, were shown as nonidentifiable in this study [Table 7.5]. The reason presumably lies on the high k<sub>m</sub> of all microorganisms 'shading' the effect of their initial concentration. For example, X<sub>pro</sub> was associated to a maximum growth rate (k<sub>m,pro</sub>) around 10 d<sup>-1</sup> [Table 7.4]. Thus, X<sub>pro</sub> doubles within 1 h (i.e.  $\frac{\log(2)}{k_m} = \frac{\log(2)\cdot 24}{10} = 0.6 h$ ), whereas HS-AD batch experiments lasted considerably longer than 20 days [Table 7.1]. Noteworthy,  $X_{ac}$  and  $X_{h2}$  are important variables to avoid batch acidification during the initial 0 - 10 days of HS-AD simulations, due to the rapid changes occurring in the bio-physical-chemistry during these days, and the influence of these two species to define the buffering capacity [Chapter 6 – Non-Ideal Manuscript](Batstone et al., 2002; Capson-Tojo et al., 2017). Nonetheless, these biomass concentrations were also rapidly disregarded by GSA in this study. On the other hand, the 'potential identifiability' of  $X_{ac,Batch7}$  suggested by GSA No. 7 was likely explained by the influence of this particular biomass content to regulate the acidification of the most extreme HS-AD condition in Experiment 7 (i.e. Batch No. 9, TS = 30 %) [Table 7.1].

With all the above, it is likely that only the initial biomass magnitude – not a precise value – was needed to calibrate the HS-AD model based on batch experiments. In other words, approximate biomass concentrations serve mainly to avoid acidification in HS-AD batch simulations, since these might not influence significantly the model calibration. The  $\theta$  influence in  $J(\theta)$  was further assessed by ABC.

## 7.3.2 Parameter Optimization

### 7.3.2.1 Second Identifiability Assessment

Figure 7.1 shows  $p(\theta'|y)$ , using  $\varepsilon = 1.05$ . The main statistics for these  $p(\theta'|y)$  are summarized in Table 7.6, including the confidence ranges (i.e. 5 - 95 % interquartile range). The correlation matrix is included as Supplementary Information. Figure 7.2 shows the 5 - 95 % interquartile range *vs.*  $\varepsilon$ , since reducing progressively  $\varepsilon$  permitted to assess the convergence of the  $\theta'$  posterior, as second identifiability assessment.



Figure 7.1 Posterior parameter distribution using 400 simulations and  $\varepsilon = 1.05$ 

|                              |                                |               | Initial Evalu | tation Range |        |        |        | Posterior Parame | ter Distribution |          |          |
|------------------------------|--------------------------------|---------------|---------------|--------------|--------|--------|--------|------------------|------------------|----------|----------|
| Parameter                    | Units                          | Initial Value | Minimum       | Maximum      | Mean   | Median | Mode   | 5% Percentile    | 95% Percentile   | Skewness | Kurtosis |
| $K_{h,pr}$                   | d-I                            | 0.050         | 0.005         | 0.095        | 0.048  | 0.048  | 0.044  | 0.040            | 0.057            | 0.217    | 2.83     |
| ${ m K}_{ m h,ch,slow}$      | d <sup>-1</sup>                | 0.0120        | 0.0012        | 0.0228       | 0.0109 | 0.0108 | 0.0102 | 0.0084           | 0.0137           | 0.171    | 2.74     |
| k <sub>m,fà</sub>            | d <sup>-1</sup>                | 10.0          | 1.0           | 19.0         | 7.3    | 6.8    | 9.9    | 4.6              | 12.0             | 1.663    | 6.93     |
| k <sub>m,c5</sub>            | d <sup>-1</sup>                | 8.0           | 0.8           | 15.2         | 10.4   | 10.3   | 8.6    | 7.2              | 13.4             | 0.084    | 2.75     |
| $k_{m,c4}$                   | d <sup>-1</sup>                | 13.0          | 1.3           | 24.7         | 19.6   | 19.5   | 20.2   | 15.8             | 23.6             | 0.001    | 2.52     |
| km,pro                       | d-1                            | 10.0          | 1.0           | 19.0         | 12.2   | 12.2   | 13.0   | 7.1              | 16.7             | -0.148   | 2.66     |
| k <sub>m,ac</sub>            | d <sup>-1</sup>                | 16.0          | 1.6           | 30.4         | 24.2   | 24.5   | 23.2   | 18.7             | 29.4             | -0.242   | 2.32     |
| $\mathbf{k}_{\mathrm{m,h2}}$ | d <sup>-1</sup>                | 20.0          | 2.0           | 38.0         | 26.0   | 25.5   | 25.1   | 17.0             | 36.1             | 0.077    | 2.19     |
| ka                           | d <sup>-1</sup>                | 0.040         | 0.004         | 0.076        | 0.040  | 0.040  | 0.045  | 0.020            | 0.059            | -0.155   | 2.83     |
| pHLLac                       |                                | 5.8           | 5.0           | 6.9          | 5.4    | 5.4    | 5.2    | 5.0              | 5.9              | 0.589    | 2.73     |
| Xac,Batch7                   | kg COD m <sup>-3</sup>         | -2.523        | -3.784        | -1.261       | -2.648 | -2.713 | -2.853 | -3.574           | -1.543           | 0.303    | 2.39     |
| fbu,su                       | kg COD kg<br>COD <sup>-1</sup> | 0.50          | 0.05          | 0.95         | 0.54   | 0.54   | 0.49   | 0.38             | 0.72             | 0.161    | 2.67     |
| fac,su                       | kg COD kg<br>COD <sup>-1</sup> | 0.29          | 0.03          | 0.55         | 0.28   | 0.29   | 0.23   | 0.12             | 0.43             | -0.180   | 2.73     |
| $f_{h2,su}$                  | kg COD kg<br>COD <sup>-1</sup> | 0.10          | 0.01          | 0.19         | 0.09   | 0.09   | 0.09   | 0.02             | 0.17             | 090.0    | 2.31     |

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**Table 7.6** Calibration of potentially-identifiable input parameters ( $\theta^{\circ}$ ): Prior and posterior distributions



**Figure 7.2** Interquartile range (percentiles 5 to 95 %) of the posterior parameter distribution using  $\varepsilon \ge 1.05$  and  $\varepsilon \le 2.50$ 

Parameter identifiability is roughly associated to the 'sharpness' of the posterior distribution (Martin et al., 2010; Toni et al., 2010). Thus, K<sub>h,pr</sub>, K<sub>h,ch,slow</sub>, k<sub>m,fa</sub>, k<sub>m,c5</sub>, k<sub>m,c4</sub>, k<sub>m,ac</sub>, pH<sub>LL,ac</sub> and f<sub>bu,su</sub> showed relatively well-defined bell-shaped distributions by ABC, suggesting an adequate identifiability [Figure 7.1]. Meanwhile, k<sub>m,pro</sub>, k<sub>m,h2</sub>, k<sub>d</sub>, X<sub>ac,Batch7</sub>, f<sub>ac,su</sub> and f<sub>h2,su</sub> showed a more uniform-like distribution, suggesting a poorer identifiability. The substantial reduction observed in the interquartile range for K<sub>h,pr</sub>, K<sub>h,ch,slow</sub>, k<sub>m,fa</sub>, k<sub>m,c5</sub>, k<sub>m,c4</sub>, k<sub>m,ac</sub>, pH<sub>LL,ac</sub> and f<sub>bu,su</sub> (i.e. 60 - 80 %) corroborated their adequate identifiability in this study, in contrast to k<sub>m,pro</sub>, k<sub>m,h2</sub>, k<sub>d</sub>, X<sub>ac,Batch7</sub>, f<sub>ac,su</sub> and f<sub>h2,su</sub> that showed a much constant interquartile range (i.e.  $\leq$  50 % reduction) [Figure 7.2]. The poor practical identifiability of these last  $\theta'$  is explained by their high correlation with the rest of  $\theta'$ . For example, the f<sub>ac,su</sub>·f<sub>bu,su</sub> correlation was -0.82, while k<sub>m,pro</sub>·k<sub>d</sub> was 0.72 – data not shown. As suggested in section 7.3.1.3, X<sub>ac,Batch7</sub> served mainly to counteract the potential acidification in Batch No. 9, since the poor reduction in the interquartile range (i.e. 23 %) alongside the high correlation with other  $\theta'$  (i.e. pH<sub>LL,ac</sub>·X<sub>ac,Batch7</sub> = 0.24) indicated that only an approximate biomass content is needed to calibrate structured HS-AD models based on batch experiments.

As expected, ABC supported the identifiability assessment by GSA. In particular,  $\theta'$  showing IE < 1.5 % in GSA No. 7 (i.e.  $k_{m,pro}$ ,  $k_{m,h2}$ , and  $f_{ac,su}$ ) [Table 7.5] were associated to a poor identifiability. However, some parameters showing IE  $\geq$  1.5 % in GSA No. 7 (i.e.  $k_d$ ,  $X_{ac,Batch7}$  and  $f_{h2,su}$ ) were also indicated as non-identifiable by ABC, in contrast to GSA, suggesting that ABC was a more sensitive methodology for parameter identifiability in this study. With all the above, a more restrictive IE threshold (i.e. 0.50 % instead of 0.20 %) could have been used in further GSA rounds, once fixing poorly-identifiable parameters to any value within the prior, as mentioned in section 7.3.1.1.

ABC is computational intensive due to the high level of  $J(\theta') - J_{min}(\theta') \le \varepsilon$  rejection, particularly when using highly-demanding  $\varepsilon$  (Filippi et al., 2013; Toni et al., 2010). For example, the acceptance ratio was 0.129 when using  $\varepsilon = 1.80$ , meaning that only 1 out of 8 simulations was accepted for posterior evaluation, whereas the acceptance ratio was 0.004 when using  $\varepsilon = 1.10$  – data not shown. Thus, ABC is not recommended to assess identifiability in complex models with a large number of  $\theta$  (i.e.  $N \ge 30$ ). Different upgrades have been proposed to increase the ABC efficiency (Beaumont et al., 2009; Filippi et al., 2013; Toni et al., 2010), though the evaluation of these upgrades for calibrating AD models was out of the scope of this study. Conversely, the GSA engine relies upon a Bayesian emulator to speed up the analysis of model outputs (Kennedy et al., 2001; Oakley et al., 2004). Therefore, GSA can be an adequate tool to reduce the global computation required for parameter optimization, by preliminarily reducing the number of  $\theta'$  to be further assessed by ABC.

### 7.3.2.2 Batch Simulations

Using the  $\theta'$  mean as  $\theta_{opt}$  [Table 7.6] led to a good approximation of both the methane production [Figure 7.3] and the rest of variables at the end of all batch experiments [Figure 7.4], used either for calibration (i.e. Batch No. 1, 3, 5, 6, 7 and 9) or cross-validation (i.e. Batch No. 2, 4 and 8) [Table 7.1]. Therefore, the  $\theta'$  mean might be a good approximation of  $\theta_{opt}$ , particularly for these  $\theta'$  where practical identifiability was likely (i.e. K<sub>h,pr</sub>, K<sub>h,ch,slow</sub>, k<sub>m,fa</sub>, k<sub>m,c5</sub>, k<sub>m,c4</sub>, k<sub>m,ac</sub>, pH<sub>LL,ac</sub> and f<sub>bu,su</sub>). Importantly, the HS-AD model was able to capture particularly well the TS and TAN contents, but also VS (data not shown), in all mono- and co-digestion experiments, confirming the suitability of the hypotheses used for model development (Pastor-Poquet et al., 2018).

Some disagreements were also observed between the simulations and the experimental results. Particularly, the implementation of a reversible NH<sub>3</sub> inhibition [Equation 7.3] in all the VFA and H<sub>2</sub> degrading populations [Table 7.2] was unable to capture the VFA accumulation at the end of HS-AD experiments [Figure 7.4]. As the most noticeable example, calibration failed to represent the S<sub>bu</sub> accumulation in Batch No. 6 [Figure 7.4e], yielding also a slight miss-adjustment in the methane production [Figure 7.3c]. More in general, S<sub>ac</sub> and S<sub>bu</sub> at the end of all experiments were poorly represented [Figure 7.4], despite the butyrate (k<sub>m,c4</sub>) and acetate (k<sub>m,ac</sub>) growth rates were adequately identified, and the NH<sub>3</sub> inhibition upon the acetate uptake is a relatively well-defined mechanism in structured AD models (Angelidaki et al., 1993; Batstone et al., 2002).

Two main reasons might explain the VFA disagreement between the model simulations and the experimental data. The first reason relates to the relatively low amount of experimental data hampering calibration, as mentioned in section 7.3.1.2. In this line, the NH<sub>3</sub> inhibition parameters in the VFA and/or H<sub>2</sub> uptakes were disregarded as unimportant by GSA to represent the experimental data [Table 7.5], mainly because only Batch No. 1 contained the VFA, pH and TAN dynamics. The second reason relates to the poor suitability of the reversible NH<sub>3</sub> inhibition [Equation 7.3] to explain the VFA accumulation in HS-AD simulations, as discussed in next section.



