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# THÈSE

Pour obtenir le grade de  
Docteur

**Délivré par Université de Montpellier**

**Préparée au sein de l'école doctorale GAIA  
Et de l'unité de recherche Laboratoire de Biotechnologie de  
l'Environnement (INRA, LBE)**

**Spécialité : APAB - Agroressources, Procédés, Aliments,  
Bioproduits**

**Présentée par Afifi AKHIAR**

**Caractérisation de la fraction liquide des digestats issus de la  
codigestion de résidus solides**

**Characterization of liquid fraction of digestates after solid-  
liquid separation from anaerobic co-digestion plants**

**Soutenance prévue le 26/04/2017 devant le jury composé de**

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## Résumé étendu

Les besoins en énergie et en électricité représentent un des besoins quotidiens les plus importants du monde moderne. Les sources énergétiques actuelles, essentiellement fossiles et nucléaires, conduisent à une augmentation des émissions de gaz à effet de serre, au réchauffement et au changement climatique, à des problèmes environnementaux et à des risques pour la santé accrues. La production d'énergie renouvelable, qui couvre actuellement de 23% de la demande énergétique mondiale, est l'option la plus durable pour pallier au réchauffement climatique (Hussain et al., 2017). De plus, lorsqu'ils ne sont pas correctement gérés, les déchets issus des activités humaines ainsi que les résidus animaux et végétaux peuvent générer, pendant leur décomposition, du méthane un gaz à effet de serre puissant.

La digestion anaérobie fait partie des solutions permettant la production d'énergie et le contrôle du réchauffement climatique. En effet, outre l'application au traitement des eaux usées, la digestion anaérobie est une technologie dont l'application est croissante pour produire de l'énergie à partir de déchets solides. Elle a le double avantage de constituer un procédé durable pour la gestion des déchets et de produire de l'énergie renouvelable et un fertilisant tout en réduisant les émissions de gaz à effet de serre (Styles et al., 2016). Dans ce procédé biologique complexe, des microorganismes dégradent la matière organique en absence d'oxygène pour produire du biogaz, composé essentiellement de méthane (55-70%) et de dioxyde de carbone (35-45%). Le biogaz peut être valorisé en chaleur, en cogénération pour la production de chaleur et électricité ou, après purification, en bio-méthane qui peut être utilisé en biocarburant ou être injecté dans le réseau de gaz naturel.

Le secteur de la méthanisation à la ferme ou territoriale connaît un fort développement, entraînant la production de grandes quantités d'un co-produit : le digestat. Ce dernier est une suspension, liquide à épaisse, contenant des composés organiques non dégradés et des microorganismes. Il est enrichi en composés minéraux tels que l'azote, le potassium et le phosphore (Madsen et al., 2011) et est généralement utilisé en tant qu'amendement organique ou fertilisant. L'augmentation drastique du volume de digestat brut produit ces dernières années peut entraîner une surproduction pour une utilisation locale (Kratzeisen et al., 2010). Par conséquent, la séparation solide-liquide du digestat brut est souvent effectuée sur place afin de réduire le coût du transport du digestat (Delzeit and Kellner, 2013).

La fraction solide des digestats est généralement utilisée, directement ou après compostage, en épandage agricole. Elle peut être séchée ou transformée en granules pour être commercialisée sous forme de bio-engrais (Drosg et al., 2015) ou utilisée comme combustible solide (Kratzeisen et al., 2010; Pedrazzi et al., 2015). De nouvelles voies ont été proposées pour la valorisation des digestats solides (Monlau et al., 2015b), comme la production de biochar (Monlau et al., 2015a; Stefaniuk and Oleszczuk, 2015) ainsi que la production de bioéthanol après fractionnement mécanique (Sambusiti et al., 2016).

Lorsque les méthaniseurs sont opérés en voie liquide, la fraction liquide des digestats peut contenir jusqu'à 90-95% de la masse totale des digestats bruts (Sheets et al., 2015). Ils présentent généralement un potentiel de biogaz résiduel très faible (Gioelli et al., 2011), mais une concentration élevée en DCO, en azote total (TN) et en azote ammoniacal ( $\text{NH}_4^+$ ) ainsi que des teneurs importantes en d'autres nutriments (Xia and Murphy, 2016). La fraction liquide des digestats est généralement éliminée par épandage, toutefois les concentrations en TN et  $\text{NH}_4^+$  peuvent limiter son application dans certains sols comme précisé dans la Directive européenne sur les nitrates (Pedrazzi et al., 2015). En effet, leur épandage peut générer des problèmes tels qu'un lessivage de l'azote (Svoboda et al., 2013) ou une infiltration dans les eaux souterraines, polluant les rivières voisines et affectant la vie aquatique. En outre, la DCO élevée des digestats est problématique pour d'autres solutions de traitement telles que des procédés biologiques qu'ils soient installés sur sites ou opérés dans une station de traitement des eaux usées urbaines.

La revue bibliographie a montré que les recherches sur la fraction liquide des digestats concernent essentiellement l'élimination, la récupération et la réutilisation des nutriments avec notamment le stripping de l'ammoniac, l'oxydation anaérobie de l'ammonium (ANAMMOX), ou la cristallisation de la struvite (Sheets et al., 2015) et la culture de microalgues (Franchino et al., 2016). Les composés organiques résiduels ont quant à eux fait l'objet de peu de publications (Ganesh et al., 2013; D. Li et al., 2015a; Xia and Murphy, 2016).

Les principaux objectifs de cette thèse sont :

- 1) Caractériser en détail la fraction liquide des digestats obtenus après séparation solide-liquide d'installations industrielles. Cette fraction liquide comprend des composés résiduels produits pendant la digestion anaérobie et des composés issus des substrats non dégradés

- 2) Etudier le fractionnement par la taille de la fraction liquide des digestats, afin de quantifier la contribution des particules en suspension, des colloïdes et de la fraction de matière dissoute sur les paramètres physico-chimiques et biologiques.
- 3) Evaluer l'origine des composés résiduels en relation avec le type de substrats utilisés, les paramètres de fonctionnement du procédé de digestion anaérobie ainsi que le type de séparation solide-liquide.

Une première partie de cette thèse a été dédiée à la caractérisation détaillée de la fraction liquide des digestats. Pour des raisons pratiques et parce que les performances des procédés de séparation solide-liquide à pleine échelle ne peuvent pas être reproduites à l'échelle du laboratoire, cette étude a été réalisée sur des digestats provenant d'installations de co-digestion de taille industrielle. Onze installations de co-digestion traitant différents types de substrats, avec différents paramètres de procédé et différents procédés de séparation ont été retenus. Les matières sèches (MS), volatiles (MO) et minérales (MM) ont été mesurées dans les digestats bruts et dans leurs fractions solide et liquide respectives. Ceci a permis d'appréhender l'efficacité des techniques de séparation solide / liquide industrielles.

Neuf filtrations successives de la fraction liquide des digestats (100  $\mu\text{m}$ , 41  $\mu\text{m}$ , 10  $\mu\text{m}$ , 1.2  $\mu\text{m}$ , 0.45  $\mu\text{m}$ , 0.2  $\mu\text{m}$ , 100 kDa, 10 kDa et 1 kDa) ont permis de définir 10 fractions : liquide brut, < 100  $\mu\text{m}$ , < 41  $\mu\text{m}$ , < 10  $\mu\text{m}$ , < 1.2  $\mu\text{m}$ , < 0.45  $\mu\text{m}$ , < 0.2  $\mu\text{m}$ , < 100 kDa, < 10 kDa et < 1 kDa. La demande chimique en oxygène (DCO) a été mesurée dans chacune de fractions. Les fractions ont ensuite été regroupées de manière à estimer les particules en suspension (> 1.2  $\mu\text{m}$ ), les colloïdes grossiers (1.2-0.45  $\mu\text{m}$ ), les colloïdes fins (0.45  $\mu\text{m}$  – 1 kDa) et la matière dissoute (< 1 kDa) comme proposé par (Ziyang and Youcai, 2007). L'azote Kjeldahl (NTK) a été mesuré sur chacune de ces quatre fractions et sur la fraction liquide des digestats brute (avant fractionnement). La demande biologique en oxygène à 5 et 21 jours, représentant respectivement les fractions rapidement et lentement biodégradables, a été mesurée sur la fraction liquide brute et sur la fraction < 1.2 $\mu\text{m}$ , c'est-à-dire comprenant les matières colloïdales et dissoutes. Le pH, l'alcalinité, la conductivité, la turbidité, la distribution de taille des particules et le temps de succion capillaire ont été déterminés sur la fraction liquide brute. D'autre part, le carbone organique et inorganique, l'azote ammoniacal, les concentrations en cations et anions, la

fluorimétrie 3 D et les absorbances UV-visible ont été mesurés sur la fraction dissoute (< 1 kDa). Ces dernières analyses ont fourni des indications sur la complexité des molécules dissoutes fluorescentes et ont permis de déterminer le ratio SUVA<sub>254</sub> obtenu à partir de l'absorbance UV spécifique à 254 nm divisée par la concentration de carbone organique total (TOC) dissous, indicateur de la teneur en carbone aromatique dans la matière organique dissoute et du degré d'humification.

Cette étude a ainsi montré que la plus grande partie de la DCO (60 à 96 % de la DCO totale) est constituée par les matières en suspension. En fonction des échantillons, les colloïdes grossiers représentent 0 à 11% de la DCO totale, les colloïdes fins représentent 2 à 16% et les matières dissoutes 2 à 18% de la DCO totale. Par ailleurs, la majeure partie du NTK se trouve soit dans les particules en suspension (11-65%) soit dans la matière dissoute (26-80%). Seulement 0,4-8,5% et 0-13% du NTK se retrouve dans les colloïdes grossiers et les colloïdes fins, respectivement. Le NTK dans la matière dissoute était composé de 62-98% de NH<sub>4</sub><sup>+</sup> et de 2-38% d'azote organique, le NH<sub>4</sub><sup>+</sup> représentant 16 à 72% du NTK total.

Les mesures de DBO<sub>5</sub> et de DBO<sub>21</sub> ont relevé la faible biodégradabilité aérobie de la fraction liquide brute, composée majoritairement de matières en suspension (BDO<sub>5</sub>/DCO ≤ 0,2 pour 10 digestats sur 11 et DBO<sub>21</sub>/DCO ≤ 0,6). De même, les mesures effectuées sur la fraction < 1.2 μm ont montré la faible biodégradabilité des composés colloïdaux et dissous (BOD<sub>5</sub>/DCO < 0,2 pour 9 digestats sur 10 et DBO<sub>5</sub>/DCO ≤ 0,5 pour 8 digestats sur 11). La DCO récalcitrante peut être due à la présence de composés complexes tels que les composés de type acides fulviques, protéines glycolées, mélanoidines et acides humiques, tels que mis en évidence en fluorimétrie 3D.

Pour élargir la base de données des digestats et essayer d'extraire des informations quant à l'origine de leurs composés résiduels présents en phase liquide, dix-huit digestats supplémentaires issus d'installations industrielles de co-digestion et un digestat de boues de station d'épuration ont été caractérisés. Toutefois, le fractionnement a été simplifié, les matières colloïdales grossières et fines ont été regroupées car elles ne représentaient pas une grande fraction des composés des digestats liquides. Ainsi les fractions liquides des digestats ont été fractionnées par 2 filtrations successives (1.2 μm et 1 kDa). Quatre fractions ont ainsi été

analysées : le liquide brut (sans filtration), les particules en suspension ( $> 1,2 \mu\text{m}$ ), les colloïdes ( $1,2 \mu\text{m}$ -1 kDa) et les composés dissous ( $<1 \text{ kDa}$ ).

Les gammes des principaux paramètres mesurés pour les digestats sont résumées dans le tableau 1. D'une manière générale, les fractions liquides des digestats provenant des installations de co-digestion présentent des concentrations très élevées en composés résiduels par rapport à la fraction liquide du digestat de boues activées. Ce point est particulièrement remarquable pour les concentrations en MS, MV, MO, DCO, NTK et  $\text{NH}_4^+$ , ces derniers étant également associés à une alcalinité, une conductivité et une turbidité plus élevées dans des digestats de co-digestion.

**Tableau 1 : Résumé des gammes de variation des principaux paramètres mesurés sur la fraction liquide des digestats**

	<b>Gamme observée pour 29 digestats - codigestion</b>	<b>Digestat de boues</b>	<b>Moyenne sur 30 digestats</b>
<b>MS (g/kg)</b>	7 – 86	$2.11 \pm 0.01$	$43 \pm 25$
<b>MV (g/kg)</b>	3 - 52	$1.15 \pm 0.05$	$26 \pm 16$
<b>MM (g/kg)</b>	4 – 38	$1.0 \pm 0.1$	$17 \pm 10$
<b>MV/MS</b>	35 - 75	55	$58 \pm 9$
<b>DCO totale (g/L)</b>	4 – 90	$1.7 \pm 0.0$	$42 \pm 26$
<b>Matières en suspension (%)</b>	60 -96	82	$80 \pm 18$
<b>Colloïdes (%)</b>	0.9 - 27	6	$8 \pm 6$
<b>Matières dissoutes (%)</b>	2 - 24	12	$12 \pm 17$
<b>NTK (g N/L)</b>	1.3 - 8.3	$1.05 \pm 0.01$	$5 \pm 2$
<b>Matières en suspension (%)</b>	11 - 67	18	$40 \pm 15$
<b>Colloïdes (%)</b>	0.2 - 19	17	$8 \pm 4$
<b>Matières dissoutes (%)</b>	21 - 95	65	$52 \pm 16$
<b><math>\text{NH}_4^+</math> (g/L)</b>	0.5 – 4.7	$0.63 \pm 0.01$	$1.9 \pm 0.9$
<b><math>\text{NH}_4^+</math>/Total NTK (%)</b>	15 - 75	60	$43 \pm 13$
<b><math>\text{K}^+</math> (g/L)</b>	0.5 - 7.1	0.1083	$3 \pm 2$
<b>Alcalinité totale (g/L <math>\text{CaCO}_3</math>)</b>	7.4 - 31.4	$3.70 \pm 0.01$	$18 \pm 8$
<b>Conductivité (S/cm)</b>	12 – 42.9	5	$28 \pm 9$
<b>Turbidité (NTU)</b>	1409 – 67067	947	$26,459 \pm 19,299$
<b>SUVA<sub>254</sub></b>	0.004 – 5.4	0.5	$2 \pm 1$
<b><math>\text{DBO}_5</math> (g <math>\text{O}_2</math>/L)</b>	0.1 - 30.1	$0.4 \pm 0.1$	$8 \pm 6$
<b><math>\text{DBO}_5</math>/DCO</b>	0.03 - 0.43	0.24	$0.2 \pm 0.1$
<b><math>\text{DBO}_{21}</math> (g <math>\text{O}_2</math>/L)</b>	0.4 - 54.2	$1 \pm 0.2$	$19 \pm 13$
<b><math>\text{DBO}_{21}</math>/DCO</b>	0.1 - 0.8	0.55	$0.5 \pm 0.2$

L'analyse en composantes principales (ACP) de l'ensemble des caractérisations des 30 fractions liquides de digestats a montré le fort impact de la technique de séparation. Les différentes techniques ont été classées selon l'indice de séparation défini par (Møller et al., 2000):

$$SI = \frac{[MS]_{solide}}{[MS]_{brut}} * \frac{[MS]_{brut} - [MS]_{liquide}}{[MS]_{solide} - [MS]_{liquide}}$$

Les résultats (tableau 2) ont permis de distinguer les techniques de séparation faiblement performantes (presse à vis, tamis vibrant ou filtre à tambour) des techniques performantes regroupant la centrifugation et diverses techniques assistées par l'ajout de polymères, flocculants et/ou coagulants.

**Tableau 2 : Classement des techniques de séparation selon leur indice de séparation croissant**

	<b>Technique de séparation faible performance</b>	<b>Technique de séparation haute performance</b>
<b>Type de séparation solide liquide</b>	Presse à vis Tamis vibrant Filtre à tambour	Centrifugation Centrifugation avec polymère Centrifugation avec flocculant Presse à vis avec flocculant + centrifugation Presse à vis avec coagulant + centrifugation Filtre presse avec coagulant inorganique et polymère
<b>Index de séparation (%)</b>	0-59	49-94

En ce qui concerne les substrats, la classification hiérarchique montre un groupe clairement lié à la présence de boues dans les substrats. Une analyse plus poussée de l'impact des substrats a été réalisée pour chaque groupe de procédé de séparation solide/liquide. Pour les fractions liquides de digestats séparées par des procédés à haute performance, des sous-groupes ont permis de distinguer les digestats contenant des boues d'épuration, ceux contenant des lisiers porcins et ceux issus de procédés piston thermophiles. Pour les fractions liquides de digestats issues des procédés de séparation faiblement performants, des sous-groupes ont séparés d'une part les digestats de lisiers porcins, de déchets d'alimentation et des industries agro-alimentaires et d'autre part les digestats issus de la co-digestion de divers déchets agricoles et industriels. Parmi les digestats séparés par des procédés faiblement performants, les teneurs en DCO, matières

sèches et volatiles de la fraction liquide ont été corrélées aux proportions en cultures énergétiques et fumiers dans l'alimentation. Finalement, pour l'ensemble des digestats, le paramètre SUVA, lié à la teneur en matières aromatiques des composés, a été corrélé au temps de séjour dans les digesteurs.

L'impact du fumier bovin sur les fortes teneurs en DCO de la fraction liquide des digestats ayant été mis en évidence lors de la première série de caractérisation (chapitre 3), des réacteurs CSTR ont été opérés au laboratoire dans des conditions maîtrisées pendant 49 semaines. L'objectif de cette expérience était de concentrer l'étude sur l'impact du substrat, en particulier celui du fumier bovin et de ses principaux constituants, sur l'origine des composés résiduels de la fraction liquide des digestats.

Un réacteur (R1) a été alimenté avec de la paille de blé, un second (R2) avec de la bouse de vache et les deux derniers (R3 et R4) avec du fumier bovin. Lors des 20 premières semaines, les substrats à teneur élevée en matières sèches (paille et fumier de vache) ont été dilués avec de l'eau du robinet pour alimenter les réacteurs R1, R3 et R4 à une concentration en MS de 15%. Le réacteur R2 a été alimenté avec la bouse de vache non diluée (concentration en MV de 14%). Au cours de la période, la charge organique (OLR) a été augmentée progressivement passant de 0,5 à 2,5 g MV / L / j. En raison de l'accumulation de matières sèches dans les réacteurs alimentés avec le fumier de vache (R3 et R4), la concentration de l'alimentation a été réduite au début de la semaine 21 à 9% MV pour R1 et R4 et à 8% VS pour R2. Les réacteurs ont ensuite été opérés avec une OLR de 2 g MV / L / d pendant 3 fois le temps de rétention hydraulique pour atteindre un état quasi stationnaire. Pour R3, l'alimentation a été arrêtée à la semaine 21 mais l'agitation a été maintenue jusqu'à la semaine 49 pour observer la dégradation des composés lentement biodégradables.

La production de méthane obtenue après stabilisation a montré que la paille avait le rendement en méthane le plus élevé suivi de la bouse de vache et du fumier. Le pH était stable pour les réacteurs alimentés par le fumier et la bouse, alors qu'il a été nécessaire de contrôler le pH dans le réacteur alimenté par de la paille qui diminuait en raison du manque en  $\text{NH}_4^+$ . Dans les réacteurs, les concentrations les plus élevées en MS ont été observées dans le réacteur alimenté avec du fumier suivi de celui alimenté avec de la bouse de vache et enfin celui avec de la paille, indiquant que le fumier avait la biodégradabilité la plus faible. Il est important de noter que le

fumier de vache utilisé dans ce travail avait une faible biodégradabilité et un faible rendement en méthane suggérant qu'il s'agissait d'un fumier âgé qui avait déjà subi une biodégradation aérobie pendant le stockage. Pour le réacteur R3-fumier de vache, l'agitation continue sans alimentation n'a pas modifié de manière significative la composition du digestat indiquant qu'il n'y avait pas de dégradation majeure de la matière organique accumulée au cours de la première période.

La caractérisation de la fraction liquide des digestats après centrifugation (Tableau 3) montre que le digestat de fumier âgé a entraîné les plus fortes concentrations en composés résiduels (exprimés en matière sèches, matières volatiles, DCO et composés azotés), suivi par celui de la bouse de vache et la paille. Les mêmes résultats ont été obtenus lors de l'analyse de la DCO transférée dans la phase liquide par une simple extraction à l'eau de chacun des trois substrats.

**Tableau 3 : Caractérisation des fractions liquides des digestats obtenus l'échelle laboratoire après stabilisation de la mono-digestion de différents substrats.**

	<b>R1-Paille</b>	<b>R2-bouse de vache</b>	<b>R4-Fumier bovin</b>
<b>MS (g/L)</b>	14,5	19,4	44,5
<b>MV (g/L)</b>	10,2	14,9	30
<b>DCO totale (g/L)</b>	15,5	23,6	48,5
<b>Matières en suspension</b>	46%	73%	80%
<b>Colloïdes</b>	35%	16%	13%
<b>Matières dissoutes</b>	20%	11%	7%
<b>DBO<sub>5</sub> (g/L)</b>	3,4	3,6	5,7
<b>DBO<sub>21</sub> (g/L)</b>	6,7	10,4	13,7
<b>DBO<sub>5</sub>/DCO</b>	0,24	0,15	0,12
<b>DBO<sub>21</sub>/DCO</b>	0,44	0,25	0,28
<b>N-NTK (g/L)</b>	N ajouté	1,54	2,68
<b>K<sup>+</sup> (g/L)</b>	0,41	0,37	5,27
<b>Na<sup>+</sup> (g/L)</b>	Na <sup>+</sup> ajouté	0,17	0,7
<b>Cl<sup>-</sup> (g/L)</b>	Cl <sup>-</sup> ajouté	0,62	1

Dans toutes les fractions liquides de tous les mono-digestats, la fraction la plus élevée de DCO était dans les solides en suspension, mais cette fraction était moindre dans le cas de la paille de blé. En revanche, l'extraction à l'eau du fumier de vache a montré une plus forte fraction de DCO dans la fraction colloïdale, qui a été partiellement biodégradée pendant la digestion anaérobie. En ce qui concerne les composés fluorescents extraits des substrats, la paille de blé et le fumier de vache ont présenté une complexité inférieure à celle des extraits de la bouse de vache. Le

changement le plus significatif après la digestion anaérobie a été observé pour le fumier de vache avec une augmentation de la complexité des composés fluorescents.

Par rapport aux digestats de paille et de bouse de vache, la concentration en ion potassium est très élevée dans le digestat de fumier. Ceci est dû à la présence d'urines, riches en potassium, dans les fumiers.

Les fractions liquides des digestats présentent une faible biodégradabilité, en particulier le digestat de fumier avec un ratio  $DBO_5/DCO$  égal à 0,12. Cette valeur est en accord avec les valeurs observées sur les digestats d'installations industrielles de co-digestion dont le fumier bovin représente plus de 50% des substrats.

Finalement, la fraction liquide du réacteur traitant le fumier présentait des teneurs en TS, VS et DCO plus faibles que celles des digesteurs industriels dont le substrat majoritaire est le fumier bovin. Ceci peut s'expliquer par des techniques de séparation différentes. En effet, le digestat des installations industrielles a été séparé par presse à vis alors que la centrifugation a été utilisée à l'échelle du laboratoire.

Ces travaux de thèse démontrent la complémentarité des deux approches utilisées pour étudier la fraction liquide des digestats : i) le prélèvement des digestats sur des installations industrielles et ii) la production de digestats à l'échelle du laboratoire. Chacune des approches présente des avantages et des limites :

A l'échelle du laboratoire, les procédés de séparation par presse à vis, tamis vibrant ou filtre à tambours ne peuvent pas être reproduits. Seule la centrifugation est disponible mais ses performances diffèrent des performances de centrifugation à l'échelle industrielle. Par ailleurs, l'obtention d'un régime stationnaire pour des réacteurs continus requiert plusieurs mois et le nombre de réacteurs en fonctionnement ne peut pas être multiplié. Cependant, la conduite de digesteurs à l'échelle du laboratoire permet de travailler dans des conditions bien maîtrisées et contrôlées.

A l'échelle industrielle, l'obtention d'un grand nombre de digestats d'origines différentes est aisée mais il est très difficile de connaître précisément les paramètres du procédé. En

particulier, si la nature des substrats est relativement bien définie, leur proportion exacte est plus approximative. De plus les flux d'alimentation des digesteurs sont exprimés en matières brutes humides, sans indication des concentrations en matières organiques. Il est alors difficile d'étudier l'impact de la charge organique, paramètre important en méthanisation.

Des pistes de recherche pour l'approfondissement de ces travaux concernent l'étude de la monodigestion des différents substrats, notamment des cultures énergétiques dont la contribution à de fortes teneurs en composés organiques dans la fraction liquide des digestats a été soulignée. L'étude de paramètres du procédé de méthanisation tels que la charge organique ou la recirculation de la fraction liquide du digestat serait également intéressante pour évaluer leur impact sur la composition du digestat. Finalement, la faible biodégradabilité des composés organiques et leur forte proportion sous forme de matières en suspension suggèrent le développement de procédés physico-chimiques de séparation tels que la coagulation pour le traitement de la fraction liquide des digestats.

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## LIST OF ABBREVIATIONS

AD	Anaerobic Digestion
AFW	Agro-food Wastes
Al <sub>2</sub> SO <sub>4</sub>	Sulphate
ANAMMOX	Anaerobic ammonium oxidation
BOD	Biochemical Oxygen Demand
BMP	Bio-methane Potential
C	Carbon
C/N	Carbon/Nitrogen
Ca	Calcium
Ca(OH) <sub>2</sub>	Calcium Hydroxide/Lime
Cer	Cereal residues
CH <sub>4</sub>	Methane
Cl <sup>-</sup>	Chloride
CO <sub>2</sub>	Carbon Dioxide
COD	Chemical Oxygen Demand
C <sub>org</sub>	Organic Carbon
CrR	Crop Residues
CST	Capillary Suction Time
CSTR	Continuous Stirred Tank Reactor
EU	European Union
EnCr	Energy crops
FA	Free Ammonia
Fe <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub>	Ferric Sulphate
FeCl <sub>3</sub>	Ferric Chloride
FOG	Fat, Oil and Grease
FVW	Fruit and vegetable wastes
GHG	Greenhouse Gas
H <sub>2</sub>	Hydrogen
H <sub>2</sub> S	Hydrogen Sulfide
HCA	Hierarchical Cluster Analysis
HPLC	High Performance Liquid Chromatography
HRT	Hydraulic Retention Time
IC	Inorganic Carbon
K <sup>+</sup>	Potassium ion
LCFA	Long Chain Fatty Acid
MBR	Membrane Bioreactor
Mg	Magnesium
Mnr	Manure

MS	Mineral Solids
MSW	Municipal Solid Waste
MW	Megawatt
N <sub>2</sub>	Nitrogen gas
Na <sup>+</sup>	Sodium ion
NaHCO <sub>3</sub>	Sodium Bicarbonate
NaOH	Sodium Hydroxide
NH <sub>3</sub>	Ammonia
NH <sub>4</sub> <sup>+</sup>	Ammonium
NH <sub>4</sub> -N	Ammonia Nitrogen
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	Ammonium Sulphate
NO <sub>2</sub> <sup>-</sup>	Nitrite
NO <sub>3</sub> <sup>-</sup>	Nitrate
O <sub>2</sub>	Oxygen gas
OFMSW	Organic Fraction of Municipal Solid Waste
OLR	Organic Loading Rate
P	Phosphorus
PCA	Principal Component Analysis
PFR	Plug Flow Reactor
PN	Partial Nitrification
PO <sub>4</sub> <sup>3-</sup>	Phosphate
POME	Palm Oil Mill Effluent
RES	Renewable Energy Sources
sCOD	Soluble Chemical Oxygen Demand
Separ. Efficiency	Separation Efficiency
SO <sub>4</sub> <sup>2-</sup>	Sulphate
sP	Soluble Phosphorus
SS	Sewage Sludge
SS-AD	Solid State Anaerobic Digestion
SUVA <sub>254</sub>	Specific Ultraviolet Absorbance at 254nm
TC	Total Carbon
TKN	Total Kjeldahl Nitrogen
TN	Total Nitrogen
TOC	Total Organic Carbon
TP	Total Phosphorus
TS	Total Solids
UV-VIS	Ultraviolet-Visible
VFA	Volatile Fatty Acid
VS	Volatile Solids
WAS	Waste Activated Sludge

## LIST OF PUBLICATION AND COMMUNICATIONS

### PUBLICATION

- 1) Akhlar, A., Torrijos, M., Battimelli, A., Carrère, H., 2017. Comprehensive characterization of the liquid fraction of digestates from full-scale anaerobic co-digestion. *Waste Management* 59, 118–128

### COMMUNICATIONS

- 1) A. Akhlar, A. Battimelli, M. Torrijos, H. Carrère, Characterisation of the liquid fraction of digestate after solid-liquid separation, 6<sup>th</sup> International Conference on Engineering for Waste and Biomass Valorisation, 23-26<sup>th</sup> May 2016, Albi, France. Short oral communication and poster.
- 2) A. Akhlar, A. Battimelli, M. Torrijos, J.P. Steyer et H. Carrère, Caractérisation approfondie de la fraction liquide de digestats industriels générés lors de la séparation liquide/solide pour une meilleure compréhension de l'impact des paramètres du procédé, Journées Recherche Innovation (JRI) biogaz méthanisation, 10-12<sup>th</sup> February 2016, Limoges, France. Oral communication.
- 3) A. Akhlar, A. Battimelli, M. Torrijos, H. Carrère, Characterisation of the liquid fraction of digestate after solid-liquid separation, 14<sup>th</sup> World Congress on Anaerobic digestion, 15-18<sup>th</sup> November 2015, Viña del Mar, Chile. Short oral communication and poster.

## INTRODUCTION

In the modern world, energy demand for electricity is really one of the most important essential of daily needs. Current energy resources which are mainly from fossil fuels and nuclear resources result in an increase of greenhouse gas (GHG) emission, global warming and climate changes, ozone depletion, environmental issues and increased health risks (Houghton, 2011; Hussain et al., 2017). To overcome this, **renewable energy** which is currently 23.7% of the total world energy demand is the best option as global warming solution (Hussain et al., 2017). In addition, when not properly managed, the **wastes** produced by human activities together with animal and plant residues can also generate methane, CH<sub>4</sub>, a powerful GHG during their decomposition.

**Anaerobic digestion** is a solution for energy generation and global warming control (Abbasi et al., 2012). Indeed, in addition to the use for wastewater treatment, anaerobic digestion is more and more used to recover energy from solid waste. It has double advantage of presenting a sustainable process for waste management and both renewable energy and fertilizer production while reducing GHG emission (Styles et al., 2016). It is a process where microorganisms degrade organic matter in the absence of oxygen in a complex biological process to produce biogas, mainly composed of methane (55-70%) and carbon dioxide (35-45%). Biogas can be used as heat, or combined heat and power generation or, after upgrading, bio-methane can be used as biofuel or injected into the natural gas grid.

Several types of **organic wastes** that are commonly used to produce energy by anaerobic digestion are waste activated sludge, municipal solid waste, livestock manure, fruit and vegetable waste, food waste, lignocellulosic biomass and slaughterhouse wastes. **Co-digestion** of more than one substrate is a successful solution to optimize anaerobic digestion of solid waste according to the specific characteristics of the substrates (Mata-Alvarez et al., 2014). For example, co-digestion makes it possible to decrease the nitrogen content of the feed, which can inhibit methanogens during the process, when substrates with high nitrogen content such as animal manures are used. On the other hand, lack of nitrogen in some agro-industrial waste and crop residues (straw for instance) may lead to nitrogen deficiency which can be avoided by the

addition of a nitrogen rich residue such as pig manure (Mata-Alvarez et al., 2014). Anaerobic co-digestion provides more advantage than mono digestion because it provides balanced nutrients (C/N ratio and macro and micronutrients) as well as reduced inhibitor accumulation (D. Li et al., 2015a, 2015b) and offers higher biogas production (Mata-Alvarez et al., 2014).

Anaerobic co-digestion plants has seen a rapid growth as i) biogas production technology; as ii) an alternative for renewable energy and; at the same time as iii) a solution for waste management. Increasing number of anaerobic digestion and co-digestion plants means simultaneous increase of the quantity of the final byproduct, the **digestate** (Kratzeisen et al., 2010). Raw digestate withdrawn from anaerobic digester is a liquid to thick slurry that contains undigested organic matter (Teglia et al., 2011) and a significant quantity of residual compounds produced during anaerobic digestion and micro-organisms; it is enriched in minerals such as nitrogen, potassium and phosphorus (Madsen et al., 2011).

Drastic increase of raw digestate volume produced daily in recent years may cause overproduction of raw digestate for local use (Kratzeisen et al., 2010). Local and regional transportation of excess raw digestate of more than 5-10 km will exceed the costs of its fertilizer value (Kratzeisen et al., 2010) and consume huge amount of fuel oil (Rehl and Müller, 2011). Therefore, **solid-liquid separation** of raw digestate is often performed on-site to reduce the cost of digestate transportation (Delzeit and Kellner, 2013). After separation, raw digestate is divided into a solid fraction and a liquid fraction.

**Solid fraction** of digestates are usually used for land application (Rehl and Müller, 2011) where they can be applied directly or after composting as organic fertilizer (Tambone et al., 2015; Zeng et al., 2015) since they contain high nutrients such as nitrogen and phosphorus (Drosg et al., 2015). Besides, solid fraction of digestates can be dried or palletized and marketed as bio-fertilizers (Drosg et al., 2015) or used as solid fuel (Kratzeisen et al., 2010; Pedrazzi et al., 2015). New routes have been proposed for solid digestate valorization (Monlau et al., 2015b) such as production of biochar (Monlau et al., 2015a; Stefaniuk and Oleszczuk, 2015), as well as bioethanol production after a mechanical fractionation (Sambusiti et al., 2016).