Figure 7.3 Methane production with mono-digestion of dried OFMSW at a) ISR = 1.00; b) ISR = 1.50; and c) ISR = 1.00; and co-digestion of dried OFMSW and sawdust at d) ISR = 0.16. Dots represent experimental data, while lines represent simulated values



**Figure 7.4** Main variables at the end of the four batch experiments: a) Total solids (TS); b) total ammonia nitrogen (TAN,  $S_{in}$ ); c) ionic strength (I); d) free ammonia nitrogen ( $NH_3$ ,  $S_{nh3}$ ); e) acetic acid ( $S_{ac}$ ); f) propionic acid ( $S_{pro}$ ); g) butyric acid ( $S_{bu}$ ); and h) valeric acid ( $S_{va}$ ). Crosses represent experimental data, while geometries represent simulated values

## 7.3.3 Main Effects of Increasing the TS Content in HS-AD of OFMSW

In this study, calibration/cross-validation served to further test the hypotheses used for model construction (e.g. mass balances), particularly regarding the TS and VS simulation. Noteworthy, the correct simulation of TS is crucial in HS-AD, as TS determines the apparent concentration of soluble compounds, subsequently affecting all the HS-AD bio-physical-chemical dynamics (Pastor-Poquet et al., 2018) [Chapter 6 – Non-ideal Manuscript]. For example, TS = 20 % supposes approximately 20 % higher apparent concentrations (i.e. kmol/kg H<sub>2</sub>O), regarding the corresponding global concentrations (i.e. kmol/kg).

The HS-AD model was also calibrated/validated to assess the effects of increasing TS upon the NH<sub>3</sub> inhibition in HS-AD. Specifically, a high solute content – potentially associated to a high TS – exacerbates the solution 'non-ideality', affecting all the HS-AD dynamics (e.g. pH, NH<sub>3</sub> concentration, CO<sub>2</sub> transfer) [Chapter 6 – Non-ideal Manuscript]. More in detail, 'non-ideality' can lower S<sub>nh3</sub>, serving as a potential source of NH<sub>3</sub> inhibition abatement in HS-AD of OFMSW. In this study, *I* ranged from 0.22 to 0.93 M [Figure 7.4c], highlighting the need for an adequate 'non-ideal' bio-physical-chemical approach (Hafner et al., 2009; Solon et al., 2015). Importantly, despite the high *I* observed, S<sub>nh3</sub> reached up to 0.13 mol N/kg in this study [Figure 7.4d] – equivalent to 0.16 mol N/kg H<sub>2</sub>O (i.e. 2.3 g N/L). Noteworthy, these S<sub>nh3</sub> were considerably high, since reactors operated at S<sub>nh3</sub> ≥ 1.0 g N/L normally show an inefficient VFA conversion (Angelidaki et al., 1993; Rajagopal et al., 2013).

The inefficient VFA conversion in HS-AD experiments was not well simulated by the reversible NH<sub>3</sub> inhibition, as mentioned in section 7.3.2.2. To understand the poor VFA simulation, it is necessary to consider the relatively-flat inhibition described by Equation 7.3, but also the COD fluxes in the HS-AD model. Noteworthy, I<sub>nh3</sub> is 0.50 when S<sub>nh3,App</sub> =  $K_{i,Snh3}$ , whereas I<sub>nh3</sub> is 0.33 when S<sub>nh3,App</sub> =  $2 \cdot K_{i,Snh3}$  [Equation 7.3]. In other words, a non-competitive reversible inhibition by NH<sub>3</sub> might be far too 'blunt' to describe the actual effect of NH<sub>3</sub> upon the anaerobic biomass. On the other hand, due to the COD fractioning used in this study, approximately 54 % of the COD from S<sub>su</sub> [Table 7.6], and 26 % of the COD from S<sub>su</sub>, S<sub>aa</sub>, and/or long-chain fatty acids (S<sub>fa</sub>) flowed through the acetate pathway (Batstone et al., 2002; Pastor-Poquet et al., 2018). Meanwhile, a considerable proportion of the initially biodegradable COD (i.e. 75 - 85 %) was assigned to X<sub>ch</sub> + S<sub>su</sub> + X<sub>pr</sub> + S<sub>aa</sub> [Table 7.3].

With all the above, the 'blunt' definition of the  $NH_3$  inhibition function, alongside the high COD flowing through the butyrate and acetate pathways, presumably favored the  $X_{bu}$  and  $X_{ac}$  growth even at considerably high  $S_{nh3}$  (i.e. up to 2.3 g N/L) during simulations. Summarizing, the high substrate content counterbalanced the effect of the  $NH_3$  inhibition,

preventing the correct simulation of  $S_{bu}$  and  $S_{ac}$  accumulation at the end of the HS-AD experiments. Therefore, the reversible NH<sub>3</sub> inhibition function [Equation 7.3] in the VFA and/or H<sub>2</sub> uptakes [Table 7.2] requires further testing to represent the VFA accumulation observed in HS-AD of OFMSW. To this particular aim, using extensive data regarding the VFA, pH and TAN dynamics in HS-AD simulations is strongly recommended.

To end up, the HS-AD model (Pastor-Poquet et al., 2018) is an invaluable platform to understand the inner mechanisms of HS-AD, whereas further model developments and/or model configurations (i.e. inhibition functions) should be also tested to enhance our understanding about the VFA accumulation within HS-AD batch experiments. Similarly, further experimental data is also needed to understand the role of NH<sub>3</sub> inhibition in HS-AD simulations. Specifically, extensive data regarding the main species driving 'non-ideality' (i.e. VFA, pH, TAN) and/or further bio-physical-chemical mechanisms (i.e. precipitation) in HS-AD might be crucial, due to the importance of 'non-ideality' for the biochemical parameter optimization [Chapter 6 – Non-ideal Manuscript]. In this scheme, the relatively simple calibration/validation methodology presented in this study can serve to test the  $\theta$ practical identifiability and/or confidence ranges, in presence of any set of experimental data and/or HS-AD model structure.

## 7.4 Conclusions

Nine different batch conditions were used to calibrate and cross-validate the HS-AD model. For parameter optimization, variance-based GSA in tandem with ABC, served to evaluate the practical identifiability of 35  $\theta_P$  and 32  $\theta_B$ . Among all these, only 8  $\theta_P$  were correctly identified with the available data, as corroborated by the convergence of  $p(\theta|y)$ . The study also showed  $S_{nh3} \ge 2.3$  g N/L and  $I \ge 0.9$  M, whereas a reversible non-competitive NH<sub>3</sub> inhibition function was not able to explain the VFA accumulation in HS-AD of OFMSW. Therefore, further datasets about the VFA, pH and TAN dynamics are required to enhance the  $\theta$  practical identifiability, whereas further model configurations should be tested to enhance the simulation of NH<sub>3</sub> inhibition in HS-AD.

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# Chapter 8

# **General Conclusions and Future Perspectives**

## 8.1 General Conclusions

Anaerobic digestion (AD) is a well-suited biotechnology to recover renewable energy and nutrients from organic waste (OW). Particularly, high-solids anaerobic digestion (HS-AD), operated at a total solid (TS) content  $\geq 10$  %, maximizes the process economy. HS-AD of the organic fraction of municipal solid waste (OFMSW) is a mature technology with multiple commercialized applications, due to the fact that OFMSW is ubiquitous, and shows a high biodegradability potential, alongside a high nitrogen and phosphorous content. Moreover, recycling minimizes the impurities of OFMSW, reducing the need for pretreatment (i.e. inert sorting, particle size reduction), and further enhancing the HS-AD economy. Nonetheless, maximizing TS in HS-AD of OFMSW exacerbates also the chances of inhibition by free ammonia nitrogen (NH<sub>3</sub>) and the risk of reactor acidification by substrate overload.

To contribute towards the optimization of HS-AD for OFMSW treatment, the understanding of the main mechanisms and kinetics of the overall HS-AD bio-physicalchemistry is essential. In this PhD research, laboratory-scale experiments highlighted the main strengths and weaknesses of HS-AD for OFMSW treatment. Simultaneously, the development of a HS-AD model condensed the gathered knowledge about the main effects occurring upon the HS-AD bio-physical-chemistry when increasing the TS content. Eventually, the experimental results served to calibrate and cross-validate the HS-AD model, highlighting the need for further target-oriented experimental data and also the need for further HS-AD model developments.

The overall methodology in this study describes a circular pathway, as mentioned in Chapter 1. Moreover, all the results obtained emphasize the need for further understanding the main mechanisms driving the acidification and NH<sub>3</sub> inhibition in HS-AD of OFMSW. This understanding should be the considered as a priority to optimize overall HS-AD economy, while maximize the methane yield and the OW stabilization. With this aim, the main achievements of this research can be summarized as follows:

# 8.1.1 Chapter 3 – Batch Experiments

- HS-AD of OFMSW requires a compromise between the initial TS content, the inoculum-to-substrate ratio (ISR), the alkalinity (ALK) and the nitrogen content. Particularly,

- An inadequately low ISR – normally associated to a high TS – leads to acidification. For example, during mono-digestion of OFMSW, a ISR  $\geq$  1.00 g VS/g VS should be used. Moreover,

- The  $NH_3$  content can be as high as 2.3 g N/L in HS-AD of OFMSW, potentially leading to volatile fatty acid (VFA) accumulation and the reduction of the methane yield. Meanwhile,

- Adding green waste to OFMSW permits to increase the TS content, simultaneously reducing the ISR and total ammonia nitrogen (TAN) in HS-AD.

Mass balances of total (TS) and volatile (VS) solids, alkalinity (ALK), total Kjeldahl (TKN) and ammonia (TAN) nitrogen need to be simultaneously fulfilled to circumvent acidification during the initial days of batch startup, but also to avoid NH<sub>3</sub> inhibition and VFA accumulation towards the end of the batch experiment, particularly at high TS contents (i.e. TS  $\geq$  15 %). Alongside those balances, the presence of gas-filled porosity ( $\epsilon$ ) should be also accounted due to the potential differences in the metabolite transport rates between a liquid and a gas media. Importantly, all these mass balances and the presence of porosity strongly determine the overall economy of HS-AD. For example, adding a lignocellulosic waste to OFMSW might lengthen considerably the biochemical treatment and reduce the methane yield.

Taking into account solely the ISR to foresee the chances of HS-AD acidification is not adequate, since the VS content of the inoculum does not condense important biochemical aspects of the inoculum-substrate mixture, as the microbial activity, the overall biodegradability potential, the biodegradability rate, and/or the inhibitory content (i.e. NH<sub>3</sub>, H<sub>2</sub>S). On the other hand, the NH<sub>3</sub> inhibition depends on the TAN and pH dynamics, which are further associated to the bio-physical-chemical composition and overall biodegradability of the substrate-inoculum mixture. Therefore, more adequate indicators are required to foresee the risk of acidification and/or NH<sub>3</sub> inhibition when increasing the substrate concentration in HS-AD of OFMSW – including or not green waste in the OFMSW composition. A robust HS-AD model might aid in this regard.

# 8.1.2 Chapter 4 – Semi-continuous Experiments

- HS-AD of OFMSW requires a reduced effluent compared to the influent to counterbalance the organic mass removal associated to the biogas production. Nonetheless,

- Uncoupling might not be sufficient to avoid overload when using mono-digestion of OFMSW.

- Substrate overload is associated to the high biodegradability rate of OFMSW in HS-AD. Moreover, overloading is exacerbated by the high NH<sub>3</sub> content resulting from the OFMSW decomposition. Thus,

- A much higher TS and lower TAN can be reached in semi-continuous HS-AD by adding green waste to OFMSW.

The organic removal in HS-AD reduces the amount of organic substrate in the digester, though further concentrates TAN, exacerbating the risk of NH<sub>3</sub> inhibition. Uncoupling the influent and effluent extends the mass retention time (MRT) in HS-AD favoring the biomass adaptation to inhibitors, while it also reduces the chances of reactor acidification. Nonetheless, increasing the TS content in semi-continuous HS-AD is roughly equivalent to increase the organic loading rate (OLR), since using a relatively low OLR with a highly-biodegradable OFMSW prevents to reach HS-AD conditions (i.e. TS  $\geq$  10 %). Meanwhile, increasing OLR also increases the TAN accumulation.

Gradually increasing OLR contributes to the biomass adaptation towards high levels of organic substrate and TAN, reducing the risk of acidification. On the other hand, the OLR increase leads to many interrelated effects including the pH modification and the VFA accumulation, but also the modification of the ionic equilibrium, ion pairing, precipitation/redissolution, and liquid-gas transfer. Meanwhile, the concentration of important methanogenic inhibitors (i.e. NH<sub>3</sub>, H<sub>2</sub>S) is also affected by changes in pH, associated to the OLR increase. However, the maximum OLR in HS-AD is difficult to predict, since the OLR depends on both the operational conditions (e.g. temperature, T; hydraulic retention time, HRT) and the OW composition (e.g. TS, TKN). Simultaneously, the OW composition also depends on external factors such as the season or the local waste management strategy.

The inherent complexity and tight interrelationship among all the dynamic variables results into continuous HS-AD of OFMSW at industrial scale being sometimes operated under conservative thresholds (i.e. low OLR, prolonged HRT) to avoid reactor acidification, though compromising the OW treatment economy. In this scheme, a HS-AD model can help to foresee the HS-AD performance under different operational conditions, permitting to optimize the process, at both laboratory and industrial scales.

# 8.1.3 Chapter 5 – Model Development

- A HS-AD model based on the Anaerobic Digestion Model No.1 (ADM1) was developed for homogenized reactors, as the main novelty of this PhD research.

- This model simulates the reactor mass/volume and TS dynamics in HS-AD, as main differences with 'wet' AD simulations (i.e. TS < 10 %). Moreover,

- The HS-AD model also considers the TS concentration effect on soluble species, in terms of apparent concentrations. With these modifications,

- The HS-AD model simulates adequately the VFA and TAN dynamics in HS-AD using OFMSW as substrate, though any other substrate can be also simulated.

ADM1 is one of the most important structured models for AD, gathering the current knowledge about the AD bio-physical-chemistry. However, the CSTR implementation of ADM1 was preliminarily conceived for 'wet' AD applications (i.e. TS < 10 %) by, for example, assuming a constant reactor content volume. In either case, ADM1 is an outstanding platform to be continuously developed, in order to extend our knowledge about HS-AD, while fostering the bioprocess optimization.

To develop a HS-AD model based on ADM1, a reduced number of hypotheses was used. These hypotheses relate to the fact that a model must be always kept as simple as possible, though well suited for the envisaged objectives. Thus, the simulation of a water-deficient environment as HS-AD needs to assess adequately the main bio-physical-chemical dynamics occurring in the process, and particularly the TS content. In this line, mass balances were conveniently adapted to simulate the TS dynamics in HS-AD simulations using OFMSW as substrate. On the other hand, apparent concentrations, as a function of TS, further influenced the kinetic rates and the NH<sub>3</sub> inhibition in HS-AD simulations.

Only these two relatively simple modifications showed a profound effect upon the HS-AD biochemistry, by affecting all the soluble compounds in the model simulations. Moreover, these modifications permitted to observe the substantial OLR increase, either in 'wet' AD or HS-AD simulations, associated to the reactor content mass/volume reduction from biogas production.

# 8.1.4 Chapter 6 – 'Non-Ideal' Effects

- In HS-AD of OFMSW, the ionic strength (*I*) might be considerably higher than 0.2 M. Thus, accounting adequately for the 'non-ideal' effects upon the bio-physical-chemistry is essential to understand the main kinetic rates in HS-AD, since

- 'Non-ideality' strongly affects the pH, NH<sub>3</sub> inhibition and CO<sub>2</sub> liquid-gas transfer, as some of the most important triggers for inhibition, VFA accumulation and/or reactor acidification in HS-AD. Importantly,

- 'Non-ideal' effects on HS-AD simulations are exacerbated at higher TS, since less water is available to dilute the ionic content in solution. Therefore,

- HS-AD simulations jointly require to account simultaneously for the 'non-ideal' biophysical-chemistry, and the reactor mass/volume and TS dynamics, as these mechanisms are mutually interdependent to define the HS-AD performance. Noteworthy,

- 'Non-ideality' might strongly affect also the HS-AD model calibration, particularly regarding the  $NH_3$  inhibition.

Since the two main setbacks in HS-AD of OFMSW are the NH<sub>3</sub> inhibition and the risk of acidification, studying 'non-ideality' in HS-AD was the most reasonable step to further develop the HS-AD model, since 'non-ideality' affects all the acid-base equilibriums and the solution pH, as two of the most important variables for the methanogenic inhibition. Moreover, the pH is strongly interwoven with all the bio-physical-chemical mechanisms and kinetics in HS-AD. Therefore, understanding the biochemical mechanisms and kinetics in HS-AD preliminarily requires the study 'non-ideality', due to the fact that 'ideal' simulations might bias the conclusions regarding the biochemical parameters, but also the risk of inhibition.

In this study, a relatively simple 'non-ideal' module was developed for assessing 'nonideality' in HS-AD. This module predominantly accounts for the modification of the acidbase equilibrium constants as a function of *I*. On the other hand, future model developments should account also for complex physical-chemical mechanisms, such as precipitation and ion-pairing, since these mechanisms simultaneously affect and are affected by the elevated number of ionic interactions in HS-AD of OFMSW.

# 8.1.5 Chapter 7 – Model Calibration/Validation

- Variance-based global sentisitivy analysis (GSA) and approximate Bayesian computation (ABC) in tandem is a well-suited strategy to evaluate the practical identifiability in structured HS-AD models, but also to obtain the best parameter estimates. Nonetheless,

- Due to the inherent complexity of an ADM1-based model, only a maximum of 8 biochemical parameter posterior distributions were correctly identified, by using all the methanogenic batch experiments obtained in Chapter 3 as a source of experimental data.

- After calibration and cross-validation, results showed that HS-AD of OFMSW might be operated at ionic strengths (I) up to 0.9 M and NH<sub>3</sub> concentrations up to 2.3 g N/L, particularly at higher TS contents (i.e. 25 - 30 %). Meanwhile,

- The use of a reversible non-competitive NH<sub>3</sub> inhibition function in all the VFA and H<sub>2</sub> kinetics for HS-AD simulations should be further tested with a more extended set of experimental data.

The use of nine different initial batch conditions permitted to calibrate up to 14 input parameters ( $\theta'$ ), from an initial set of 35 biochemical/structural parameters ( $\theta_P$ ) and 32 initial conditions ( $\theta_B$ ). Among these, only 8 biochemical parameters were considered as correctly identified, due to the considerable reduction observed in the posterior distribution (i.e.  $\geq 60$ %). The overall practical identifiability was mainly related to the HS-AD model structure and the informativeness of the available experimental data used for calibration. Therefore, calibrating more parameters, aiming predominantly to discern about the potential NH<sub>3</sub> inhibition upon all the VFA and H<sub>2</sub> degraders, requires multiple experimental datasets predominantly containing the individual TAN, VFA and pH dynamics in HS-AD of OFMSW, in order to circumvent the parameter non-identifiability. In the same line, further HS-AD model developments were also recommended, in order to address the relative complexity of the HS-AD bio-physical-chemistry (i.e. precipitation, ion pairing), and the high influence of 'non-ideality' in the parameter estimation, as highlighted before.

To end up, performing laboratory-scale HS-AD experiments while simultaneously developing the HS-AD model in this PhD research permitted to feedback the gathered knowledge from the experimental setups to the mathematical model and *vice versa*. More in particular, HS-AD experiments highlighted the importance of the overall substrate biodegradability, alongside the high risk of NH<sub>3</sub> inhibition and acidification, suggesting a potential strategy to operate HS-AD of OFMSW: to counterbalance the TS increase with the risk of acidification and NH<sub>3</sub> inhibition. Subsequently, the HS-AD model development and calibration highlighted the need for a 'non-ideal' physical-chemical approach, and the need for further experimental data, to upgrade the model and further explore the inner mechanisms in HS-AD of OFMSW. To this particular aim, gathering adequate experimental data should be considered as a milestone.

HS-AD experimental data can be difficult and/or time-consuming to obtain due to the complexity of the laboratory-scale experiments, but also the inherent complexity of the semisolid sample and the need for sample pretreatment. Nevertheless, further experimental data about HS-AD of OFMSW will permit to test further hypotheses and/or model configurations, enhancing our understanding about the highly-complex interactions across the biochemistry and the physical-chemistry in HS-AD. Noteworthy, the HS-AD model development and calibration is an inherently continuous process, as highlighted before, aiming to extend our understanding will yield with no doubt substantial improvements regarding the industrial performance of HS-AD for OFMSW treatment. Meanwhile, the enhancement in the HS-AD performance will broad the environmental benefits, including the greenhouse gases emission abatement and the recycling of nutrients back to agriculture. With all those aims, the next section provides a glimpse of those areas that might further contribute to extend our knowledge about HS-AD.

# 8.2 Future Perspectives

During this decade, research will likely continue to evolve towards HS-AD optimization, due to the fact that HS-AD is the most environmental friendly technology for OW treatment. Among the most important HS-AD aspects requiring further development, the instability issues associated to acidification and/or NH<sub>3</sub> inhibition in HS-AD of OFMSW will need to be mitigated, as repeatedly highlighted thorough this PhD research.

To this aim, further understanding about the main bio-physical-chemical dynamics and kinetics in HS-AD is crucial, as also highlighted before. This understanding will require the simultaneous development of both adequate experimental setups, including the bio-physical-chemical analyses, for experimental data collection, and an adequate HS-AD model. In this scheme, it must be considered also the need for model calibration, in order to test hypotheses about the most important HS-AD mechanisms. All these developments will benefit from the multidisciplinary involvement of researchers in different areas, including mathematics, engineering, physics, chemistry, geochemistry and statistics, among others. To get further insight about the HS-AD bio-physical-chemistry, some guidelines are proposed next.

# 8.2.1 Experimental Data Collection

In HS-AD, particulate solids, solutes, ion-pairs, redox species, liquids, gases and microorganisms are simultaneously interacting. Thus, due to the great number of variables in HS-AD, as much experimental data as possible, regarding the characterization of the substrate and the reactor content dynamics, is needed to test hypotheses and develop adequate predictive tools. More in particular, extensive experimental datasets are necessary to build, calibrate and validate a robust HS-AD model, that can ease the bioprocess optimization, as highlighted in Chapter 7. In this line, either batch or continuous experiments will need to be frequently (i.e. daily) sampled to assess the main HS-AD dynamics (i.e. pH, TS, TAN), since these dynamics potentially avoid parameter (practical) non-identifiability within structured HS-AD models. Needless to say, the experimental setup must be also adequately designed for collecting representative samples, due to the inherent heterogeneity and complex rheology of a semi-solid environment as HS-AD.

The experimental analyses required in HS-AD include all these performed in this PhD research (e.g. TS, VS, TAN, TKN, biogas production and composition, see Chapters 3 and 4), but also many others providing further information about the main bio-physical-chemical mechanisms in HS-AD. Some of the important analyses not performed in this PhD research, but being potentially interesting for developing a much complex HS-AD model, might include elementary (i.e. CHNS), metal ions (e.g. Fe<sup>2+</sup>), microbial populations and rheological analyses. Among these, addressing adequately the main microbial dynamics might be particularly important to further understand the link between the biochemistry and the physical-chemistry in HS-AD, despite the difficulties to translate microbial measurements into adequate model units.

Multiple slightly-different HS-AD experiments might need to be also performed to assess the influence of the different operational variables. At this point, it must be remarked the high chances of 'undesired' acidification in HS-AD experiments, observed in Chapters 3 and 4. In short, the occurrence of acidification will require to restart/reevaluate some of those biochemical reactors. On top of that, the high complexity of the HS-AD matrix, including biochemical and physical-chemical species, normally requires sample pre-treatment (i.e. centrifugation, filtration, chemical addition and/or adsorption/desorption), as highlighted before, to minimize the interferences within the semi-solid matrix, though lengthening considerably the process of data assimilation – while increasing the chances of experimental errors. With all the above, representative experimental data collection in HS-AD is simply an enormous task. Nonetheless, an adequate methodology must be envisaged to collect all the necessary datasets supporting further HS-AD model developments.

## 8.2.2 Further HS-AD Model Developments

Developing a robust structured model for HS-AD might lead towards significant improvements about the industrial HS-AD performance for OFMSW treatment, as mentioned before. In particular, a well-suited HS-AD model can help to highlight non-linear patterns existing in the experimental datasets. Moreover, a HS-AD model can help also to foresee the HS-AD performance under slightly different initial and/or influent conditions, permitting to explore further optimization scenarios for HS-AD of OFMSW, while reducing the chances of acidification and/or NH<sub>3</sub> inhibition – including the associated economic losses.

Other advantages of a HS-AD model include the development of control strategies for HS-AD reactors and/or the possibility to condense the HS-AD knowledge in a single tool to train researchers and plant operators. Moreover, the HS-AD model can be used to explore and test hypotheses about some aspects of the HS-AD bio-physical-chemistry that might not be 'easily' assessed from experimental measurements as, for example, the ionic interactions, the 'actual' concentration of inhibitors, the concentration of bioavailable trace elements and/or microbial interactions. Importantly, a structured HS-AD model cannot be successfully calibrated/validated without the aid of well-defined experimental setups and targeted bio-physical-chemical analyses, as highlighted in section 8.2.1. Taking as a reference the HS-AD model developed and calibrated along Chapters 5 to 7, some upgrades are recommended below.

### Particulate Compounds and Model Units

ADM1 was preliminarily proposed in chemical oxygen demand (COD) units, though some authors also expressed this model in molar units. Nonetheless, both modelling approaches are somehow limited by the poor definition of the elementary composition of all the species in AD, particularly regarding the most complex species (i.e. particulates), as highlighted in Chapter 3. Noteworthy, particulate compounds, including carbohydrates, proteins and lipids, strongly determine the VFA and biogas composition in ADM1, though these are rarely measured in AD experiments, likely due to the laboratory analysis complexity, as mentioned next. Furthermore, complex compounds (i.e. carbohydrates, proteins, lipids) are composed by simpler – though still relatively complex – units (i.e. sugars, amino-acids, long-chain fatty acids), which potentially determines the overall molecular weight, redox state, biodegradability rate and/or inhibitory potential of these compounds. For example, proteins might be composed of multiple amino acids (i.e.  $\geq 23$ ) showing a completely different carbon-to-nitrogen ratio for each substrate. Particulate compounds determine also the specific weight ( $\rho$ s), density ( $\rho$ ) and porosity ( $\epsilon$ ) within HS-AD. Thus, enhancing the characterization of complex particulates will likely enhance also the simulation of the reactor content mass/volume, specific weight and TS. In this line, the mass/volume and TS dynamics strongly influence the bio-physical-chemistry in HS-AD, as mentioned in Chapter 5. Therefore, the explanatory potential of HS-AD models could be enhanced by progressively increasing the model complexity. In the same line, other aspects of the digestate characterization, as the viscosity and/or rheology, being two of the most important drivers for the economy of digestate post-treatment, could be approximated by a HS-AD model, once  $\rho$ s and  $\epsilon$  were adequately simulated.