**Liquid fraction** after solid liquid separation represents 90-95% of total mass of digestate for most full-scale liquid anaerobic digestion plants (Sheets et al., 2015). They generally contain very low residual biogas potential (Gioelli et al., 2011) but high concentration of Chemical Oxygen Demand (COD), total nitrogen (TN) and ammonia nitrogen ( $\text{NH}_4^+$ ) as well as other nutrient concentrations (Xia and Murphy, 2016). It is generally eliminated by land application but TN and  $\text{NH}_4^+$  have limits for its application into soils as reported in the European Nitrates Directive (Pedrazzi et al., 2015). When mismanaged, the disposal of liquid fraction of digestates by land application can generate issues such as nitrogen leaching (Svoboda et al., 2013) or infiltration into the groundwater, polluting nearby rivers and affecting aquatic life. Furthermore, high COD causes problems for other treatment solutions such as biological treatments or discharged to wastewater treatment plant.

Several researches on the **nutrients removal, recovery and reuse** for liquid fraction of digestates have been conducted. Some examples of research on nutrients removal, recovery and reuse from liquid fraction of digestates are ammonia stripping, struvite crystallization and anaerobic ammonium oxidation (ANAMMOX) (Sheets et al., 2015). However, the researches were only focused on nutrients and there is little knowledge on organic matter of liquid fraction of digestates.

An example of digestate management at industrial scale is presented in Figure 1 for the co-digestion plant of Auch, France. This plant has an installed capacity of 1.067 MWelec/year and receives 44 000 tons of waste per year. After maturation, the raw digestate undergoes a solid-liquid separation by screw press. The solid fraction produced (12 000 t/y) is eliminated by land application on 3 733 ha. The designers of the plant have chosen quite an advanced solution for the treatment of the liquid fraction with a first phase of centrifugation followed by stripping and by an aerobic treatment in a membrane bioreactor for nitrogen and COD treatment. The treated effluent is then discharged to the municipal wastewater treatment plant of the city of Auch for polishing.

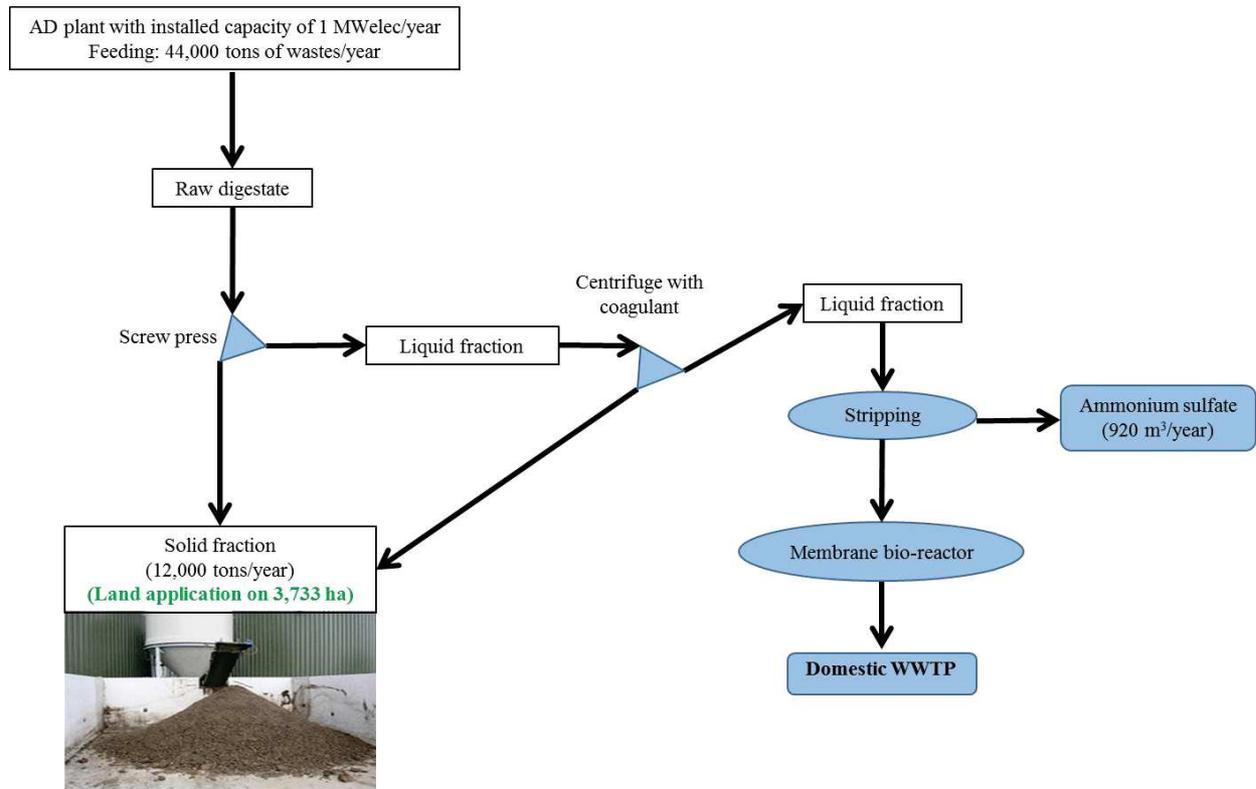


Figure 1: Digestate management at the co-digestion plant of Auch, France (Environnement, 2014)

The design of the plant in Auch has highlighted a general lack of information on the composition of the liquid fraction of digestate after solid liquid separation, in particular with regard to its organic matter composition and concentration making its design problematic and uncertain. Hence, the aim of this work was to bring knowledge on the liquid fraction of digestates which will be useful for both researchers and engineers for the design of innovative treatment solutions. The specificity of the experimental work carried out during this thesis is that it focused on both digestate from industrial-scale plants and laboratory scale experiments in order to have a comprehensive characterization of the liquid fraction of digestates and to better understand the origin of the residual organic matter.

The main **objectives** of this study are:

- 1) To fully characterize liquid fraction of digestates obtained after solid-liquid separation from full-scale co-digestion plants made-up of both the residual compounds produced during anaerobic digestion and the untreated compounds from the substrates.
- 2) To study the size fractionation of the liquid fraction of digestates, to quantify the contribution of suspended particles, colloids and dissolved matter fraction on the physicochemical and biological parameters.
- 3) To try to understand the origin of the residual compounds in relation with the kind of substrates used, operating conditions of the anaerobic digestion process and solid-liquid separation performed.

This thesis consists of 5 **chapters**.

Chapter 1 provides a literature review on anaerobic digestion plants throughout the world, anaerobic digestion processes focusing on co-digestion, digestate characteristics and solid-liquid separation.

Chapter 2 presents materials and methods which include digestate collection, filtration and size fractionation of liquid fraction of digestates from full-scale co-digestion plants, lab-scale reactors to produce digestate from mono-substrate, extraction of mono-substrate with water to identify the compounds already contained in the substrate, analytical methods (chemical, physical and biological) and statistical analysis.

Chapter 3 discusses on full characterization (chemical, physical and biological) and fractionation of liquid fraction of digestates after solid-liquid separation from 11 full scale anaerobic co-digestion plants.

Chapter 4 discusses on simplified characterization of 18 more liquid fraction of digestates from full scale anaerobic co-digestion plants and 1 liquid fraction of digestate from waste activated sludge (WAS). Statistical analysis was performed on the total 30 digestates using principal component analysis (PCA), hierarchical cluster analysis (HCA) and correlation matrix. Such analysis will investigate the relationships between characteristics (chemical, physical and biological) of liquid fraction of digestates in relation to the substrates, operating parameters and type of solid-liquid separation.

Chapter 5 focuses on the impact of mono-substrate on residual compounds produced in liquid fraction of digestate after anaerobic digestion. For this purpose, lab-scale reactors were operated up to 48 weeks and fed by mono-substrate chosen based on the findings from Chapter 3 and Chapter 4. Besides, extraction with water for each mono-substrate chosen will also be discussed in order to understand the residual compounds already present in the substrate.

The outcome and perspective of the findings in this research based on the objectives aimed for this thesis will finally be presented in Conclusion and Perspective.

## **1. Literature Review**

### **1.1. Anaerobic digestion**

Anaerobic digestion is a process where microorganisms degrade organic matter in the absence of oxygen to produce biogas, mainly composed of methane and carbon dioxide. Biogas can be used as heat, or combined heat and power generation or after upgrading bio-methane can be used as biofuel or injected into the natural gas grid. The residual material, called digestate, contains undigested organic matter from the substrate, residual compounds produced during anaerobic digestion and micro-organisms. It is rich in minerals such as nitrogen and phosphorus and can be used as organic fertilizer. Anaerobic digestion has thus the double advantage of presenting a sustainable process for waste management and both renewable energy and fertilizer production while reducing greenhouse gas (GHG) emission (Styles et al., 2016).

### **1.2. Anaerobic digestion industries progress throughout the world**

Recent years have seen a strong development of anaerobic digestion units worldwide, especially in USA, Europe and China (Table 1). USA presents the highest total biogas production but originating from landfill at 75% (Deng et al., 2014). In this country there are 1,497 anaerobic plants in 2013 (Edwards et al., 2015) treating sewage sludge, biowaste, agricultural and industrial wastes. On the other hand, biogas production in Germany is mostly based on agricultural and industrial wastes (Table 1). China, with 26.5 million plants in 2007 (Deng et al., 2014; Mao et al., 2015) produces biogas which mainly originates from sewage treatment (Table 1) but there are about 40 million domestic small biogas plants (Baidya and Ghosh, 2016) and these provide households with gas for cooking and lightening. Such domestic biogas plants are also spread in India (4 millions), Nepal (0.25 million) and the rest of Asia (0.25 million) (Baidya and Ghosh, 2016; Halder et al., 2016; Raheem et al., 2016).

Table 1: Production of biogas in 2013 in USA, Germany, China, Britain, France and Italy  
 adapted from Deng et al (Deng et al., 2014)

	Biogas production (ktoe)	Landfill (%)	Sewage (%)	Others (%)
USA	5095	75	2	23
Germany	4213.4	6.3	9.2	84.5
China	3727.5	24	70	6
Britain	1723.9	85.5	14.5	-
France	526.2	84	8	8

In India, according to Rao et al (Rao et al., 2010), the total installed capacity of energy generation from solid biomass and waste till 2007 was 1227 MW against a potential of 25700 MW from municipal solid wastes, crop residues and agricultural wastes, sewage sludge, animal manure and industrial wastes (distilleries, dairy plants, pulp and paper, poultry, slaughter houses, sugar) which could reduce the energy supply deficit in the country (Rao et al., 2010). In Malaysia, the study of the potential and challenges of anaerobic digestion technology implementation for biogas production from various waste water treatment and waste management industries in Malaysia has been investigated (Kumaran et al., 2016). In the study, Malaysia has the potential of electricity generation capacity of 2135 MW with the reduction of 11.35 Mt of CO<sub>2</sub> equivalent by the year 2020 through anaerobic digestion (Kumaran et al., 2016). The potential substrates for anaerobic digestion plants in Malaysia are palm oil mill effluent (POME), sewage sludge (SS), chicken manure, swine manure, dairy manure, sheep manure, goat manure, banana, animal blood, animal rumen and food waste (Abdeshahian et al., 2016; Hosseini and Wahid, 2013; Kumaran et al., 2016; May et al., 2013; Tock et al., 2010; Umar et al., 2014).

In Africa, anaerobic digestion is still at an early stage even though recent initiatives have shown an increase of interest for the technology (Roopnarain and Adeleke, 2017). National biogas programs have been implemented in Kenya, Uganda, Ethiopia, Tanzania, Rwanda, Cameroon, Burkina Faso and Benin where these countries are seen as the model for startup for other African countries without assistance from other countries (Roopnarain and Adeleke, 2017). The biogas technology in Africa is not just seen as an option for renewable source for electricity but also for solving waste management problems (Roopnarain and Adeleke, 2017). However, there are still problems to overcome for anaerobic digestion implementation in Africa such as requirement for

stronger commitment from the government, more communications, awareness and knowledge to be expanded to the public, government and private sectors as well as more commercialization especially from stakeholders and investors (Bundhoo et al., 2016; Mengistu et al., 2016; Roopnarain and Adeleke, 2017).

In Europe, 17,358 plants were counted in 2015 (European Biogas Association, 2016). Commitment to European Union (EU) requirements to increase renewable energy sources (RES) for electricity by 2020 as well as the high feed-in-tariffs and high state incentives for renewable energies has led to a rapid increase of agricultural anaerobic digestion plants (Frantál and Prousek, 2016; Haas et al., 2011; Martinát et al., 2016; Piwowar et al., 2016; Torrijos, 2016). Therefore, an increase of 65% of biogas plants in Europe can be seen within five years from 2010 to 2015 with an increase of 111% of installed electric capacity (MW) as shown in Figure 2. With more than 10,000 plants (Figure 3), Germany presents the highest biogas production in Europe, the main part originating from agricultural biogas plants (Table 1). This is also similar to Italy with the second highest number of biogas plants in Europe (Figure 3), with 80% biogas plants fed with substrates from agriculture (Torrijos, 2016). In France and the United Kingdom (Britain), biogas production mainly originates from landfills (Table 1), however a growing number of agricultural plants in these countries and the ban of organic waste landfilling might reverse these trends (Torrijos, 2016).

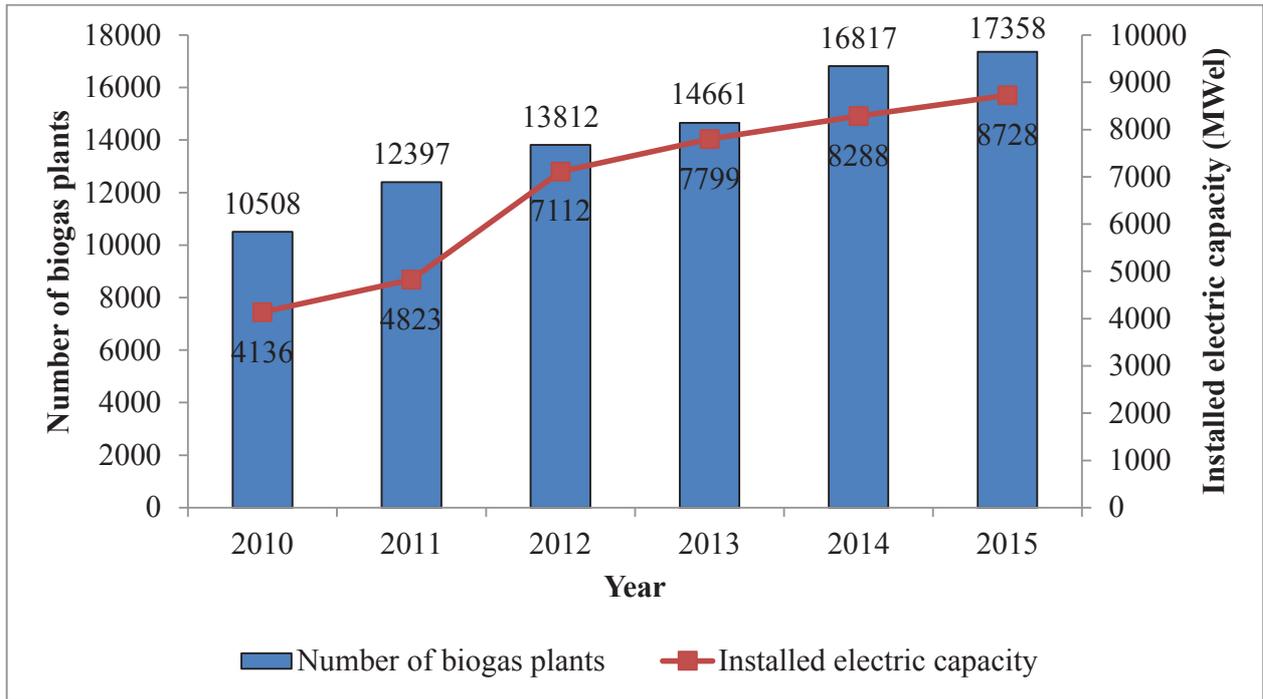


Figure 2: Number of biogas plants in Europe from 2010 to 2015 with installed electric capacity (European Biogas Association, 2016)

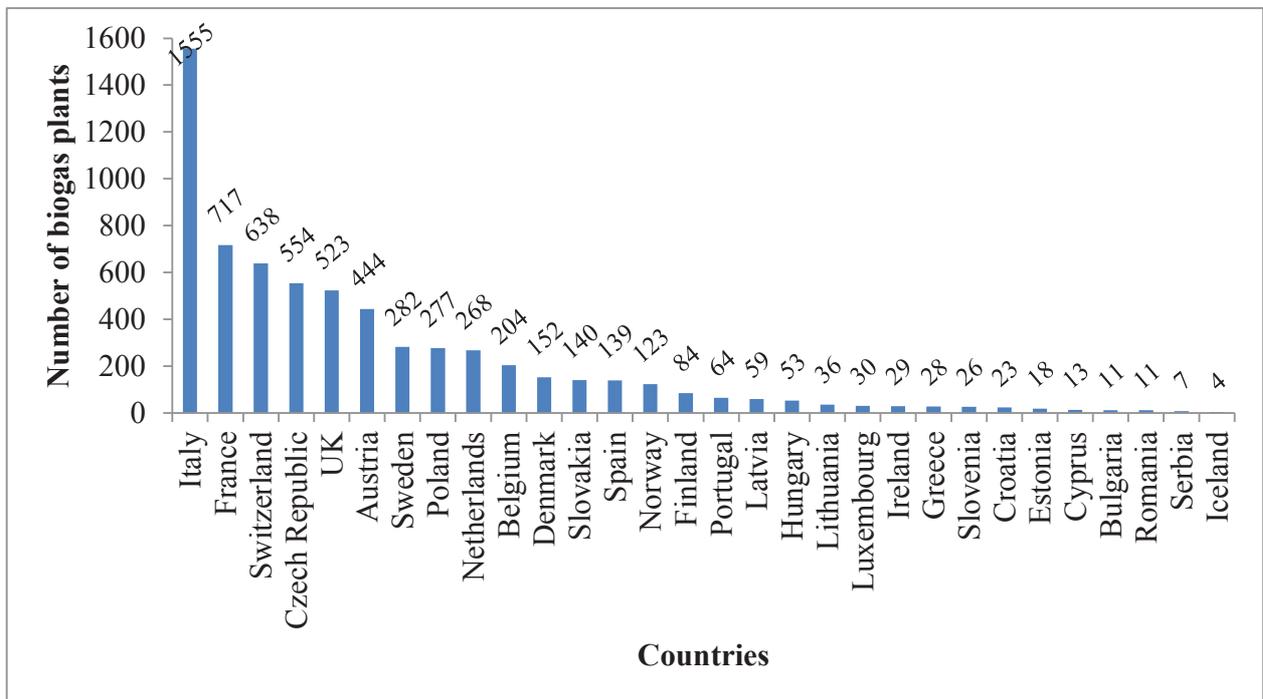


Figure 3: Number of biogas plants in Europe in 2015 excluding Germany with 10846 biogas plants (European Biogas Association, 2016).

Overall, worldwide, the increase of biogas technology implementation and the development of anaerobic digestion shows that this technology is seen as a solution for waste management and as an alternative for renewable energy. More works are on-going to improve the facilities and research in anaerobic digestion. Countries like China, Germany, USA, Italy, UK and France are seen leading in the biogas sector in the world due to long establishment, intensive research and government incentives for renewable energy as well as waste management solution option. Asian countries have also shown their interests in the biogas technology. With more proper research and studies on-going, Asia will see the growth of biogas sector in the next few coming years. Even though African countries are in their primary phase to develop this biogas technology and there are still more hurdles to overcome, there have been interests, development and implementation work to apply this technology for its potential renewable energy production as well as its waste management solution.

### **1.3. Mechanisms in anaerobic digestion**

Anaerobic digestion (AD) undergoes organic matter biodegradation in a complex biological process which takes four main steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis as shown in Figure 4. Each step requires the activity of its own specific group of microorganisms (Appels et al., 2008; Chandra et al., 2012; Kangle et al., 2012; Madsen et al., 2011):

1. **Hydrolysis** – Hydrolysis occurs with hydrolytic microorganisms converting high molecular weight compounds and insoluble organic substrates such as carbohydrates, proteins and lipids into smaller molecular materials and soluble organic substrates such as sugars, amino acids and fatty acids (Appels et al., 2008).
2. **Acidogenesis** – Acidogenesis process occurs after hydrolysis where acidogenic microorganisms convert small molecular materials into volatile fatty acids (VFAs) (e.g., acetic, propionic and butyric) along with the generation of carbon dioxide (CO<sub>2</sub>), hydrogen (H<sub>2</sub>), ammonia (NH<sub>3</sub>), ammonium (NH<sub>4</sub><sup>+</sup>) (Appels et al., 2008).

3. **Acetogenesis** – Acetogenesis process occurs after acidogenesis where acetogens bacteria convert organic acids (VFAs) into acetic acid along with  $H_2$ ,  $CO_2$  (Appels et al., 2008). The mixture of  $CO_2/H_2$  is transformed into acetate through homoacetogenesis (Monlau et al., 2013).
  
4. **Methanogenesis** – The final process, methanogenesis uses methanogenic archaea (mostly *Methanobacteriales* and *Methanosarcinales*) to convert acetic acid,  $H_2$ ,  $CO_2$  into methane ( $CH_4$ ) and carbon dioxide ( $CO_2$ ) (Appels et al., 2008; Guo et al., 2015; Madsen et al., 2011; Yu et al., 2014a). The mixture of  $CO_2/H_2$  is transformed by hydrogenophilic methanogens into methane while acetate is transformed into methane by acetoclastic methanogens (Monlau et al., 2013).

The biogas produced from the four steps of anaerobic digestion consists of mainly methane (55–75%) and  $CO_2$  (25–40%) (Fabbri and Torri, 2016; Monlau et al., 2013; Yang et al., 2015).

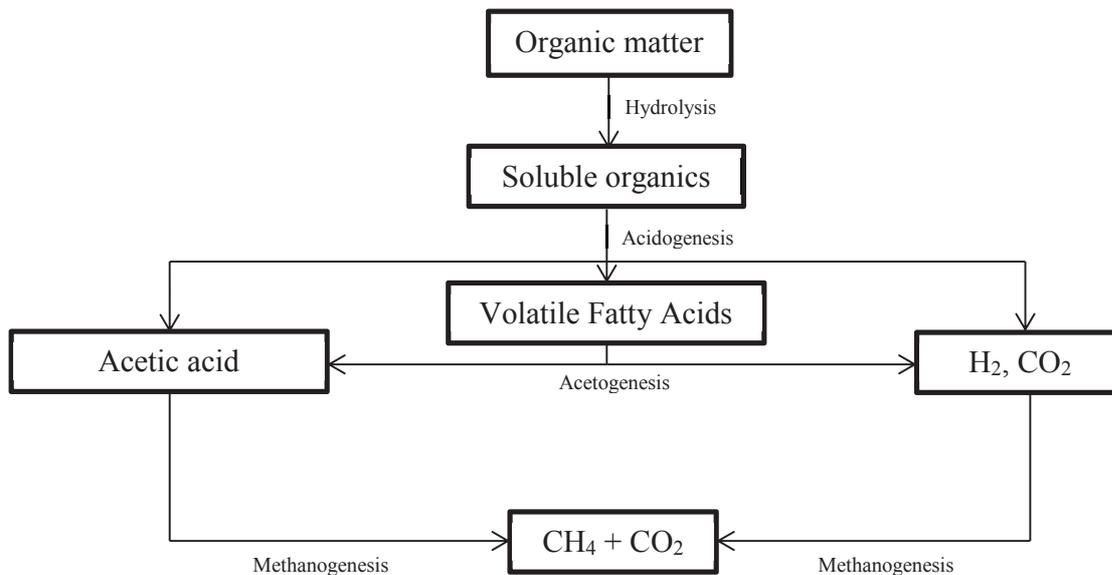


Figure 4: Biodegradation process in anaerobic digestion (Appels et al., 2008; Chandra et al., 2012)

## **1.4. Optimum conditions required for anaerobes metabolic activity**

There are factors which affect the biodegradation process in anaerobic digestion. The main factors observed are temperature, pH, organic loading rate (OLR), hydraulic retention time (HRT), carbon/nitrogen (C/N) ratio and trace elements concentration (Mao et al., 2015).

### **1.4.1. Temperature**

Temperature is one of the most significant parameters influencing the performance of anaerobic digestion because it has influence on the activity of enzymes and co-enzymes thus has also influences on methane yield and digestate quality as mentioned in a review (Zhang et al., 2014). Temperature for anaerobic microorganisms can either be psychrophilic (10–30 °C), mesophilic (30–40 °C) or thermophilic (50–60 °C) (Zhang et al., 2014).

Psychrophilic anaerobic digestion did not receive as much interests as mesophilic or thermophilic anaerobic digestion due to lower methane yield, longer retention time required and lower volatile solids reduction (Nkemka and Hao, 2016). However, research on psychrophilic anaerobic digestion is growing recently especially in cold climate country like Canada (Massé and Saady, 2015; Rajagopal et al., 2016; Saady and Massé, 2015), China (Wei et al., 2014) and Bolivia (Martí-Herrero et al., 2015).

Mesophilic anaerobic digestion is often preferred to thermophilic anaerobic digestion due to better process stability and higher diversity of microbial activity (Bayr et al., 2012b; Labatut et al., 2014; Mao et al., 2015).

Thermophilic anaerobic digestion has some advantages which are higher reaction rates, higher efficiency of organic wastes biodegradation, better efficiency for solid-liquid separation process and higher destruction of pathogenic organisms (Buhr and Andrews, 1977; Labatut et al., 2014; Mao et al., 2015). Methane production under thermophilic condition is slightly higher than under mesophilic condition (Gou et al., 2014; Mata-Alvarez et al., 2014; Yu et al., 2014b). However, under thermophilic condition, higher chance of acidification can occur compared to mesophilic

condition, which results in some disadvantages such as decreased stability, long-chain fatty acid (LCFA) accumulation, less diverse microbial community, decrease in biogas production, low quality effluent as well as increase in soluble chemical oxygen demand (sCOD), toxicity and susceptibility to the environment (Jiang et al., 2013; Labatut et al., 2014; Mao et al., 2015; Yu et al., 2014b). Besides, thermophilic anaerobic digestion requires higher energy for heating and maintaining the temperature at 50–60 °C (Buhr and Andrews, 1977) .

Therefore, the temperature chosen for the system is very important in anaerobic digestion process to ensure process stability. In addition, the control of the temperature should always be implemented (Jimenez et al., 2015b).

#### **1.4.2. pH**

pH monitoring and control in anaerobic digestion process are really important (Jimenez et al., 2015b) because the pH in the digester affects the performance and efficiency of anaerobic digestion process. This is due to the correlation of pH with the inhibition due to acid form of VFAs (Charnier et al., 2016). Accumulation of VFA will cause acidification and thus cause drop in pH, slow the process and eventually cause digester failure (Charnier et al., 2016). Besides, pH variations in the digester depends on many parameters such as alkalinity and  $\text{NH}_4^+$  concentration (Kondusamy and Kalamdhad, 2014).

The ideal pH for an effective anaerobic digestion process ranges from 6.8 to 7.4 (Mao et al., 2015). pH optimum for acidogenesis ranges from pH 5.5 to 6.5 while pH optimum for methanogenesis ranges from 6.5 to 8.2 with optimal pH 7 (Mao et al., 2015; Zhang et al., 2014). pH below pH 6.5 or above 8.2 will decrease the growth rate of methanogens which therefore, can cause digester failure (Jha et al., 2011; Mao et al., 2015). Usually, for maintaining constant pH during the process, sodium bicarbonate ( $\text{NaHCO}_3$ ) and sodium hydroxide ( $\text{NaOH}$ ) can be added during startup period for continuous process (Kondusamy and Kalamdhad, 2014).

### 1.4.3. Organic Loading Rate (OLR)

OLR indicates an amount of organic matter (in terms of COD or VS) of substrates fed per volume of digester per day (Mao et al., 2015). A lot of researches have shown the impact of increasing OLR in anaerobic digestion, which will increase the biogas and methane productivity until at a certain level of OLR where biogas and methane productivity decreases (Ganesh et al., 2013; Gou et al., 2014; Jabeen et al., 2015; Krishnan et al., 2016; LANZA et al., 2015; D. Li et al., 2015a, 2015b; Liu et al., 2012; Noutsopoulos et al., 2013), indicating that the degradation capacity of the reactor has been exceeded. This is due to the increase of VFAs concentration and soluble chemical oxygen demand (sCOD) with increasing OLR (Jiang et al., 2013; D. Li et al., 2015b; Liu et al., 2012). Therefore, exceeding the maximum OLR that the reactor can stand will cause accumulation of VFAs as well as high sCOD which will cause decrease in degradation efficiency, drop of pH, decrease in specific methane potential and thus failure in digester (Mao et al., 2015; Mata-Alvarez et al., 2014).

Depending on the kind of substrate and co-substrates and on the type of technology, a stable biogas production can be achieved at different range of OLR (D. Li et al., 2015a, 2015b). Besides, the temperature also has an effect on maximum OLR for the same substrate and co-substrate used (Gou et al., 2014). Different maximum OLRs achieved before biogas decrease with relation to different substrate and co-substrate used as well as operating parameters are summarized in Table 2.

Table 2: The effect of different substrate and co-substrate and temperature on maximum OLR when biogas drop

Substrate and co-substrate	Temperature	Reactor type	Liquid/high solids anaerobic digestion	OLR tested (g VS/L/day)	Maximum OLR (g VS/L/day) when biogas drop	Reference
Grease sludge and sewage sludge	mesophilic	semi-continuous	liquid	1.9, 2.0, 3.1, 3.5, 4.1, 8.3	4.1	(Noutsopoulos et al., 2013)
Waste activated sludge and municipal biomass waste	mesophilic	CSTR	liquid	1.2, 2.4, 3.6, 4.8, 6, 8	8	(Liu et al., 2012)
Waste activated	mesophilic	CSTR	liquid	1, 2, 3, 4,	5	(Gou et al.,

sludge and food waste				5, 6, 7, 8		(2014)
Waste activated sludge and food waste	thermophilic	CSTR	liquid	1, 2, 3, 4, 5, 6, 7, 8	7	(Gou et al., 2014)
High-solids co-digestion of food waste and rice husk	mesophilic	pilot scale plug flow	high solids	5, 6, 9	> 6	(Jabeen et al., 2015)
Cow manure and rice straw	mesophilic	CSTR	liquid	3, 3.6, 4.2, 4.8, 6, 8, 12	6	(D. Li et al., 2015a)
Pig manure and rice straw	mesophilic	CSTR	liquid	3, 3.6, 4.2, 4.8, 6, 8, 12	8	(D. Li et al., 2015b)
Cow manure with straw, grass and fruit and vegetables	mesophilic	plug flow	high solids	1.5, 2.5, 5, 7.5	> 2.5	(Ganesh et al., 2013)
Dry co-digestion of dairy manure and wheat straw	psychrophilic	Sequential batch reactor	high solids	2.9, 3.7 and 4.4	> 2.9	(Saady and Massé, 2015)
Swine manure and olive mill wastewater	mesophilic	CSTR	liquid	1.2, 2, 2.8, 3.8 to 4.4	-	(Kougias et al., 2014)
Grass silage	thermophilic	CSTR	liquid	3, 4, 5, 6, 7	> 4	(M.A. Voelklein et al., 2016)
Spirulina platensis	mesophilic	CSTR	liquid	1, 1.6, 2.0, 2.7, 4	> 1	(Aramrueang et al., 2016)

Therefore, the appropriate OLR is linked to the type of substrates used, the technology selected and the operating parameters chosen. Depending on the temperature and other operating parameters, some substrates can be digested at high OLR while others can only be digested at low OLR.

#### 1.4.4. Hydraulic Retention Time (HRT)

Increasing OLR in anaerobic digestion means decreasing HRT (Di Maria et al., 2015; Liu et al., 2012). The problem with lower HRT, corresponding to high OLRs, is the possibility of VFAs accumulation (Dareiotti and Kornaros, 2014). Higher HRT means higher total volatile solid mass reduction which will result in higher biogas and methane yields (Aramrueang et al., 2016; Chandra et al., 2012; Dareiotti and Kornaros, 2014). Besides, it provides buffering capacity for protection against the effects of shock loadings and toxic compounds and helps to permit

biological acclimation to toxic compounds (Chandra et al., 2012). Therefore, longer HRT is suggested for an efficient bio-degradation of the substrates in the digester (Ruile et al., 2015).

#### **1.4.5. Carbon–nitrogen (C/N) ratio**

Carbon and nitrogen are two important sources of food for anaerobic bacteria where carbon is required for energy and both carbon and nitrogen are important for building the new cell structure (Jain et al., 2015). Anaerobic digestion process is sensitive to carbon/nitrogen (C/N) ratio which indicates the level of nutrients of a substrate (Mao et al., 2015). High C/N ratio will cause insufficient nitrogen to maintain cell biomass and lack of ammonia nitrogen supply in the digester (Mao et al., 2015). In contrast, low C/N ratio will lead to possible  $\text{NH}_4^+$  inhibition of Archae in the digester (Mao et al., 2015). The C/N ratio optimum in anaerobic digestion was reported to be in the range from 20 to 35 with ratio 25 being most commonly used (D. Li et al., 2015a, 2015b; Mao et al., 2015; Panichnumsin et al., 2010).