In spite of some physical-chemical analyses permit to approximate the carbohydrate, protein and lipid content of semi-solid samples, the complexity of the matrix prevents to fully distinguish among them and/or express these compounds in adequate COD/mass fractions. Therefore, physical-chemical analyses for complex substances are recommended to be further developed/established, and thoroughly performed along HS-AD experiments, whereas a more extended Petersen matrix must be also envisaged for ADM1-based models, to enhance the representativeness of the particulate module to simulate HS-AD. Noteworthy, these developments can enhance not only our understanding about the HS-AD biochemistry, but also the model performance for industrial optimization. Needless to say, the high-concentration of the particulate compounds in HS-AD exacerbates their influence in the model outputs.

### Variable Stoichiometry and Microbial Dynamics

The high organic content exacerbates the risk of reactor acidification, while also favors the simultaneous co-existence AD and dark fermentation (DF) in HS-AD reactors. Moreover, the progressive shift among diverse environmental conditions (i.e. pH) can determine the occurrence of variable stoichiometry in HS-AD, as suggested in Chapters 2 and 7. Noteworthy, this phenomenon might strongly determine the overall pH dynamics and biomass inhibition, but also the biogas production and composition, and even the microbial populations dynamics in HS-AD. In the same line, it must be noted that slightly different environmental conditions can favor the adaptation of slightly different biomass populations, potentially influencing the capabilities of a mathematical model to foresee the HS-AD performance. Therefore, the coupling between the acidogenic and methanogenic steps should be further explored, with particular emphasis on forecasting the risk of acidification in HS-AD of OFMSW.

It is important to note also that stepping further towards the understanding of variable stoichiometry in HS-AD will simultaneously extend our knowledge about high-solids DF (HS-DF). Thus, not only the transition from HS-DF to HS-AD could be explored, but also

the recovery of H<sub>2</sub> and valuable intermediates (i.e. VFA) from high-solids OW. In this line, HS-DF can benefit from similar advantages of HS-AD (i.e. reducing the reactor volume and operational costs). Moreover, the overall economy of OW treatment might be substantially enhanced, by broadening the diversity of end-products to be recovered. This would be a substantial step towards the bio-refinery concept, serving simultaneously for waste treatment, alongside valuable organic intermediate, renewable energy and nutrient recovery. Importantly, the variable stoichiometry and microbial dynamics can influence (and be influenced) by the bio-physical-chemistry and spatial effects within HS-AD reactors.

### Extending the Non-Ideal Bio-Physical-Chemistry Module

The 'non-ideal' bio-physical-chemistry needs to be considered in all HS-AD models. Particularly, apart from the effect of 'non-ideality' on the acid-base and liquid-gas transfer equilibriums, the mechanisms of precipitation, ion pairing and surface adsorption need to be also considered within HS-AD simulations, as indicated in Chapter 6. Noteworthy, all these mechanisms are strongly interwoven with pH, but also with the biochemical rates and the biogas composition.

'Non-ideal' HS-AD simulations might be also associated to some setbacks. Firstly, more (ionic) species should be accounted in the model structure increasing the uncertainty of the model simulations in absence of adequate experimental data. Secondly, including further physical-chemical mechanisms in an ADM1-based model potentially exacerbates the model stiffness, due to the need for coupling biochemical and physical-chemical processes occurring at different time-scales (i.e. seconds *vs.* days). Finally, even if 'non-ideality' software is yet available (i.e. Phreeqc), experimental data might be rarely available regarding the complex ionic interactions in HS-AD of OFMSW and/or the involved equilibrium constants. Therefore, the author recommends to include only these physical-chemical modules significantly influencing 'non-ideality' in HS-AD, while also being associated to experimental data, while avoiding a 'potentially-unnecessary' increase in the model complexity.

# Need for Spatial Simulations

The compositional heterogeneity within HS-AD reactors mainly depends on the operational TS content and/or the presence of homogenizing devices, as mentioned in Chapter 2. As a general rule, HS-AD reactors do not include stirrer. Instead, HS-AD homogenization depends on the recirculation of the solid/liquid digestate and/or the external mixing of substrate and inoculum. In these cases, compositional gradients should be
expected within HS-AD reactors, particularly at industrial scale. Needless to say, these compositional gradients affect the bio-physical-chemistry and *vice versa*.

Computational fluid dynamics (CFD) software can serve to understand the effects of rheology, but also organic intermediates and microorganisms transport in HS-AD reactors. The main disadvantages of CFD models are the considerable increase on computational demands, and the need for establishing preliminary hypotheses about the rheology of semisolid mixtures (e.g. viscosity dependence with TS). On the other hand, it must be noted that an adequate bio-physical-chemical framework needs to be also embedded in CFD models, further increasing the computational demands. Unfortunately, calibration might require an extremely high number of simulations (i.e.  $\geq$  1,000), as suggested in Chapter 7. Therefore, to enhance the simulation of all the HS-AD dynamics, while keeping the computational cost at a minimum, both the bio-physical-chemistry and the fluid dynamics should be recommended to be studied as separated aspects during a 'preliminary' approach. Only then, the coupling between both modules should be explored. Needless to say that validating CFD applications depends also on the availability of experimental data about the flow patterns in HS-AD.

The above guidelines can be summarized as: "A much complex model will require much complex experimental data". These guidelines do not pretend to be exhaustive, since the scientific development will always rely on the envisaged objectives, and the continuously-evolving research paradigm. Instead, the results, conclusions and future guidelines presented along this PhD research aim to contribute towards the current understanding of the inner mechanisms driving the biochemical kinetics in HS-AD. Particularly, the overall PhD research might aid to (thoroughly) plan further experimental setups, obtain highly-informative experimental data and eventually develop mathematical tools with sound applications regarding the understanding and optimization of HS-AD. The global aim is to enhance the industrial applicability of this anaerobic biotechnology for OW treatment, as a remarkable source of renewable energy, valuable end-products and nutrient recycling.

> Vicente Pastor Poquet Naples, 5<sup>th</sup> December 2018

## Annex

# Thesis Overview: From the Experimental Setup to Further Model Requirements

This chapter presents unpublished results:

Pastor-Poquet, V., Papirio, S., Steyer, J.-P., Trably, E., Rintala, J., Escudié, R., & Esposito, G. (*in preparation*). Developing a High-Solids Anaerobic Digestion Model: From the Experimental Setup to Further Model Requirements.

#### Abstract

The objective of this study was to understand and simulate the main effects in the biophysical-chemical mechanisms and kinetics, when increasing the total solid (TS) content in high-solids anaerobic digestion (HS-AD) of the organic fraction of municipal solid waste (OFMSW). Batch and semi-continuous experiments highlighted the importance of increasing TS to exacerbate the risk of ammonia inhibition and reactor acidification. Meanwhile, the development of a HS-AD model for homogenized conditions permitted to condense the experimental knowledge, and further explore the inhibition/acidification mechanisms. As a main novelty, the HS-AD model was calibrated using one batch experiment for monodigestion and two semi-continuous experiments, one for mono-digestion and other for codigestion of OFMSW and sawdust. Particularly, the model calibration indicated that the maximum growth rates of acetogens and methanogens are up to one order of magnitude lower than the values proposed in the Anaerobic Digestion Model No.1 (ADM1) for thermophilic conditions (55°C), corroborating the high risk of HS-AD acidification in case of overload. The model calibration also suggested that the NH<sub>3</sub> inhibition might be more important for hydrogenotrophic than acetoclastic methanogens. Moreover, the calibration permitted to highlight some of the main aspects requiring further assessment for HS-AD simulations, as the 'non-ideal' bio-physical-chemical corrections, the variable stoichiometry and the mass transfer effects. These developments will enhance the predictive capabilities of the HS-AD model for OFMSW treatment.

**Keywords**: HS-AD Model; ADM1; OFMSW; Batch and Semi-Continuous Experiments; Ammonia Inhibition; Acidification.

### A.1. Introduction

Anaerobic digestion (AD) is a well-suited treatment biotechnology for the organic fraction of municipal solid waste (OFMSW), which includes food waste (FW) and green/lignocellulosic waste (GW), allowing the recovery of biogas (i.e.  $CH_4 + CO_2$ ) as a source of renewable energy, and an organic digestate rich in nutrients (e.g. N, P) (De Baere et al., 2013; Karthikeyan et al., 2013). However, the difficulties to start the process at industrial scale and/or the risk of accumulation of inhibitory substances, limit the applicability of AD for OFMSW treatment (Angelidaki et al., 2006; Chen et al., 2008). In particular, the presence of undesirable levels of inhibitors in AD can lead to volatile fatty acid (VFA) accumulation, and even reactor failure by acidification (i.e.  $pH \le 6.0$ ). Among these, the ammonium ion (NH<sub>4</sub><sup>+</sup>) and in particular the free ammonia (NH<sub>3</sub>), jointly defining the total ammonia nitrogen (TAN), and resulting from the protein/amino-acid decomposition, are two of the most important inhibitors in AD of OFMSW (Astals et al., 2018; De Vrieze et al., 2012).

'High-solids' anaerobic digestion (HS-AD) is a specific AD operation at a total solid (TS) content  $\geq 10$  %, permitting to reduce the reactor volume, the water addition and the digestate dewatering, in contrast to 'wet' AD (i.e. TS < 10 %) (Pastor-Poquet et al., 2018). However, HS-AD can be subjected to a greater risk of substrate overload and acidification than 'wet' AD due to the higher organic content, while the inhibition might be exacerbated due to the lower amount of free water available (Abbassi-Guendouz et al., 2012; Benbelkacem et al., 2015). Adding GW to OFMSW is an adequate strategy to increase TS in HS-AD, since the reduced hydrolysis rate alongside the reduced protein content of lignocellulosic materials reduces the TAN and VFA accumulation (Brown et al., 2013; Pastor-Poquet et al., 2018, In Press). Unfortunately, co-digestion can also reduce the methane yield, regarding readily biodegradable substrates (i.e. FW). Therefore, understanding the practical limitations of HS-AD (i.e. overload, inhibition and acidification) is crucial to enhance the overall OFMSW treatment economy.

Batch experiments yield invaluable information about the main operative parameters in HS-AD, such as the initial TS and the inoculum-to-substrate ratio (ISR), being these parameters preliminary indicators of the operational values at industrial scale. The initial TS is related to the TS balance, whereas the ISR (i.e. g VS/g VS) relates to the volatile solids (VS) balance of the inoculum-substrate mixture. In general, TS needs to be maximized, whereas the ISR needs to be minimized, to enhance the specific biogas yield (i.e. mL/L<sub>Reactor Content</sub>). To optimize the TS-ISR pair, different strategies can be followed, such as drying the substrate, centrifuging the inoculum and/or using different OFMSW:GW ratios (Brown et al., 2013; Capson-Tojo et al., 2017; Forster-Carneiro et al., 2008).

Semi-continuous experiments also yield important information about the HS-AD operational parameters, such as the maximum TS, the organic loading rate (OLR) and the hydraulic retention time (HRT). The optimal OLR-HRT pair ensures an adequate waste stabilization, while minimizes the treatment costs. Moreover, the OLR and HRT in HS-AD of OFMSW depend on the substrate TS and biodegradability, since a TS removal up to 80 % occurs, as a consequence of the biogas production (Angelidaki et al., 2006; Mata-Álvarez, 2003). In this line, the reactor content mass/volume often decreases during HS-AD, while the mass/volumetric effluent needs to be relative lower than the influent, to maintain the reactor content mass/volume constant.

Alongside batch and semi-continuous experiments, a mathematical model is required to evaluate the non-linear patterns in the HS-AD bio-physical-chemistry - not easily observed by HS-AD experiments. Moreover, a HS-AD model can serve for HS-AD optimization, similarly to the Anaerobic Digestion Model No. 1 (ADM1) for 'wet' AD (Batstone et al., 2002; Batstone et al., 2015; Xu et al., 2015). ADM1 gathers the main biochemical and physical-chemical mechanisms in AD, including the disintegration, hydrolysis, acidogenesis, acetogenesis and methanogenesis of organic substrates, the ionic equilibrium of VFA, inorganic carbon (i.e.  $HCO_3^{-}$ ) and inorganic nitrogen (i.e.  $NH_4^{+}$ ), and the liquid-gas transfer of CH<sub>4</sub>, CO<sub>2</sub> and H<sub>2</sub>. However, ADM1 was primarily conceived for 'wet' AD, where the reactor content volume (V<sub>Reactor</sub>) can be assumed constant. On the other hand, ADM1 did not consider the effects of a high TS content on the HS-AD bio-physical-chemistry. For example, a higher organic content can result into a higher ionic strength (I, i.e.  $\geq 0.2$  M), promoting 'non-ideal' effects on the HS-AD bio-physical-chemistry (e.g. ionic equilibrium and liquid-gas transfer modifications) (Tait et al., 2012). Therefore, some modifications are required in ADM1 to address the main bio-physical-chemical processes occurring in HS-AD of OFMSW (Pastor-Poquet et al., 2018) [Chapter 6 - Non-ideal Manuscript].