#### **1.4.6. Trace elements concentration**

Addition of trace elements can supply nutrients to microbial community in anaerobic digestion and thus improve the efficiency of anaerobic digestion process and enhance biogas and methane production (Choong et al., 2016; Janke et al., 2016; Nordell et al., 2016; Ortner et al., 2015, 2014; W. Zhang et al., 2015). Besides, addition of trace elements can also improve the stability of anaerobic digestion process by reducing VFAs and allow higher OLR to be used (Bayr et al., 2012a; Q. Zhang et al., 2016). In a study (Nordell et al., 2016), addition of the following trace elements, Fe (9%), Co (<100  $\mu\text{g/L}$ ), Ni (<100  $\mu\text{g/L}$ ), Se (<100  $\mu\text{g/L}$ ) and W (<100  $\mu\text{g/L}$ ) has shown to increase biogas production by 24% in co-digestion of manure and industrial waste. Addition of either 1000 to 5000 ppm (dry basis) of iron, 0.148 to 0.580 mg/L or <1 to 3 ppm (dry basis) cobalt, 5 to 25 ppm (dry basis) or 0.801 to 5.362 mg/L nickel, <0.1 ppm (dry basis) selenium, <0.1 ppm (dry basis) or 0.658 to 40.39 mg/L tungsten was recommended (Bayr et al., 2012a; Kayhanian and Rich, 1996, 1995).

## **1.5. Problem in process**

In anaerobic digestion processes, some problems can occur due to inhibitory substances and intermediate products such as VFAs,  $\text{NH}_4^+$  and free ammonia (FA) accumulation, presence of sulfide/sulfate, light metal ions and heavy metals which will inhibit methanogenesis process even though they are essential nutrients for bacterial growth at low concentrations (Chen et al., 2008; Kougias et al., 2013; Yuan and Zhu, 2016; Q. Zhang et al., 2016). The most commonly reported inhibitions are VFAs and  $\text{NH}_4^+$  accumulation in the digester. The inhibition caused by specific inhibitors depends widely on the anaerobic inocula, substrate composition and operating conditions of anaerobic digestion (Chen et al., 2008). Therefore, co-digestion with other substrates, adaptation of microorganisms to inhibitory substances, and integration of methods to remove or reduce toxic compounds before anaerobic digestion can prevent inhibition during anaerobic digestion process and therefore, improve the efficiency of anaerobic digestion (Chen et al., 2008). Beside inhibition, another problem related to anaerobic digestion process is foaming which can occur, for example, due to an increase of biogas production (Kougias et al., 2013).

### **1.5.1. Volatile fatty acids (VFAs)**

VFAs are one of the control parameters in anaerobic digestion as it indicates the activity of the methanogenic consortia (Madsen et al., 2011). Indeed, VFAs are the main intermediate compounds produced during anaerobic digestion and are transformed into  $\text{CH}_4$  and  $\text{CO}_2$  by acetogens and methanogens microorganisms (Buyukkamaci and Filibeli, 2004). VFAs are mainly acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, caproate and heptanoate (Ahring et al., 1995; Jiang et al., 2013; Rebecchi et al., 2016). In anaerobic digestion, major VFAs present are acetate and propionate (Buyukkamaci and Filibeli, 2004). However, depending on the substrate, acetate and butyrate can be the main VFAs; for example they represented 60% of total VFAs in anaerobic digestion of food waste (Jiang et al., 2013) with the highest concentration and yield obtained at pH 6 (Jiang et al., 2013; Wang et al., 2014). Besides, VFAs can also be affected by the pH in the digester. At low pH, dominating VFAs are acetate and butyrate. Meanwhile, at high pH 8, VFAs are mainly acetate and propionate (Zhang et al., 2014).

VFA<sub>s</sub> accumulation can be interpreted as either organic over-loading or inhibition of the methanogenic microorganisms (Madsen et al., 2011). High concentration of VFA<sub>s</sub> in the digester causes microbial stress, decrease in pH which leads to digester failure (Buyukkamaci and Filibeli, 2004). Increasing OLR or decreasing hydraulic retention time (HRT) (Ruile et al., 2015) is seen as the main contribution of VFA<sub>s</sub> accumulation in the digester. For example, VFA<sub>s</sub> concentration was observed low at 0.16 g/L when OLR was 8 g VS/L/day and increased up to 13 g/L when OLR was 12 g VS/L/day for mesophilic co-digestion of rice straw and cow manure (D. Li et al., 2015a). Similar situation was also observed with mesophilic co-digestion of rice straw and pig manure where VFA<sub>s</sub> concentration was 0.12 g/L at OLR 8 g VS/L/day but increased to 8.5 g/L at OLR 12 g VS/L/day (D. Li et al., 2015b).

Therefore, VFA<sub>s</sub> concentration is an important parameter to monitor in anaerobic digestion to have an indication of the performance of the process. VFA concentration provides consistent information on the status of the process along with other commonly used indicators such pH, alkalinity, gas production and gas composition (Jimenez et al., 2015b). On top of that, individual VFAs (acetate, propionate and butyrate) can provide more information of the process status which can easily be measured using GC or HPLC after removing all particulate matter from the sample (Jimenez et al., 2015b).

The ratio of VFAs concentration to alkalinity, also known as free organic acids/total inorganic carbon (FOS/TAC), describes buffering capacity in anaerobic digestion process (M.A. Voelklein et al., 2016). During anaerobic digestion process, the reaction of ammonia with carbon dioxide and water produces ammonium bicarbonate which contributes to increases of alkalinity. Alkalinity provides an essential buffer during the process which holds the pH at the optimum 6.8 to 7.4 (Zhao and Viraraghavan, 2004). The optimum VFA/alkalinity ratio for methanogens activity is from 0.1 to 0.3 (T. Zhang et al., 2015; Zhao and Viraraghavan, 2004). The VFA/alkalinity ratio from 0.3 to 0.4 requires immediate corrective action such as increasing buffering capacity of the medium by addition of sodium bicarbonate while the ratio more than 0.8 causes pH depression which will cause digester failure (Ros et al., 2013; Zhao and Viraraghavan, 2004).

### 1.5.2. Ammonium (NH<sub>4</sub><sup>+</sup>)

NH<sub>4</sub><sup>+</sup> is produced when nitrogenous matter, mostly in the form of proteins and urea is biologically degraded during the process (Chen et al., 2008; Monlau et al., 2015b; Yenigün and Demirel, 2013). This is in the case of protein-rich wastes, such as slaughterhouse wastes and food processing industries (Labatut et al., 2014) as well as organic fraction of municipal solid waste (OFMSW) and manure (Yenigün and Demirel, 2013). NH<sub>4</sub><sup>+</sup> is considered as an essential nutrient for bacterial growth to maintain the stability and performance during anaerobic digestion (Monlau et al., 2015b; Rajagopal et al., 2013). The two principal forms of inorganic ammonia nitrogen in aqueous solution are NH<sub>4</sub><sup>+</sup> and free ammonia (FA) (NH<sub>3</sub>). FA is the main cause of inhibition compared to NH<sub>4</sub><sup>+</sup> since it is freely membrane-permeable (Chen et al., 2008).

The concentration from 50 to 200 mg NH<sub>4</sub><sup>+</sup>-N/L is necessary to supplement nutrients for microorganisms for an efficient anaerobic digestion process (Chen et al., 2008; Rajagopal et al., 2013). The concentration from 200 to 1000 mg NH<sub>4</sub><sup>+</sup>-N/L has no antagonistic effect (Rajagopal et al., 2013). However, high concentration from 1500 to 3000 mg NH<sub>4</sub><sup>+</sup>-N/L can be an inhibitor during methanogenesis process during anaerobic digestion especially at higher pH which will cause digester failure (Rajagopal et al., 2013; Yenigün and Demirel, 2013). Higher concentration than 3000 mg NH<sub>4</sub><sup>+</sup>-N/L can cause total inhibition or toxic at any pH (Rajagopal et al., 2013). This is because methanogens are the least tolerant to high ammonia concentration. For example, a concentration from 4 to 5.7 g NH<sub>4</sub><sup>+</sup>-N/L led to a loss of 56.5% of methanogens activity (Chen et al., 2008). Moreover, concentrations from 1.7 to 14 g NH<sub>4</sub><sup>+</sup>-N/L had shown to have 50% less methane production (Chen et al., 2008). This wide range of total ammonia concentration as inhibitory can be due to the differences in substrates and inocula, environmental conditions (pH and temperature) and acclimation periods (Chen et al., 2008).

There is no available simple strategy to mitigate ammonia-toxicity once it has exceeded threshold inhibition level (Chen et al., 2008). Therefore, to operate a reactor at high ammonia level, the factors required to be considered are the choice of the temperature, control of pH, C/N ratio and utilization of acclimatized-microflora (Mao et al., 2015; Rajagopal et al., 2013). For example, an appropriate C/N in anaerobic digestion makes possible the increased of OLR

without having ammonia inhibition (D. Li et al., 2015a, 2015b). Recirculation of liquid digestate from the reactor originating from low C/N ratio substrate is not recommended as it will increase  $\text{NH}_4^+$  and free ammonia FA concentrations in the reactor (S. Wu et al., 2016).

### **1.5.3. Foaming**

One of the major problems which can occur in anaerobic digestion plants is foaming (Kougias et al., 2013) which can often be related to an increase of biogas production (Kougias et al., 2013). Increase of OLR is also the main factor which affects foaming in anaerobic digestion (Kougias et al., 2013). Besides, the substrate composition in particular the content of proteins or lipids also contributes to foaming in combination with OLR (Kougias et al., 2013). At low OLR, proteins initiated more foaming than lipids (Kougias et al., 2013). For foaming prevention, OLR 3.5 g VS/L/day was suggested in manure-based anaerobic digestion plant (Kougias et al., 2013). However, other finding only observed foaming at high OLR of  $\geq 8$  kg VS/(m<sup>3</sup> d) in a co-digestion of cow manure and rice straw and under mesophilic condition (D. Li et al., 2015a).

### **1.6. Types of reactors**

The type of reactor chosen plays really an important role in anaerobic digestion of solid residues as it has an impact on the efficiency of the process (Mao et al., 2015). The type of reactor chosen depends on the type of anaerobic digestion performed either liquid anaerobic digestion (wet) or solid-state anaerobic digestion (dry) (Mata-Alvarez, 2003). Liquid anaerobic digestion is usually operated at solid concentrations between 0.5 to 12-15% while solid-state anaerobic digestion is operated at solid concentrations higher than 15% (Li et al., 2011a). Continuously stirred tank reactor (CSTR) is usually used for liquid anaerobic digestion of solid residues. Dry anaerobic digestion is carried out either in batch reactor (leach bed reactors or garage-style digester) (André et al., 2016; Degueurce et al., 2016) or in continuous reactors such as plug-flow reactor (PFR) (Mata-Alvarez, 2003). In this thesis, focus will be more on CSTR reactor, and in a lesser extent, on PFR. The schematic diagram of CSTR and PFR reactors are shown in Figure 5.

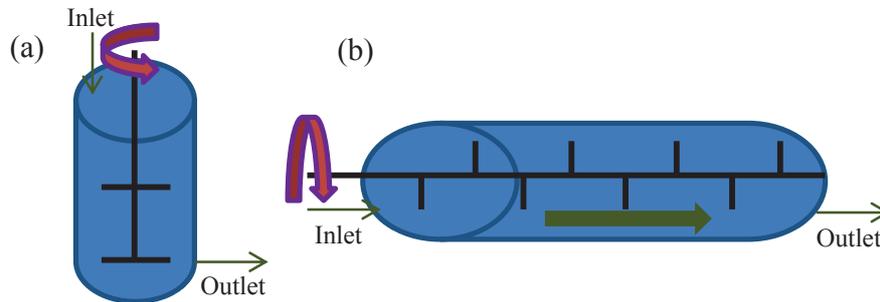


Figure 5: Schematic diagram of (a) CSTR and (b) PFR reactors

CSTR has been used to treat wastewaters containing high suspended particles during anaerobic digestion process (Mao et al., 2015). However, the use of CSTR for anaerobic digestion and co-digestion of organic fraction of municipal solid waste (OFMSW), manures, food wastes and other agro-industrial wastes became common in the last decade (Capson-Tojo et al., 2016; Mata-Alvarez et al., 2014). In CSTR system, continuous mixing provides good contact between substrate and anaerobic sludge (Mao et al., 2015).

PFR is a type of reactor which is long and horizontal tank usually situated above ground (Mao et al., 2015). It features no internal agitation (Mao et al., 2015) where the contents is not completely mixed but moves as a plug through the reactor from the inlet to the outlet (Li et al., 2011b). PFR is more efficient than CSTR in terms of overall bioconversion (Mao et al., 2015). PFR is generally used for solid state anaerobic digestion (SS-AD) with higher solids input between 25 to 35% total solids (TS) (Li et al., 2011b) and later maintains 20% TS in the tank (Li et al., 2011b) with HRT from 15-20 days (Mao et al., 2015). Moreover, it provides low concentrations of VFA in the effluent, high degree of sludge retention and stable reactor performance (Mao et al., 2015). PFR works at both conditions mesophilic and thermophilic and is suitable for many types of substrates such as pig manure, distillery wastewater, cattle residues and OFMSW (Mao et al., 2015). However, the disadvantage of PFR is that at lower solids content, accumulation of sediment in the reactors can occur (Li et al., 2011b).

## **1.7. Substrates**

The type of substrates and their characteristics have large contribution to the efficiency of anaerobic digestion process. Moreover, the characteristics of the substrates were observed to have largest impact on degradation time (Ruile et al., 2015). Several types of substrates that are commonly used to produce biogas by anaerobic digestion are waste activated sludge, municipal solid waste, livestock manure, fruit and vegetable waste, food waste and lignocellulosic biomass.

### **1.7.1. Sewage sludge**

Anaerobic digestion of sewage sludge has long been established for stabilization of sewage sludge produced from wastewater treatment plants as well as for methane production (Appels et al., 2008; Astals et al., 2013a). Sewage sludge usually contains low TS and VS concentrations with low C/N ratio as shown in Table 4. A lot of efforts have been made to improve anaerobic digestion of sludge including optimization of process conditions, multi stage digester application and pretreatment to improve biodegradability (Appels et al., 2008; Cao and Pawłowski, 2012; Carrère et al., 2010). Moreover, there have also been recent studies such as thermophilic digestion of waste activated sludge coupled with solar pond (G. Zhang et al., 2016), microbial electrolysis contribution to anaerobic digestion of waste activated sludge (W. Liu et al., 2016), influence of applied voltage on the performance of bioelectrochemical anaerobic digestion of sewage sludge (Feng et al., 2016), grease trap sludge addition to anaerobic digestion of sewage sludge (Grosser and Neczaj, 2016), single and two-phase thermophilic anaerobic digestion of waste activated sludge (Leite et al., 2016) and trace element addition on anaerobic digestion of sewage sludge with in-situ carbon dioxide sequestration (Linville et al., 2016).

### **1.7.2. Organic fraction of municipal solid waste (OFMSW)**

OFMSW can be defined as either a mixture of wastes from kitchens, parks and gardens or a mixture of food, garden wastes and paper wastes (Campuzano and González-Martínez, 2016; Cesaro and Belgiorno, 2014). The production of OFMSW and its composition depends on the geographic region, number of inhabitants and their social condition, predominant economic

activities, regional food habits, seasonal variations and recollection system (Campuzano and González-Martínez, 2016; Li et al., 2011a). The characteristics of OFMSW can be classified as physical (particle size, density), chemical (humidity, TS, VS, TKN, total phosphorus (TP), carbon) and bromatological (carbohydrates, proteins, fat, oil and grease (FOG)) (Campuzano and González-Martínez, 2016). The density of OFMSW ranges from 328 to 1052 kg/m<sup>3</sup>. Higher the density, lesser the contents of unwanted substances and materials (Campuzano and González-Martínez, 2016). The characteristics of OFMSW are summarized in Table 4.

Research on anaerobic digestion of OFMSW has been carried out for 3 decades (Cecchi et al., 1993). In Europe, commercialization of MSW anaerobic digestion plants which started in early 1990s have grown rapidly until 2011 with the total installed capacity reaching almost 8.5 million tons/year across 205 plants with an average of 42,000 tons/plant/year (Iacovidou et al., 2013). 56% of MSW anaerobic digestion plants convert biogas into heat and electricity that is directly used, 16% purify the biogas and inject it in the gas grid, 28% trade it as a transport fuel (Iacovidou et al., 2013).

Bio-methane potential of OFMSW has a high variation ranging from 61-580 L/kg VS (Table 4) which is due to high variation in TS and VS contents of OFMSW (Table 4). High VS/TS ratio from 75 to 95% has shown high methane production range from 300 and 600 L/kg VS (Campuzano and González-Martínez, 2016). Besides, the ratio of C/N of OFMSW has also high variation from 11-27 (Table 4). Therefore, co-digestion with other substrates could balance C/N ratio for efficient anaerobic digestion.

Besides, the particle sizes of OFMSW have influence on anaerobic digestion process. The larger the particles, the longer it takes to produce biogas (Campuzano and González-Martínez, 2016). The source sorted OFMSW and a mixture of the grey fraction of MSW resulted in higher specific biogas production of 200 m<sup>3</sup>/ton of treated waste with specific methane production of 0.4 m<sup>3</sup>/kg of volatile solids (VS) feed. Meanwhile, mechanically sorted OFMSW and sludge resulted in specific biogas production of 60 m<sup>3</sup>/ton of treated waste with a specific methane production of 0.13 m<sup>3</sup>/kg of volatile solids (VS) feed (Li et al., 2011a). Grinding or chopping

process of OFMSW is suggested before being fed to the digester due to its heterogeneity characteristics (Campuzano and González-Martínez, 2016).

Due to high solids concentration, municipal solid waste can be treated in solid-state anaerobic digestion (Li et al., 2011a). Due to increasing interest of anaerobic digestion facilities for OFMSW treatment as well as for renewable energy recovery, more research have also been developed on the chemical (ozonation, alkali and acid addition), physical (mechanical, thermal, microwave irradiation and ultrasound) and biological pre-treatment of OFMSW for improving anaerobic digestion process as mentioned in a review (Cesaro and Belgiorno, 2014).

### **1.7.3. Animal manures**

Increasing of livestock production increased the production of by-products, manures and slurries. Previously, composting of animal manure was the best way to recycle surplus manure (Bernal et al., 2009). However, high amounts of manure produced cause impossible direct land application for fertilization purpose (Bernal et al., 2009).

Research on anaerobic digestion of manure and its co-substrates has been reported in a review with most of the publications referring to only pig manure and cow manure (Mata-Alvarez et al., 2014). However, recent researches have also focused on other types of manure such as chicken manure (Abouelenien et al., 2010; Dalkılıç and Ugurlu, 2015; Nie et al., 2015; Niu et al., 2015; S. Wu et al., 2016), goat manure (Kafle and Chen, 2016; Tauseef et al., 2013) and horse manure (Hadin et al., 2016; Hadin and Eriksson, 2016). The characteristics and bio-methane potential of animal manures with respect to the type of animal are summarized in Table 4. Depending on type of animal, some manures have low TS content while others have higher. Some manure has already been mixed with straw that are used for bedding which increase its TS content. Besides, manure also contains high nitrogen making its C/N ratio low (Table 4).

Manure based anaerobic digestion plants are often implemented in two main models which are centralized (co-digesting manures from several farms with organic residues from township and

industry and on-farm plants) and on-farm plants (co-digesting manures with other farm waste such as crop residues) (Madsen et al., 2011; Mata-Alvarez et al., 2014).

#### **1.7.4. Lignocellulosic biomass**

Lignocellulosic biomass is herbaceous material (Chandra et al., 2012) that is comprised of holocelluloses (cellulose (30–70%), hemicelluloses (15–35%) and lignin (10–25%)) along with smaller quantities of pectin, proteins, lipids, soluble non-structural materials such as sugars, nitrogenous material, chlorophyll and waxes) and inorganic compounds which vary in terms of quantitative and qualitative depending on the plant material (Monlau et al., 2013; Sawatdeenarunat et al., 2014).

Lignocellulosic biomass used for anaerobic digestion can be grasses or gramineae residues (wheat straw, wheat bran, corn stover, rice straw, barley straw, maize stover, maize bran and rye straw), energetic plant (miscanthus), cellulosic energy crops (corn silage, maize silage, summer switchgrass, grass silage) and yard wastes (Chandra et al., 2012; Monlau et al., 2013; Mosier et al., 2005). Other examples of lignocellulosic biomass are coconut fibers, newsprint, wheat grass, sisal fiber, corn fiber, grass hay, bagasse, sugar beet tops, winter rye, office paper and corn kernels (Monlau et al., 2013; Mosier et al., 2005).

Anaerobic digestion is seen as a promising technology to degrade lignocellulosic biomass for renewable energy production since it does not only convert simple organic compounds such as pentoses, hexoses, and VFAs into methane, the technology also converts polymers (cellulose, starch, hemicelluloses) into methane (Monlau et al., 2013). However, anaerobic digestion of lignocellulosic biomass is limited by the accessibility of holocelluloses that are embedded in the lignin network (Monlau et al., 2013). This is due to the interactions of cellulose, hemicellulose and lignin that form a rigid structure which creates a highly resistant and recalcitrant biomass structure which makes hydrolysis of lignocellulose the rate-limiting step during anaerobic digestion (Sawatdeenarunat et al., 2014).

Therefore, pretreatments of lignocellulosic biomass have been applied as upstream unit operations to break the complex structure of biomass. Main pretreatment objectives are to remove lignin, increase biomass porosity and surface area, solubilize hemicelluloses and cellulose as well as to reduce the cellulose crystallinity (Monlau et al., 2013; Mosier et al., 2005). This is in order to improve the rate of enzyme hydrolysis for facilitating the biological conversion of biomass into bioenergy or bio-based products (Monlau et al., 2013; Mosier et al., 2005). The pretreatments are generally divided into four categories: physical (chipping, grinding, milling and irradiation), chemical (alkali, acid, oxidative, organic solvents and ionic liquids), thermal (CO<sub>2</sub> explosion, hot water pretreatment, SO<sub>2</sub> explosion, steam explosion; ammonia fiber explosion (AFEX)), biological (fungi and bacterial) and enzymatic, or a combination of two of them (Chandra et al., 2012; Monlau et al., 2013; Mosier et al., 2005). Several review papers have reported an increase of methane yield from 17% to 600% by pretreatments of lignocellulosic biomass (Frigon and Guiot, 2010; Ge et al., 2016; Yang et al., 2015). However a lot of these pretreatments have economical and environmental limitations due to the high cost of enzymes or energy required as well as the production of solid/liquid waste streams (Sawatdeenarunat et al., 2014).

Several suggestions for an improved anaerobic digestion of lignocellulosic biomass was made: adjustment of the C/N ratio with addition of nutrients or trace metals, integration of cost-effective thermochemical or/and enzymatic pre-treatments, improvement of the hydrolytic functions of the in situ microbial populations (either by enrichment of naturally present hydrolytic micro-organisms or by the addition and retention of de novo hydrolytic capabilities) (Frigon and Guiot, 2010), replacing microbial inoculums with rumen microorganisms (Sawatdeenarunat et al., 2014; Yang et al., 2015; Yue et al., 2013). The characteristics and bio-methane potential of lignocellulosic biomass with respect to the type of biomass are summarized in Table 4.

#### **1.7.5. Food waste**

Food waste is defined as end products discarded from various foods processing and supply for human consumption that have not been recycled or used for other purposes (Capson-Tojo et al.,

2016; Giroto et al., 2015; Lin et al., 2013). Food waste has an economic value that is generally less than the cost of collection and recovery for reuse (Lin et al., 2013). There are almost 89 million tons and 1.3 billion tons of foods wastes produced per year in Europe and around the world respectively (Capson-Tojo et al., 2016; Chen et al., 2015; Lin et al., 2013). China, USA, UK, South Korea, India, Japan and Canada are the top 7 countries for food waste production (Chen et al., 2015). From 30 to 40 % of the total food generated is discarded in USA, United Kingdom or Japan (Capson-Tojo et al., 2016) making the proportion of food waste in municipal solid waste ranged from 12 to 30 % (Zhang et al., 2014). The composition of food waste varies between countries and some examples are shown in Table 3. The characteristics of food waste are shown in Table 4.

Table 3: Composition of food waste from different countries (adapted from Capson-Tojo et al (Capson-Tojo et al., 2016))

% Wet weight	UK	Finland	Portugal	Italy	Europe	China	Thailand	Asia
Fruit and vegetables	60.9	44.5	59.2	69	58.4	30.2	13.1	31.8
Pasta/rice/cereals	1.5	0.4	0.2	12.4	3.6	57.5	46.5	27
Meat and fish	6.7	4.3	7.3	6.2	6.1	-	0.3	0.1
Dairy products	1.7	2.0	0.7	1.4	1.4	1.7	0.6	3.9
Mixed meals	12.3	6.3	29.0	1.4	12.2	-	-	-
Other foods	16.9	42.5	3.6	9.6	18.3	10.6	39.5	37.2

The problems associated with food wastes are variations of pH and chemical composition due to seasonal variations and changes in food processing, bacterial contamination and high production rate leading to problems for management and disposal (Lin et al., 2013). The disposal of food wastes without proper treatment has caused severe environmental pollution in many countries (Zhang et al., 2014). Current food waste valorization practices include animal feed, composting, incineration and landfilling (Lin et al., 2013; Melikoglu et al., 2013). However, these types of valorization have disadvantages such as direct and indirect emission of CH<sub>4</sub> and CO<sub>2</sub> by landfill disposal, energy loss to evaporate water content by incineration and high transportation cost of food waste to be composted (Lin et al., 2013).

Therefore, anaerobic digestion is a feasible and efficient food waste treatment compared to other treatment routes (Zhang et al., 2007). Moreover, food wastes which are lipid rich resources (lipid concentration about 5.0 g/L) contain macromolecular organic matter as well as various trace elements as nutrients which are suitable for anaerobic microorganisms (Zhang et al., 2014). As a result, there has been recently an increase of the number of publications on food waste anaerobic digestion from 64 articles in 2002 to 437 articles in 2014 (Chen et al., 2015).

Due to increasing interests of research on food waste anaerobic digestion, more recent researches have been developed such as, for example, food waste anaerobic digestion using yeasts for more efficient fermentative breakdown (Suwannarat and Ritchie, 2015), two-stage food waste anaerobic digestion system with increasing OLR from 6 to 15 g VS/L/day on a first stage hydrolysis reactor (HRT 4 days) followed by a second stage methanogenic reactor with increasing OLR from 2 to 5 g VS/L/day (HRT 12 days) (M. A. Voelklein et al., 2016) and a study on the effect of recirculation and temperature on performance and microbiology of food waste anaerobic digestion (Zamanzadeh et al., 2016). Moreover, a pilot scale study has also been carried out for scale up food waste mesophilic anaerobic digestion (Fiore et al., 2016).

The addition of trace element in food waste anaerobic digestion either under mesophilic or thermophilic conditions can increase performance efficiency up to 40% in biogas production (Menon et al., 2016; W. Zhang et al., 2015). Besides, pre-treatment such as thermal, thermophilic, mechanical, ultrasonic, ozonation, alkali and biological of food waste for anaerobic digestion have also shown more efficient process with improved in methane production (Ariunbaatar et al., 2015; Kondusamy and Kalamdhad, 2014).

#### **1.7.6. Fruit and vegetable wastes (FVW)**

FVW wastes contain easily biodegradable organic matter with high moisture content (Bouallagui et al., 2009, 2004). The organic fraction of FVW wastes includes 75% easy biodegradable matter (sugars and hemicelluloses), 9% cellulose and 5% lignin (Bouallagui et al., 2005). The characteristics of FVW wastes are shown in Table 4.

The production of fruit and vegetable waste is also very high and becoming a source of concern in municipal landfills because of its high biodegradability (Bouallagui et al., 2005). Due to that, anaerobic digestion is the best option for FVW treatment as well as renewable energy recovery. Decades ago, FVW were only disposed by dumping, land spreading or fed to the animals (Lane, 1984; Viturtia et al., 1989). However, research on anaerobic digestion of FVW has started in 1980's (Lane, 1984; Ranade et al., 1987; Viturtia et al., 1989) and have been developed since then (Bouallagui et al., 2003; Ganesh et al., 2014; Garcia-Peña et al., 2011; Mtz.-Viturtia et al., 1995; Sitorus and Panjaitan, 2013; Viswanath et al., 1992; Y. Wu et al., 2016). More recently, pilot scale anaerobic digestion of food waste has also been studied and obtained optimum organic loading rate ranged from 2.5 to 3.0 g VS/L d for a full-scale power plant (Scano et al., 2014).

#### **1.7.7. Slaughterhouse waste**

Slaughterhouse wastes are animal by-products generated from slaughterhouse such as bones, fats and skins as well as wastewater from cleaning operations that are usually discarded (Cuetos et al., 2008). Slaughterhouse and rendering wastes contain high concentration of organic matter and are rich in proteins and lipids (Bayr et al., 2012b; Cuetos et al., 2008).

Due to high protein and lipid contents, anaerobic digestion of slaughterhouse and rendering wastes could cause problem during the process due to the production of VFAs,  $\text{NH}_4^+$  and long chain fatty acids (LCFAs) (Bayr et al., 2012b; Cuetos et al., 2010, 2008; Hejnfelt and Angelidaki, 2009). In consequence, anaerobic digestion of slaughterhouse waste can only be operated at low OLR from 0.8–1.7 kg VS/m<sup>3</sup>/d with HRT 25-100 days (Bayr et al., 2012a, 2012b; Cuetos et al., 2008; Salminen and Rintala, 2002) under mesophilic condition. Thermophilic anaerobic digestion seems not possible for slaughterhouse waste even at low OLR due to inhibition by VFAs,  $\text{NH}_4^+$  and LCFAs (Bayr et al., 2012b; Hejnfelt and Angelidaki, 2009). For psychrophilic anaerobic digestion of slaughterhouse waste, the process performance is only stable at low OLR of 0.4 kg VS/L/day (Ortner et al., 2014). Under mesophilic condition, it is possible to operate slaughterhouse anaerobic digestion at higher OLR up to 2.5 kg VS/L/day with addition of trace elements (Bayr et al., 2012a; Ortner et al., 2015, 2014). The characteristics

of slaughterhouse wastes are shown in Table 4. Methane production from anaerobic digestion of slaughterhouse has a high variation from 225 to 720 L/kg VS (Table 4).

#### **1.7.8. Summary of the substrates characteristics**

The various possible feedstocks for anaerobic digestion present high variations in TS, VS TC, TN, C/N and methane yield which are summarized in Table 4.

Table 4: Total solids (TS), volatile solids (VS), total carbon (TC), total nitrogen (TN), C/N ratio and methane yield of different substrates

Substrates	TS (%)	VS (%)	TC (% of TS)	TN (% of TS)	C/N	Methane yield (L/kg VS)	References
<b>Sewage sludge</b>							
Sewage sludge	1.6-12.7	1.2-5.2	24-37	3-6.6	5.6-8.3	159-300	(Astals et al., 2013a; Cheng et al., 2016b; Gou et al., 2014; K. Li et al., 2015; Peng et al., 2016; Silvestre et al., 2015; Xie et al., 2017; G. Zhang et al., 2016)
<b>Organic fraction of municipal solid waste (OFMSW)</b>							
Organic fraction of municipal solid waste (OFMSW)	15-95	7-55	8-51.3	0.53-9.8	11.4-27	61-580	(Campuzano and González-Martínez, 2016; Cecchi et al., 1993; Dhar et al., 2015)
<b>Animal manures</b>							
Cow dung	9.2-29	7.3-20.2	26-42	1.2-5.1	6-24	136-302	(Aboudi et al., 2016; Anitha et al., 2015; Ashekuzzaman and Poulsen, 2011; Dhamodharan et al., 2015; D. Li et al., 2015a; K. Li et al., 2015; Miah et al., 2016; Møller et al., 2004; Rico et al., 2011; J. Wei et al., 2015; Y. Wei et al., 2015)
Cow manure (mixed with straw)	31	20	14.6	0.38	39	84	(Buendía et al., 2009)
Liquid fraction of cow manure	5.8	4.2	ND	6.2	ND	206-223	(Rico et al., 2011)
Pig manure	47.5	36.5	39	3.9	10	356-410	(K. Li et al., 2015; Møller et al., 2004)
Horse manure	20-37	17-31	-	-	22-42	-	(Hadin and Eriksson, 2016)
Rabbit manure	28	25	37.7	2.1	17.9	323	(K. Li et al., 2015)
Sheep manure	53.6	48.7	30.3	1.4	22.5	99	(Liu et al., 2015)
Chicken manure	42-50	35-45	18.1-43	2.2-9	3.8-8.9	118-377	(K. Li et al., 2015; Liu et al., 2015; Sun et al., 2016)
<b>Lignocellulosic biomass</b>							
Corn stover	87-93	76-85	39-44	1-1.1	29-63	114-239	(Ge et al., 2016; Y. Li et al., 2016; Monlau et al., 2013; Sawatdeenarunat et al., 2014; Y. Wei et al., 2015)
Corn silage	29	27	48	2.7	18	194-370	(Frigon and Guiot, 2010; Monlau et al., 2013; Sun et al., 2016)
Garden waste	37	25	35	1.6	22	-	(Fitamo et al., 2016)
Grass clippings	21	18	46	3.9	11.8	-	(Fitamo et al., 2016)

Maize straw	-	-	45.6	0.3	152	291-338	(Chandra et al., 2012; Sawatdeenarunat et al., 2014)
Rice straw	94	89	36-41.8	0.8	47-47.5	190-302	(Chandra et al., 2012; D. Li et al., 2015a; Monlau et al., 2013; Sawatdeenarunat et al., 2014)
Sugar beet pulp	24.2	22.6	43	1.8	23.8	283-355	(Suhartini et al., 2014)
Wheat straw	89.1-90.1	86.3-88.2	39.4-48.5	0.4-0.9	55-109	83-378	(Chandra et al., 2012; Liu et al., 2015; Monlau et al., 2013; Peng et al., 2016; Sawatdeenarunat et al., 2014)
<b>Food waste</b>							
Food waste	16-34	15-28	45-57.4	1.9-3.5	9.8-24.5	260-372	(Capson-Tojo et al., 2016; Cheng et al., 2016a; Fitamo et al., 2016; J. Kim et al., 2016; C. C. Liu et al., 2016; Yong et al., 2015; Zhang et al., 2014; W. Zhang et al., 2015)
<b>Fruit and vegetable wastes</b>							
Fruit and vegetable wastes	3.4-21.8	2.8-15.6	29.65	1.57	18.88	160-470	(Bouallagui et al., 2005; Callaghan et al., 2002; Ganesh et al., 2014; Scano et al., 2014; Shen et al., 2013)
Tomato residues	12.5	10.2	38	2.4	12-16		(Y. Li et al., 2016)
<b>Slaughterhouse wastes</b>							
Slaughterhouse wastes	11.1-53.2	10.23-52.6	ND	0.8-6.9	ND	225-720	(Bayr et al., 2012b; Hejnfelt and Angelidaki, 2009; Ortner et al., 2015; Wu et al., 2009)

## 1.8. Anaerobic co digestion

Anaerobic digestion of mono-substrate has some drawbacks which are related to characteristics of the substrate (Mata-Alvarez et al., 2014). Examples of some disadvantages of anaerobic digestion of mono-substrate are low organic content in sewage sludge, high concentration of heavy metals and other improper materials in organic fraction of municipal solid waste (OFMSW), high nitrogen content in animal manures which can inhibit methanogens, lack of nitrogen in some agro-industrial waste and crop residues and high nitrogen and long chain fatty acids (LCFA) content in slaughterhouse waste (Mata-Alvarez et al., 2014). Therefore, anaerobic co-digestion of more than one substrates is a solution to overcome this drawbacks (Mata-Alvarez et al., 2014). Besides, anaerobic co-digestion provides more advantage than mono digestion because it provides balanced nutrients (C/N ratio and macro and micronutrients) as well as reducing inhibitors accumulation (D. Li et al., 2015a, 2015b) and offers higher biogas production (Mata-Alvarez et al., 2014).