The HS-AD model complexity depends on the envisaged objectives, since HS-AD can be excessively complex compared to 'wet' AD (Batstone et al., 2015; Bollon et al., 2013; Mata-Álvarez, 2003). For example, HS-AD can be highly heterogeneous, showing both acidified and methanogenic zones simultaneously within the reactor. Moreover, at a very high TS (e.g.  $\geq 25$  %), the presence of gas-filled porosity ( $\epsilon$ ) or the reactor content stratification can slow down significantly the metabolite transport. On the other hand, the model complexity depends on the experimental data available, since highly-complex models are usually associated to an elevated number of parameters (over-parameterization) that might not be practically identified (Donoso-Bravo et al., 2011; Gupta et al., 2008). Practical non-identifiability issues arise when the model parameters, including the initial/influent conditions, are strongly correlated among themselves, and only a reduced set of experimental data is available to assess the individual contribution of each parameter on the bio-physicalchemical dynamics. In these cases, different model parameters and/or model structures can lead to similar model outputs. Particularly, ADM1-based models are only partially identifiable in presence of multiple and highly-informative datasets (Batstone et al., 2015).

The objective of this study was to understand and simulate the main effects in the biophysical-chemical mechanisms and kinetics when increasing the TS content in HS-AD of OFMSW. To this aim, batch and semi-continuous experiments were performed, in parallel to the development of a HS-AD model for homogenized (i.e. completely mixed) conditions. The joint development of model and experiments brings new insights about the main instability issues in HS-AD of OFMSW, such as the risk of ammonia inhibition and reactor acidification. Moreover, the comparison between the experimental and simulated values suggests the need for further HS-AD model developments, in order to enhance our understanding about the HS-AD bio-physical-chemical dynamics, the predictive capabilities of the model, and the recovery of renewable energy and nutrients from OFMSW.

### A.2. Methodology

### A.2.1. Experimental Section

The HS-AD experiments used throughout this study consisted on batch experiments [Chapter 3 – Batch Manuscript], as well as semi-continuous experiments (Pastor-Poquet et al., 2018, In Press), both performed at thermophilic conditions (55°C). In both experimental configurations, OFMSW was the main substrate, while beech sawdust was used to simulate the GW addition to OFMSW. OFMSW was also dried at 55°C to increase the TS of some batch experiments. 'Wet' inoculum was obtained from a methanogenic reactor fed with OFMSW, while centrifuged (high-solids) inoculum served to increase simultaneously the TS and ISR of some batch experiments. Six batch experiments, including two sacrifice tests, were performed at different TS, ISR and/or co-digestion ratios [Table A.1]. On the other hand, semi-continuous experiments for mono-digestion of OFMSW or co-digestion of OFMSW and sawdust were started at TS < 5 % and were progressively loaded to reach HS-AD conditions (i.e. TS  $\geq$  10 %). In these semi-continuous reactors, the effluent mass was reduced in comparison to the influent mass to maintain constant the reactor mass content (M<sub>Reactor</sub>).

All the above substrates, inoculum, experiments and physical-chemical analyses performed are summarized in [Chapter 3 – Batch Manuscript] and (Pastor-Poquet et al., 2018, In Press). The physical-chemical analyses included the TS and VS, total Kjeldahl nitrogen (TKN), TAN, pH, carbonate (ALK<sub>P</sub>) and intermediate (ALK<sub>I</sub>) alkalinity, VFA (acetic, propionic, butyric and valeric), chemical oxygen demand (COD), gas volume and

composition (CH<sub>4</sub>, CO<sub>2</sub> and H<sub>2</sub>), mono-valent ions (Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup>), density ( $\rho$ ) and specific weight ( $\rho_s$ ) analyses. The experimental NH<sub>3</sub> was approximated as shown by Capson-Tojo et al. (2017).  $\epsilon$  was obtained as 1 -  $\rho/\rho_s$ . A bio-methane potential (BMP) test for OFMSW and for sawdust were also performed and expressed per unit of substrate VS added (VS<sub>subs</sub>). The methane yield of batch experiments was evaluated as for the BMP. The overall COD conversion was expressed per unit of global VS added (i.e. substrate + inoculum).

### Table A.1 High-solids anaerobic digestion (HS-AD) batch experiments at different inoculum-to-substrate ratio (ISR) and initial total solids (TS) for the organic fraction of municipal solid waste (OFMSW) including in some cases beech sawdust as a co-substrate. Adapted from [Chapter 3 – Batch Manuscript]

| Test No. | Objective                                       | Substrate                   | Inoculum<br>* | ISR<br>(g VS/g<br>VS) | Operational TS<br>(%)                           | Reactor<br>Volume<br>(mL) | Replicates |
|----------|-------------------------------------------------|-----------------------------|---------------|-----------------------|-------------------------------------------------|---------------------------|------------|
| 1        |                                                 |                             |               | 0.5                   | 10.2, 12.6, 15.6,<br>19.2, 23.3, 28.3<br>& 33.6 | 280                       | 3          |
| 2        | Evaluate Main<br>Biodegradability<br>Indicators | OFMSW                       | HS            | 1.0                   | 9.5, 13.6, 18.4 &<br>24.0                       | 280                       | 3          |
| 3        |                                                 |                             |               | 1.5                   | 10.8, 13.4, 16.4<br>& 19.6                      | 160                       | 3          |
| 4        |                                                 | Dried<br>OFMSW +<br>Sawdust | HS            | 0.2                   | 10.0, 15.0, 20.0,<br>24.7 & 30.2                | 280                       | 3          |
| 5        | Evaluate Main                                   | Dried<br>OFMSW              | HS            | 1.0                   | 15.0                                            | 280                       | 15         |
| 6        | HS-AD<br>Dynamics                               | Dried<br>OFMSW +<br>Sawdust | HS            | 0.6                   | 19.4                                            | 280                       | 15         |
| -        | Evaluate                                        | OFMSW                       | W             | 2.0                   | 2.9                                             | 280                       | 6          |
| -        | Maximum<br>Methane Yield                        | Sawdust                     | W             | 1.0                   | 4.1                                             | 160                       | 3          |

\* HS = High-Solids; W = 'Wet' Inoculum

### A.2.2. HS-AD Model

The above batch and semi-continuous experiments eased the development of a HS-AD model for homogenized conditions based on ADM1, as described elsewhere (Pastor-Poquet et al., 2018) [Chapter 6 – Non-ideal Manuscript]. To reduce the HS-AD model complexity, four hypotheses were used: the  $\rho_s$  of solids and solvents (i.e. H<sub>2</sub>O) is constant, mass transfer processes (i.e. diffusion) are negligible,  $\varepsilon$  can be disregarded, and the main bio-physical-chemical reactions occur predominantly in water. These hypotheses were based on a reasoned assessment of the batch/semi-continuous experimental results, in order to keep the model as simple as needed, but also as informative as possible, regarding the effects of a TS increase in HS-AD for OFMSW.

The HS-AD model accounts for the reactor content mass/volume modification from biogas production. Thus, the global, solids and inert mass balances were implemented as a function of the biogas outflow, allowing also the simulation of the TS and VS dynamics. All soluble compounds were associated to the concentration effect of a high-TS-content matrix via apparent concentrations (i.e. kmol/m<sup>3</sup> H<sub>2</sub>O) [Equation A.1] (Pastor-Poquet et al., 2018). With these modifications, the HS-AD model can simulate indistinctly high-solids and 'wet' AD applications, and the transition between these operational regimes. A 'non-ideal' bio-physical-chemical module was subsequently coupled to the HS-AD model, to simulate the main effects of a high I (i.e. > 0.2 M) upon the main ionic equilibriums [Chapter 6 – Non-Ideal Manuscript]. Precipitation and ion pairing were not included to keep simple the 'non-ideal' routine.

$$S_{T,i,App}\left(\frac{kg \text{ or } kmol}{m^3 \text{ Solvent}}\right) = \frac{S_{T,i}\left(\frac{kg \text{ or } kmol}{m^3 \text{ Total}}\right)}{(1 - TS)\left(\frac{kg \text{ Solvent}}{kg \text{ Total}}\right)} \cdot \frac{\rho_{Solvent}\left(\frac{kg \text{ Solvent}}{m^3 \text{ Solvent}}\right)}{\rho_{Global}\left(\frac{kg \text{ Total}}{m^3 \text{ Total}}\right)}$$
(A.1)

### A.2.3. Kinetic Rates

The original ADM1 biochemical rates (Batstone et al., 2002) were used for model verification, while some modifications were needed to fit the experimental data by calibration [Table A.2]. First, carbohydrates were split between rapidly ( $X_{ch}$ ) and slowly ( $X_{ch,slow}$ ) biodegradable carbohydrates, to simulate the slower hydrolysis of lignocellulosic substrates. Secondly, the valerate uptake was assumed to be carried by valerate degraders

 $(X_{c5})$ , instead of butyrate and valerate being jointly degraded by butyrate degraders  $(X_{bu})$ . Finally, the reversible (non-competitive) inhibition by soluble NH<sub>3</sub> (S<sub>nh3</sub>) [Equation A.2] was removed from the acetic acid uptake, while a double-threshold inhibition by S<sub>nh3</sub> [Equation A.3] and a double-threshold inhibition by TS [Equation A.4] were added to the hydrogen (H<sub>2</sub>) uptake.

| D                                              | Biochemical Rate (r <sub>j</sub> , kg COD m <sup>-3</sup> d <sup>-1</sup> )                                                                                    |                                                                                                                                                                                                       |  |  |
|------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|--|
| Process                                        | ADM1                                                                                                                                                           | This study                                                                                                                                                                                            |  |  |
| Hydrolysis of Readily Biodeg.<br>Carbohydrates | $k_{h,ch} {\cdot} X_{ch}$                                                                                                                                      | kh,ch' Xch,fast                                                                                                                                                                                       |  |  |
| Hydrolysis of Slowly Biodeg.<br>Carbohydrates  | -                                                                                                                                                              | $k_{h,ch,slow} {\cdot} X_{ch,slow}$                                                                                                                                                                   |  |  |
| Hydrolysis of Proteins                         | $k_{h,pr}$ · $X_{pr}$                                                                                                                                          | $k_{h,pr} \cdot X_{pr}$                                                                                                                                                                               |  |  |
| Hydrolysis of Lipids                           | $k_{h,li}$ ·X <sub>li</sub>                                                                                                                                    | $k_{h,li} \cdot X_{li}$                                                                                                                                                                               |  |  |
| Sugar Uptake                                   | $\begin{array}{c} k_{m,su} \cdot S_{su,App} / (K_{s,Xsu} + S_{su,App}) \cdot X_{su} \cdot I_{pH} \\ \cdot I_{in} \end{array}$                                  | $\substack{k_{m,su}\cdot S_{su,App}/(K_{s,Xsu}+S_{su,App})\cdot X_{su}\cdot I_{pi}\\\cdot I_{in}}$                                                                                                    |  |  |
| Aminoacid Uptake                               | $\begin{array}{l} k_{m,aa} \cdot S_{aa,App} / (K_{s,Xaa} + S_{aa,App}) \cdot X_{aa} \cdot I_{pH} \\ \cdot I_{in} \end{array}$                                  | $\substack{k_{m,aa}\cdot S_{aa,App}/(K_{s,Xaa}+S_{aa,App})\cdot X_{aa}\cdot I_{p}}{\cdot I_{in}}$                                                                                                     |  |  |
| LCFA Uptake                                    | $k_{m,fa} \cdot S_{fa} / (K_{s,Xfa} + S_{fa}) \cdot X_{fa} \cdot I_{pH} \cdot I_{in} \cdot I_{h2}$                                                             | $k_{m,fa} \cdot S_{fa}/(K_{s,Xfa}+S_{fa}) \cdot X_{fa} \cdot I_{pH} \cdot I_{in} \cdot I_{h2}$                                                                                                        |  |  |
| Valerate Uptake                                | $\begin{array}{l} k_{m,c5} \cdot S_{va,App} / (K_{s,Xc5} + S_{va,App}) \cdot X_{c5} \cdot I_{pH} \\ \cdot I_{in} \cdot I_{h2} \end{array}$                     | $\begin{array}{c} k_{m,c5} \cdot S_{va,App} / (K_{s,Xc5} + S_{va,App}) \cdot X_{c5} \\ \cdot I_{pH} \cdot I_{in} \cdot I_{h2} \end{array}$                                                            |  |  |
| Butyrate Uptake                                | $\begin{array}{l} k_{m,c4} \cdot S_{bu,App} / (K_{s,Xc4} + S_{bu,App}) \cdot X_{c4} \cdot I_{pH} \\ \cdot I_{in} \cdot I_{h2} \end{array}$                     | $\begin{array}{c} k_{m,c4} \cdot S_{bu,App} / (K_{s,Xc4} + S_{bu,App}) \cdot X_{c4} \\ \cdot I_{pH} \cdot I_{in} \cdot I_{h2} \end{array}$                                                            |  |  |
| Propionate Uptake                              | $k_{m,pro} \cdot S_{pro,App} / (K_{s,Xpro} + S_{pro,App}) \cdot X_{pro} $<br>$\cdot I_{pH} \cdot I_{in} \cdot I_{h2}$                                          | $k_{m,pro} \cdot S_{pro,App} / (K_{s,Xpro} + S_{pro,App}) \cdot X_{pro} \cdot I_{pH} \cdot I_{in} \cdot I_{h2}$                                                                                       |  |  |
| Acetate Uptake                                 | $k_{m,ac} \cdot S_{ac,App} / (K_{s,Xac} + S_{ac,App}) \cdot X_{ac} \cdot I_{pH} $<br>$\cdot I_{in} \cdot I_{nh3}$                                              | $k_{m,ac} \cdot S_{ac,App} / (K_{s,Xac} + S_{ac,App}) \cdot X_{ac} \cdot I_{pi}$                                                                                                                      |  |  |
| Hydrogen Uptake                                | $\begin{array}{c} k_{m,h2} \! \cdot \! S_{h2,App} \! / \! (K_{s,Xh2} \! + \! S_{h2,App}) \! \cdot \! X_{h2} \! \cdot \! I_{pH} \\ \cdot \! I_{in} \end{array}$ | $\begin{array}{c} k_{m,h2} \!\cdot \! S_{h2,App} \! / \! (K_{s,Xh2} \!\!+ \!\! S_{h2,App}) \!\cdot \! X_{h2} \\ \!\cdot \! I_{pH} \!\cdot \! I_{in} \!\cdot \! I_{nh3} \!\cdot \! I_{TS} \end{array}$ |  |  |
| Sugar Degraders Decay                          | $k_d \cdot X_{su}$                                                                                                                                             | $k_{d} \cdot X_{su}$                                                                                                                                                                                  |  |  |
| Aminoacid Degraders Decay                      | $k_d \cdot X_{aa}$                                                                                                                                             | $k_d \cdot X_{aa}$                                                                                                                                                                                    |  |  |
| LCFA Degraders Decay                           | $k_d \cdot X_{fa}$                                                                                                                                             | $k_d \cdot X_{fa}$                                                                                                                                                                                    |  |  |
| Valerate Degraders Decay                       | -                                                                                                                                                              | $k_d \cdot X_{c5}$                                                                                                                                                                                    |  |  |
| <b>Butyrate Degraders Decay</b>                | $k_d \cdot X_{c4}$                                                                                                                                             | $k_d \cdot X_{c4}$                                                                                                                                                                                    |  |  |
| Propionate Degraders Decay                     | $k_d \cdot X_{pro}$                                                                                                                                            | $k_d \cdot X_{pro}$                                                                                                                                                                                   |  |  |
| Acetate Degraders Decay                        | $k_d$ · $X_{ac}$                                                                                                                                               | $k_d \cdot X_{ac}$                                                                                                                                                                                    |  |  |
| Hydrogen Degraders Decay                       | kd·Xh2                                                                                                                                                         | kd·Xh2                                                                                                                                                                                                |  |  |