Previously, co-digestion of sewage sludge and the organic fraction of the municipal solid waste (OFMSW) has been the most reported co-digestion mixture (Mata-Alvarez et al., 2014). However, co-digestion of sewage sludge with fats, oils and greases has increased in the literature between 2010 and 2013 (Mata-Alvarez et al., 2014). Besides, publications on co-digestion of sewage sludge with other co-substrates such as fruit and vegetable waste, slaughterhouse waste, crude glycerol and algae have also been reported in recent years (Fonoll et al., 2015; Mata-Alvarez et al., 2014). From 2010 to 2013, animal manures became the most reported main substrate for anaerobic co-digestion with other substrates followed by sewage sludge and OFMSW. Industrial waste, agricultural waste and OFMSW stand as the most reported co-substrate in the review (Mata-Alvarez et al., 2014).

One of the advantages of co-digestion is providing nutrient balance (Kangle et al., 2012) especially for carbon-rich, poor buffer capacity, high C/N ratio substrate (Mata-Alvarez et al., 2014) such as rice straw (D. Li et al., 2015a, 2015b; Ye et al., 2013). Co-digestion with another nitrogen-rich, low C/N ratio and high buffer capacity substrate such as animal manure is shown as a good solution to balance the C/N ratio and increase in biogas yield (Comino et al., 2010; D.

Li et al., 2015a, 2015b; Y. Li et al., 2016; Mata-Alvarez et al., 2014; Ye et al., 2013). For example, co-digesting rice straw (RS) and cow manure (CM) provides optimum C/N of 22.4 with ratio of 1RS:1CM based on volatile solids (VS) (D. Li et al., 2015a) and co-digestion of pig manure (PM) and rice straw (RS) gives optimum C/N ratio of 23.4 with ratio of 1PM:1RS based on VS (D. Li et al., 2015b). Other examples, co-digestion of lignocellulosic biomass with other substrates such as expired dog food, paper mill sludge, pig wastewater, solid slaughterhouse, kitchen waste, yard waste, food waste, soybean processing waste, chicken manure, cow manure, pure organic co-substrates or spent mushroom substrate provides C/N ratio from 11-21 and has shown an increase from 9-691% of methane yield compared to mono-digestion of lignocellulosic biomass (Ge et al., 2016; Yang et al., 2015). In addition, co-digestion of straw with food waste provides C/N ratio of 31 in a mixing ratio of straw to food waste of 1:5 which has shown to increase methane production yield up to 150% compared to mono-digestion of only straw (Yong et al., 2015). The optimum proportion of the co-substrate is also important to avoid digester failure (Comino et al., 2010) as an increase of proportion of the co-substrate may lead to the system breakdown (Astals et al., 2013b).

Moreover, anaerobic co-digestion can also improve performance of food waste anaerobic digestion (Zhang et al., 2014). Co-digestion of food waste with other substrates such as straw, dairy manure, pig manure, garden wastes, sludge has also shown to increase methane production (Dennehy et al., 2016; Ebner et al., 2016; Fitamo et al., 2016; Yong et al., 2015). Co-digestion of manure with crude glycerol, by-product of the biodiesel production, cheese whey, sugar by-products, olive mill waste and slaughterhouse waste led to promising methane yield (Mata-Alvarez et al., 2014).

High effort has been put to enhance process performance of anaerobic co-digestion which will increase biogas production such as the study on the use of new co-substrates such as cyanide containing cassava pulp (Glanpracha and Annachhatre, 2016), residues from enzymatic saccharification (Borowski et al., 2016), sorghum stem (Z. Zhang et al., 2016), Miscanthus and seagrass (Li et al., 2017), microalgae (Herrmann et al., 2016b)(Jung et al., 2016), spent poppy straw (Bayrakdar et al., 2016), tall fescue (Chen et al., 2016), kraft mill fibre sludge (Ekstrand et

al., 2016), aloe peel waste (Huang et al., 2016), casein whey (Brown et al., 2016) and spent coffee grounds (J. Kim et al., 2016).

## **1.9. Digestate**

Increasing number of anaerobic digestion and co-digestion plants means simultaneous increase of the quantity of the final byproduct, the digestate (Kratzeisen et al., 2010). Raw digestate withdrawn from anaerobic digester is a liquid to thick slurry that contains a significant quantity of solids. A common anaerobic digestion plant with 500 kW power produces more than 10,000 t of digestate per year with a dry matter of about 10% (Kratzeisen et al., 2010).

### **1.9.1. Digestate composition**

The composition of digestate varies depending on the composition of substrates, source of inoculum, anaerobic digestion operating conditions (pH, temperature, organic loading rate (OLR), hydraulic retention time (HRT)) and anaerobic digestion configurations (without or with post digester). Besides, pretreatment of the substrate before digestion has also an impact on the composition of the digestates (Mata-Alvarez et al., 2014; Monlau et al., 2015b; Tampio et al., 2016b). Digestate contains high variation of undigested organic matter with VS/TS from 39 to 85% (Teglia et al., 2011). Moreover, high OLR and short HRT during anaerobic digestion caused up to 35% undigested organic matter in the digestates even if pretreatment is applied on the substrates (Monlau et al., 2015b).

Digestate which is originated from co-digestion of manure and industrial organic waste contains nutrients such as nitrogen, phosphorus, potassium which can be useful for land application as fertilizer (Madsen et al., 2011). Besides, the concentration of ammonia in the digestate can vary depending on type of substrate and of manure. This was shown in a batch study where at OLR 8 g VS/L, chicken manure contributed to highest amount of ammonia (1.07 g N/L) in the digestate followed by pig manure (0.56 g N/L), dairy manure (0.45 g N/L) and rabbit manure (0.35 g N/L) (K. Li et al., 2015).

Parameters such as pH, salinity, nutrients and heavy metals are important chemical properties that needs to be considered in digestates (Mata-Alvarez et al., 2014). pH of digestates are generally slightly alkaline in the range from pH 6.7 to pH 8.4 (Tampio et al., 2016b) due to degradation of VFA and the production of ammonia during the process. Besides, addition of strong bases or carbonates to control both the pH and buffer capacity of the system also contributes to alkalinity of digestate (Monlau et al., 2015b). The composition of the digestates with respect to different substrates and co-substrates at the input are shown in Table 5.

Table 5: Digestates compositions

Substrates and co-substrates	pH	TS (%)	VS (%)	VS/TS (%)	TKN (% on TS)	NH <sub>4</sub> <sup>+</sup> /TKN (%)	C/N	COD (g/L)	Residual methane yields (mL CH <sub>4</sub> /g VS)	Reference
Sludge based anaerobic digestates										
Waste activated sludge (WAS) and vegetable wastes	7.6	3.4	2.4	70	6.4	79	6.1	26.7	-	(Tampio et al., 2016b)
Manure based anaerobic digestates										
Cow manure (100%)	-	7.4	-	-	4.6	65	10	-	-	(Risberg et al., 2016)
Cow manure (99%), fodder and silage wastes	-	6.1	-	-	6.7	68	6.3	-	-	(Risberg et al., 2016)
Manure (95%), organic wastes (5%)	-	3.1	-	-	14.5	82	2.4	-	-	(Risberg et al., 2016)
Cow manure (90%), food wastes (10%)	-	4.3	-	-	8.1	69	4.8	-	-	(Risberg et al., 2016)
Manure (81%), slaughterhouse wastes (15%), food wastes (3%), energy crops (1%)	-	4.8	-	-	7.9	63	5.3	-	-	(Risberg et al., 2016)
Cow manure (80%), organic wastes (20%)	-	4.1	-	-	13.9	74	2.8	-	-	(Risberg et al., 2016)
Cow manure (75%), slaughterhouse wastes (20%), grease (3%), soybean and silage wastes (2%)	-	5.2	-	-	11	72	3.7	-	-	(Risberg et al., 2016)
Manure (cow and pig) (75%), industrial wastes (25%)	-	6.5	-	-	8.3	61	5	-	-	(Risberg et al., 2016)
Manure (cow, pig, chicken) (75%), food wastes (25%)	-	3.9	-	-	12.3	75	3.1	-	-	(Risberg et al., 2016)
Animal manure (70%), energy crops (20%), food industries by-products (10%)	7.9	9.6	7.4	77	4.4	46	-	-	38	(Menardo et al., 2011)
Animal manure (55%), energy crops (45%)	7.8	5.4	5	74	5.9	52	-	-	19	(Menardo et al., 2011)
Animal manure (37%), energy crops (47%), food industries by-products (16%)	8.0	3.7	2.5	67	14	58	-	-	4	(Menardo et al., 2011)
Cattle slurry (96%), glycerine (4%)	5.6	3.8	2.6	69	4.9	52	9.5	-	-	(Albuquerque et al., 2012)
Cattle slurry (94%), glycerine (6%)	7.4	7.3	5.6	77	3.2	38	18.5	-	-	(Albuquerque et al., 2012)
Cattle slurry (84.1%), cattle manure (4.3%), maize-oat	7.5	9	6.6	74	4.4	61	8.5	-	-	(Albuquerque et al.,

silage (11.6%)										2012)
Cattle slurry (95%), orange peel waste (5%),	7.9	2.4	1.8	74	5.9	53	6.6	-	-	(Albuquerque et al., 2012)
Pig slurry (96%), cabbage (4%)	-	6.0	-	-	7.2	72	6.3	-	-	(Risberg et al., 2016)
Pig slurry (92.5%), sludge (1%), biodiesel wastewater (6.5%)	8.2	2	0.9	44	20.3	87	1.5	-	-	(Albuquerque et al., 2012)
Pig slurry (99.4%), pasteurized slaughterhouse (0.6%)	8.0	2.1	1.2	54	13.8	77	2.0	-	-	(Albuquerque et al., 2012)
Pig slurry (87%), energy crops (13%)	8.1	1.7	1.1	62	11.2	78	-	-	3	(Menardo et al., 2011)
Dairy manure, biowaste	7.4-7.9	2.8-4.4	2.1-3.3	69-76	5-6.2	52-63	-	-	-	(Paavola and Rintala, 2008)
Liquid fraction of dairy manure	7.7-7.9	3.8-4.5	2.2-2.8	59-61	7.7-8.5	58-72	-	29.3-36.9	15-103	(Rico et al., 2011)
Cattle slurry (12%), farmyard manure (31%), poultry manure (8%), maize silage (27%), drying maize residues (21%), rice chaffs (1%)	7.9-8.1	8.8-9.6	6.7-7.6	75-82	-	-	-	-	262 L methane /m2 surface/day	(Gioelli et al., 2011)
Cattle slurry (50.5%), energy crops (49.5%)	8.1	5.7	4.3	76	6.3	51	-	-	-	(Riva et al., 2016)
Food wastes based anaerobic digestates										
Food wastes	7.6-8.3	2-7.9	1.2-6.4	62-81	9.9-23.6	21-82	1.5-3.3	21.8-100.3	-	(Tampio et al., 2016b)
Food wastes	7.7-8.0	6.7-7.9	4.6-5.1	68-77	9.3-11.6	26-52	3.3-4.5	-	80-135	(Tampio et al., 2015)
Food wastes (Source segregated domestic food wastes)	7.89-7.91	6.6-6.7	4.8-4.9	73	13.1-13.3	55-56	-	-	-	(Serna-Maza et al., 2015)
Food wastes (45%), organic wastes (30%), slaughterhouse wastes (15%), manure (7%), grease (3%)	-	4.2	-	-	12.1	67	2.9	-	-	(Risberg et al., 2016)
Food wastes (45%), slaughterhouse wastes (40%), organic wastes (15%)	-	1.7-6.1	-	-	12.5-20	66-82	2.1-2.5	-	-	(Risberg et al., 2016)
Food wastes and garden wastes	-	2.4	1.32	55	10	83	-	-	-	(Stoknes et al., 2016)
Organic wastes of food industry and WAS	7.6	2.4	1.6	69	-	-	-	24.6	169.4	(Boni et al., 2016)
Fruit and vegetable wastes	7.1-7.6	-	-	-	-	25-45	2.7-13.3	-	-	(Di Maria et al., 2014)
Food wastes, slaughterhouse wastes, municipal wastes	8.4	3.9	2.7	69	22	63	1.3	-	-	(Köster et al., 2015)
Food wastes, slaughterhouse wastes, source separated household wastes, kitchen wastes and garden wastes	7.9-8.2	1.4-6.1	0.5-4.3	38-71	11.2-15.7	46-69	2-4	-	-	(Sheets et al., 2015)
Lignocellulosic based anaerobic digestates										

Corn and wheat	6.9	2.03	1.15	57	20.3	70	-	19.1	-	(Meixner et al., 2015)
Maize silage (25%), sorghum silage (11%), olive waste (11%), cow manure (8%), pig manure (18%), and turkey poultry manure on coconut chips (26%)	8.1	8.3	6.03	73	6.4	76	-	74.8	70	(Sambusiti et al., 2015)
Grass (45%), cow manure (33%) and fruit and vegetables (22%)	7.4-7.7	7.1-15.3	4.9-11.6	69-76	-	-	-	-	-	(Ganesh et al., 2013)
Corn and wheat	6.9	2.03	1.15	57	20.3	70	-	19.1	-	(Meixner et al., 2015)
OFMSW based anaerobic digestates										
OFMSW	8.3	3.2	1.9	59	14	71	2.3	30.6	-	(Tampio et al., 2016b)
Organic fraction of residual household waste	8.2	-	-	-	1.9	22	-	-	-	(Zeng et al., 2015)
Organic wastes (78%), silage (12%), grease (10%)	-	5.9	-	-	9.3	64	4.4	-	-	(Risberg et al., 2016)
Organic wastes (75%), manure (25%)	-	1.1	-	-	23.6	81	1.7	-	-	(Risberg et al., 2016)
Organic wastes (55%), food wastes (45%)	-	2.2	-	-	20.9	78	1.7	-	-	(Risberg et al., 2016)
Slaughterhouse wastes based anaerobic digestates										
Slaughterhouse wastes (95%), manure (5%)	-	1.4	-	-	17.1	79	2.6	-	-	(Risberg et al., 2016)
Slaughterhouse wastes (69%), cow manure (21%), whey (9%), others (1%)	-	3.3	-	-	20	80	2.3	-	-	(Risberg et al., 2016)
Other digestates										
Distillery wastes (100%)	-	6.6	-	-	8.5	55	5	-	-	(Risberg et al., 2016)
Distillery wastes (80%), cereals (10%)	-	3.7	-	-	17.3	64	1.4	-	-	(Risberg et al., 2016)

### **1.9.2. Digestate utilization**

Digestate is usually managed through several ways for optimal transport and application. Digestate management operations include storage and application on agricultural land, solid-liquid separation, stabilization process (composting), physical-chemical treatment (combination of separation, ultra-filtration, reverse osmosis and ionic exchanger), mechanical drying (belt dryer, drum dryer and solar dryer) and thermal vaporization (concentration) (Rehl and Müller, 2011). Digestate is commonly used as fertilizer (Kratzeisen et al., 2010). This is due to the properties of digestates which is a mixture of partially degraded organic matter, anaerobic biomass and inorganic matter which is suitable to be used as organic fertilizer or soil conditioner since it contains considerable amount of nutrients (Albuquerque et al., 2012; Astals et al., 2013b; Mata-Alvarez et al., 2014). In a review of 13 anaerobic digestion plants originating from processing source-segregated MSW, 77% of digestate are recycled on land while the remaining is landfilled (Iacovidou et al., 2013).

However, undigested organic matter retained in digestate causes residual biogas emission during storage or land application which contributes to greenhouse gases (GHG) emission and pollution to the atmosphere (Gioelli et al., 2011). For example, digestate from storage emitted 12% methane of the annual methane produced in the digester from co-digestion of dairy manure and food industry (Baldé et al., 2016). Moreover, some digestates have high content of ammonium, salinity, COD, phosphate and colour which will cause high risk to environment and all organisms if it is improperly exposed to agriculture land (Tigini et al., 2016).

Some recent research on post-treatment of digestate has been conducted including ultrasonication which has shown 25% gain of methane compared to unsonicated digestate (Boni et al., 2016), using “active” bulking agent like dry wood chips which serves as water and nitrogen sinks to improve composting (Zeng et al., 2015), using digestates as cheaper substitute for synthetic fillers for solid surface materials (digestates-composites) (Taurino et al., 2016), cultivating vegetables on digestates (Stoknes et al., 2016) and producing new quality pyrolysis oil, char and syngas from digestate through thermo-catalytic reforming process (Neumann et al., 2014).

According to Baldé et al., the potential best practices for further digestate use include covered storage and solid-liquid separation (Baldé et al., 2016).

### **1.10. Solid-liquid separation of digestate**

Drastic increase of raw digestate volume produced daily in recent years causes overproducing raw digestate for local use (Kratzeisen et al., 2010). Local and regional transportation of excess raw digestate of more than 5-10 km will exceed the costs of its fertilizer value (Kratzeisen et al., 2010) and consumes huge amount of fuel oil (Rehl and Müller, 2011). Therefore, solid-liquid separation of raw digestate is often performed on-site to reduce the cost of digestates transportation for disposal (Delzeit and Kellner, 2013). Solid-liquid separation of digestate can be done by using either bow sieve, double circle bow sieve, sieve belt press, sieve drum press, press screw/auger separator, sieve centrifuge or decanter centrifuge (Burton and Turner, 2003). However, the most common solid-liquid separation applied in full-scale plants are screw press, screening drum press (vibrating screen) and centrifuge (Al Seadi et al., 2013; Delzeit and Kellner, 2013) as shown in the schematic diagrams in Figure 6. The addition of precipitating agents (such as aluminium sulphate ( $\text{Al}_2\text{SO}_4$ ), ferric chloride ( $\text{FeCl}_3$ ), ferric sulphate ( $\text{Fe}_2(\text{SO}_4)_3$ ) and lime ( $\text{Ca}(\text{OH})_2$ )), flocculants or organic polymers (acrylamide) ((Drosig et al., 2015; Meixner et al., 2015) will enhance the performance of solid-liquid separation. Moreover, recent research has also suggested the uses of functionalized chitosan to improve solid-liquid separation efficiency (David et al., 2016).

After separation, raw digestate is divided into a solid fraction and a liquid fraction (Gioelli et al., 2011). The distributions of the mass, total solids (TS), volatile solids (VS), ash, total nitrogen (TN), ammonia nitrogen ( $\text{NH}_4\text{-N}$ ), phosphorus (P), potassium (K) and carbon (C) of the digestate after solid-liquid separation are shown in Table 6.

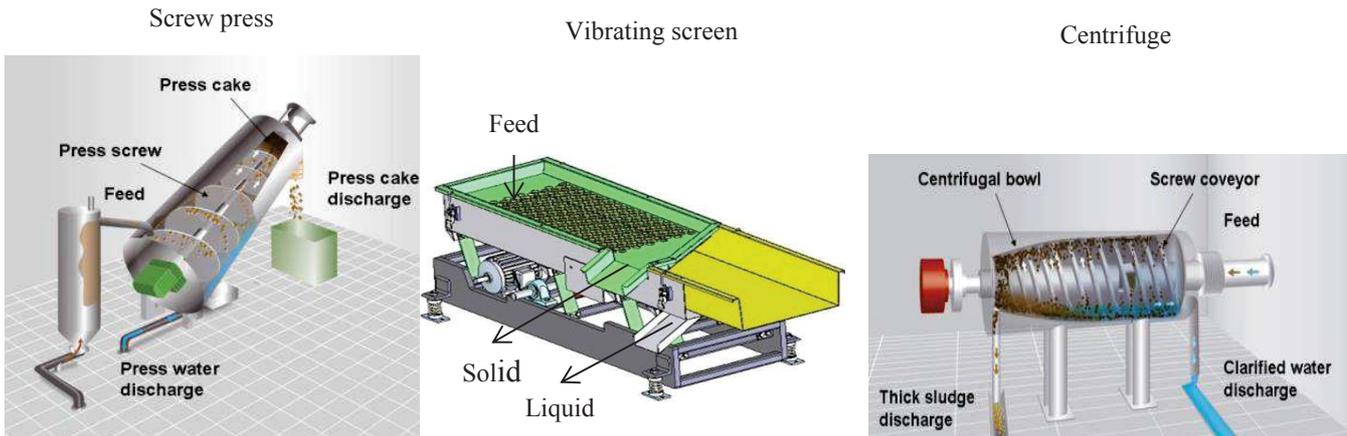


Figure 6: Schematic diagram of solid-liquid separation by screw press, vibrating screen and centrifuge (adapted from Hjorth et al. (Hjorth et al., 2010) and Panca Desain (Desain, 2017)).

Table 6: Distribution of the principal constituents after solid-liquid separation (adapted from Drogg et al. (Drogg et al., 2015))

	General range		Screw press		Centrifuge	
	Liquid (%)	Solid (%)	Liquid (%)	Solid (%)	Liquid (%)	Solid (%)
Mass	80-92	8-20	90	10	92	8
Total solids (TS)	14-52	48-86	52	48	14	86
Volatile solids (VS)	35-45	55-65	-	-	-	-
Ash	50-60	40-50	-	-	-	-
Total nitrogen (TN)	65-83	17-35	83	17	75	25
Ammonia-Nitrogen (NH <sub>4</sub> -N)	70-93	7-30	82	9.2	92.5	7.5
Phosphorus (P)	22-45	55-78	-	-	22	78
Potassium (K)	70-93	7-30	90	10	93	7
Carbon (C)	30-40	60-70	-	-	-	-

### 1.11. Solid fraction of digestate

Solid fraction of digestates are usually used for land application (Rehl and Müller, 2011) where they are applied, directly or after composting, as organic fertilizer (Tambone et al., 2015; Zeng et al., 2015). Moreover, it contains high percentage of phosphorus after solid-liquid separation (Table 6). Besides, solid fraction of digestate can be dried either using belt dryer, drum dryer, feed-and turn dryer, fluidized bed dryer or solar drying system or palletized to be marketed as bio-fertilizers (Drogg et al., 2015). In addition, it can also be pelletized and used as solid fuel (Kratzeisen et al., 2010).

Recently, solid fractions of digestates have gained more interest in research and development due to its characteristic of high organic fraction (Table 6) as well as high nutrients contents. New routes have been proposed for solid digestate valorization (Monlau et al., 2015b) such as production of bio-fuel in domestic furnaces (Pedrazzi et al., 2015), production of biochar (Monlau et al., 2015a; Stefaniuk and Oleszczuk, 2015), post treatments (thermal, alkaline and enzymatic) for methane recovery (Sambusiti et al., 2015) as well as bioethanol production after a mechanical fractionation such as centrifugal milling (Sambusiti et al., 2016).

## **1.12. Liquid fraction of digestate**

### **1.12.1. Characteristics of liquid fraction of digestate**

Liquid fraction after solid-liquid separation contain 90-95% of total mass of digestate in most liquid anaerobic digestion plants (Sheets et al., 2015). They generally contain very low residual biogas potential (Gioelli et al., 2011) but high concentrations of chemical oxygen demand (COD) (Ganesh et al., 2014, 2013; D. Li et al., 2015a; Xia and Murphy, 2016), total nitrogen (TN) and ammonia nitrogen as well as nutrient (Xia and Murphy, 2016). Besides, they contain lower TS and VS concentrations as well as lower VS/TS ratio than raw digestate (Gioelli et al., 2011). The compositions of liquid fraction of digestate found in the literature based on types of substrates are shown in Table 7. pH ranges from 7.5 to 9.4. The range of total solids (TS) and volatile solids (VS) of liquid fraction of digestate can be from 1.1 to 8% and from 0.5 to 4.8%, respectively. COD is high in variation from 0.3 to 17.6 g O<sub>2</sub>/L. Organic carbon (C<sub>org</sub>) ranges from 7.4 to 20.6 g C/L. C/N ratio of liquid fraction of digestate ranges from 1.6 to 11.9 with high variation of NH<sub>4</sub><sup>+</sup> concentration from 0.37 to 5.1 g N/L. Moreover, TN concentration of liquid fraction of digestate is also high in variation from 0.1 to 8.0 g N/L. Total phosphorus (TP) concentration ranges from 0.03 to 1.2 g P/L. Phosphate (PO<sub>4</sub><sup>3-</sup>) and magnesium (Mg) concentrations range from 0.01 to 0.3 g/L and 0.01 to 0.54 g/L respectively. Potassium (K) concentration ranges from 0.1 to 5 g K/L.

The concentrations of residual compounds found in liquid fraction of digestate are higher in comparison to concentrations of leachate from anaerobic sanitary landfills which contains 0.5 to

1.3 % of TS, 0.45 % of VS, 0.6 to 12 g O<sub>2</sub>/L COD, 0.1 to 2.6 g N/L NH<sub>4</sub><sup>+</sup>, 1.6 to 1.7 g K/L potassium, 0.1 to 2.1 g N/L TN and 0.01 to 0.04 g P/L phosphorus (Aziz et al., 2010; Li et al., 2009; Naveen et al., 2017; Ziyang and Youcai, 2007).

Taken as a whole, Table 7 shows that no study has considered the full characterization of liquid fraction of digestate. In addition, very little information on the co-digestion plants is available in the literature, except the nature of substrates. Most of published analysis focused on nitrogen and phosphorus concentrations and were carried out for in the frame of researches on nutrients removal, recovery and reuse.

Table 7: Compositions of liquid fraction of digestates

Substrate	Feeding (m <sup>3</sup> /d)	OLR (kg VS/m <sup>3</sup> /d)	HRT (d)	Digester	Solid-liquid separation	T (°C)	pH	TS (%)	VS (%)	COD (g/L)	sCOD (g/L)	C <sub>org</sub> (g/L)	TC (g/L)	TN (g/L)	C/N	NH <sub>4</sub> <sup>+</sup> (g/L)	TP (g/L)	sP (g/L)	PO <sub>4</sub> <sup>3-</sup> (g/L)	Mg <sup>2+</sup> (g/L)	K <sup>+</sup> (g/L)	Reference
Sludge and wastewater based liquid fraction of digestates																						
Sludge	NS	NS	NS	NS	Centrifuge and 0.45 mm filtration	NS	7.8	-	-	0.3	-	-	-	0.39	-	0.37	0.2	-	0.19	0.03	0.1	(Huang et al., 2015)
Swine wastewater	NS	NS	NS	NS	Centrifugation (4000 rpm, 15 min, 5°C)	NS	8.4	1.1	0.5	3.97	-	-	-	1.7	-	1.65	0.17	-	0.15	0.01	2.1	(Obaja et al., 2003)
Livestock wastewater	150	NS	NS	NS	Lab-scale centrifugation (2000 rpm, 10 min)	NS	NS	-	-	1.61	-	-	-	0.74	-	0.73	0.03	-	0.013	-	-	(S. Kim et al., 2016)
OFMSW based liquid fraction of digestate																						
Organic waste, energy crops and animal slurry	NS	NS	NS	NS	Mechanical separation	NS	-	8	2.9	-	-	-	-	2.7	-	-	1.2	-	-	-	5	(Vanden Nest et al., 2015)
OFMSW	NS	NS	30	NS	NS	NS	8.2	1.9	1.1	-	-	7.4	-	2.5	8.3	1.7	-	-	-	-	-	(Michelle et al., 2015)
Manure based liquid fraction of digestate																						
Pig manure	NS	0.4-0.8	35	Semi-continuous	Centrifugation	Ambient	7.5	1.9	0.4	5.6	3	-	-	-	-	1.36	-	0.05	-	0.08	-	(X. Li et al., 2016)
Manure	NS	NS	NS	NS	NS	NS	-	NS	NS	7.5	-	-	-	3.6	-	3.3	0.12	-	-	-	-	(Gong et al., 2013)
Cattle slurry (90%) and corn silage (10%)	NS	NS	NS	NS	Roller press	NS	8.1	4.2	2.7	-	-	-	-	3.1	-	1.8	-	-	-	-	-	(Perazzolo et al., 2015)







## **1.12.2. Nutrients removal, recovery and reuse from liquid fraction of digestate**

Researches on nutrients removal, recovery and reuse from liquid fraction of digestate have intensively been conducted.

### **1.12.2.1. Nutrients removal**

#### **1.12.2.1.1. Ammonia stripping**

Ammonia stripping is one of the most effective physicochemical nitrogen removal and recovery (Sheets et al., 2015). Ammonia stripping is a process where  $\text{NH}_3$  in liquid sample converts to gas when comes in contact with air or steam that contains no or few  $\text{NH}_3$  (Sheets et al., 2015). The factors that affect the process are pH, temperature, air/liquid ratio and pressure (De la Rubia et al., 2010; Guštin and Marinšek-Logar, 2011; Sheets et al., 2015). High pH level has the most influence on ammonia stripping followed by air flow rate and temperature (Guštin and Marinšek-Logar, 2011). In a research, continuous ammonia stripping on liquid fraction of digestate after centrifugation originating from pig slurry showed up to 92.8% and 88.3% removal yield of ammonia and total nitrogen respectively (Guštin and Marinšek-Logar, 2011). Another study, coupling an anaerobic digester (fed with source segregated food waste) with side-stream ammonia stripping columns operated semi-continuously using biogas as stripping medium, had shown 48% of  $\text{NH}_4^+$ -N removal at high temperature  $\geq 70^\circ\text{C}$  and high pH of 10 (Serna-Maza et al., 2014). Bousek et al. studied side stream ammonia stripping from liquid fraction of digestate after sieving at 1 mm originating from a mixture of pig manure, maize silage, sugar and pig fodder (Bousek et al., 2016). The effect of oxygen contact during air stripping showed 86%  $\text{NH}_4$ -N removal after 4 hours. Flue gas can be chosen as another option to avoid inhibition of anaerobic microflora with 45%  $\text{NH}_4$ -N removal after 4 hours compared to biogas with 16 %  $\text{NH}_4$ -N removal after 4 hours. This is due to the stripping performance which is negatively correlated to  $\text{CO}_2$  level in the strip gas (Bousek et al., 2016). Other study showed ammonia stripping with optimum  $\text{Ca}(\text{OH})_2$  addition of 12 g/L at  $\text{pH} > 7$  resulted in 89.9% of  $\text{NH}_4^+$ -N removal in liquid fraction of digestate originating from pig manure (X. Li et al., 2016). The

addition of  $\text{Ca}(\text{OH})_2$  can also remove 97.2% of soluble phosphorus due to precipitation (X. Li et al., 2016).

#### 1.12.2.1.2. Anaerobic ammonium oxidation (ANAMMOX)

Anaerobic ammonium oxidation (ANAMMOX) is a process that uses Planctomycetes-like bacteria under anoxic conditions to oxidize ammonium ( $\text{NH}_4^+$ ) to nitrogen gas ( $\text{N}_2$ ) using nitrite ( $\text{NO}_2^-$ ) as an electron acceptor and inorganic carbon as an electron donor (Magrí et al., 2013; Sheets et al., 2015). The factors which affect ANAMMOX process are substrate composition (nitrite, ammonium and inorganic carbon), organic carbon (organic molecules such as acetate, propionate, glucose, fructose and lactate), pH, temperature, dissolved oxygen, phosphate ( $\text{PO}_4^{3-}$ ), sulfide ( $\text{S}^{2-}$ ) and salinity (Magrí et al., 2013). Moreover, these parameters have their concentration range limits which affect the efficiency of nitrogen removal as shown in Table 8.

Table 8: ANAMMOX process concentration range limit (adapted from Magrí et al (Magrí et al., 2013) and Sheets et al (Sheets et al., 2015))

Parameter	Concentration range
pH	6.5 - 8.8
Temperature ( $^{\circ}\text{C}$ )	35 - 40
Dissolved oxygen (DO)	1%
Salinity	8800 – 30,000
Ammonium ( $\text{NH}_4^+$ ) (g/L)	0.6 – 5.9
Ammonia ( $\text{NH}_3$ ) (g/L)	0.002 – 0.15
Nitrite ( $\text{NO}_2^-$ ) (g/L)	0.1 – 0.182
Phosphate ( $\text{PO}_4^{3-}$ ) (g/L)	0.031 – 0.620
Sulfide ( $\text{S}^{2-}$ ) (g/L)	0.001 – 0.064
Inorganic carbon (g/L)	0.15 – 0.295
Organic carbon (g/L)	0.142 – 0.242

Complete autotrophic nitrogen removal (ANR) which is the combination of partial nitrification (PN) and ANAMMOX was suggested for the subsequent treatment of liquid fraction of digestate from livestock for nitrogen removal (Magrí et al., 2013). However, there is no full-scale treatment by ANR of liquid fraction of digestate applied for manure (Magrí et al., 2013). The energy requirement and effectiveness of ANR depends on the composition of liquid fraction of digestate (Magrí et al., 2013). The dilution is necessary to avoid inhibition by  $\text{NH}_3$ , phosphates and sulfides (Sheets et al., 2015). PN-ANAMMOX is more competitive than other alternatives

on nutrient recovery due to lower specific energy requirement compared to traditional biological nitrogen removal, air ammonia stripping/ $(\text{NH}_4)_2\text{SO}_4$  absorption, steam ammonia stripping/ $(\text{NH}_4)_2\text{SO}_4$  absorption, struvite crystallization, concentration by vacuum evaporation and concentration by reverse osmosis particularly at concentrations of up to  $2 \text{ kg NH}_4^+\text{-N m}^3$  (Magrí et al., 2013; Sheets et al., 2015). However, at higher concentrations, other processes may become more advantageous (Magrí et al., 2013). The disadvantage of ANAMMOX is that phosphorus, heavy metals or antibiotics in liquid fraction of digestate can negatively affect the ANAMMOX process. Therefore, phosphorus should be precipitated before the ANR stage (Magrí et al., 2013).

#### **1.12.2.1.3. Direct contact membrane distillation process**

Direct contact membrane distillation process was applied for liquid digestate originating from livestock wastewater and after lab-scale centrifugation. The treatment successfully removed 99% COD and Total Phosphorus while the removal of Total Nitrogen was from 85 to 96% depending on the extent of cake layer formed on the membrane surface (S. Kim et al., 2016).

#### **1.12.2.2. Nutrients recovery**

##### **1.12.2.2.1. Vacuum evaporation**

Vacuum evaporation process consists in boiling a liquid sample at a temperature lower than typical boiling temperature at atmospheric conditions under negative pressure (Chiumenti et al., 2013). Vacuum evaporation of liquid fraction of digestate has been studied using 0.100 and  $0.025\text{m}^3$  pilot scale plants (Chiumenti et al., 2013). In this study, liquid fraction of digestate after screw press originating from swine manure, corn silage and other biomasses was used. Two-stage vacuum evaporation with acidification has shown to effectively concentrate 1688%, 1850% and 1527% of TS, VS and TKN, respectively. The two-stage system has successfully removed 94.4% of mass which contained 2.5% mass of TKN which can either be discharged into surface water, after purification or used as dilution water for the input of digester (Chiumenti et al., 2013). The reduction of pH to 5 is necessary to prevent from vaporization of ammonia to form

condensate and therefore, remained in the solid concentrate (Chiumenti et al., 2013). In comparison, other study on vacuum evaporation of liquid fraction of digestate originating from pig manure has shown at optimum pH 6, there were increase of only 114% and 224.8% in  $\text{NH}_4^+$ -N and soluble phosphorus concentrations respectively (X. Li et al., 2016).

#### **1.12.2.2.2. Struvite recovery**

Precipitation via the formation of struvite (combination of magnesium ions, ammonium and phosphate ( $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$ )) is seen as a promising method to recover high magnesium, ammonium and phosphate concentrations in liquid fraction of digestate due to its simple but high efficiency and environmental friendly method (Escudero et al., 2015; Moerman et al., 2009; Tao et al., 2016). Struvite precipitation processes can be affected by the source of  $\text{PO}_4^{3-}$ ,  $\text{Mg}^{2+}$ , effluent solid content, pH and  $\text{Mg}:\text{NH}_4:\text{PO}_4$  molar ratio (Escudero et al., 2015; Tao et al., 2016). However, the limits to efficiency of struvite process are high  $\text{Ca}^{2+}$  concentration, high ionic strength, high suspended solids concentration, high alkalinity and complex chemical composition (Tao et al., 2016).

Liquid fraction of digestate originating from manure especially rich in ammonium, nitrogen and orthophosphates can give high market potential for struvite recovery as well as ammonia recovery ( $(\text{NH}_4)_2\text{SO}_4$ ) (Tao et al., 2016). For example, Tampio et al. used ammonia stripping combined with  $\text{H}_2\text{SO}_4$  scrubbing to recover ammonium sulfate  $(\text{NH}_4)_2\text{SO}_4$  which is a chemically pure product with no TS, VS, phosphorus and potassium (Tampio et al., 2016a). The same author also combined ammonia stripping (with  $\text{H}_2\text{SO}_4$  scrubbing) with reverse osmosis and succeeded to obtain  $(\text{NH}_4)_2\text{SO}_4$  and remove TS, VS, total nitrogen,  $\text{NH}_4$ -N, phosphorus and potassium from liquid fraction of digestate (Tampio et al., 2016a).

Besides, struvite precipitation can also be used for ammonium removal from liquid fraction of digestate. A research obtained 95% removal from initial  $\text{NH}_4^+$  concentration of 2.5 g/L in only 30 seconds after addition of  $\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$  and  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  with molar ratio of 1:1:1 for  $\text{Mg}:\text{NH}_4:\text{PO}_4$  without pH adjustment (Escudero et al., 2015).

#### **1.12.2.2.3. Vacuum thermal stripping with acid absorption**

Vacuum thermal stripping with acid absorption is a recent innovative technology which allows ammonia recovery at higher flow rate in recirculation line of a mesophilic anaerobic digester compared to thermal stripping with higher temperature (Ukwuani and Tao, 2016). Ammonia is stripped out and absorbed to a sulfuric acid solution and later forms ammonium sulfate crystals which have market values. At an optimum boiling point of 65°C and pressure of 25.1 kPa, more than 95% of ammonia can be stripped out of liquid digestate in one and half hour (Ukwuani and Tao, 2016).

#### **1.12.2.2.4. Combined evaporation and reverse osmosis**

Tampio et al studied the combination of evaporation and reverse osmosis for nutrients recovery. The study showed 99.7, 99.1, 100 and 100% recovery of total nitrogen,  $\text{NH}_4^+\text{-N}$ , phosphorus and potassium, respectively (Tampio et al., 2016a). The same author also combined stripping before evaporation and reverse osmosis and obtained 100% recovery of total nitrogen,  $\text{NH}_4^+\text{-N}$ , phosphorus and potassium (Tampio et al., 2016a).

#### **1.12.2.3. Nutrients reuse**

Nutrients from liquid fraction of digestate can also be reused. This was shown in a study where liquid fraction of digestate originating from biomass (*Tectona Grandis*, *Syzygium cumini* and *Ficus aurea* leaves) fed at 1 kg TS/m<sup>3</sup> into a plug flow reactor was filtered at 50 µm and autoclaved and later used as nutrient supplement and maintaining moisture content for mushroom (*Pleurotus florida*) cultivation (Malayil et al., 2016). The study had shown to increase from 66 to 100% yield of mushroom growth. An increase of 20% N supply was found to increase 40% of mushroom yields (Malayil et al., 2016). Another study on nutrient reuse was from diluted digestate originating from pig slurry and corn to cultivate green algae *Chlorella vulgaris*. Result obtained was very promising with 90% removal of ammonia, total nitrogen and phosphate (Franchino et al., 2016).

Other study which recycled liquid digestate has proven to improve methane production in a low nitrogen mono-digestion of straw. With supplementary of co-digestion with sewage sludge or addition of macronutrient (N and P), it was shown to increase process performance with higher methane production yield with low VFAs accumulation and stable pH (Peng et al., 2016).

### **1.13. Conclusion**

Anaerobic digestion, its different processes, substrates, and co-digestion have been discussed in this chapter. The final by-product, the digestate, has been described before and after solid-liquid separation. After separation, solid fraction of digestate has been studied for its valorization. For liquid fraction of digestate, a lot of researches have been conducted on nutrient removal, recovery and reuse. However, the researches were mainly focused on nutrients; and this literature review has pointed out a lack of knowledge on the organic fraction of the liquid fraction of digestate. Only few studies have highlighted their high COD content but low residual methane potential. Besides, the aerobic biodegradability of liquid fraction of digestate has not been considered in the literature for a possible aerobic post-treatment. This thesis aims to provide a full characterization of several liquid fractions of digestates in order to give the first elements for their further post-treatment and valorization. Another objective of the thesis is to explain the variability of the residual compounds in liquid fraction of digestates in relation to the origins of the substrates and to the operating parameters or type of solid-liquid separation performed.



## **2. Materials and Methods**

### **2.1. Characterization of digestates from full-scale biogas plants**

#### **2.1.1. Digestate collection**

Raw digestates, solid and liquid fractions of digestates after full-scale separation were collected from 29 co-digestion plants treating solid wastes. In order to have representative samples, plants with different types of feedstocks were selected as shown in Table 9. Each plant was operated at different operating parameters as shown details in Table 10. The digestates were separated on-site into solid and liquid fractions either by screw press, centrifugation or vibrating screens as shown in Table 10. Besides, to have a comparison of the samples from co-digestion plants with a common anaerobic digestion plant treating Waste Activated Sludge (WAS), raw digestate, solid and liquid fractions were collected from a WAS anaerobic digestion plant (Plant G). In this study, raw digestate and solid fraction of digestates were collected for determination of total solids (TS) and volatile solids (VS) contents in order to have some information on the efficiency of on-site solid-liquid separation.

Table 9: Feedstock compositions and proportions

Plants	Substrates composition
A	Sludge (36%), fats (22%), manure (16%), crop residues (7%), blood (7%), fruits and vegetables (7%), pet food (5%)
B	Cow manure (60%), pig manure (20%), cereal residues (10%), fats, (8%), rainwater (2%)
C	Fruits and vegetables (100%)
E	Sludge (40%), fats (30%), food waste (30%)
F	Liquid cow manure (50%), crop residues (18%), cereal residues (11%), fruits and vegetables (11%), poultry manure (8%), cow manure (2%)
H	Sludge (50%), pig manure (28%), fats (22%)
I	Bio-waste (100%)
J	Corn silage (95%) and poultry manure (5%)
K	Wastewater (25-30%), fats (20-30%), energy crops (20%), cereal residues (10-15%), sludge (10-12%), bio-waste and some slaughterhouse waste (3-4%)
L	Pig manure (64%), fruits and vegetables (11%), cereal residues (9%), crop residues (5%), grass (5%), sludge (5%)
M	Cow manure (33.3%), corn silage (33.3%), liquid fraction from the farm (33.3%)
G	Waste activated sludge (100%)
N	Solid sludge (28.8%), liquid sludge (14.8%), liquid cow manure (13.3%), liquid pig manure (10.3%), thick liquid grease (7.4%), glycerin (5.5%), liquid grease (5.4%), horse manure (3.3%), catch crops (2.5%), sheep manure (2.2%), grass silage (1.9%), cereal residues (1.9%), cow manure (1.5%), wastewater (0.6%), glucose (0.5%), poultry litter (0.2%)
O	Duck manure (62%), cow manure (9%), fats (8%), mixture of cream milk (7%), glycerin (7%), pig manure (4%), blood (3%)
P	Slurry (55%), manure (20%), sweetcorn cobs (10%), cheese plant sludge (5%), filtration sediment (5%), fats (5%)
Q	Liquid cow manure (35.2%), pig manure (22.3%), cow manure (19%), sorghum silage (16.6%), whey (5.2%), cereal residues (0.85%), tomato leaves (0.85%)
R	Pig manure (53.6%), apple pomace (14.3%), cereal residues (10.7%), grass silage (10.7%), corn withers (10.7%)
S	Sludge (50%), pig manure (28%), fats (22%)
T	Pig manure (48%), fats (40%), corn silage (12%)
U	Liquid manure (38.1%), grass silage (29.5%), manure (17.4%), corn silage (12.6%), cereal residues (2.4%)
V	Grass silage (24.1%), corn silage (20.7%), liquid manure (20.7%), solid manure (a little of chicken manure) (13.8-17.2%), whole grain plants (10.3-13.8%), crushed grains (6.9%)
W	Corn silage (85-90%), rye (10-15%), crushed grains (1 ton/year)
X	Corn silage (70%), rye or barley (25%), grass (5%), chicken manure (small quantity)
Y	Bio-waste (43.2%), digestate recirculation (37.8%), fats (16%), cereal residues (3%)
Z	Water (24%), duck manure (22.4%), cattle manure (22%), goat manure (10.2%), poultry manure (7.7%), grass silage (6.7%), cereal residues (3.8%), corn silage (3.2%)
I2	Municipal bio-waste (100%)
AA	Liquid fraction of digestate recirculation (53.1%), pig manure (18.2%), grass silage (7.6%), fats (7.4%), duck manure (5.5%), bull manure (4.5%), cereal residues (2.9%), corn silage (0.8%)
AB	Pig manure (33%), milk industry residues (20%), fats (20%), corn silage (10%), energy crop silage (10%), cattle feed residues (7%)
AC	Pig manure (100%)
AD	Food waste (48%), contents from garbage and septic tanks (48%), fats (4%)

Table 10: Operating parameters (temperature range, type of reactor, size of reactor, size of post reactor, feeding, retention time and methane production) of the full-scale plants

Plants	Temperature range	Type of reactor	Size of reactor (m <sup>3</sup> )	Size of post reactor (m <sup>3</sup> )	Feeding (tons/d)	Retention time (days)	Methane production (m <sup>3</sup> /d)
A	Mesophilic	CSTR	2800	-	120	-	-
B	Mesophilic	CSTR	1370	-	15	60	1500-1600
C	Mesophilic	CSTR	350	450	16.6	21 + 27	558
E	Mesophilic	CSTR	3300	1800	90	37 + 20	4500
F	Mesophilic	CSTR	1200	-	15	63	1230
H	Mesophilic	CSTR	930	-	30	31	1550
I	Thermophilic	Plug flow	3150	-	100	30	10000
J	Thermophilic	Plug flow	1200	-	35	35	3500
K	Mesophilic	CSTR	2800	1360	80	35 + 17	-
L	Mesophilic	CSTR	2350	-	55	46	1450
M	Mesophilic	CSTR	400	400	10	40 + 40	550
G	Mesophilic	CSTR	10000	-	19.1	20	5583
N	Mesophilic	CSTR	1500	650	15.8	95 + 41	691
O	Mesophilic	CSTR	2 x 7500	2 x 3500	290	52	17085
P	Mesophilic	CSTR	1000	1000	30.5	65	-
Q	Mesophilic	CSTR	3900	3900	30	-	3915
R	Mesophilic	CSTR	2300	-	28	85	1450
S	Mesophilic	CSTR	920	640	30-35	26 + 18	-
T	Mesophilic	CSTR	2600	-	34.2	50	2381
U	40-45	CSTR	2 x 718.5	682	2 x 10.6	(2 x 68) + 32.5	-
V	52	CSTR	2 x 1500	3000	29	37	-
W	50-60	CSTR	10000	-	70-100	100-120	6240
X	40-41	CSTR	2400	-	27-30	70-80	-
Y	Mesophilic	CSTR	3400	1600	57 + 35 recirculation	37	12400
Z	Mesophilic	CSTR	1200	1200	31.3	66	1418
I2	Thermophilic	Plug flow	3150	-	100-150	20-30	5500-8500
AA	Mesophilic	CSTR	1300	-	65.9	45	2300
AB	Mesophilic	CSTR	2900	-	50	57	3090
AC	Mesophilic	CSTR	500	-	7-8	40	-
AD	Mesophilic	CSTR	1500	3000	75	20 + 40	2790

Table 11: Types of solid-liquid separation

Plants	Types of solid-liquid separation
A	Screw Press
B	Screw Press
C	Vibrating screen
E	Centrifuge
F	Screw press
H	Centrifuge
I	Screw press with coagulant, followed by centrifuge
J	Screw press with coagulant
K	Screw press
L	Screw press
M	Vibrating screen
G	Centrifuge with addition of polymer
N	Screw press
O	Centrifuge with flocculant
P	Screw press
Q	Screw press
R	Centrifuge
S	Centrifuge
T	Rotary drum. Solid fraction was later dried.
U	Screw press
V	Screw press
W	Screw press
X	Screw press
Y	Screw press
Z	Screw press
I2	Screw press with flocculant + centrifuge
AA	Screw press
AC	Centrifuge
AD	Filter press (150 plates) + inorganic coagulant + polymer. Solid fraction was later dried

### 2.1.1. Filtration and size fractionation

Liquid fraction of digestates (Figure 7) were diluted with milliQ water in order to facilitate filtration and prevent a rapid clogging of the filters. The dilution factor varied from 0 to 1/20 and was chosen based on final COD range from 1 to 5 g/L.

### **2.1.1.1. Detailed fractionation**

Full size fractionation was carried out on 11 liquid fraction of digestates (A, B, C, E, F, H, I, J, K, L and M) (first collection). Coarse filtration (100  $\mu\text{m}$ , 41  $\mu\text{m}$ , 10  $\mu\text{m}$ ) followed by microfiltration (1.2  $\mu\text{m}$ , 0.45  $\mu\text{m}$ , 0.2  $\mu\text{m}$ ) (Figure 8) and ultrafiltration (100 kDa, 10 kDa, 1 kDa) (Figure 9) were successively performed on 11 liquid fraction of digestates at room temperature. This was to have 10 fractionation sizes which are raw liquid, < 100  $\mu\text{m}$ , < 41  $\mu\text{m}$ , < 10  $\mu\text{m}$ , < 1.2  $\mu\text{m}$ , < 0.45  $\mu\text{m}$ , < 0.2  $\mu\text{m}$ , < 100 kDa, < 10 kDa and < 1 kDa in order to have detailed characterization of liquid fraction of digestates. Coarse and microfiltration were performed using Merck Millipore Ltd membrane nylon filters. Ultrafiltration was performed using Ultrafiltration Amicon with maximum pressure,  $P_{\text{max}}$  75 psi (5.3kg/cm<sup>2</sup>). Ultrafiltration filters used were regenerated cellulose with diameter 76 mm from Amicon Bioseparations, EMD Millipore Corporation. All permeates of each size were collected and stored at 4°C for chemical, physical, biological characterization.

From detailed characterization of 11 liquid fraction of digestates, the fractionation sizes were further grouped as suspended particles (> 1.2  $\mu\text{m}$ ), coarse colloids (1.2-0.45  $\mu\text{m}$ ), fine colloids (0.45  $\mu\text{m}$ -1 kDa) and dissolved matter (< 1 kDa) (Ziyang and Youcai, 2007).

### **2.1.1.2. Simplified fractionation**

In order to understand the link between substrates fed, operating parameters and types of solid liquid separation used on liquid fraction of digestates and to have enough data to carry out statistical analyses, 18 more liquid fraction of digestates from co-digestion plants (N, O, P, Q, R, S, T, U, V, W, X, Y, Z, I2, AA, AB, AC and AD) (second collection) were further characterized. However, these liquid fraction of digestates were only filtered at 1.2  $\mu\text{m}$  and 1kDa in order to have fractionation sizes of raw liquid, < 1.2  $\mu\text{m}$  and < 1 kDa which represent suspended particles, colloids (coarse and fine) and dissolved matter. Same chemical, physical and biological analyses for characterization were performed as previous detailed characterization.



Figure 7: Liquid fraction of digestates

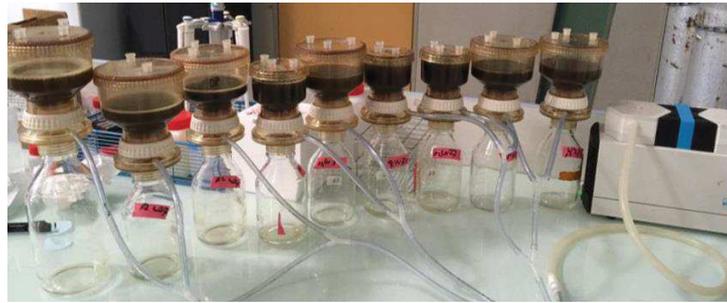


Figure 8: Coarse and microfiltration



Figure 9: Ultrafiltration

## 2.2. Statistical analysis

Statistical analysis was performed in order to classify the parameters analysed on 29 liquid fraction of digestates from full-scale co-digestion plants in separate groups. This is to investigate the relationships between characteristics (chemical, physical and biological) of liquid fraction of digestates in relation to the feedstocks, operating parameters (loading rate, type of reactor, hydraulic retention time, temperature), methane production and type of solid-liquid separation on residual compounds in liquid fraction of digestate. Chemical, physical and biological characteristics of liquid fraction of digestates included in PCA are based on simplified fractions described in Section 2.1.1.2.

In order to perform this, principal component analysis (PCA), hierarchical cluster analysis (HCA) and correlation matrix were performed using R version 3.3.2 (Sincere Pumpkin Patch) (2016-10-31) (Team, 2014). PCA was performed with center-scaled variables using function 'FactoMineR' package version 1.35 (Husson et al., 2011) with PCA plots package 'factoextra' version 1.0.4. HCA was performed using 'hclust' function from 'stats' package version 3.3.2 with distances calculated by Euclidean distance of center-scaled variables. The clustering algorithm was according to Ward (Ward, 1963). The dendrogram was plotted using function 'dendextend' package version 1.4.0. Correlation matrix was performed using 'rcorr', the function of 'Hmisc' package version 4.0.2. The correlation matrix were plotted by 'corrplot' function, package 'corrplot' version 0.77.

### **2.3. Study of impact of mono-substrates on liquid fraction of digestates**

#### **2.3.1. Reactors**

Reactors were operated in this study in order to have controlled conditions for mono substrate. Besides, this will give better understanding on the mechanisms of formation of residual compounds produced in liquid fraction of digestate from a chosen substrate. From previous detailed characterization, cow manure was observed to have a major impact on liquid fraction of digestates. Therefore, cow manure (mixture of cow dung and straw) along with straw and fresh cow dung respectively were selected as substrates as shown in Figure 10 and taken from Saint Denis, a small village in the south of France. Cow manure taken was a mixture of cow dung and straw used as bedding material that was already stored on-site for a certain period of time. The initial characteristics of straw, cow dung and cow manure are shown in Table 12. Straw and cow manure were grounded into size between 3-5cms. Cow dung was well mixed to be to take homogenous samples.

Four continuous stirred-tank reactors (CSTR) (Figure 11) with working volume of 6 litres were operated for up to 48 weeks at temperature 37°C. Mixers used for all reactors consist of three propellers purchased from Avld Toulouse, France. The mixers were operated using Heidolph

mechanical stirrer Model RZR 2021 at 100-300 rpm depending on the OLR and foaming while maintained at 300 rpm at steady state condition. Higher RPM was used due to higher TS content in the reactor and to avoid foaming due to biogas production. The temperatures of all reactors were controlled with water bath using Heating Immersion Circulator model ED (v.2) purchased from Julabo GmbH Germany. The reading of biogas production was taken using Ritter MilliGascounter® Type MGC-1 V3.3 PMMA with properties: minimum flow of 1 mL/hr, maximum flow of 1 L/hr, maximum pressure of 100 mbar, pressure loss of 5.0 mbar and packing liquid of silox. pH for each reactor was measured daily during weekdays using pH meter Mettler Toledo InPro® 3250i/SG/425 that was immersed throughout reactor operation and the reading was taken from Transmitter pH M300 purchased from Mettler Toledo, Switzerland. Reactor 1 (R1) was fed with straw, reactor 2 (R2) with cow dung, reactor 3 (R3) and reactor 4 (R4) with cow manure. Details on organic loading rate (OLR) and hydraulic retention time (HRT), mass of raw substrates and volume of water added per day, 5 days per week in weekdays are shown in Table 13. OLR in all reactors was increased from 0.5 to 2.5 g VS/L/day from week 1 to week 14 (Table 13). OLR was then decreased to 2 g VS/L/day to avoid inhibition and foaming from week 15. In week 21, VS fed to R1, R2 and R4 was reduced, whereas for R3, feeding was stopped but reactor was continuously stirred until beginning of week 49 to see the degradation of organic matter in its liquid fraction of digestate. Three hydraulic retention times (HRT) were achieved at OLR of 2 g VS/L/day with the new VS fed for R1, R2 and R4 (Table 13). Total solids (TS) and volatile solids (VS) fed (%) per day in R1, R2, R3 and R4 are shown in Table 13. R1, R2, and R4 were supplemented in micronutrients using 150 µL of Vithane™ solution per day (a commercial mix of trace elements purchased from Biothane Systems International) diluted 200 times (1 µL/g feeding) from week 38 until the end of reactor operation. R3 was injected only once in week 38 with 9ml of Vithane™ solution diluted 200 times.

Monitoring pH and biogas production was performed daily while monitoring of volatile fatty acids and ammonium concentrations was performed weekly. Methane content of the biogas was analysed weekly. All biogas and methane productions were expressed in normal conditions (0 °C, 1 atm). Digestates from the four reactors were withdrawn weekly. Physical, chemical and biological characterizations were performed once in 3 weeks. All digestates were separated into solid and liquid fractions by centrifugation at the speed 18,000 g for 20 minutes at 4°C. Liquid

fraction of digestate was diluted 20 times with milli-Q water. Raw liquid after dilution was filtered into 1.2  $\mu\text{m}$  and 1 kDa according to simplified fractions in Section 2.1.1.2. The permeates were collected and stored at 4°C for chemical, physical, biological characterization similar to in Section 2.1.1.2.



Figure 10: Substrate of reactors: (a) straw (R1), (b) cow dung (R2) and (c) cow manure (R3, R4)

Table 12: Initial characteristics of straw, cow dung and cow manure

	Straw	Cow dung	Cow manure
<b>Total solids (TS) (g TS/kg)</b>	901 $\pm$ 0.3	176 $\pm$ 8	365 $\pm$ 29
<b>Volatile solids (VS) (g VS/kg)</b>	819 $\pm$ 6	144 $\pm$ 7	182 $\pm$ 7
<b>VS/TS (%)</b>	91	82	50
<b>Total Kjeldahl Nitrogen (TKN) (g TKN/kg)</b>	3.8 $\pm$ 0.9	4.3 $\pm$ 0.6	8.7 $\pm$ 0.4



Figure 11: Four 6 litres CSTR reactors

Table 13: Organic loading rate (OLR), hydraulic retention time (HRT), mass of raw substrates added per day , volume of water added per day, total solids (TS) and volatile solids (VS) fed (%) per day for reactor R1 with straw feeding, reactor R2 with cow dung feeding, reactors R3 and R4 with cow manure feeding

Week	Organic loading rate (OLR) (g VS/L/day)				Hydraulic retention time (HRT) (days)				Mass of raw substrate added (g) per day				Volume of water added (mL) per day				Total solids (TS) fed (%) per day				Volatile solids (VS) fed (%) per day																				
	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4																	
1-2	0.5				420	392	420		3.66	21.43	16.67		16.3	-				17				18				29				15				14				15			
3-5	1				210	196	210		7.32	42.86	33.33		32.7																												
6-8	1.5				140	131	140		10.98	64.29	50		49																												
9-11	2				105	98	105		14.63	85.71	66.67		65.4																												
12-14	2.5				84	78	84		18.29	107.14	83.33		81.7																												
15-20	2				105	98	105		14.63	85.71	66.67		65.4																												
21-44	2	-	2		63	56	-	56			-	66.67	118.7		64	-	83.3	10	10	-	15	9	8	-	8																
45-48	2	-				63	-			-				-				-				-																			

### 2.3.2. Water extraction

Extraction of cow manure, cow dung and straw with water was performed to observe the organic matters already contained in the substrates which were transferred to the liquid phase without the presence of inoculum. Extraction was performed in 500 mL glass bottles for 0, 0.25, 1, 3, 6, 9 and 24 hours using mechanical shaker in 35°C room to determine the time needed for steady state. The mass of the substrates, volume of Milli-Q water, total solids (TS) and volatile solids (VS) added in the bottle were the same as in the reactors and are shown in Table 14. The supernatant of water extraction was obtained after centrifugation at condition 18,000 g, 20 minutes and 4°C for chemical oxygen demand (COD) analysis. Supernatant obtained at steady state time was further characterized in the same simplified fractions described in Section 2.1.1.2.

Table 14: Mass of substrate, volume of Milli-Q water, total solids (TS) and volatile solids (VS) added in the bottle

Substrate	Mass of substrate (g)	Volume of Milli-Q water (mL)	TS in the bottle (%)	VS in the bottle (%)
Straw	27.9	222.1	5.02	4.58
Cow dung	138.5	111.5	4.99	3.88
Cow manure	106.4	143.6	7.45	3.83

## 2.4. Analytical methods

### 2.4.1. Chemical analysis

#### 2.4.1.1. Determination of pH

pH was measured using WTW series inoLab pH720 pH meter calibrated with pH 4 and pH 7 buffer solutions. pH measurement was performed on raw digestates, liquid fraction of digestates and supernatant of water extraction.

#### 2.4.1.2.Determination of alkalinity

Alkalinity is the capability of an alkaline substance (also called base) to neutralize an acid. The reaction of acid and  $\text{HCO}_3^-$  is shown in equation (1).



Alkalinity measurement was performed by acid titration as described elsewhere (APHA, 2012). 10 mL sample were pipetted into a small glass container. The glass container containing sample was then mixed with a magnetic stirrer on a Fisher Scientific FB15045 Magnetic Stirrer. Initial pH of the sample was measured with WTW series inoLab pH720 pH meter and recorded. The sample was titrated with Sigma Aldrich 0.1 N hydrochloric acid (HCl) using Brand Titrette Burette. The titration stopped when the pH of the sample reached pH 4.3. The volume of the acid used for titration was recorded for calculation of total alkalinity. Calculation for total alkalinity is shown in equation (2). Alkalinity was performed in duplicate with less than 3% error.

$$\begin{aligned} \text{Total alkalinity } \left(\frac{\text{mg}}{\text{L}}\right) & \quad (2) \\ = & \frac{\text{Volume of acid used (ml)} \times \text{normality of acid } \left(\frac{\text{eq}}{\text{L}}\right) \times 50,000 \left(\text{mg} \frac{\text{CaCO}_3}{\text{eq}}\right)}{\text{Volume of sample (ml)}} \end{aligned}$$

#### 2.4.1.3.Determination of total solids (TS), volatile solids (VS) and mineral solids (MS)

TS are dry solids present in a given sample. VS are solids that are vaporized at the temperature of 550°C, they represent the organic matter. The ratio of VS/TS represents organic matter content in dry solids. MS are the solids remaining after ignition at 550°C. TS, VS and MS were determined using standard methods (APHA, 2012). Clean porcelain crucibles were initially dried in the 105°C oven for minimum one hour. The crucibles were then placed inside desiccators for cooling until room temperature for about one hour. For TS analysis, the initial weight of crucible was recorded ( $W_C$ ) as well as the weight of the sample ( $W_S$ ). Crucibles were then placed inside the oven at 103-105°C for 24 hours, cooled for about one hour in a desiccator. The weight of the

crucibles containing dried sample were then recorded ( $W_{C+S1}$ ). The calculation of TS concentration in g/kg is shown in equation (3):

$$\text{Total solids (TS)} \left( \frac{g}{kg} \right) = \frac{W_{C+S1} - W_C}{W_S} \times 1000 \left( \frac{g}{kg} \right) \quad (3)$$

( $W_C$ ): Weight of initial crucible (g)

( $W_S$ ): Weight of sample (g)

( $W_{C+S1}$ ): Weight of crucible + weight of dried sample after 24 hours in the oven at 105°C (g)

For VS analysis, crucibles containing dried sample ( $W_{C+S1}$ ) were placed in the furnace (Figure 8) and were ignited at 550°C for 3 hours. The crucibles were then placed in the 105°C oven for one hour cooling at the oven temperature before placed in the desiccator for cooling until room temperature. The weight of the crucibles containing remaining solid were then recorded ( $W_{C+S2}$ ). The calculation of VS concentration was obtained with the calculation shown in equation (4) below:

$$\text{Volatile solids (VS)} \left( \frac{g}{kg} \right) = \frac{W_{C+S1} - W_{C+S2}}{W_S} \times 1000 \left( \frac{g}{kg} \right) \quad (4)$$

( $W_S$ ): Weight of sample (g)

( $W_{C+S1}$ ): Weight of crucible + weight of dried sample at 105°C for 24 hours (g)

( $W_{C+S2}$ ): Weight of crucible + weight of ignited sample at 550°C for 3 hours (g)

MS concentration (g/kg) was obtained from calculation as shown in equation (5) below:

$$\text{Mineral solids (MS)} \left( \frac{g}{kg} \right) = \frac{W_{C+S2} - W_C}{W_S} \times 1000 \left( \frac{g}{kg} \right) \quad (5)$$

( $W_S$ ): Weight of sample (g)

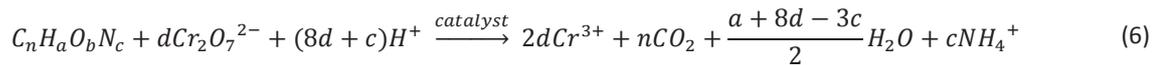
( $W_C$ ): Weight of initial crucible (g)

( $W_{C+S2}$ ): Weight of crucible + weight of ignited sample at 550°C for 3 hours (g)

All TS, VS and MS analyses were performed in triplicate with less than 10% error.