Table A.2 Biochemical rates used in the high-solids anaerobic digestion model

$$I_{nh3} = \frac{K_{i,nh3}}{K_{i,nh3} + S_{nh3,App}}$$
(A.2)

$$If S_{nh3,App} \ge S_{nh3,min}, \ I_{nh3} = e^{\left(-2.77259 \cdot \left(\frac{S_{nh3,App} - S_{nh3,min}}{S_{nh3,max} - S_{nh3,min}}\right)^2\right)} \ else \ I_{nh3} = 1$$
(A.3)

$$If TS \geq TS_{min}, \ I_{TS} = e^{\left(-2.77259 \cdot \left(\frac{TS - TS_{min}}{TS_{max} - TS_{min}}\right)^2\right)} \ else \ I_{TS} = 1$$
(A.4)

### A.2.4. Model Verification

The 'ideal' and 'non-ideal' mathematical implementation of the HS-AD model was verified for a continuous operation example, using simulated OFMSW at different influent TS (i.e. 10-40 %) as substrate [Chapter 6 – Non-Ideal Manuscript]. To compensate the organic removal from biogas production, while stabilizing the continuous operation at steady-state, a proportional controller was added for the volumetric effluent ( $Q_{Effluent}$ ) (Pastor-Poquet et al., 2018).

### A.2.5. Model Calibration

The full model calibration was outside the scope of this study. Noteworthy, due to the high number of parameters (i.e. > 30) and initial/influent conditions (i.e. > 20), the HS-AD model needs to be calibrated in presence of extensive experimental data to avoid parameter practical non-identifiability, as also discussed in sections A.3.4 and A.3.5. Instead, the HS-AD model calibration served to thoroughly validate the hypotheses used for model construction (e.g. mass balances), permitting also to assess the effect of ammonia build-up in batch and semi-continuous HS-AD of OFMSW. Moreover, specific areas of the HS-AD model requiring further development could be highlighted. To this aim, the kinetic rates were modified as shown in section A.2.3, whereas a potential set of initial conditions and model parameters was set by trial-and-error to match the experimental data available.

The experimental data used to calibrate the HS-AD model consisted of one sacrifice experiment using mono-digestion of OFMSW [Test 5, Table A.1], and two semi-continuous experiments; one for mono-digestion of OFMSW, and other for co-digestion of OFMSW and sawdust. The experimental data included the TS, VS, pH, COD, TKN, TAN, VFA, mono-valent ions, biogas production and composition. The M<sub>Reactor</sub> and the influent/effluent masses were available also for semi-continuous experiments.

The biochemical parameters for thermophilic AD were extracted from Batstone et al. (2002). Some of these parameters were subsequently modified to fit the experimental data [Table A.3]. Importantly, the same biochemical parameters were used for all calibration datasets. Table A.4 shows the initial reactor contents and substrate compositions. These compositions were based on the experimental data available (e.g. BMP, TS, COD). Meanwhile, the initial biomass content was also approximated by trial-and-error, since some of these (e.g.  $X_{ac}$ ,  $X_{h2}$ ) seemed strongly correlated to the biochemical parameters under study. The semi-continuous influent/effluents were induced as 40-minutes input steps, to simulate the effect of the manual operation on the reactor dynamics.

| Parameter                      | ADM1 (55°C) | This Study | Units                  |
|--------------------------------|-------------|------------|------------------------|
| K <sub>h,ch</sub>              | 10.000      | 0.060      | d <sup>-1</sup>        |
| $K_{\rm h,ch,slow}$            | -           | 0.001      | d <sup>-1</sup>        |
| $\mathbf{K}_{\mathrm{h,pr}}$   | 10.000      | 0.060      | d <sup>-1</sup>        |
| $K_{h,li}$                     | 10.000      | 0.060      | d <sup>-1</sup>        |
| k <sub>m,c5</sub>              | 30.0        | 3.0        | d <sup>-1</sup>        |
| k <sub>m,c4</sub>              | 30.0        | 2.8        | d <sup>-1</sup>        |
| k <sub>m,pro</sub>             | 20.0        | 5.0        | d <sup>-1</sup>        |
| k <sub>m,ac</sub>              | 16.0        | 4.0        | d <sup>-1</sup>        |
| k <sub>m,h2</sub>              | 35.0        | 14.0       | d <sup>-1</sup>        |
| $\mathbf{k}_{d}$               | 0.04        | 0.10       | d <sup>-1</sup>        |
| K <sub>i,nh3,Xac</sub>         | 0.011       | -          | kmol N m <sup>-3</sup> |
| Snh3,min,Xh2                   | -           | 0.05       | kmol N m <sup>-3</sup> |
| Snh3,max,Xh2                   | -           | 0.10       | kmol N m <sup>-3</sup> |
| $TS_{min,Xh2}$                 | -           | 22         | %                      |
| $TS_{max,Xh2}$                 | -           | 32         | %                      |
| $pH_{LL,ac}$                   | 6.0         | 5.7        |                        |
| $\mathbf{f}_{bu,su}$           | 0.13        | 0.19       | kg COD kg COD-1        |
| $\mathbf{f}_{\mathrm{pro,su}}$ | 0.27        | 0.11       | kg COD kg COD-1        |
| $\mathbf{f}_{\mathrm{ac,su}}$  | 0.41        | 0.60       | kg COD kg COD-1        |
| $\mathbf{f}_{h2,su}$           | 0.19        | 0.10       | kg COD kg COD-1        |
| $\mathbf{N}_{i,subs}$          | -           | 0.001      | kmol N m <sup>-3</sup> |

Table A.3 Modified parameters in this study

| N                           | Batch          |                | TT                  |        |          |                        |
|-----------------------------|----------------|----------------|---------------------|--------|----------|------------------------|
| name                        | Sacrifice Test | Mono-digestion | <b>Co-digestion</b> | OFMSW  | Sawdust  | - Units                |
| $\mathbf{S}_{su}$           | 8.243          | 1.636          | 0.560               | 45.496 | 0.156    | kg COD m <sup>-3</sup> |
| $\mathbf{S}_{aa}$           | 3.010          | 2.029          | 0.205               | 40.079 | 0.153    | kg COD m <sup>-3</sup> |
| $\mathbf{S}_{\mathrm{fa}}$  | 1.980          | 0.304          | 0.089               | 19.903 | 0.327    | kg COD m <sup>-3</sup> |
| $\mathbf{S}_{va}$           | 0.791          | 0.000          | 0.002               | 2.709  | 0.000    | kg COD m <sup>-3</sup> |
| $\mathbf{S}_{bu}$           | 0.500          | 0.875          | 0.247               | 2.049  | 3.408    | kg COD m <sup>-3</sup> |
| $\mathbf{S}_{\mathrm{pro}}$ | 2.059          | 0.713          | 0.745               | 4.884  | 5.549    | kg COD m <sup>-3</sup> |
| $\mathbf{S}_{\mathrm{ac}}$  | 0.103          | 2.417          | 0.021               | 3.186  | 0.257    | kg COD m <sup>-3</sup> |
| $\mathbf{S}_{h2}$           | 0.000          | 0.000          | 0.000               | 0.000  | 0.000    | kg COD m <sup>-3</sup> |
| $\mathbf{S}_{\mathrm{ch4}}$ | 0.000          | 0.000          | 0.000               | 0.000  | 0.000    | kg COD m <sup>-3</sup> |
| $\mathbf{S}_{\mathrm{ic}}$  | 0.029          | 0.050          | 0.050               | 0.000  | 0.000    | kmol C m-3             |
| $\mathbf{S}_{\mathrm{in}}$  | 0.186          | 0.243          | 0.214               | 0.068  | 0.010    | kmol N m <sup>-3</sup> |
| $\mathbf{S}_{i}$            | 0.000          | 0.000          | 0.000               | 0.000  | 0.000    | kg COD m <sup>-3</sup> |
| $\mathbf{S}_{i,subs}$       | 34.706         | 12.000         | 4.400               | 52.114 | 21.216   | kgCOD m <sup>-3</sup>  |
| $\mathbf{X}_{ch}$           | 32.971         | 1.964          | 0.280               | 36.397 | 15.444   | kg COD m <sup>-3</sup> |
| $X_{\mathrm{ch,slow}}$      | 0.000          | 0.000          | 7.000               | 63.281 | 460.200  | kg COD m <sup>-3</sup> |
| $\mathbf{X}_{\mathrm{pr}}$  | 27.090         | 6.086          | 1.842               | 40.078 | 7.485    | kg COD m <sup>-3</sup> |
| $\mathbf{X}_{\mathrm{g}}$   | 17.823         | 1.217          | 0.799               | 9.952  | 6.221    | kg COD m <sup>-3</sup> |
| $\mathbf{X}_{\mathrm{su}}$  | 0.200          | 0.200          | 0.100               | 0.000  | 0.000    | kg COD m <sup>-3</sup> |
| $\mathbf{X}_{aa}$           | 0.200          | 0.100          | 0.100               | 0.000  | 0.000    | kg COD m <sup>-3</sup> |
| $\mathbf{X}_{\mathrm{fa}}$  | 0.010          | 0.020          | 0.020               | 0.000  | 0.000    | kg COD m <sup>-3</sup> |
| $X_{c5}$                    | 0.020          | 0.050          | 0.050               | 0.000  | 0.000    | kgCOD m <sup>-3</sup>  |
| $X_{c4}$                    | 0.100          | 0.100          | 0.200               | 0.000  | 0.000    | kg COD m <sup>-3</sup> |
| $X_{\text{pro}}$            | 0.020          | 0.010          | 0.020               | 0.000  | 0.000    | kg COD m <sup>-3</sup> |
| $\mathbf{X}_{\mathrm{ac}}$  | 0.250          | 0.080          | 0.150               | 0.000  | 0.000    | kg COD m <sup>-3</sup> |
| $X_{h2}$                    | 0.070          | 0.200          | 0.200               | 0.000  | 0.000    | kg COD m <sup>-3</sup> |
| $\mathbf{X}_{\mathrm{i}}$   | 0.000          | 0.000          | 0.000               | 0.000  | 0.000    | kg COD m <sup>-3</sup> |
| $X_{i,subs}$                | 86.765         | 6.000          | 11.000              | 52.114 | 1039.584 | kg COD m <sup>-3</sup> |
| $\mathbf{S}_{cat}$          | 0.10           | 0.08           | 0.10                | 0.15   | 0.00     | kmoleq m-3             |
| $\mathbf{S}_{\mathrm{an}}$  | 0.05           | 0.04           | 0.04                | 0.10   | 0.00     | kmoleq m-3             |
| $M_{\mathrm{Global}}$       | 37             | 3910           | 3410                | -      | -        | g                      |
| $\rho_{Global}$             | 1078           | 1000           | 1000                | 1128   | 1300     | kg m <sup>-3</sup>     |
| TS                          | 15.5           | 3.7            | 3.1                 | 27.5   | 93.6     | %                      |
| VS                          | 13.0           | 2.8            | 2.2                 | 25.7   | 92.8     | %                      |
| V <sub>Reactor</sub>        | 280            | 5000           | 5000                | -      | -        | mL                     |