#### 2.4.1.4. Determination of chemical oxygen demand (COD)

COD is a measurement of oxidizable organic matter in a given sample. Potassium dichromate ( $K_2Cr_2O_7$ ) is used as chemical to oxidize the organic matter of the sample in an acidic medium at high temperature ( $150^\circ C$ ). The chemical reaction for oxidation of organic matter is shown in equation (6) below:



where  $d = 2n/3 + a/6 - b/3 - c/2$

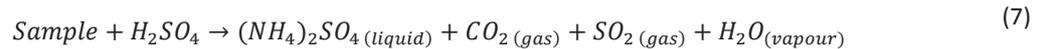
COD was analyzed using commercial Aqualytic 420721 COD Vario Tube Test middle range (MR) 0-1,500 mg/L. For COD analysis, few different dilutions of the sample were made in order to have the range between 0 to 1,500 mg  $O_2/L$ . 2 mL sample was pipetted into each tube and was performed in triplicate. 2 mL of Milli-Q water was pipetted into one tube and used as blank sample. The tubes were left in HACH COD reactor at  $150^\circ C$  for 2 hours for oxidizing organic matter in the acidic medium. The samples were then left cooled at room temperature for 1 hour. The COD readings were taken using Aqualytic Multi Direct with middle range (MR) programme of 0-1500 mg/L.

#### 2.4.1.5. Determination of total Kjeldahl nitrogen (TKN)

TKN is a measurement of a sum of organic nitrogen, ammonia ( $NH_3$ ), and ammonium ( $NH_4^+$ ) in a given sample. TKN value with addition of nitrate and nitrite values will sum up to Total Nitrogen (TN). For TKN analysis, sample was previously diluted so the TKN range will be between 80 to 540 mg N/L. 5mL Milli-Q water was pipetted into the first glass tube as blank sample. 5 mL of 1 g/L ammonium sulfate ( $(NH_4)_2SO_4$ ) were pipetted into the second glass tube as reference solution. 5 mL of each sample were pipetted into the next glass tube. All samples were prepared in triplicate and an average error is estimated to 3%.

Determination of TKN undergoes three steps: digestion, distillation and titration. In digestion process, nitrogen is decomposed with addition of 10 mL concentrated sulfuric acid ( $H_2SO_4$ )

(99.7%) with catalyst into each test tube containing sample and boiled at 340-370°C temperature using Buchi Digest Automat K-438. 915 mg of copper selenite was used as catalyst and was previously added in 2.5 L of concentrated sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) (99.7%) and mixed with magnetic stirrer in the fume cupboard 24 hours before the experiment. The process will produce an ammonium sulfate solution as shown in the equation (7):



The mineralization is completed when the initial dark colour of the sample turned colourless. The mineralization usually takes 4 to 5 hours. The next processes are distillation and titration which were performed using Buchi AutoKjeldahl Unit K-370. During distillation process, excess base (NaOH (32%)) is added to acid digestion mixture to convert (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> to NH<sub>3</sub> as shown in equation (8) below:



In titration process, NH<sub>3</sub> gas is captured and condensed into boric acid solution (4%) (pH of 4% boric acid solution was previously adjusted to 4.65 using NaOH (32%)) and produced NH<sub>4</sub><sup>+</sup> as shown in the equation (9) below:



During this process, NH<sub>3</sub> reacted with the boric acid and the produced borate ion was then titrated with 0.02 N HCl acid. The results obtained were valid if the reference solution of 1 g/L ammonium sulfate ((NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>) gives reading of 212.8 mg N/L with an error less than 10%.

#### 2.4.1.6. Determination of ammonium (NH<sub>4</sub><sup>+</sup>)

(NH<sub>4</sub><sup>+</sup>) was determined in dissolved matter of the sample by distillation and titration processes with Buchi AutoKjeldahl Unit K-370. The processes are similar with the process of TKN measurement except the measurement of NH<sub>4</sub><sup>+</sup> was performed without digestion process. Only distillation and titration processes take place for ammonium (NH<sub>4</sub><sup>+</sup>) determination. All samples were previously filtered at 1kDa to remove solids and colloids. All samples were prepared in triplicate and average error is estimated to 5%.

#### 2.4.1.7. Determination of total organic carbon (TOC) and inorganic carbon (IC)

TOC is a measurement of carbon content in a given sample. Carbon reacts with oxygen in a high temperature oxidizing furnace as shown in equation (10).



IC is a measurement of inorganic carbon which is the sum of carbon dioxide, carbonic acid, carbonate and bicarbonate. IC is measured by acidification process with hydrochloric acid (HCl (2N)) of a given sample which drives the equilibrium to CO<sub>2</sub>. CO<sub>2</sub> is then emitted from solution and measured by infrared spectroscopy. TOC and IC measurements were performed using Shimadzu TOC-V<sub>CSN</sub> Total Organic Carbon Analyzer with Shimadzu ASI-V auto sampler. All samples were previously diluted and filtered at 1.2 μm and 1kDa. 8 mL and 5 mL were required for TOC and IC measurements respectively for both <1.2 μm and 1kDa fractions. The range of concentration for both TOC and IC is from 0 to 1000 mg/L. The measurement was performed in triplicate with error less than 3%. The reference solution for TOC used in this experiment was Volusol Etalon COT 1000 mg/L purchased from VWR Prolabo. The reference solution for IC used was a mixture of 3.5 g of reagent grade sodium hydrogen carbonate (previously dried for 2 hours in a silica gel desiccator) and 4.41 g of sodium carbonate (previously dried for 1 hour at 280 - 290°C) in a 1 L volumetric flask filled up with milli-Q water up to the mark which corresponds to 1 g/L of IC.

#### 2.4.1.8. Determination of metabolites and simple sugars

Metabolites and simple sugars (carboxylic acids, sugars and alcohols, i.e. saccharose, lactose, glucose, pyruvate, xylose, arabinose, succinate, lactate, glycerol, formate, fumarate, acetate, 1,3-propanediol, propionate, ethanol and butyrate) were measured using High Performance Liquid Chromatography (HPLC) with a refractive index detector (Waters R410) at a flow rate of 0.7 mL/min on an Aminex HPX-87H, 300 x 7.8 mm (Bio-Rad) column at temperature 35°C. H<sub>2</sub>SO<sub>4</sub>, 4mM was used as the mobile phase. For measurement, all samples were previously diluted and filtered at 1kDa. 500 to 850 μL of sample were pipetted into each vial. HPLC injected 40 μL of the sample for the analysis. The range of each metabolite and simple sugar that can be measured is from 0.2 to 10 g/L. All samples were prepared in triplicate.

#### **2.4.1.9. Determination of cations and anions**

Cations (potassium ( $K^+$ ), sodium ( $Na^+$ ), calcium ( $Ca^{2+}$ ), magnesium ( $Mg^{2+}$ )) and anions (chloride ( $Cl^-$ ), phosphate ( $PO_4^{3-}$ ), sulfate ( $SO_4^{2-}$ ), nitrate ( $NO_3^-$ ), nitrite ( $NO_2^-$ )) were analyzed using ion chromatograph (ICS 3000, Dionex, USA) equipped with pre-columns and separation columns CG 16 and CS16 (3 mm  $\varnothing$ ) for cations and AG15 and AS15 (2 mm  $\varnothing$ ) for anions, respectively. The eluents used for this analysis were methanesulphonic acid (MSA) 25 mM pumped at 0.35 mL/min for cations and KOH 10 mM pumped at 0.3 mL/min for anions at initial time and MSA 40 mM at 24 minutes and KOH 74 mM at 28 minutes. The temperature of the columns was at 35°C. All samples were previously diluted and filtered at 1 kDa. 500  $\mu$ L of each sample were used for measurements. The analysis was performed in duplicate with different dilutions with an average error less than 5%. The reference solution used for this measurement was “Etalon Multi Chromato, Ionique à façon” purchased from SCP SCIENCE. The range for cations and anions measurement for this device is from 1 to 80 mg/L.

#### **2.4.1.10. Determination of volatile fatty acids (VFA)**

VFA concentration (acetic (C2), propionic (C3), butyric and iso-butyric (C4 and iC4), valeric and iso-valeric (C5 and iC5), caproic and isocaproic (C6 and iC6) acids concentrations were measured using Perkin Elmer Clarus 580 Gas Chromatograph with an Elite-FFAP cross-bond®carbowax® 15 m column connected to a flame ionization detector at temperature of 280°C. Nitrogen gas ( $N_2$ ) at 3 mL/min was used as carrier gas. All samples were previously diluted and filtered at 1kDa. 500  $\mu$ L of each sample was pipetted into each analytical vial. 500  $\mu$ L of standard internal solution (1g/L Diethylacetic acid ( $C_6H_{12}O_2$ ) acidified to 5% with  $H_3PO_4$ ) was then pipetted into each vial containing sample. All samples were prepared in duplicate. The control solution used was a mixture prepared with concentration of 1 g/L of each C2, C3, C4, iC4, C5, iC5, C6 and iC6 acids. The volume of the sample injected was 1  $\mu$ L. The range for the measurement of this device is from 0.1 to 1 g/L of each C2, C3, C4, iC4, C5, iC5, C6 and iC6. The margin error of this measurement was from 2 to 5% with a quantification threshold of 0.1 g/L.

#### **2.4.1.11. Biogas measurement**

Gas measurement for carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>), hydrogen sulfide (H<sub>2</sub>S), nitrogen (N<sub>2</sub>) and oxygen (O<sub>2</sub>) was performed using Perkin Elmer® Clarus 480 Gas Chromatograph, Waltham, USA. 200 µL of each sample was injected in a capillary R-Q column separating CO<sub>2</sub> from other gases. The remaining gases were separated by another capillary Rt-Molsieve 5Å column. The temperature of the injector was 250°C and the thermic conductivity detector was at 150°C and the vector gas was Argon (350 kPa, 34 mL/min). Calibration was performed on this machine with a standard gas with 25% CO<sub>2</sub>, 0.1% H<sub>2</sub>S, 0.5% O<sub>2</sub>, 10% N<sub>2</sub> and 64.4% CH<sub>4</sub> in composition. Biogas measurement was performed for measuring gas composition in the reactors once a week after steady state.

#### **2.4.2. Physical analysis**

##### **2.4.2.1. Determination of particle size distribution**

Particle size distribution was analyzed using Beckman Coulter LS200 granulometer. The device uses the Fraunhofer method of light diffraction for particle sizes measurement in the range from 0.375 to 2,000 µm. Sample was added into the vessel containing water which is equipped with sensors to detect the percentage concentration of the sample. The addition of the sample was stopped when the percentage concentration of the sample in the vessel was from 8 to 12%. The scattered light was detected by a device which converts the signal to a size distribution based on volume.

##### **2.4.2.2. Determination of turbidity**

Turbidity is a measure of the degree of a radiant light energy which is scattered or absorbed by suspended particles in water. Turbidity was performed using HACH model 2100P portable turbidimeter with formazin for standard calibration. The portable turbidimeter uses the nephelometric principle of turbidity measurement. This apparatus meets the design criteria established by the US Environmental Protection Agency, Method 180.1. Dilution was previously

made on raw sample and 20 mL of diluted sample were used for measurement. The range for the measurement of this device is from 0.01 to 1000 NTU.

#### **2.4.2.3. Determination of absorbance spectra**

Absorbance spectra were measured using a double beam recording spectrophotometer (Shimadzu, reference UV-2501PC UV-VIS) equipped with software UVProbe Version 2.10 for data acquisition. The cuvettes used to put the samples and the reference were  $10.00 \pm 0.01$  mm pathlength cell with Spectrosil Quartz material. The absorbance spectra measured was from 190 nm to 600 nm. Milli-Q water was used as blank in the reference cuvette. All samples were previously diluted and filtered at 1 kDa and re-diluted to measure absorbance less than 1.5 unit of absorbance. 3500  $\mu$ L of each sample was required for the measurement. All measurements were performed in duplicate.  $SUVA_{254}$  (L/mg.m) ratio was obtained from the specific UV absorbance at wavelength 254 nm divided by dissolved total organic carbon (TOC) concentration ( $A_{254}/TOC$ ) of the sample.  $SUVA_{254}$  ratio was used as an indicator of the aromatic carbon content in the dissolved organic matter as well as the degree of humification. This ratio is also linked to biological degradability (Zheng et al., 2014).

#### **2.4.2.4.3D Fluorescence spectroscopy**

Fluorescence is a light emitted by molecule excitation usually by absorption of a photon followed by a spontaneous emission. Organic and inorganic compounds (mainly aromatic or polyaromatic) in solution emit light when they are excited by photons, in order to come back to their fundamental state following the Stockes' law. Molecules at rest in the vibrational level of fundamental state electronic state  $S_0$  are excited to the state  $S_1$  under the effect of light radiation absorption (excitation wavelength). Fluorescence spectra can be obtained in 2D with one excitation wavelength scan or 3D scan (200 to 600 nm with an increment of 10 nm) obtained for tryptophan amino acid. In 3D scan, excitation wavelength ( $\lambda_{ex}$ ) constitutes the Y-axis, emission wavelength ( $\lambda_{em}$ ) the X-axis and fluorescence intensity the Z-axis. 3D Fluorescence spectroscopy was analysed using a Perkin Elmer LS55 fluorescence spectrometer. The emission and excitation's slit width was fixed at 10 nm in the monochromator. The speed was 1200 nm/s

in the scanning monochromator and the fluorescence values were recorded every 0.5 nm between 200 and 600 nm.

Cuvette used to put the sample was High Precision Cell made of Quartz SUPRASIL with light path 10x10 mm purchased from Hellma Analytics. The definition of the fluorescence zones obtained by 3D fluorescence spectroscopy is shown in Figure 12.

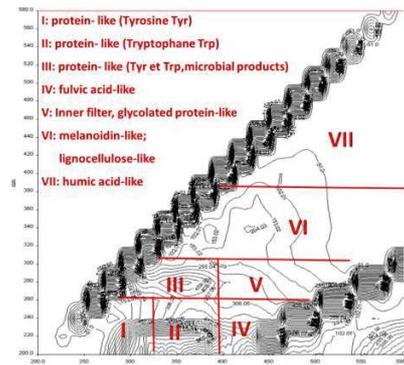


Figure 12: The fluorescence spatialization integration for spectra interpretation and quantification (Jimenez et al., 2014)

The fluorescence spatialization integration for spectra interpretation and quantification was according to: 1) Protein-like (Tyrosine, Tyr); 2) Protein-like (Tyrptophane, Trp); 3) Protein-like (Tyr and Trp, microbial products); 4) Fulvic acid-like; 5) glycolated protein-like; 6) Melanoidin-like; lignocellulose-like; 7) Humic acid-like (He et al., 2013, 2011, Jimenez et al., 2015a, 2014; Muller et al., 2014). Fluorescence 1, 2 and 3 were less complex compounds while 4, 5, 6 and 7 were more complex compounds. Fluorescence 1, 2 and 3 were grouped as protein-like in Chapter 3 and 4 for the characterization of liquid fractions of digestates from full scale co-digestion plants. All samples were previously diluted and filtered at 1 kDa and re-diluted to be in the wavelength range from 200 to 600 nm. 3500 to 4000  $\mu$ L of each sample were required for each analysis. All analyses were performed in duplicate.

#### 2.4.2.5. Determination of conductivity

Conductivity in liquid is a measure of liquid capability to conduct electrical flow. This ability is linked to the concentration of ions and ionized species in the liquid. The measurement of

conductivity was performed using WTW multi 3410 digital multi parameter meters equipped with a probe TretraCon ® 925 at the reference temperature of 25°C. All analyses were performed on raw sample without dilution.

#### **2.4.2.6. Determination of Capillary Suction Time (CST)**

Capillary Suction Time (CST) is a measurement of filterability and conditionability of a given liquid sample containing suspended and colloidal particles. CST is obtained from the time taken in seconds to draw a known volume of filtrate from a suspension by the capillary suction pressure generated from a standard CST filter paper. A CST test unit comprises of test head, control unit and filter papers. The pressure of capillary suction is between 5,000 Pa to 10,000 Pa depending on the viscosity of the filtrate while the hydrostatic head is 100 Pa. The CST was obtained from two electrodes placed at a standard interval from the funnel. The time taken for the water to pass between two electrodes constitutes the CST. CST was performed using Type 304B CST timer purchased from Triton Electronics Ltd. The funnel used was 18mm of diameter. Filter paper used for CST measurement was CST papers with size 7 x 9 cm, basis weight of 440 g/m<sup>2</sup>, thickness of 0.92 mm, porosity of 9 sec/100mL/sqin and tensile strength of 4525 m/d g/15mm purchased from Triton Electronics Ltd. All raw samples were previously diluted to the same total solids concentration of 10 g TS/kg. For measuring purpose, volume of 2 mL was used for each analysis.

#### **2.4.3. Biological analysis**

##### **2.4.3.1. Biochemical Oxygen Demand (BOD)**

BOD after day 5 (BOD<sub>5</sub>) and day 21 (BOD<sub>21</sub>) were performed using WTW OxiTop® control system (WTW GmbH, 2010, 2004). BOD<sub>5</sub> measurement was performed to measure the amount of easily biodegradable compounds while BOD<sub>21</sub> accounts for both easily and slowly biodegradable compounds. Recalcitrant or non-biodegradable compounds were obtained by subtracting BOD<sub>21</sub> from COD. BOD<sub>5</sub>/COD and BOD<sub>21</sub>/COD ratios give an indication of relative biodegradability in 5 and 21 days respectively. OxiTop®-C heads and OxiTop® OC100

controller were used to measure the pressure in the bottles, and auto-converted the pressure to BOD (mg/L O<sub>2</sub>) as shown in equation (11).

$$BOD \left( \frac{mg \ O_2}{L} \right) = \frac{M(O_2)}{R \cdot T_m} \cdot \left( \frac{V_{tot} - V_I}{V_I} + \alpha \frac{T_m}{T_o} \right) \cdot \Delta p(O_2) \quad (11)$$

M (O<sub>2</sub>) = Molecular weight of oxygen (32000 mg/mol)

R = Gas constant (83.144 L.hPa/(mol.K))

T<sub>o</sub> = Reference temperature (273.15 K)

T<sub>m</sub> = Measuring temperature (293.15 K)

V<sub>tot</sub> = Bottle volume (mL)

V<sub>I</sub> = Sample volume (mL)

α = Bunsen absorption coefficient (0.03103)

Δp(O<sub>2</sub>) = Difference of the partial oxygen pressure (hPa)

For preparation of inoculum, fresh inoculum was taken from raw municipal wastewater in the treatment plant of Narbonne (France). Large solids were removed using coarse filter paper. The filtrate was aerated at least one day before use at temperature 20°C protected from any light source to allow the biological activity to restart or continue. (The wastewater sample used for biological inoculation must not exceed a COD of 300 mg / L or 100 mg TOC / L).

For preparation of dilution water, 4 nutrient salt solutions were prepared. Salt solution 1 (S1) (phosphate buffer solution pH 7.2) was prepared by addition of 8.5 g of potassium dihydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>), 21.75 g of dipotassium hydrogen phosphate (K<sub>2</sub>HPO<sub>4</sub>), 17.688 g of disodium hydrogen phosphate (Na<sub>2</sub>HPO<sub>4</sub>) and 1.7 g ammonium chloride (NH<sub>4</sub>Cl) in a 1 L volumetric flask. (The pH of the buffer should be 7.2 without adjustment). Salt solution 2 (S2) was magnesium sulphate heptahydrate (MgSO<sub>4</sub>·7H<sub>2</sub>O), solution at 22.5 g/L. Salt solution 3 (S3) was calcium chloride (CaCl<sub>2</sub>), solution at 27.5 g/L. Salt solution 4 (S4) was iron (III) chloride (FeCl<sub>3</sub>), solution at 0.15 g/L. All salt solutions S1, S2, S3 and S4 were prepared using Milli-Q water and were mixed on magnetic stirrer to ensure complete dissolution. Dilution water was then prepared by adding 5 mL of each S1, S2, S3 and S4 in a 5 L volumetric flask. 2.5 mL of the

solution allylthiourea inhibition (N-Allylthiourea (5 g/L)) was added to the flask to prevent microbiological oxidation of nitrogen. Milli-Q water was added to the mark of 5L. Dilution water was properly mixed on magnetic stirrer before used (dilution water should be freshly prepared at the start of the workday and discarded at the end of the day or it can be kept 1-2 days if it is stored at 5°C). In order to validate the performance of BOD analysis, reference solution (glucose glutamic acid solution) was prepared by adding 5.25g of glucose (C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>) and 5.25g of glutamic acid (C<sub>5</sub>H<sub>9</sub>NO<sub>4</sub>) in a 1 L volumetric flask and filled up with Milli-Q water up to the mark of 1L and mixed on magnetic stirrer.

The quantity of sample volume and total filling volume to be used were calculated on the basis of COD value of the sample as shown in equation (12) and Table 15. BOD estimated was based on COD measured with an estimation of BOD/COD ratio of 0.5. The sample volume and total filling volume to be used was decided depending on the sufficiency of the available sample. The volume of prepared dilution water to be used in each bottle was determined by using equation (13), where the volume of inoculum was based on 0.02 mL inoculum per mL of total filling volume.

$$\text{Sample volume (mL)} = \frac{\text{Initial Oxygen (O}_2\text{) in the bottle (mg)} \times \frac{2 \text{ COD}}{1 \text{ BOD}}}{\text{COD of the sample } \left(\frac{\text{mg}}{\text{L}}\right)} \times 1000 \left(\frac{\text{mL}}{\text{L}}\right) \quad (12)$$

Table 15: Total filling volume (mL) and initial oxygen (O<sub>2</sub>) in the bottle (mg)

Total filling volume (mL)	Initial Oxygen (O <sub>2</sub> ) in the bottle (mg)
450	15.9
400	29.9
350	43.9
300	57.8
250	71.8
200	85.8
150	99.8

$$\begin{aligned} \text{Prepared dilution water (mL)} \\ = \text{Total filling volume(mL)} - \text{sample (mL)} - \text{inoculum (mL)} \end{aligned} \quad (13)$$

For the preparation of BOD analysis, 507mL brown glass bottle was used for each sample. Each sample was prepared in triplicate. Blank sample (only prepared with dilution water and inoculum) and reference sample (prepared with dilution water, reference solution of glucose glutamic acid and inoculum) were performed to validate the performance of BOD analysis. For blank sample bottle, 343 mL of prepared dilution water from a total filling volume of 350 mL was added. Reference sample bottle was filled with 333 mL of prepared dilution water and 10 mL prepared reference solution from a total filling volume of 350 mL.

For sample preparation in detailed characterization (first collection), liquid fraction of digestates before filtration and after filtration at 1.2  $\mu\text{m}$  (after suspended particles removal) were analysed. For simplified characterization (second collection), only liquid fraction of digestates before filtration was analysed. For the biodegradability of liquid fraction of digestate from the reactors, only liquid fraction of digestates after centrifugation at the speed 18,000 g for 20 minutes at 4°C were analysed. The volume of the sample filled into the bottle according to the sample volume was determined based on COD value as shown in equation (12).

The prepared dilution water was filled before adding the sample with the volume previously calculated in equation (13). All bottles were added with magnetic stirrer to allow mixing during the analysis. 2 pellets of sodium hydroxide were added in the rubber sleeve at the neck of each bottle to absorb carbon dioxide produced during the biodegradation. Inoculum was added into each bottle at the final step (0.02 mL inoculum per mL of total filling volume) and the bottle was closed immediately with OxiTop®-C head to prevent from reaction of microorganisms with extra oxygen outside the bottle. OxiTop®-C heads were then programmed using OxiTop® OC100 controller to start BOD analysis. All bottles were left on WTW Oxitop IS 12 Inductive Stirring System in a temperature controlled Gallenkamp incubator at  $20 \pm 0.5$  °C for 21 days.

### **3. Comprehensive characterization of the liquid fraction of digestates from full-scale anaerobic co-digestion**

*This chapter is adapted from Akhilar et al., 2017. Comprehensive characterization of the liquid fraction of digestates from full-scale anaerobic co-digestion. Waste Management 59, 118–128.*

The most common solution used for the elimination of digestates from farm or territorial co-digestion plants is direct land application of the raw digestate or of only the solid fraction after solid/liquid separation, in order to limit transportation costs. However, solid-liquid separation of digestate generates a liquid fraction which volume represents quite a high fraction of the initial mass of the raw digestate and its elimination by land application can be problematic, especially for big size centralized plants, due to low organic and nutrients content, high volume produced, high transportation costs making land application not the best solution. Hence, alternatives might be required when land application cannot be implemented at reasonable costs. The centralized co-digestion plant of Auch, France, is a good illustration of this problematic. Indeed, this unit is fed with 44 000 t of different kind of waste per year (waste activated sludge, fats, manure, crop residues, blood, fruits and vegetables and pet food). After maturation, the raw digestate undergoes a solid-liquid separation by screw press. The solid fraction produced represents 12 000 t/y which are eliminated by land application on 3 733 ha located 15 km around the plant. Land application of the liquid fraction was difficult and the designers have implemented the following solution: centrifugation of the liquid fraction followed by valorization of the nitrogen content by stripping, with the production of 920 m<sup>3</sup>/y of ammonium sulfate, and a final aerobic treatment by MBR in order to decrease the organic load of the liquid fraction. The treated effluent is discharged in the sewage network for final treatment at the municipal wastewater treatment plant of the city of Auch. During the design of the treatment solution for the liquid fraction, the designers faced a lack of information on the composition of the liquid fraction of digestate after solid-liquid separation, especially on its organic matter composition, concentration and biodegradability making its design problematic and uncertain.

Literature review on the liquid fraction of digestates has shown that their studies mainly focused on nutrients recovery, reuse or removal with little information on COD or organic components.

However, full characterization of liquid fraction of digestate is necessary to design proper and efficient post treatment and valorization solutions when land application is not feasible. Management of the liquid fraction of digestate can thus be improved by thorough knowledge of this effluent composition and the aim of this chapter was to carry out a comprehensive characterization of the liquid fraction of digestate after solid-liquid separation.

We can assume that the composition of the liquid fraction of digestate will vary according to the origins of substrates, operating parameters used in the digester and type of solid-liquid separation. For practical reasons and because performance of full-scale solid-liquid separation processes cannot be reproduced at lab-scale, this study was carried out on digestates originating from full-scale co-digestion plants. Eleven co-digestion plants treating different types of substrates (Table 9, samples A to M), with different process parameters (Table 10) and different separation processes (Table 11) were selected.

In a first part, total, volatile and mineral solids were measured in raw digestates and in their solid and liquid fractions. This made it possible to discuss the efficiency of solid-liquid separation techniques. In a second part, the liquid fractions of digestates were fully characterized. In particular, we focused on size fractionation of the liquid fraction of digestate, to quantify the contribution of suspended particles, colloids and dissolved matter fraction on the physico-chemical and biological parameters.

### **3.1. Comprehensive characterization of the liquid fraction of digestates from the first collection**

#### **3.1.1. Solids characterization**

Figure 13 presents TS, VS and MS concentrations of raw (a), solid fraction (b) and liquid fraction (c) of digestates. Figure 13a shows that solid concentrations of the raw digestates were highly variable from one sample to the other.

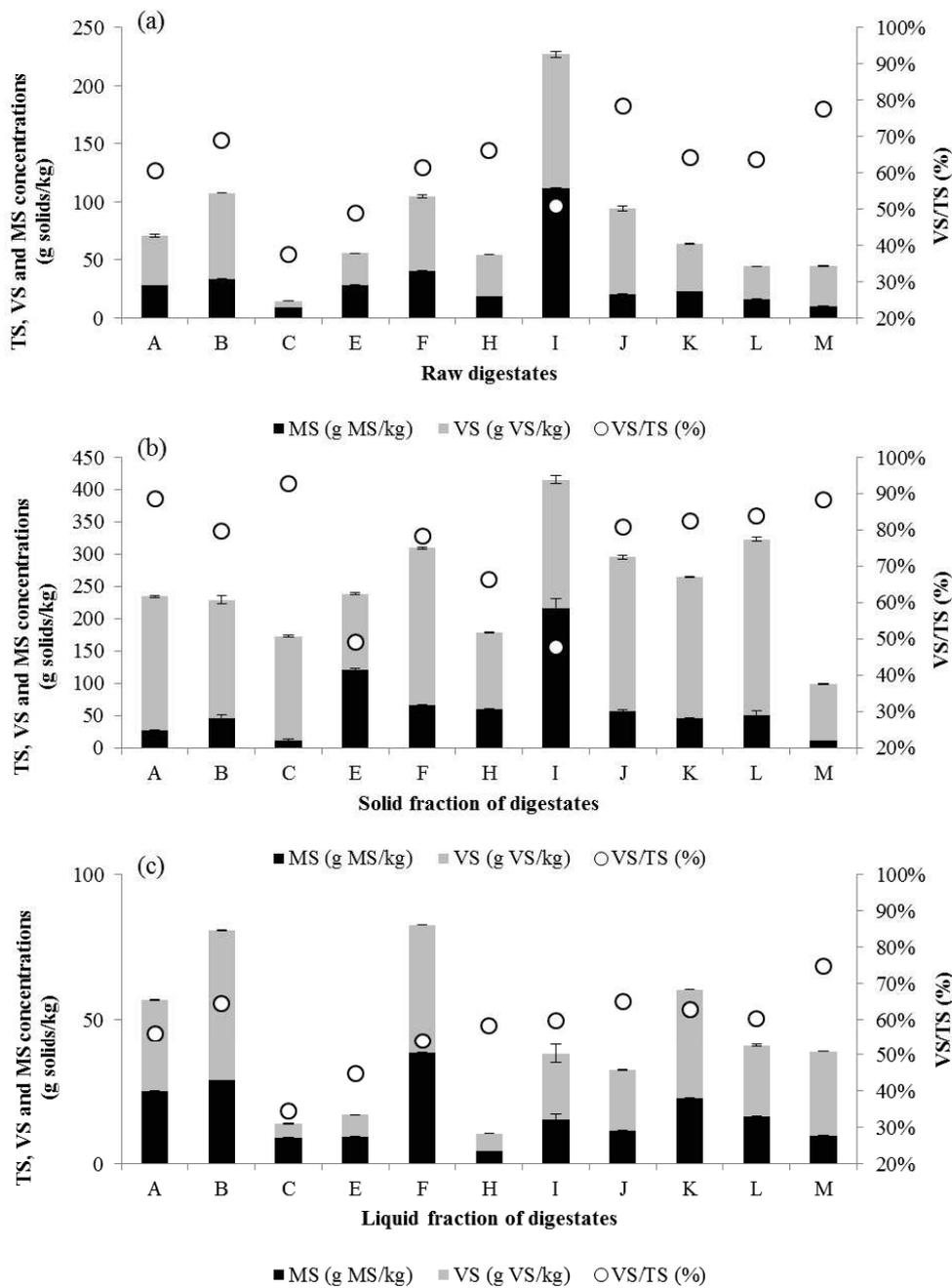


Figure 13: TS, VS and MS concentrations (g/kg) in: (a) raw digestates, (b) solid fraction of digestates after separation and (c) liquid fraction of digestates after separation

Indeed, for raw digestates originating from co-digestion from CSTR digesters, TS concentrations varied from 14.4 to 107 g TS/kg while VS concentrations varied from 5.4 to 74.1 g VS/kg with an organic fraction (VS/TS) ranging from 37% to 77%. TS concentrations obtained in this study

were in the range of values previously found by other researchers (Monlau et al., 2015b; Tampio et al., 2016b). For 3 digestates, the organic fraction (VS/TS) measured were lower than values generally reported i.e. 62–77% for agricultural digestates (Monlau et al., 2015b) and 59–81% for digestates originating from food waste and organic fraction of municipal solid waste (Tampio et al., 2016b). The lowest values (VS/TS = 37%) corresponded to digestate from substrates presenting a high biodegradability (fruits and vegetables). For the two raw digestates from plug flow (PF) digesters (I and J), TS concentrations were 227 g TS/kg and 94 g TS/kg while VS concentrations were 115 g VS/kg and 74 g VS/kg with VS/TS of 51 and 78%, respectively. Figure 13b shows that the solid fractions contained higher TS and VS concentrations than in the corresponding raw digestates with TS concentrations from 99.2 g TS/kg to 416 g TS/kg and VS concentrations from 87.6 to 272 g VS/kg with VS/TS ranging from 48% to 93%. Liquid fractions contained TS and VS concentrations from 10.3 to 82.7 g TS/kg (Figure 13c) and from 4.7 to 52.3 g VS/kg, respectively with VS/TS from 35 to 75%.

Even though it was not possible to make mass balances at the industrial plants, the ratio of TS concentration in liquid fraction over TS concentration in raw digestate ( $[TS_{\text{liquid}}]/[TS_{\text{raw}}]$ ) can give an indication on the efficiency of the solid-liquid separation. Indeed, a low ratio suggests a good separation while on the contrary, a high ratio indicates that the quantity of solids retained by the separation device is low. A significant difference was observed in the values of  $[TS_{\text{liquid}}]/[TS_{\text{raw}}]$  ratio according to the kind of separation device. This ratio was equal or below 0.3 for 4 samples showing an efficient separation with: 0.2 for sample I after screw press with coagulant followed by centrifugation; 0.2–0.3 after centrifugation only (samples E and H) and; 0.3 after screw press with coagulant addition (sample J). On the contrary, high  $[TS_{\text{liquid}}]/[TS_{\text{raw}}]$  ratios over 0.8 were calculated after screw press only (samples A, B, F, K and L) and vibrating screen (samples C and M) suggesting a poor separation efficiency.