Table A.4 Initial and influent conditions used for simulations

### A.3. Results and Discussion

### A.3.1. Bio-Physical-Chemical Characterization of Substrates and Inoculum

An average composition of the organic substrates and inocula is shown in Table A.5. OFMSW showed TS = 26 % and BMP = 497 NmL CH<sub>4</sub>/g VS<sub>subs</sub>, in agreement with literature values (Angelidaki et al., 2006; Mata-Álvarez, 2003). The 55°C-dried OFMSW showed TS = 92 %. The beech sawdust showed TS = 94 % and BMP = 161 NmL CH<sub>4</sub>/g VS<sub>subs</sub>, in agreement with GW (Brown et al., 2013). Noteworthy, the BMP of sawdust was considerably lower than the BMP of OFMSW, indicating a lower biodegradability of sawdust under anaerobic conditions (Pastor-Poquet et al., 2018, In Press). Moreover, sawdust showed  $\varepsilon = 0.76$  in contrast to the raw OFMSW (i.e.  $\varepsilon = 0.01$ ). The TS of 'wet' and high-solids inocula were 3 and 16 %, respectively.

|                                            | Organic Substrates |                  |               | Inoculum      |                    |  |
|--------------------------------------------|--------------------|------------------|---------------|---------------|--------------------|--|
|                                            | OFMSW              | Dried OFMSW      | Sawdust       | Wet           | <b>High-Solids</b> |  |
| TS <sub>0</sub> (%)                        | $26.3\pm0.1$       | $92.2\pm1.7$     | $93.6\pm0.6$  | $2.8 \pm 1.1$ | $15.6\pm2.0$       |  |
| VS <sub>0</sub> (%)                        | $24.1\pm0.4$       | $85.7\pm1.7$     | $92.9\pm0.3$  | $1.9\pm0.8$   | $12.4\pm1.4$       |  |
| TAN (g N/kg)                               | $1.29\pm0.06$      | $3.45\pm0.09$    | $0.11\pm0.00$ | $3.23\pm0.60$ | $3.24\pm0.65$      |  |
| TKN (g N/kg)                               | $6.50 \pm 1.50$    | $25.45 \pm 1.12$ | $0.67\pm0.45$ | $4.13\pm0.84$ | $8.66 \pm 1.35$    |  |
| COD (g O <sub>2</sub> /g)                  | $0.43\pm0.02$      | $1.38\pm0.09$    | $1.16\pm0.00$ | $0.04\pm0.02$ | $0.21\pm0.05$      |  |
| pН                                         | $4.4\pm0.1$        | $4.4\pm0.2$      | $5.6\pm0.1$   | $8.4\pm0.2$   | $8.4\pm0.5$        |  |
| ALK <sub>P</sub> (g CaCO <sub>3</sub> /kg) | -                  | -                | -             | $11.4\pm0.8$  | $8.7\pm2.4$        |  |
| ALK <sub>I</sub> (g Acetic/kg)             | $0.8\pm0.7$        | $0.7\pm0.6$      | $2.2\pm0.7$   | $7.7\pm1.3$   | $3.5\pm1.5$        |  |
| Specific Weight (g/mL)                     | $1.09\pm0.01$      | 1.43             | 1.30          | 1.00          | $1.08\pm0.02$      |  |
| Density (g/mL)                             | $1.08\pm0.00$      | 0.59             | 0.31          | 1.00          | $1.08\pm0.02$      |  |
| Gas-Filled Porosity, <i>ɛ</i>              | $0.01\pm0.01$      | 0.59             | 0.76          | 0.00          | $0.00\pm0.01$      |  |
| BMP (NmL CH <sub>4</sub> /g VS)            | $497\pm58$         | -                | $161\pm12$    | -             | -                  |  |

 Table A.5 Physical-chemical characterization of organic wastes and inoculum. Adapted from [Chapter 3 – Batch Manuscript] and Pastor-Poquet et al. (2018, In Press)

# A.3.2. Batch Experiments – Dealing with Acidification and Ammonia Inhibition

HS-AD batch experiments for mono-digestion of OFMSW using an ISR of 0.5 (Test 1) and 1.0 g VS/g VS (Test 2), and showing a maximum TS of 33.6 and 24.0 %, respectively, resulted in acidification. With mono-digestion of OFMSW using an ISR = 1.5 g VS/g VS (Test 3), all TS conditions showed methanogenesis, though the maximum TS was 19.6 %. Interestingly, the methane yield standard deviation increased alongside the initial TS in Test 3 [Figure A.1], potentially due to mass transfer effects [Chapter 3 – Batch Manuscript]. With co-digestion of OFMSW and sawdust using ISR = 0.2 g VS/g VS and a maximum TS = 30.2 % (Test 4), methanogenesis succeeded only at TS of 10.0 and 15.0 % TS. The sacrifice test for mono-digestion of OFMSW using an ISR = 1.0 g VS/g VS and TS = 15 % (Test 5) resulted in methane production, while the sacrifice test for co-digestion of OFMSW and sawdust using an ISR = 0.6 g VS/g VS and a TS = 20 % TS (Test 6) resulted in acidification.



**Figure A.1** Batch experiment results obtained during the mono-digestion of the organic fraction of municipal solid waste (OFMSW) using an inoculum-to-substrate ratio of 1.5 g VS/g VS: Chemical oxygen demand (COD) conversion, and total (TAN) and free (NH<sub>3</sub>) ammonia nitrogen at the end of the experiment (day 92)

All these results indicate that the optimum TS-ISR pair showing methanogenesis in HS-AD of OFMSW depends on the overall set of physical-chemical characteristics of the substrate-inoculum mixture (e.g. TS, ALK<sub>P</sub>, biodegradability) [Chapter 3 – Batch Manuscript]. For example, the presence of sawdust in co-digestion permitted to reduce the ISR (e.g. 80 %) in comparison to mono-digestion [Table A.1], due to the slower hydrolysis of lignocellulosic substrates. Meanwhile, the final VFA in mono-digestion of OFMSW using an ISR = 1.5 g VS/g VS (Test 3) increased alongside a higher initial TS [Figure A.1]. This VFA accumulation was likely associated to ammonia inhibition, since both TAN and NH<sub>3</sub> increased progressively at higher TS, due to the lower amount of water available. Therefore, an adequate trade-off must be found between maximizing the TS or the overall substrate biodegradability in HS-AD, since the TS increase can reduce the methane yield of OFMSW via ammonia inhibition.

Unfortunately, due to the inherent complexity of the HS-AD matrix, the overall substrate-inoculum biodegradability, risk of acidification or ammonia inhibition are extremely complex to predict solely with batch experiments. Noteworthy, both NH<sub>4</sub><sup>+</sup> and NH<sub>3</sub> have been reported as inhibitory for acetoclastic and hydrogenotrophic methanogens (Astals et al., 2018; Chen et al., 2008). However, the contribution of each species, as well as the specific inhibitory thresholds, depends on the biomass acclimation and/or some AD operative parameters (e.g. TS, pH). On the other hand, the valerate, butyrate and propionate uptakes might be either affected by ammonia inhibition and/or by thermodynamic constrains associated to the build-up of organic intermediates (i.e. acetate, H<sub>2</sub>) (Batstone et al., 2002; Saady, 2013) [Chapter 6 – Non-Ideal Manuscript]. With all the above, a HS-AD model might help to discern about the different aspects crucially influencing HS-AD of OFMSW.

# A.3.3. Semi-continuous Experiments – Counterbalancing the TS removal

### and VFA accumulation

Semi-continuous experiments showed VFA accumulation as a consequence of substrate overload and the presence of inhibitory compounds in OFMSW (i.e. NH<sub>3</sub>), leading to a progressive drop on the pH and CH<sub>4</sub> content, and the eventual bioprocess failure by acidification (i.e.  $pH \le 6.0$ ) (Pastor-Poquet et al., 2018, In Press). The TS with mono-digestion of OFMSW started at 2.8 % and reached HS-AD conditions (i.e.  $TS \ge 10$  %) only under extreme overloading. The maximum OLR was 8.5 g VS/kg·d, while TAN increased up to 4.9 g N/kg. In short, the high biodegradability and TKN of OFMSW resulted in a rapid TS removal, alongside a rapid TAN build-up, preventing to increase further the TS content with mono-digestion. Importantly, the weekly-averaged mass effluent was averagely 18 % lower

regarding the mass influent in these reactors, permitting to extend relatively the mass retention time. However, this last strategy was not sufficient to fully counteract overloading.

In contrast, the semi-continuous co-digestion of OFMSW and sawdust permitted to increase TS above 30 % before the reactors acidified, being this TS content considerably higher than the lower HS-AD threshold (i.e. 10 % TS). Thus, the lower biodegradability and TKN of sawdust permitted to use a maximum OLR up to 16.0 g VS/kg·d, with a OFMSW:sawdust ratio of 0.4-0.5 g TS/g TS, while TAN was maintained constant around 3.0 g N/kg. Noteworthy, the addition of sawdust lowered also the biogas yield in co-digestion compared to mono-digestion (i.e. 229 vs. 86 L/g VS<sub>subs</sub>, respectively). Meanwhile, despite sawdust reduced the ammonia build-up, an important VFA accumulation was still observed. At this point, assessing further environmental aspects, such as the apparent concentrations [Equation A.1] and the ionic strength (*I*), was considered crucial to correctly understand the HS-AD bio-physical-chemical mechanisms. Moreover, a given degree of  $\varepsilon$  could be observed (though not measured) in the upper layer of co-digestion reactors as TS reached approximately 25 %, due mainly to the  $\varepsilon$  difference between OFMSW and sawdust [Table A.5]. With all the above, the link among the different variables in HS-AD (e.g. TS, pH, TAN, VFA) should be further assessed by a mathematical model.

### A.3.4. HS-AD Model – Condensing the Lessons Learnt

### A.3.4.1. Model Verification for OFMSW treatment

Batch and semi-continuous experiments highlighted some of the most important aspects to be considered in HS-AD simulations for OFMSW treatment, as the organic mass/volume removal from biogas production, the ammonia inhibition, and the risk of reactor acidification. In the same line, the HS-AD model verification examples [Chapter 6 – Non-Ideal Manuscript] required a reduced  $Q_{Effluent}$  (i.e. up to 5 %) to compensate the organic removal, while the apparent concentrations levered up the soluble concentrations in all kinetic rates [Table A.2], and all the acid-base equilibriums were affected by the high *I* (i.e.  $\geq$  0.2 M). Particularly, a lower NH<sub>3</sub> concentration (i.e. up to 50 %) was repeatedly observed under the 'non-ideal' than the 'ideal' HS-AD model implementation, yielding a considerable reduction also in the acetate accumulation (i.e. up to 90 %). On the other hand, the Henry's constant for CO<sub>2</sub> (K<sub>H,co2</sub>) was also reduced (i.e. up to 10 %), exacerbating the CO<sub>2</sub> stripping and the risk of acidification in 'non-ideal' HS-AD model verification. Importantly, the overall set of bio-physical-chemical results in the HS-AD model verification were highly non-linear, indicating a strong effect of the model structure on the parameter calibration.

#### A.3.4.2. Parameter Calibration – Where the HS-AD Model Performed Adequately

The simultaneous simulation of one sacrifice and two semi-continuous experiments required the calibration of 20 biochemical parameters [Table A.3]. These parameters were mainly associated to the hydrolysis (K<sub>h</sub>), maximum growth (k<sub>m</sub>) and biomass decay (k<sub>d</sub>) rates, and the sugar COD fractioning (f<sub>su</sub>). Meanwhile, the lower pH thresholds of the acetate uptake (pH<sub>LL,ac</sub>), but also the upper and lower NH<sub>3</sub> thresholds (S<sub>nh3,max,Xh2</sub> and S<sub>nh3,min,Xh2</sub>, respectively) and upper and lower TS thresholds (TS<sub>max,Xh2</sub> and TS<sub>min,Xh2</sub>, respectively) for the H<sub>2</sub> uptake, served as main inhibitory mechanisms to drive the overall VFA accumulation in all simulations. Particularly, the NH<sub>3</sub> inhibition upon the H<sub>2</sub> uptake [Equation A.3] permitted to reproduce the propionate, butyrate and valerate accumulation predominantly in the sacrifice test, since all these VFA uptakes are associated to H<sub>2</sub> inhibition by thermodynamic constrains [Table A.2]. Importantly, the overall set of modified parameters indicates a considerably slower growth for acetogens and methanogens, in contrast to acidogens, highlighting the elevated risk of acidification within HS-AD of OFMSW in case of substrate overload.

The calibration of these biochemical parameters permitted to simulate all the HS-AD dynamics in the mono-digestion sacrifice [Figure A.2]. In this experiment, the biogas production, pH, TS, VS and TAN were well simulated, though some differences were observed in the VFA simulation (mainly acetate and butyrate). All the other dynamics, such as the biogas composition, COD and  $M_{Reactor}$  were also adequately described (data not shown). Importantly, *I* rose up to 0.6 M, corroborating the need for 'non-ideal' corrections to enhance HS-AD simulations (Tait et al., 2012)[Chapter 6 – Non-ideal Manuscript].

Using the same biochemical parameters, the HS-AD model was also able to reproduce all the semi-continuous dynamics for mono-digestion of OFMSW [Figure A.3] and codigestion of OFMSW and sawdust [Figure A.4], where overloading conditions and the lignocellulosic material addition, respectively, allowed the TS increase against the TAN and VFA accumulation. Thus, the TS, VS and TAN were again well simulated in these reactors, confirming the adequacy of the hypotheses used for the HS-AD model construction. Meanwhile, the simulations also represented adequately the pH and VFA, though some differences were still observed between these experimental and simulated values. More in particular, the differences in the acetate dynamics strongly determined the overall 28 and 33 % difference in the cumulative biogas production for mono-digestion and co-digestion, respectively. Summarizing, all the differences between the experimental and simulated values in this study were predominantly associated to the acetate consumption and the overall VFA accumulation, as further discussed in section A.3.4.4.



**Figure A.2** Experimental and simulated values for the sacrifice test using mono-digestion of the organic fraction of municipal solid waste (Test 6): a) Cumulative biogas production; b) total (TS) and volatile (VS) solids; c) total ammonia nitrogen (TAN), ammonium ion  $(NH_{4^+})$ and free ammonia  $(NH_3)$ ; d) total volatile fatty acids (VFA), in chemical oxygen demand (COD) units; e) pH; and f) ionic strength (I)



**Figure A.3** Experimental and simulated values for the semi-continuous mono-digestion of the organic fraction of municipal solid waste: a) Cumulative biogas production; b) total (TS) and volatile (VS) solids; c) total ammonia nitrogen (TAN), ammonium ion (NH<sub>4</sub><sup>+</sup>) and free ammonia (NH<sub>3</sub>); d) total volatile fatty acids (VFA), in chemical oxygen demand (COD) units; e) pH; and f) ionic strength (I)



**Figure A.4** Experimental and simulated values for the semi-continuous co-digestion of the organic fraction of municipal solid waste and beech sawdust: a) Cumulative biogas production; b) total (TS) and volatile (VS) solids; c) total ammonia nitrogen (TAN), ammonium ion (NH4<sup>+</sup>) and free ammonia (NH3); d) total volatile fatty acids (VFA), in chemical oxygen demand (COD) units; e) pH; and f) ionic strength (I)

## A.3.4.3. The Influence of the HS-AD Model Structure for Simulating the Ammonia Inhibition

Both the apparent concentrations and the 'non-ideal' conditions can significantly influence the kinetic rates and overall dynamics of the HS-AD model, either by modifying the soluble substrate concentration and/or the inhibitory content [Table A.2], as mentioned in section A.3.4.1. Specifically, the average TAN concentrations were 3.9, 3.6 and 3.0 g N/kg, while the corresponding apparent concentrations were 4.2, 3.8 and 3.5 g N/kg H<sub>2</sub>O, for the sacrifice test, the semi-continuous mono-digestion and the semi-continuous co-digestion, respectively. In other words, the apparent TAN concentrations were much closer whether semi-continuous mono-digestion or co-digestion was used, suggesting that co-digestion of OFMSW with GW can be used to increase the TS, but not to significantly alleviate the TAN build-up in HS-AD – as might be concluded by only assessing the HS-AD experimental results (Brown et al., 2013; Mata-Álvarez, 2003; Pastor-Poquet et al., 2018, In Press). In the same line, the average I was 0.34 and 0.32 M for semi-continuous mono-digestion and codigestion, respectively, permitting to alleviate in a similar magnitude the NH<sub>3</sub> build-up in HS-AD with or without GW addition. Therefore, the joint inclusion of both apparent concentrations and 'non-ideal' conditions is crucial to calibrate the HS-AD model, particularly regarding the ammonia inhibition [Chapter 6 – Non-ideal Manuscript].

To correctly evaluate the ammonia inhibition (i.e. by  $NH_4^+$  and/or  $NH_3$ ), the simulation of the pH dynamics was also needed [Figures A.2, A.3 & A.4]. Thus, due to the higher pH, the  $NH_3$  concentration was much higher in the sacrifice than in the semi-continuous simulations: the average  $NH_3$  concentration was 0.74 and 0.13 g N/kg H<sub>2</sub>O for the sacrifice experiment and the semi-continuous reactors, respectively. Importantly, the  $NH_3$ concentration was higher, despite of the *I* in the mono-digestion sacrifice (i.e. 0.48 M) was also higher than the average *I* observed in semi-continuous experiments (i.e. 0.32-0.34 M) – note that an acidifying system releases  $ALK_P$  by  $CO_2$  stripping (Lahav et al., 2002), as an outstanding contributor to the *I* calculations. These results corroborate the non-linear interrelationships existing between the pH, *I* and the  $NH_3$  concentration, and the need for an adequate HS-AD model for their evaluation, since all these variables depend on the overall HS-AD dynamics (e.g. TS, TAN, VFA,  $ALK_P$ ) but also on the particular operational variables (e.g. ISR, OLR, HRT).

Interestingly, simply the addition of  $NH_3$  inhibition upon the  $H_2$  uptake and a much lower  $k_m$  in all VFA and  $H_2$  uptakes [Tables A.2 & A.3], in contrast to the  $NH_3$  inhibition upon the acetate uptake, permitted to reproduce simultaneously all the experimental datasets. In this line, an important acetate accumulation was mainly observed in the semi-continuous experiments, despite the lower  $NH_3$  concentration. These results suggest that the  $NH_3$ inhibition might be stronger for hydrogenotrophic than acetoclastic methanogens. Moreover, these results indicate that there might be other environmental aspects potentially affecting the acetate uptake, as mentioned next.

## A.3.4.4. Main Differences between the Experimental Data and Model Simulations, and Some Potential Reasons

As mentioned in section A.3.4.2., the VFA accumulation defined the main differences between the experimental data and the HS-AD model simulations in this study. The reason relates to the fact that the acetate uptake strongly regulated the biogas production and pH in these simulations, due to the elevated COD flowing through the acetate pathway (i.e. 80 %) and the high influence of the acetoclastic methanogenesis for releasing inorganic carbon (i.e. HCO<sub>3</sub><sup>-</sup>), as main pH buffer in AD.

To simulate the acetate dynamics in all experimental datasets, the acetate uptake was only associated to a slow growth (i.e.  $k_{m,ac} = 4.0 d^{-1}$ ) and the pH inhibition (i.e. down to pH = 5.7). However, neither the acetate accumulation, nor the biogas production, could be perfectly fitted for the initial 30 days of semi-continuous simulations [Figures A.3 and A.4]. On the other hand, the pH drop during the last 30 days in the semi-continuous simulations could not be reproduced without the addition of other inhibition factor(s) in the acetoclastic uptake [Table A.2]. For example, a  $NH_4^+$  double-threshold inhibition in the acetate uptake could simulate the aforementioned pH drop, as well as the overall cumulative biogas production in semi-continuous mono-digestion (data not shown). Nonetheless, with this last strategy, the soluble acetate could not be correctly simulated simultaneously in the sacrifice and mono-digestion experiments, since both the NH4<sup>+</sup> concentration during the initial 20 days in the sacrifice test and the last 30 days of the semi-continuous mono-digestion were around 4.0 g N/kg [Figures A.2 and A.3]. Therefore, other environmental aspects could be influencing the acetate uptake in HS-AD of OFMSW, such as the lack of essential nutrients (e.g. phosphorous, trace elements), the presence of further inhibitory compounds (e.g. Na<sup>+</sup>), the existence of different acetoclastic populations, and/or a given degree of variable stoichiometry (Chen et al., 2008; De Vrieze et al., 2012; Jabłonski et al., 2015; Mata-Álvarez, 2003; Saady, 2013).

The maximum growth rates of VFA/H<sub>2</sub> degraders being substantially lower than the proposed values in ADM1 [Table A.3] permit to speculate about the lack of nutrients and/or the presence of further inhibitory compounds reducing the HS-AD kinetics. For example, these low  $k_m$  values could be associated to a simultaneous NH<sub>3</sub> and NH<sub>4</sub><sup>+</sup> inhibition (Astals et al., 2018) – though more parameters would need to be included (and calibrated), exacerbating the model over-parameterization. On the other hand, the hypothesis of variable stoichiometry is also likely, since the H<sub>2</sub> uptake inhibition (triggering H<sub>2</sub> accumulation) and/or the low pH levels observed might drive the COD fractioning of complex substances

(e.g. sugars, proteins) away from acetate (Saady, 2013). This last hypothesis would be also supported by the fact that a fixed sugar-to-butyrate fractioning in this study (i.e.  $f_{bu,su} = 0.19$ ) could not represent simultaneously the butyrate build-up in the sacrifice and semi-continuous experiments. All the above hypotheses need to be tested with further experimental data and/or HS-AD model configurations, to discern about the main factors driving the VFA accumulation and risk of reactor acidification in HS-AD of OFMSW.

### A.3.5. Future HS-AD Model and Data Requirements

The HS-AD model for homogenized reactors is an adequate tool to simulate OFMSW with a medium-to-low proportion of GW (i.e. TS  $\leq$  25-30 %), since an elevated substrate biodegradability often results into a considerably lower TS within the digester (e.g. 10 %), fulfilling the hypotheses used for model construction. However, further HS-AD model developments are strongly encouraged to understand the intricacies of HS-AD for OFMSW treatment.

Along this study, the main aspects determining the HS-AD experiments and simulations for the anaerobic degradation of OFMSW were the ammonia and pH inhibition, but also the high *I*, the CO<sub>2</sub> stripping, the COD fractioning and the mass transfer effects. Thus, some guidelines can be proposed to ease further HS-AD model developments. First of all, the elevated *I* (i.e.  $\geq 0.5$  M) might require a much complex bio-physical-chemical module, specifically including precipitation and ion-pairing mechanisms, to address the inherent complexity of HS-AD [Chapter 6 – Non-Ideal Manuscript]. In particular, the bio-physicalchemistry of multivalent ions (e.g. HPO<sub>4</sub><sup>2-</sup>, Ca<sup>2+</sup>) can be crucial, due to the high influence of these species in the *I* calculations (Batstone et al., 2015; Tait et al., 2012). These mechanisms might enhance the pH, TAN and biogas production/composition simulations, while helping to discern about the relative importance of the NH<sub>3</sub> and/or NH<sub>4</sub><sup>+</sup> inhibition in HS-AD of OFMSW.

Understanding the effects of ammonia inhibition is essential to avoid the VFA accumulation in HS-AD of OFMSW. Among the most important aspects to be correctly simulated is the methanogenesis inhibition by  $NH_3$  and pH, as shown in section A.3.4.4, including the potential adaptation of different methanogenic populations to the modification of the environmental conditions (e.g. pH, *I*,  $NH_3$ ). Moreover, the relative effect of  $NH_3$  and/or  $H_2$  inhibition must be also studied in depth for acetogens, since these populations strongly influence the acetate production and the biogas production/composition. In the same line, the elevated organic content and inherent risk of reactor acidification might require also to understand the phenomena of variable stoichiometry, since these mechanisms can strongly

regulate the VFA composition/dynamics alongside thermodynamic constrains. Similarly, correctly addressing the mass transfer effects in HS-AD can be crucial to simulate acidogenic-methanogenic zones and the resistance against acidification under overloading conditions.

To simulate mass transfer effects, the volumetric dynamics of solids, liquids and gases (i.e.  $\varepsilon$ ) should be adequately considered, since the organic intermediate transport can be significantly reduced through solid and gaseous, in contrasts to liquid media (Bollon et al., 2013; Xu et al., 2015). These mechanisms are predominantly related to the viscosity and pressure as some of the main variables, and depend on the TS content and the substrate composition, but also the existence of homogenising devices (i.e. stirrer, recirculation). For example, rising the TS content over 25 % in semi-continuous co-digestion, a given degree of  $\varepsilon$  was observed, due both to the low water in the HS-AD reactor and the structuring properties of lignocellulosic materials. In this study, the TS inhibition [Equation A.4] upon the H<sub>2</sub> uptake permitted to approximate the VFA accumulation during the last 30 days of co-digestion sacrifice [Figure A.4]. Nonetheless, the correct simulation of mass transfer limitations might require computational fluid dynamics (CFD) and, more in particular, the integration of the biochemistry, physical-chemistry and rheology/hydrodynamic mechanisms within HS-AD simulations (Batstone et al., 2015; Sadino-Riquelme et al., 2018).

To end up, extending the model complexity, as well as assessing the suitability of different model structures to simulate HS-AD of OFMSW, requires extensive target-oriented experimental data to test further hypotheses, while avoiding parameter non-identifiability (Donoso-Bravo et al., 2011; Gupta et al., 2008). The experimental data required would be associated to the different HS-AD dynamics (i.e. biochemistry, physical-chemistry, rheology) to be simulated, as well as the initial conditions, as mentioned thorough this study. Ideally, further HS-AD model and experimental setups developments should be conducted in parallel, as in this study, to ponder simultaneously the intricacies/limitations of both approaches, while posing realistic hypotheses to address the interrelationship between the required model complexity and the experimental data available.

### A.4. Conclusions

HS-AD experiments were conducted in parallel to the development of a HS-AD model, to evaluate the main effects of increasing the TS content in HS-AD of OFMSW. The experimental assessment indicated that a high TS exacerbates the risk of ammonia inhibition and reactor acidification, whereas permitted to pose a preliminary set of hypotheses to deal with the inherent complexity of HS-AD simulations. The HS-AD model calibration highlighted the importance of NH<sub>3</sub> inhibition upon the hydrogenotrophic methanogens, and the strong interrelationship among the pH, *I* and NH<sub>3</sub> concentration in HS-AD of OFMSW. Therefore, to further enhance the understanding and optimize HS-AD, the precipitation, variable stoichiometry and mass transfer mechanisms should be also simulated.

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