### **3.1.2. Particle size distribution in the liquid fraction of digestates**

Table 16 shows that particle sizes were below 1500  $\mu\text{m}$  for all samples except A, C, E, H, J which were below 600  $\mu\text{m}$ . Most of the particles are either in the range from 10 to 50  $\mu\text{m}$  (from 30.76% to 57.14% in volume) or from 100 to 500  $\mu\text{m}$  (from 29.43% to 44.6% in volume) or

both. The mean and median sizes of the particles varied from 31 to 257  $\mu\text{m}$  and from 14 to 116  $\mu\text{m}$  respectively. No correlation between mean and median of particles sizes with solid-liquid separation technique, TS, and origins of the substrates was observed. Further experiment is required to find the correlation between the particle sizes and the origins of the substrates, operating parameters in the digester and type of solid-liquid separation.

Table 16: Particle size range, particle size range representing the highest volume in the sample, mean and median size in the liquid fraction of digestates.

Liquid fraction of digestate	Particle size range ( $\mu\text{m}$ )	Particle size ( $\mu\text{m}$ ) range representing the highest volume in the sample (%)	Mean ( $\mu\text{m}$ )	Median ( $\mu\text{m}$ )
A	<400	10–50 $\mu\text{m}$ (55.4%)	48	30
B	<1000	10–50 $\mu\text{m}$ (30.76%) and 100–500 $\mu\text{m}$ (32.13%)	138	53
C	<400	10–50 $\mu\text{m}$ (57.14%)	31	14
E	<200	100–500 $\mu\text{m}$ (39.31%)	76	89
F	<1000	10–50 $\mu\text{m}$ (32.01%) and 100–500 $\mu\text{m}$ (29.43%)	105	44
H	<400	10–50 $\mu\text{m}$ (49.1%)	61	49
I	<1500	100–500 $\mu\text{m}$ (44.6%)	208	112
J	<600	100–500 $\mu\text{m}$ (43.04%)	121	80
K	<1000	10–50 $\mu\text{m}$ (54.25%)	82	30
L	<1300	100–500 $\mu\text{m}$ (31.55%)	257	116
M	<1300	10–50 $\mu\text{m}$ (32.07%)	179	46

### 3.1.3. COD characterization and size fractionation of the liquid fraction of digestates

Liquid fraction of digestates from 11 co-digestion plants were further characterized. Coarse filtration (100  $\mu\text{m}$ , 41  $\mu\text{m}$ , 10  $\mu\text{m}$ ) followed by microfiltration (1.2  $\mu\text{m}$ , 0.45  $\mu\text{m}$ , 0.2  $\mu\text{m}$ ) and ultrafiltration (100 kDa, 10 kDa, 1 kDa) were successively performed and the permeates obtained were analyzed.

Total COD of liquid fraction of digestates from the 11 co-digestion plants varied from 9.2 to 78 g/L (Figure 14). Fractionation of liquid fraction showed that 60–96% (7.2–67.3 g/L) of the COD was in the fraction >1.2  $\mu\text{m}$  which corresponded to the suspended particles. Depending on the samples, 0–11% (0–4 g/L) of the COD was in coarse colloids and 2–16% (0.16–6.2 g/L) was in fine colloids. 2–18% (0.48–8.6 g/L) of the COD was in dissolved matter indicating a rather low impact of dissolved matter on the total COD. COD in dissolved matter was neither due to VFA, carboxylic acids, sugars nor alcohols, because none of these compounds were detected.

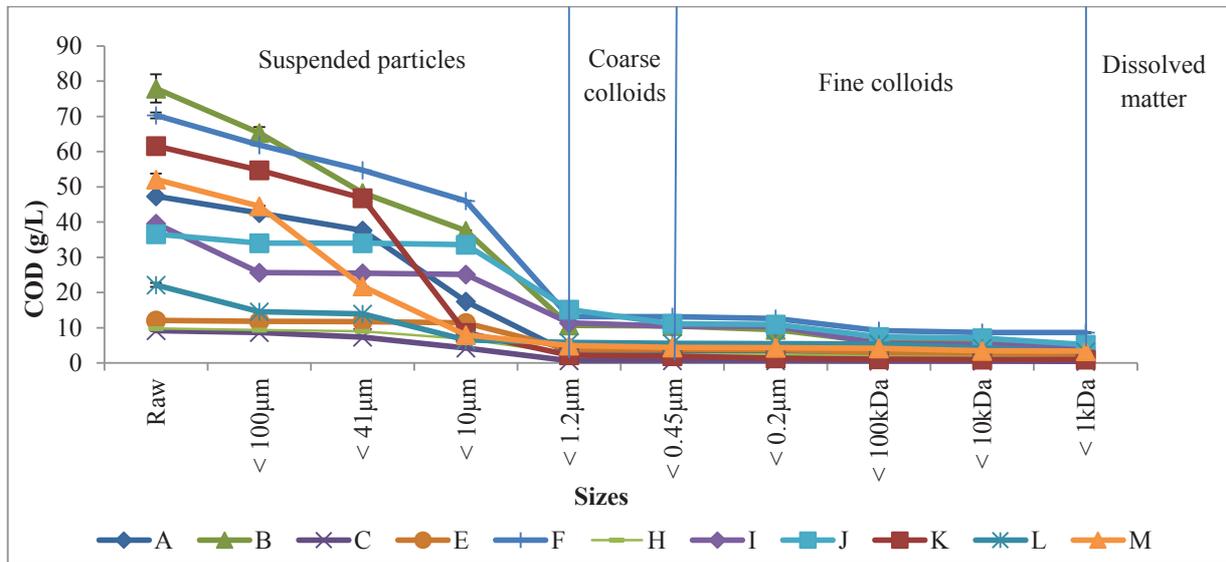


Figure 14: Evolution of the COD (g/L) of permeates during successive filtrations for the liquid fraction of digestates.

The concentrations of total COD were quite well correlated to the VS concentrations (Figure 15a). The impact of solid-liquid separation on total COD in liquid fraction of digestates is shown in Figure 15b. A group with low total COD was found after centrifugation while another group with high total COD was found after screw press separation. Another impact on total COD in liquid fraction of digestate seemed to be due to the origin of substrates. This can be seen with the relation of COD with the percentage of cow manure in the substrate for liquid fraction of digestates after screw press from mesophilic CSTR digester, as shown in Figure 15c. A relation between COD in coarse colloids, fine colloids and dissolved matter and the percentage of cow manure in mesophilic CSTR digesters was also observed ( $R^2 = 0.7553$ , data not shown). In comparison, lower COD concentration was found in liquid fraction of digestate C originating from 100% fruits and vegetables. However, it is difficult to identify the specific role of the solid-liquid separation, or the type of substrate, as this later parameter has also an influence on the type of liquid-solid separation which can be used.

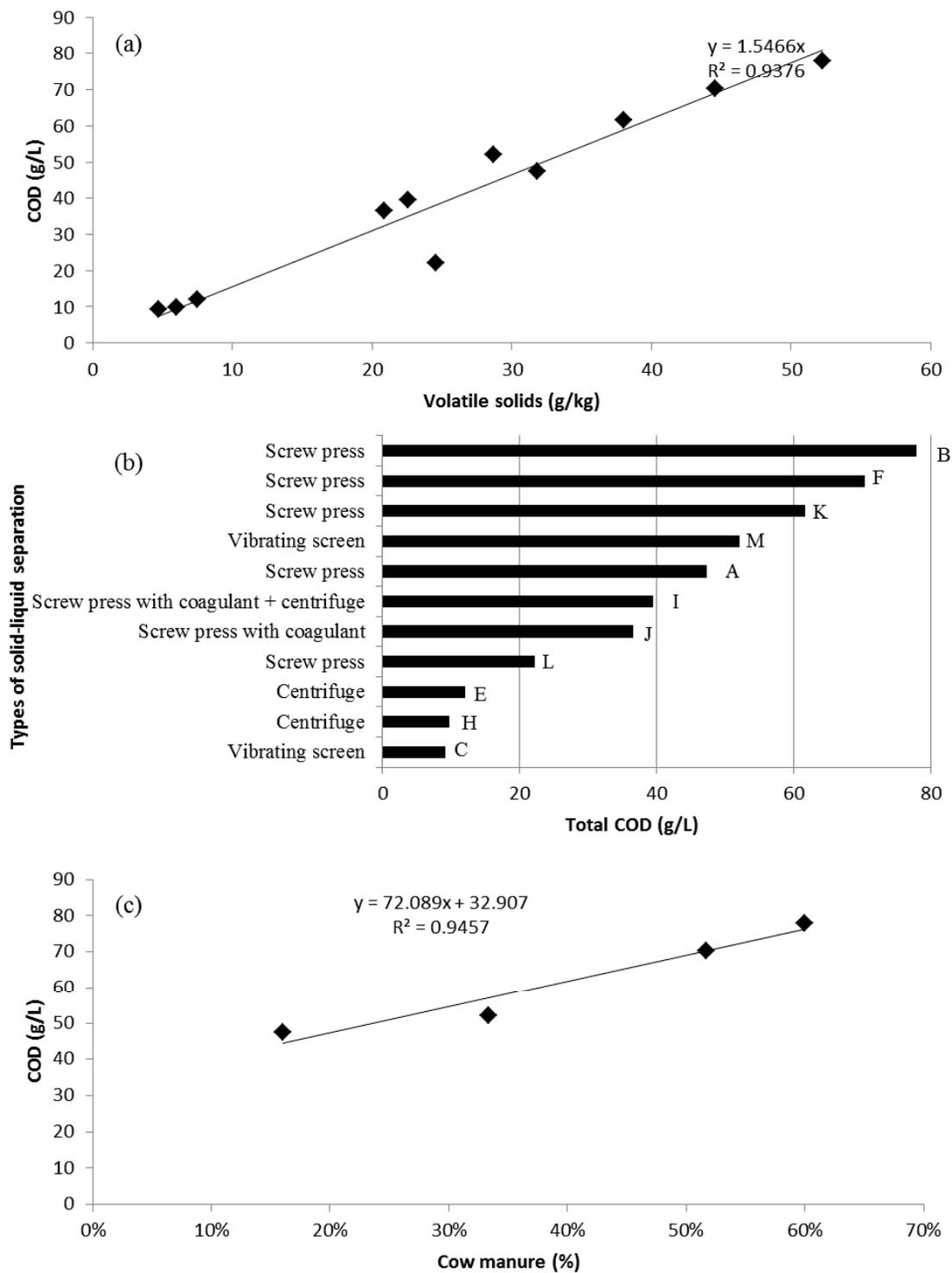


Figure 15: (a) Total COD (g/L) vs VS (g VS/kg) in liquid fraction of digestates, (b) solid-liquid separation vs total COD (g/L) in liquid fraction of digestates, (c) total COD in liquid fraction of digestates (g/L) vs cow manure in the digester feeding (%)

The highest total COD in liquid fraction of digestate after separation by centrifugation or screw press with coagulant were observed in samples I and J which were both from thermophilic plug flow digesters. These conditions also increased COD percentage in coarse colloids and fine colloids: highest percentage of 3% and 11% in coarse colloids and 15% and 16% in fine colloids were only found in I and J, respectively. Both I and J also contained high COD in dissolved matter. The temperature effect on COD was previously studied by other researchers who confirmed higher COD of 17.2 g/L under thermophilic condition compared to COD of 10.9 g/L under mesophilic condition at Organic Loading Rate (OLR) of 1.5 kg VS/m<sup>3</sup>day by co-digesting cow manure and dry dog food in CSTR digester (Labatut et al., 2014). Another study also confirmed higher COD of 3–6 g/L under thermophilic condition compared to COD of 1–2 g/L under mesophilic condition in coarse colloids, fine colloids and dissolved matter from CSTR digesters co-digesting rendering plant and slaughterhouse wastes at OLR of 1.5 kg VS/m<sup>3</sup>day (Bayr et al., 2012b).

#### **3.1.4. TKN and NH<sub>4</sub><sup>+</sup> concentrations in liquid fraction of digestates**

Nitrogen (N) and NH<sub>4</sub><sup>+</sup> concentrations in liquid fraction of digestate from co-digestion is important to consider to avoid excessiveness in case of land application where maximum N application is 150–250 kg N/ha/y according to the European Commission (Saveyn and Eder, 2014). Figure 16a shows TKN concentrations and its distribution into suspended particles, coarse colloids, fine colloids and dissolved matter (NH<sub>4</sub><sup>+</sup> and dissolved organic nitrogen). Since there was no nitrate and nitrite, TKN equals to total nitrogen (TN). TKN concentrations from 1.5 to 6.5 g N/L were measured, with TKN concentrations above 4 g N/L except for C and M. Depending on the sample, main part of TKN was either in suspended particles (11–65% (0.5–3.0 g/L)) or in dissolved matter (26–80% (0.74–3.41 g/L)). Only 0.4–8.5% (0.01–0.38 g/L) and 0–13% (0.01–0.61 g/L) of TKN was in coarse colloids and fine colloids, respectively. TKN in dissolved matter was composed of 62–98% (0.5–3.1 g N/L) of NH<sub>4</sub><sup>+</sup> and 2–38% (0.06–0.97 g N/L) of organic nitrogen. NH<sub>4</sub><sup>+</sup> represented 16–72% of total TKN. High NH<sub>4</sub><sup>+</sup> concentration in the liquid fraction of digestates can be removed through ammonia stripping or evaporation (X. Li et al., 2016). The ratio of TKN in suspended particles contained in total TKN ( $[\text{TKN}_{\text{susp}}]/[\text{totalTKN}]$ ) (%) was calculated (Figure 16b). Higher ratio from 46 to 65% was

observed after either screw press or vibrating screen. Lower ratio from 11 to 38% was observed after either screw press with coagulant or centrifuge. This confirmed that separation technique has an impact on TKN concentrations.

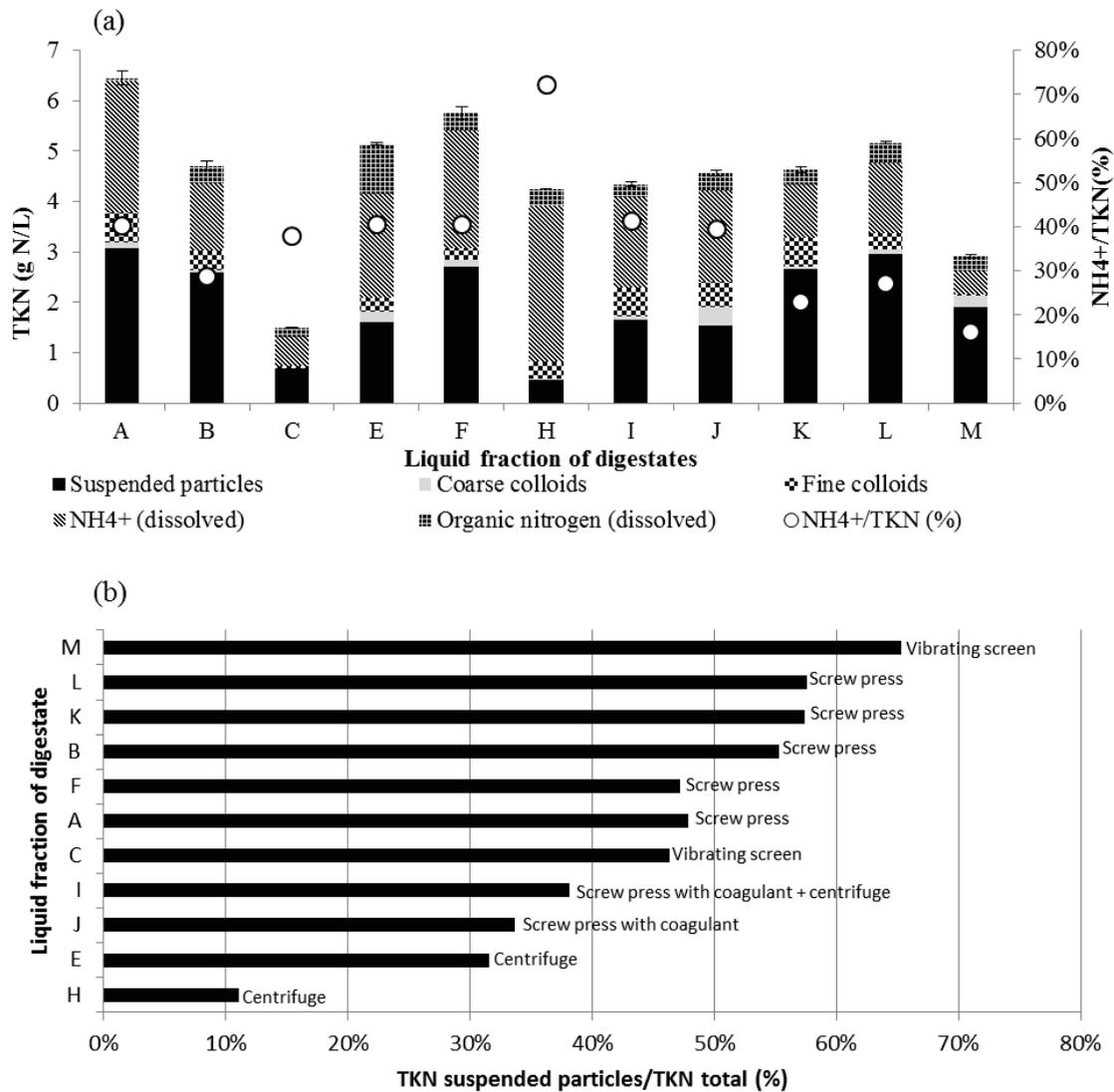


Figure 16: (a) TKN concentrations (g N/L) in suspended particles, coarse colloids, fine colloids and dissolved matter (NH<sub>4</sub><sup>+</sup> and organic nitrogen) and NH<sub>4</sub><sup>+</sup>/TKN (%), (b) TKN suspended particles/TKN total (%) of liquid fraction of digestates with solid-liquid separation technique

### 3.1.5. Ion concentrations in dissolved matter of liquid fraction of digestates

Ions concentration is important to consider to avoid exceeding maximum land application of 100 kg/ha/y of potassium and 60 kg/ha/y of phosphate (Rollett et al., 2015). The most concentrated ion present was potassium with concentrations between 1.05 and 5.48 g/L (Figure 17). Chloride, sodium and phosphate concentrations were relatively high in some samples and ranged from 0.54 to 3.81 g/L, 0.08 to 3.02 g/L and 0 to 2.13 g/L, respectively. Magnesium, calcium and sulfate had concentrations lower than 1 g/L and were in the range of 0–0.26 g/L, 0.05–0.44 g/L and 0– 0.67 g/L, respectively.

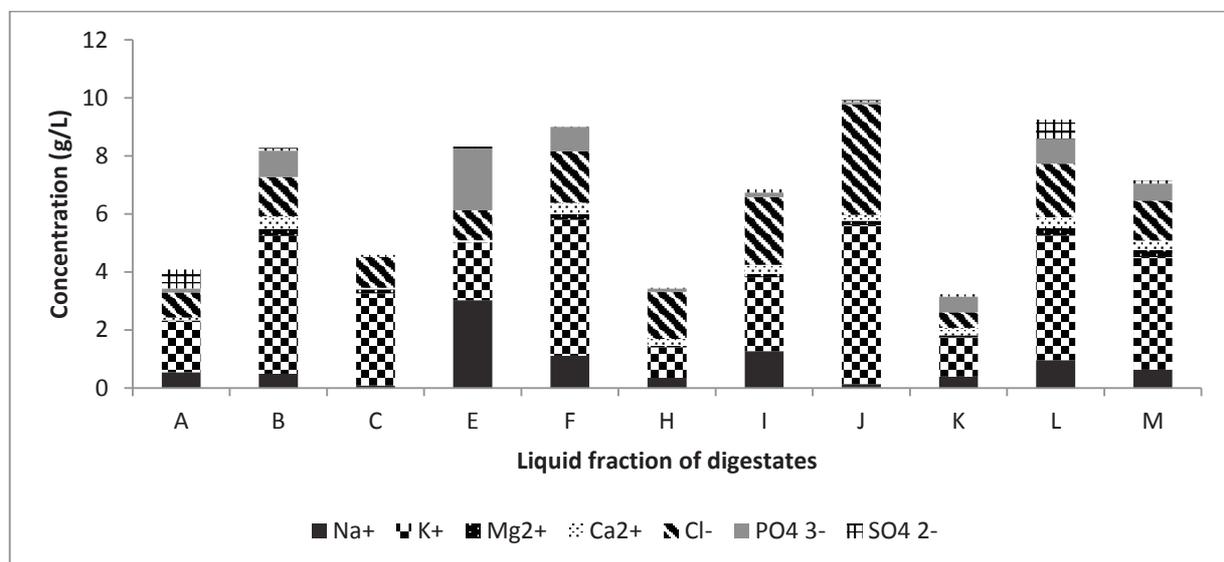


Figure 17: Concentration of sodium (Na<sup>+</sup>), potassium (K<sup>+</sup>), magnesium (Mg<sup>2+</sup>), calcium (Ca<sup>2+</sup>), chloride (Cl<sup>-</sup>), phosphate (PO<sub>4</sub><sup>3-</sup>) and sulfate (SO<sub>4</sub><sup>2-</sup>) in the fraction of dissolved matter of the liquid fraction of digestates

### 3.1.6. Other physico-chemical parameters

Table 17 presents pH, total alkalinity, IC, TOC, COD/TOC, C/N, turbidity, conductivity and SUVA<sub>254</sub> in the liquid fraction of digestates. The pH ranged from 7.6 to 8.4 and is within the range of 6.7 to 9.2 found in other liquid fraction of digestates as previously reviewed (Xia and Murphy, 2016). Total alkalinity was high and ranged from 7.4 to 24.8 g/L CaCO<sub>3</sub>. Co-digestion with manure or food waste was observed to contribute to higher total alkalinity compared to

digestion of only fruits and vegetables. Total alkalinity was observed to be linked to IC ( $R^2 = 0.65$ , data not shown). IC and TOC varied from 1.3 to 3.6 g/L and from 0.5 to 3.2 g/L, respectively. High TOC was observed in liquid fraction of digestates co-digesting either manure or food waste. However, TOC in liquid fraction of digestates originating from co-digestion with manure were still very low compared to TOC in solid fraction of digestate co-digesting cattle slurry with cattle manure and maize-oat silage with TOC of 422 g/kg (Bustamante et al., 2012). The ratio of COD/TOC varied from 2.2 to 3.2  $\text{gO}_2/\text{gC}$  except for C, E and H which were less than 1.1. COD/TOC ratio less than 1.1 was observed in liquid fraction of digestate co-digesting 100% fruits and vegetables or >40% sludge with highly biodegradable waste (fats and food wastes) and had no or low percentage of manure. The C/N ratio in dissolved matter varied from 0.2 to 1.6 and were lower than C/N ratio from 1.5 to 6.1 found in the raw digestates (Tampio et al., 2016b). This was due to major part of organic matter in the substrates was degraded during AD process. The accumulation of  $\text{NH}_4^+$  during AD process also contributed to lower C/N ratio in the digestates (Monlau et al., 2015b). This high concentration of ammonium can be beneficial for balancing low nitrogen feedstock such as lignocellulosic biomass.

Table 17: pH, total alkalinity, inorganic carbon (IC), total organic carbon (TOC), COD/TOC, organic carbon/organic nitrogen (C/N), turbidity, conductivity and SUVA<sub>254</sub> of liquid fraction of digestates

Sample	pH	Total alkalinity (g/L $\text{CaCO}_3$ )	IC (g/L)	TOC (g/L) <1 kDa	COD/TOC ( $\text{gO}_2/\text{gC}$ ) <1 kDa	C/N <1 kDa	Turbidity (NTU)	Conductivity (mS/cm)	SUVA <sub>254</sub> (L/mg.m)
A	8.2	24.80 ± 0.03	2.329 ± 0.001	0.470 ± 0.002	3.2	0.2	43,300	26	1.6
B	7.9	17.07 ± 0.04	2.696 ± 0.003	1.532 ± 0.004	2.8	0.9	51,400	29	2.6
C	8.1	7.40 ± 0.04	1.297 ± 0.001	0.791 ± 0.003	0.6	1.1	6160	14	0.2
E	8.4	21.5 ± 0.1	3.159 ± 0.003	3.164 ± 0.004	0.7	1	3780	31	0.004
F	8.3	23.6 ± 0.4	3.566 ± 0.003	2.66 ± 0.05	3.2	1	49,400	38	1.6
H	8.3	14.13 ± 0.02	2.40 ± 0.03	1.23 ± 0.01	1.1	0.4	2960	27	0.7
I	8.1	13.83 ± 0.06	2.250 ± 0.006	1.454 ± 0.006	3.0	0.7	12,840	30	1.9
J	8.2	13.2 ± 0.1	2.380 ± 0.001	1.74 ± 0.01	3.0	0.8	7590	35	2.6
K	7.6	14.04 ± 0.02	1.598 ± 0.002	0.452 ± 0.002	2.2	0.3	41,800	16	1.9
L	8.3	22.598 ± 0.004	2.941 ± 0.001	1.104 ± 0.004	2.9	0.6	29,640	33	2.4
M	8.2	8.98 ± 0.04	1.66 ± 0.01	1.21 ± 0.02	2.8	1.6	38,160	16	3.0

Turbidity was high from 2960 to 51,400 NTU and as expected was linked to suspended particles ( $R^2 = 0.84$ , data not shown). More than 98% of turbidity was in suspended particles except for I

(95%) and J (94%). 5% and 6% of turbidity in I and J respectively were in coarse and fine colloids. High turbidity in liquid fraction of digestate can be the main problem if liquid fraction of digestate is used for microalgae cultivation. This is due to one of the most important requirement for microalgae growth is light penetration. High turbidity will have a negative impact on microalgae growth and removal of nitrogen (Marcilhac et al., 2014; Xia and Murphy, 2016). The conductivity was high and ranged from 14 to 38 mS/ cm. Conductivity was observed to be linked to IC ( $R^2 = 0.8$ , data not shown) and total ions ( $R^2 = 0.8$ , data not shown) concentration in dissolved matter.  $SUVA_{254}$  ranged from 1.6 to 3 L/mg/m and was in the range of fulvic acid-like compounds (0.6–3.9 L/mg/m) (Zheng et al., 2014) except for E, C and H which was 0.004, 0.2 and 0.4, respectively. No liquid fraction of digestate in this study had  $SUVA_{254}$  value higher than 5.0 L/mg/m which referred to humic acid-like compounds (Zheng et al., 2014). Lower  $SUVA_{254}$  values as shown by C, E, and H indicate lower humification degree (Zheng et al., 2014) and confirmed the COD/TOC less than 1.1 for liquid fraction of digestates originating from highly biodegradable waste. Similar range of  $SUVA_{254}$  values from 0.9 to 3.1 L/mg/m for liquid fraction of 30 digestates were also obtained by other researchers indicating the presence of fulvic acid-like compounds rather than humic acid-like (Zheng et al., 2014).

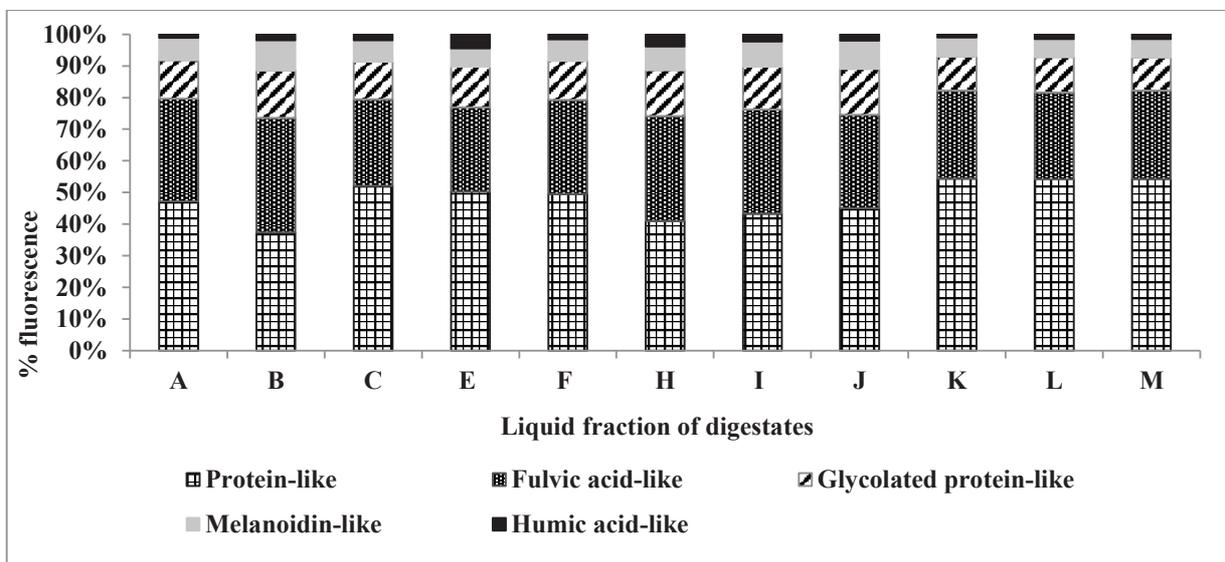


Figure 18: 3D fluorescence spatialization quantification from 3D spectra of liquid fraction of digestates

Analyses with 3D fluorescence spectroscopy confirmed the presence of high fulvic-acid like compounds from 27 to 36% compared to other complex compounds, glycolated protein-like (11–15%), melanoidin-like (6–10%) and humic acid-like (1–4%) as shown in Figure 18. Protein-like was from 37 to 54%.

### 3.1.7. Biological analysis

BOD<sub>5</sub> and BOD<sub>21</sub> were performed to determine the possibility of aerobic biological post treatment for liquid fraction of digestates from co-digestion plants. Figure 19a shows that BOD<sub>5</sub> and BOD<sub>21</sub> varied from 1.7 to 9 g/L and from 3.14 to 32 g/L, respectively. BOD<sub>5</sub>/COD was ≤0.2 except H with BOD<sub>5</sub>/COD <0.4 and BOD<sub>21</sub>/COD was ≤0.6.

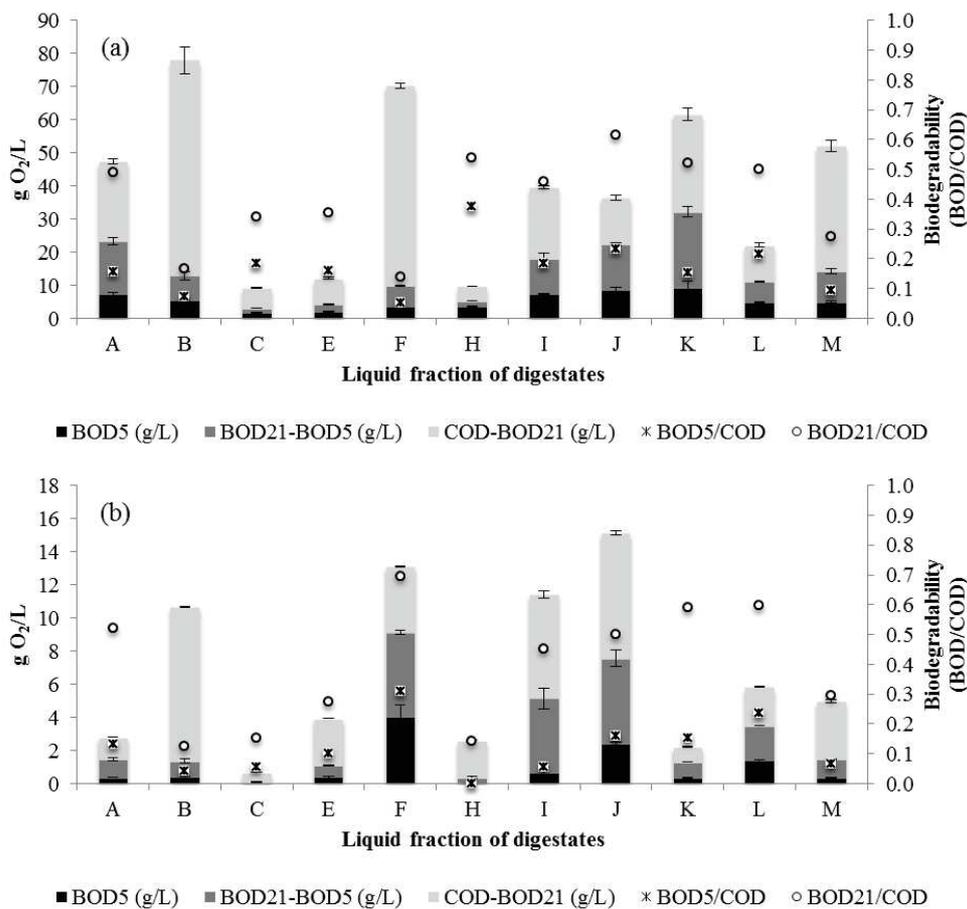


Figure 19: (a) BOD<sub>5</sub>, BOD<sub>21</sub> and COD in liquid fraction of digestates and (b) BOD<sub>5</sub>, BOD<sub>21</sub> and COD in liquid fraction of digestates (<1.2 μm)

Since 59–96% of COD in liquid fraction of digestates analyzed in this study were in suspended particles, BOD analyses were also performed in the fraction  $<1.2 \mu\text{m}$  (after suspended particles removal) (Figure 19b).

$\text{BOD}_5$  ( $<1.2 \mu\text{m}$ ) and  $\text{BOD}_{21}$  ( $<1.2 \mu\text{m}$ ) varied from 0 to 4 g/L and 0.1 to 9.1 g/L respectively.  $\text{BOD}_5/\text{COD}$  ( $<1.2 \mu\text{m}$ ) ratio was  $<0.2$  except F and L with  $\leq 0.3$ .  $\text{BOD}_{21}/\text{COD}$  ( $<1.2 \mu\text{m}$ ) was  $\leq 0.5$  except F, K and L with  $\text{BOD}_{21}/\text{COD}$  ( $<1.2 \mu\text{m}$ )  $\leq 0.7$ .

All these results indicate poor biodegradability for liquid fraction of digestates from co-digestion plants for the raw liquid fraction as well as for the soluble fraction after suspended particles removal. The recalcitrant COD can be due to the presence of complex compounds such as fulvic acid-like, glycolated protein-like, melanoidin-like and humic acid-like compounds as previously discussed.

### **3.2. Conclusion**

This study shows the high range of variation of chemical, physical and biological characteristics of liquid fraction of digestates from full-scale co-digestion plants, depending on the type of solid-liquid separation along with the type of substrates used to feed the digester. Centrifugation and screw press with coagulant were the most efficient separation techniques which resulted in the lower TS concentration in liquid fraction of digestate. On the contrary, screw press only and vibrating screen were the least efficient separation techniques which resulted in almost the same TS concentration in liquid fraction of digestate than in the raw digestate. The origin of substrates, especially manure, seems to have major impact on characteristics of liquid fraction of digestates. In this study, variation of COD from 9.2 to 78 g/L was observed in liquid fraction of digestates from co-digestion plants with 60–96% of the COD was in suspended particles, 2–27% in colloids and 2–18% in dissolved matter. With the characteristics of poor biodegradability in aerobic condition, due to the presence of complex compounds such as fulvic acid-like compounds, only physico-chemical post treatment can be proposed for the treatment of organic carbon in liquid fraction of digestates from co-digestion plants. From the detail characterization performed, the size fractionation of the liquid fraction of digestates was simplified in order to group the organic matter into 3 groups: suspended particles, colloids and dissolved matter. The next chapter will discuss on the data analysis of the characteristics of liquid fractions of digestates in order to have

better understanding of the link between these characteristics and the types of substrates fed, digester operating parameters and solid-liquid separation performed. In order to have a better representative sample, 18 more liquid fractions of digestate from co-digestion plants and 1 from waste activated sludge (WAS) were characterized with the same analyses than those performed in this chapter but this a simplified distribution of raw liquid into 3 fractions that is to say after successive filtrations at 1.2  $\mu\text{m}$  and then 1 kDa which makes it possible to separate the fractions of suspended particles ( $> 1.2 \mu\text{m}$ ), colloids (1.2  $\mu\text{m}$  to 1 kDa) and dissolved matter ( $< 1 \text{ kDa}$ ). All the characteristics of all liquid fractions of 30 digestates as well as the types of the substrates at the input, operating parameters of the digesters as well as type of solid liquid separation performed will be included in the statistical analysis and discussed in Chapter 4.



#### **4. Relationship between characteristics of liquid fractions of digestates and types of solid-liquid separation, substrates and operating parameters of reactors**

In the previous chapter, high variability in the liquid fraction composition and characteristics of digestates from co-digestion plants was shown. In order to have enough data to carry out statistical analysis and get information on the links between the characteristics of liquid fractions of digestates and both feedstocks nature and process parameters, 18 more liquid fractions of digestates from full scale anaerobic co-digestion plants were characterized. However, the fractionation of these samples was simplified on the base of the results obtained from previous detailed characterization in Chapter 3. Thus successive filtrations at 1.2  $\mu\text{m}$  and 1 kDa were carried out and they allow to obtain four fractions: raw liquid (without any filtration), suspended particles ( $> 1.2 \mu\text{m}$ ), colloids (1.2  $\mu\text{m}$ -1 kDa) and dissolved matter ( $< 1 \text{ kDa}$ ). All the simplified characteristics will be compared to the characteristics of liquid fraction of 11 digestates previously characterized in Chapter 3.

The statistical analysis using principal component analysis (PCA) on the total of 29 liquid fractions of digestates is presented in this chapter to investigate the relationships between the characteristics (chemical, physical and biological) of liquid fraction of digestates in relation to the feedstocks, operating parameters (loading rate, type of reactor, hydraulic retention time, temperature), biogas production and type of solid-liquid separation.

#### **4.1. Characterization of the liquid fraction of digestates from the second collection**

##### **4.1.1. Total solids concentrations**

Table 18 shows TS, VS and MS concentrations as well as the organic fraction (VS/TS) (%) in the raw, solid fraction and liquid fraction of 18 digestates. TS concentrations in raw digestates ranged from 18.9 to 117.9 g TS/kg except for I2 which was 282.8 g TS/kg. Such a high TS concentration in I2 was due to high MS concentration: 185.0 g MS/kg compared to the range from 10.2 to 39.2 g MS/kg for other digestates. VS concentrations ranged from 8.7 to 78.7 g VS/kg except I2 with 97.9 g VS/kg where these organic fractions ranged from 35 to 71% of TS.

These results confirm the ones obtained in Chapter 3 with more or less similar ranges for TS, VS and MS concentrations (Table 18). Samples I2 and I were originated from the same plant treating bio-waste in a plug flow reactor whereas other digestates were from liquid anaerobic digestion from CSTR, which explains the high TS, MS and VS concentrations for these samples. It is worth noting that sample J from Chapter 3 also originated from plug flow reactor but with corn silage and poultry manure as feedstock presented TS, VS and MS concentrations in the same range as digestates from CSTR.

For solid fractions of digestates, TS concentrations ranged from 179.9 to 374.0 g TS/kg except for T, I2 and AD with 893.9, 439.7 and 879.6 g TS/kg respectively. Such high concentrations were due to drying process after solid-liquid separation of digestates T and AD. For I2 and I samples, a high TS concentration in solid fraction was due to both high TS and MS concentration in raw digestate and to a 2-step separation process by screw press with coagulant addition followed by centrifugation. The range of TS concentrations of solid digestates from CSTR was slightly higher than previously found from 99.2 to 323.9 g TS/kg. If dried digestates (T and AD) are excluded, VS concentrations varied from 114.5 to 267.4 g VS/kg which is in the range as previously found in Chapter 3 from 87.6 to 272.4 g VS/kg. These organic fractions represented 38 to 87% of TS which were slightly lower than previously found from 48 to 93%. MS concentrations ranged from 29.2 to 111.8 g MS/kg except T, I2 and AD with higher MS concentrations of 356.4, 272.9 and 453.7 g MS/kg respectively. The range of MS concentrations was in the range as previously found from 11.5 to 121.5 g MS/kg except I with 217.2 g MS/kg.

For liquid fractions of digestates, TS, VS and MS concentrations ranged from 6.7 to 85.5 g TS/kg, 2.9 to 51.3 g VS/kg and 3.7 to 34.2 g MS/kg respectively. VS/TS (%) ranged from 37 to 73%. TS, VS and MS concentrations ranges were close to the ranges in previous characterization from 10.3 to 82.7 g TS/kg, from 4.7 to 52.3 g VS/kg and from 4.3 to 38.1 g MS/kg for TS, VS and MS respectively. The organic fraction represented in TS were also close to the range from 35 to 75% as previously found.

Table 18: Total solids (TS), volatile solids (VS), mineral solids (MS) concentrations (g/kg) and VS/TS (%) in raw digestates, solid fractions of digestates and liquid fractions of digestates (\* dried solid fractions)

Plants	Raw digestates				Solid fractions of digestates				Liquid fractions of digestates			
	TS (g TS/kg)	VS (g VS/kg)	MS (g MS/kg)	VS/TS (%)	TS (g TS/kg)	VS (g VS/kg)	MS (g MS/kg)	VS/TS (%)	TS (g TS/kg)	VS (g VS/kg)	MS (g MS/kg)	VS/TS (%)
N	93 ± 2	62 ± 2	30.9 ± 0.2	67	218 ± 14	189 ± 15	29 ± 3	87	76.7 ± 0.1	46.0 ± 0.2	30.7 ± 0.1	60
O	34 ± 2	21 ± 2	12.8 ± 0.7	62	277 ± 3	165 ± 4	112 ± 6	60	13.10 ± 0.04	7.12 ± 0.02	5.99 ± 0.03	54
P	68.0 ± 0.2	39 ± 1	29 ± 1	58	202 ± 9	163 ± 8	39 ± 2	81	61.6 ± 0.2	35 ± 2	27 ± 2	56
Q	71.20 ± 0.03	43 ± 1	28 ± 1	61	267 ± 3	203 ± 4	65 ± 7	76	72.6 ± 0.8	48.1 ± 0.8	24 ± 2	66
R	47.5 ± 0.4	33.5 ± 0.4	14.0 ± 0.6	70	247 ± 2	151 ± 5	96 ± 4	61	26.66 ± 0.02	18.63 ± 0.06	8.02 ± 0.07	70
S	48.4 ± 0.3	31.4 ± 0.3	16.94 ± 0.09	65	187.6 ± 0.3	114 ± 2	73 ± 2	61	6.72 ± 0.02	3.04 ± 0.02	3.68 ± 0.01	45
T	71.6 ± 0.5	42.1 ± 0.7	29.5 ± 0.2	59	*893.9 ± 0.4	538 ± 7	356 ± 7	60	46.86 ± 0.07	27.34 ± 0.04	19.52 ± 0.05	58
U	78.1 ± 0.3	42.0 ± 0.6	36.0 ± 0.7	54	244 ± 7	173 ± 5	70 ± 3	71	66.2 ± 0.8	42 ± 1	25 ± 2	63
V	73.8 ± 0.4	45 ± 1	29 ± 2	61	180 ± 3	125 ± 4	55 ± 3	69	54.35 ± 0.02	32 ± 2	22 ± 2	59
W	82 ± 2	58 ± 1	24.1 ± 0.5	71	244 ± 15	209 ± 6	35 ± 11	85	57 ± 1	41.1 ± 0.2	15 ± 1	73
X	94.7 ± 0.9	59 ± 2	36 ± 2	62	209 ± 3	166.4 ± 0.8	42 ± 2	80	85.5 ± 0.7	51 ± 1	34.2 ± 0.7	60
Y	52.7 ± 0.6	33.7 ± 0.2	19.0 ± 0.7	64	374 ± 4	267 ± 6	107 ± 4	71	44.6 ± 0.3	26.2 ± 0.2	18.4 ± 0.4	59
Z	118 ± 2	79 ± 5	39 ± 6	67	246 ± 9	205 ± 21	41 ± 13	83	67.27 ± 0.09	41 ± 1	26 ± 1	61
I2	283 ± 7	98 ± 1	185 ± 7	35	440 ± 10	167 ± 4	273 ± 8	38	37 ± 2	20.7 ± 0.6	17 ± 2	56
AA	61.5 ± 0.3	39.9 ± 0.8	21.6 ± 0.8	65	314 ± 31	256 ± 23	58 ± 8	82	34.49 ± 0.05	22.09 ± 0.09	12.4 ± 0.1	64
AB	67.4 ± 0.8	36.6 ± 0.8	31 ± 2	54	297 ± 2	202.6 ± 0.9	94 ± 2	68	46.3 ± 0.3	23.4 ± 0.2	22.8 ± 0.1	51
AC	32 ± 1	19.7 ± 0.9	12.0 ± 0.2	62	315.4 ± 0.9	213 ± 1	102.6 ± 0.4	67	16.12 ± 0.06	8.73 ± 0.06	7.39 ± 0.01	54
AD	18.9 ± 0.3	8.7 ± 0.2	10.2 ± 0.2	46	*880 ± 5	426 ± 39	454 ± 34	48	7.8 ± 0.2	2.9 ± 0.2	4.88 ± 0.06	37
First collection (chapter 3)	14.4 - 227.2	5.4 - 115.4	9.0 - 111.8	37 - 78	99.2 - 416.2	87.6 - 272.4	11.5 - 217.2	48 - 93	10.3 - 82.7	4.7 - 52.3	4.3 - 38.1	35 - 75

\*Samples were dried after solid-liquid separation

#### 4.1.2. Particle sizes distribution

Figure 20 shows the distribution of particle sizes in liquid fraction of 18 digestates. Particle sizes between 0 to 50  $\mu\text{m}$  have high variation from 19 to 93%. Particle sizes between 50 to 100  $\mu\text{m}$  ranged from 5 to 41% while particle sizes between 100 to 500  $\mu\text{m}$  ranged from 0 to 43%. Bigger particle sizes from 500 to 1000  $\mu\text{m}$  and from 1000 to 2000  $\mu\text{m}$  ranged from 0 to 16% and from 0 to 11% respectively. This shows that particle sizes of liquid fractions of digestates are mostly in the range from 0 to 50  $\mu\text{m}$  followed by the range from 100 to 500  $\mu\text{m}$  which confirms results from Chapter 3.

The mean size of the particles ranged from 24 to 388  $\mu\text{m}$  which were slightly higher than in previous characterization from 31 to 257  $\mu\text{m}$ . The median sizes of the particles ranged from 7 to 199  $\mu\text{m}$  which was also higher than in previous characterization from 14 to 116  $\mu\text{m}$ .

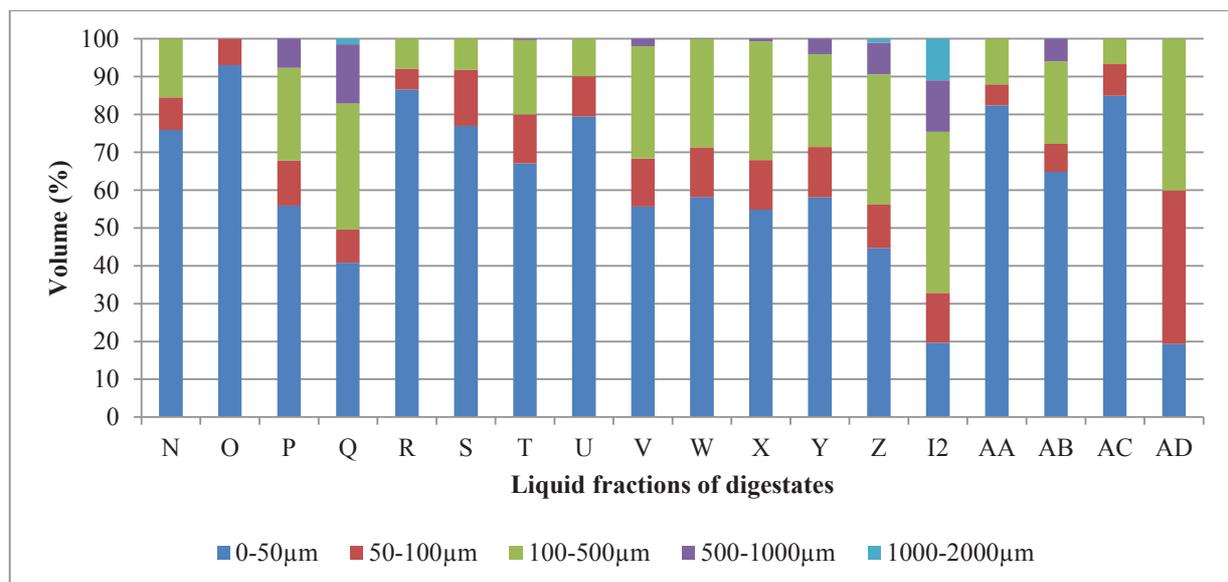


Figure 20: Particle sizes distribution in liquid fraction of 18 digestates

#### 4.1.3. COD

Figure 21 shows COD in suspended particles, colloids and dissolved matter in liquid fraction of 18 digestates. First of all, it can be seen that sample AD presents very low COD concentration

(0.84 g O<sub>2</sub>/L), which is due to highly efficient solid-liquid separation with addition of coagulant and polymer. However, this sample presented high chloride concentration and was not diluted contrary to other samples, which led to chloride interferences in COD measurement (Greenberg and Eaton, 1999). COD concentration of sample AD will not be considered in further discussion. COD concentration in liquid fractions of digestates had a high variation from 4.4 to 90.1 g O<sub>2</sub>/L. The COD range observed was slightly higher than the previous COD range from 9.2 to 78 g O<sub>2</sub>/L in Chapter 3.

60 to 96% (2.6 to 82.7 g O<sub>2</sub>/L) of COD was in suspended matter. This range is the same to the range in previous characterization from 60–96% (7.2–67.3 g/L). COD in colloids ranged from 0.9 to 16% (0.6 to 8.8 g O<sub>2</sub>/L). The percentage range of COD in colloids was slightly lower than previously found from 2 to 27% but the concentration range was similar to previous characterization from 0.2 to 9.9 g O<sub>2</sub>/L. COD percentage in dissolved matter ranged from 3 to 24% (1 to 8.2 gO<sub>2</sub>/L). The percentage range of COD in dissolved matter was slightly higher than previously found from 2–18% (0.48–8.6 g/L). Similar to previous characterization, COD in dissolved matter was neither due to VFA, carboxylic acids, sugars nor alcohols, because none of these compounds were detected.

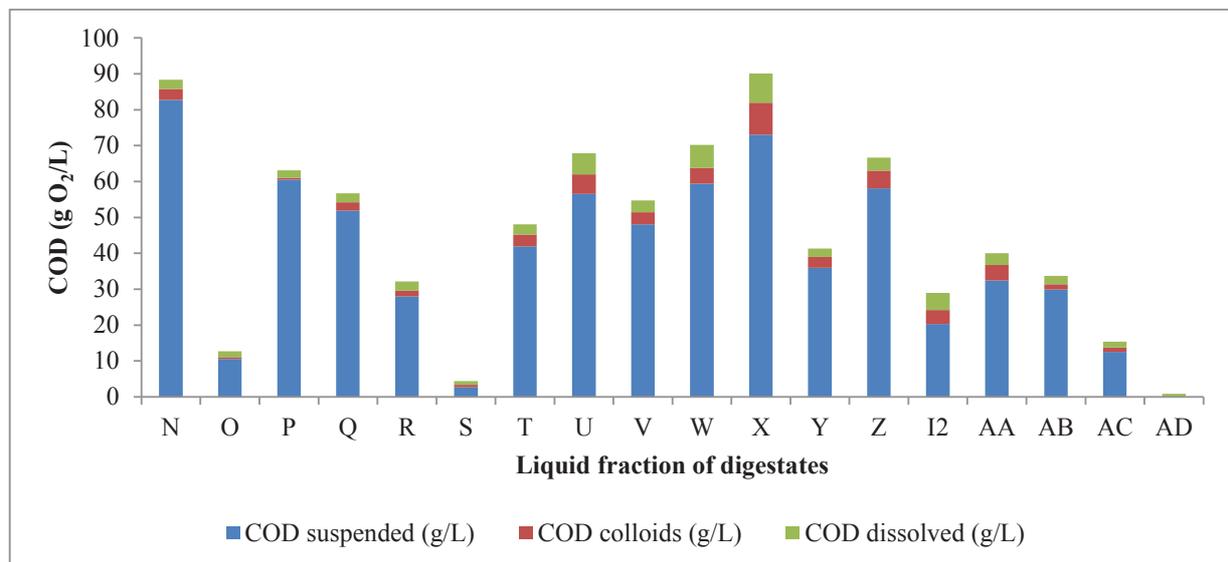


Figure 21: COD in suspended particles, colloids and dissolved matter in liquid fraction of 18 digestates

#### 4.1.4. Total nitrogen (TN)

Table 19 shows Total nitrogen (TN) concentrations in suspended particles, colloids, dissolved organic nitrogen (dissolved Norg) and  $\text{NH}_4^+$  in liquid fraction of 18 digestates. TN concentrations (g/L) ranged from 1.3 to 8.3 g N/L and was slightly higher compared to previous characterization from 1.5 to 6.5 g N/L. TN percentage in suspended particles ranged from 27 to 67% (0.7 to 4.0 g N/L) except for AD with 0%. This range was similar but slightly higher to the range from 11–65% (0.5–3.0 g/L). Absence of suspended TN in sample AD confirms that solid-liquid separation with addition of coagulant will lead to lower TN in the suspended particles.

TN in colloids represented 0.2 to 12% colloids (0.005 to 0.5 g N/L). The range was similar but lower than the range from previous characterization from 4 to 19% (0.1 to 0.9 g N/L). TN percentage in dissolved matter was from 21 to 95% (0.6 to 5.2 g N/L) which was slightly broader than the range from previous characterization from 26–80% (0.74–3.41 g/L). TN in dissolved matter was composed of 66 to 100% (0.5 to 4.7 g N/L) of  $\text{NH}_4^+$  and 0 to 34% (0 to 1.1 g N/L) of dissolved Norg. The range is close to the range previously found from 62–98% (0.5–3.1 g/L) of  $\text{NH}_4^+$  and 2–38% (0.06–0.97 g/L) of dissolved Norg.  $\text{NH}_4^+$  represented 15 to 75% of TN similar to previously found from 16–72%  $\text{NH}_4^+$  of TN.

Table 19: TKN concentrations total, <1.2 µm and <1kDa (g N/L) with their distribution into suspended, colloids and dissolved matter (composed of NH<sub>4</sub><sup>+</sup> and organic nitrogen (Norg) dissolved)

	TKN total (g N/L)	TKN (<1,2 µm) (g N/L)	TKN dissolved (< 1 kDa) (g N/L)	TKN suspended (g N/L)	TKN colloids (g N/L)	NH <sub>4</sub> <sup>+</sup> (g N/L)	NH <sub>4</sub> <sup>+</sup> /TKN total (%)	NH <sub>4</sub> <sup>+</sup> /TKN dissolved (%)	Norg dissolved (g N/L)	Norg dissolved/ TKN total (%)	Norg dissolved/ TKN dissolved (%)
N	6.27 ± 0.07	3.04 ± 0.06	2.79 ± 0.03 (44%)	3.2 (52%)	0.3 (4%)	2.2 ± 0.1	36	80	0.55	9	20
O	2.69 ± 0.01	1.97 ± 0.02	1.83 ± 0.02 (68%)	0.7 (27%)	0.1 (5%)	1.24 ± 0.02	46	68	0.59	22	32
P	3.04 ± 0.07	0.99 ± 0.02	0.64 ± 0.02 (21%)	2.0 (67%)	0.4 (12%)	0.47 ± 0.01	15	73	0.17	6	27
Q	3.79 ± 0.07	1.8 ± 0.1	1.64 ± 0.07 (43%)	2.0 (52%)	0.2 (5%)	1.69 ± 0.05	45	103	0	0	0
R	3.20 ± 0.07	1.93 ± 0.03	1.8 ± 0.1 (56%)	1.3 (40%)	0.1 (4%)	1.55 ± 0.06	48	87	0.24	7	13
S	3.87 ± 0.07	3.10 ± 0.06	2.9 ± 0.1 (74%)	0.8 (20%)	0.2 (6%)	2.2 ± 0.1	56	75	0.71	18	25
T	8.3 ± 0.2	5.5 ± 0.1	5.22 ± 0.08 (63%)	2.8 (34%)	0.2 (3%)	4.67 ± 0.08	56	89	0.56	7	11
U	5.8 ± 0.2	3.52 ± 0.07	3.3 ± 0.1 (57%)	2.2 (39%)	0.3 (4%)	2.7 ± 0.1	46	81	0.61	11	19
V	4.33 ± 0.05	2.32 ± 0.02	2.13 ± 0.05 (49%)	2.0 (46%)	0.2 (4%)	1.76 ± 0.04	41	83	0.37	9	17
W	5.67 ± 0.04	3.3 ± 0.1	2.87 ± 0.03 (51%)	2.4 (42%)	0.4 (7%)	2.97 ± 0.04	52	104	0	0	0
X	7.6 ± 0.1	3.68 ± 0.01	3.1 ± 0.1 (41%)	4.0 (52%)	0.5 (7%)	3.21 ± 0.05	42	102	0	0	0
Y	7.7 ± 0.2	4.7 ± 0.1	4.20 ± 0.08 (55%)	3.0 (39%)	0.5 (6%)	3.1 ± 0.4	41	74	1.07	14	26
Z	5.2 ± 0.2	3.2 ± 0.2	2.8 ± 0.1 (53%)	2.1 (39%)	0.4 (8%)	2.24 ± 0.08	43	82	0.51	10	18
I2	3.62 ± 0.09	2.20 ± 0.03	1.93 ± 0.04 (53%)	1.4 (39%)	0.3 (8%)	1.6 ± 0.1	44	83	0.33	9	17
AA	5.01 ± 0.03	3.46 ± 0.03	3.11 ± 0.05 (62%)	1.5 (31%)	0.3 (7%)	2.04 ± 0.05	41	66	1.07	21	34
AB	4.8 ± 0.3	2.59 ± 0.09	2.48 ± 0.07 (51%)	2.2 (46%)	0.1 (2%)	1.96 ± 0.05	41	79	0.52	11	21
AC	2.79 ± 0.06	1.86 ± 0.09	1.9 ± 0.1 (67%)	0.9 (33%)	0.0 (0%)	1.51 ± 0.08	54	81	0.35	13	19
AD	1.31 ± 0.03	1.34 ± 0.05	1.25 ± 0.03 (95%)	0.0 (0%)	0.1 (6%)	0.97 ± 0.01	74	77	0.28	22	23
First Collection (chapter 3)	1.49 – 6.46	0.80 – 3.78	0.74 – 3.41 (26 – 80%)	0.47 – 3.09 (11 – 65%)	0.01 – 0.38 (0.4 – 8.5%)	0.5 - 3.1	16 - 72	62 – 98%	0.05 – 0.97	1-19	2-38

#### 4.1.5. Ion concentrations

Ions concentrations in liquid fraction of 18 digestates are shown in Table 20. Highest concentration observed was  $K^+$  which ranged from 0.5 to 7.09 g/L followed by  $Cl^-$  which ranged from 0.88 to 4.4 g/L. The range of  $K^+$  concentrations was slightly higher compared to previous characterization which ranged from 1.05 to 5.48 g/L (Table 20). Similar to  $Cl^-$ , the range was slightly higher than the range as previous characterization from 0.54 to 3.81 g/L. The range of  $Na^+$  concentrations from 0.13 to 3.24 g/L was close to the range in Chapter 3 from 0.08 to 3.02 g/L. For  $PO_4^{3-}$ , the concentrations ranged from 0 to 1 g/L and were lower compared to the range from 0 to 2.13 g/L in previous characterization. The lowest ion concentrations observed was  $SO_4^{2-}$  which ranged from 0 to 0.9 g/L and the range was lower than 1 g/L similar to previous characterization which ranged from 0 to 0.67 g/L.

Table 20: Sodium ( $Na^+$ ), potassium ( $K^+$ ), chlorine ( $Cl^-$ ), phosphate ( $PO_4^{3-}$ ) and sulfate ( $SO_4^{2-}$ ) concentrations

	$Na^+$	$K^+$	$Cl^-$	$PO_4^{3-}$	$SO_4^{2-}$
N	0.43 ± 0.02	3.53 ± 0.05	1.01 ± 0.05	0.35 ± 0.01	0.9 ± 0.0
O	0.80 ± 0.01	1.55 ± 0.03	1.02 ± 0.00	0.11 ± 0.00	0.30 ± 0.01
P	0.70 ± 0.01	2.53 ± 0.02	0.88 ± 0.02	0.80 ± 0.01	0.08 ± 0.00
Q	0.59 ± 0.01	3.65 ± 0.05	1.17 ± 0.00	0.53 ± 0.02	0.21 ± 0.01
R	0.29 ± 0.00	2.81 ± 0.04	0.96 ± 0.01	0.49 ± 0.00	0
S	0.54 ± 0.02	1.41 ± 0.00	1.87 ± 0.01	0.06 ± 0.00	0.03 ± 0.00
T	1.4 ± 0.2	3.3 ± 0.3	2.94 ± 0.01	0.44 ± 0.01	0
U	0.34 ± 0.02	6.69 ± 0.01	1.7 ± 0.2	1.0 ± 0.1	0.23 ± 0.07
V	0.22 ± 0.01	5.4 ± 0.2	1.52 ± 0.04	0.91 ± 0.02	0.18 ± 0.04
W	0.13 ± 0.01	5.02 ± 0.01	0.95 ± 0.03	0.42 ± 0.01	0.3 ± 0.1
X	0.17 ± 0.01	7.09 ± 0.05	0.95 ± 0.02	0.81 ± 0.01	0.24 ± 0.02
Y	2.44 ± 0.01	2.85 ± 0.04	3.77 ± 0.06	0.49 ± 0.01	0.33 ± 0.02
Z	0.47 ± 0.03	5.2 ± 0.4	1.47 ± 0.05	0.64 ± 0.01	0.08 ± 0.01
I2	1.20 ± 0.06	3.57 ± 0.05	2.3 ± 0.2	0.2 ± 0.0	0.05 ± 0.01
AA	0.65 ± 0.02	4.3 ± 0.1	2.11 ± 0.05	0.39 ± 0.01	0.10 ± 0.01
AB	3.24 ± 0.03	3.86 ± 0.09	3.83 ± 0.02	0.6 ± 0.0	0.03 ± 0.00
AC	0.80 ± 0.05	2.5 ± 0.2	1.28 ± 0.01	0.06 ± 0.00	0.12 ± 0.00
AD	1.31 ± 0.03	0.5 ± 0.0	4.4 ± 0.2	0	0
First collection (chapter 3)	0.08 - 3.02	1.05 - 5.48	0.54 - 3.81	0 - 2.13	0 - 0.67

#### 4.1.6. Other physico-chemical parameters

Table 21 shows pH, total alkalinity, inorganic carbon (IC), dissolved total organic carbon (TOC), COD/TOC (< 1 kDa), C/N, turbidity (NTU), conductivity and SUVA<sub>254</sub>. pH ranged from 7.7 to 9.09. Alkalinity was from 10.0 to 31.4 g CaCO<sub>3</sub>/L except AD with 2.3 g CaCO<sub>3</sub>/L. IC and dissolved TOC ranged from 0.5 to 4.4 g/L and from 0.1 to 1.6 g/L respectively. Dissolved COD/TOC and C/N ratios ranged from 1.6 to 5.9 g O<sub>2</sub>/g C and from 0.5 to 3, respectively. For turbidity, the range was from 1,409 to 67,067 NTU except AD with extremely low with 12 NTU. AD was previously separated using filter press with addition of coagulant and polymer. Conductivity ranged from 12 to 42.9 mS/cm. Taken as a whole, Table 21 shows that the range of all these parameters is slightly broader than the range observed for the liquid fraction of 11 digestates analyzed in Chapter 3.

Table 21: pH, total alkalinity, inorganic carbon (IC), dissolved total organic carbon (TOC), COD/TOC (< 1 kDa), C/N, turbidity (NTU), conductivity, SUVA<sub>254</sub>

Liquid fraction of digestates	pH	Total alkalinity (g CaCO <sub>3</sub> /L)	IC (g/L)	Dissolved TOC (g/L)	COD/TOC (g O <sub>2</sub> /g C) (<1 kDa)	C/N (< 1 kDa)	Turbidity (NTU)	Conductivity (mS/cm)	SUVA <sub>254</sub> (L/mg. m)
N	8.12	24.0	2.7	0.9	2.7	1.3	67,067	33.4	2.7
O	8.39	10.3	1.6	1.0	1.6	1.4	6835	20.0	1.6
P	7.7	10.0	1.3	0.6	3.3	3.0	33,552	15.9	2.9
Q	7.93	23.3	2.1	0.9	2.7	1.8	48,940	24.9	2.8
R	7.82	16.3	1.7	0.9	3.0	1.4	20,712	20.5	2.6
S	8.08	13.8	1.8	0.4	2.9	0.7	1409	21.7	2.4
T	8.49	31.4	3.7	1.6	1.8	1.0	25,160	46.3	1.5
U	8.04	23.4	3.7	1.3	4.4	1.5	42,030	39.0	5.0
V	7.92	27.6	2.5	0.9	3.8	1.6	36,260	28.9	3.7
W	8.2	20.2	3.7	1.1	5.6	1.7	30,015	36.0	5.4
X	8.32	29.1	4.4	1.4	5.9	1.9	60,800	40.8	4.4
Y	8.51	26.0	3.2	0.8	2.8	1.0	27,000	43.0	2.4
Z	7.99	29.0	2.9	1.3	2.8	1.5	42,420	33.3	3.0
I2	8.24	12.7	2.0	1.5	3.0	1.8	10,100	27.5	2.6
AA	8.28	18.1	2.8	1.2	2.7	1.3	20,027	33.9	2.6
AB	7.89	20.4	2.9	0.8	3.1	1.5	23,093	38.9	2.4
AC	8.02	12.0	1.7	1.0	1.7	1.5	10,367	21.1	1.1
AD	9.09	2.3	0.5	0.1	-	0.5	12	17.4	2.2
First collection (chapter 3)	7.6-8.4	7.4 - 24.8	1.3 - 3.6	0.5 - 3.2	0.6 - 3.2	1.1 - 3.8	2,960 - 51,400	15.8 - 41.6	0.004 - 3

SUVA<sub>254</sub> ranged from 1.1 to 3 L/mg/m except U, V, W and X with 5.0, 3.7, 5.4 and 4.4 L/mg/m. The range of SUVA<sub>254</sub> was in the range as previously found in Chapter 3 from 0.004 to 3 L/mg/m as well as in the range from 0.9 to 3.1 L/mg/m for 30 liquid fractions of digestates found by other researcher which were in the range of fulvic acid-like compounds from 0.6 to 3.9 L/mg/m (Zheng et al., 2014). High SUVA<sub>254</sub> for U, V, W and X can be due to the origins of the substrate which was from corn silage. Anaerobic digestion of corn silage in mesophilic condition can produce large amount of residual structural polysaccharides and undigested lignin (Santi et al., 2015). Moreover, U and V which were also originated from grass silage can contain high lignin which was poorly degraded during anaerobic digestion process (Lehtomäki et al., 2008).

3D fluorescence spectroscopy analyses of liquid fraction of 18 digestates are shown in Figure 22.

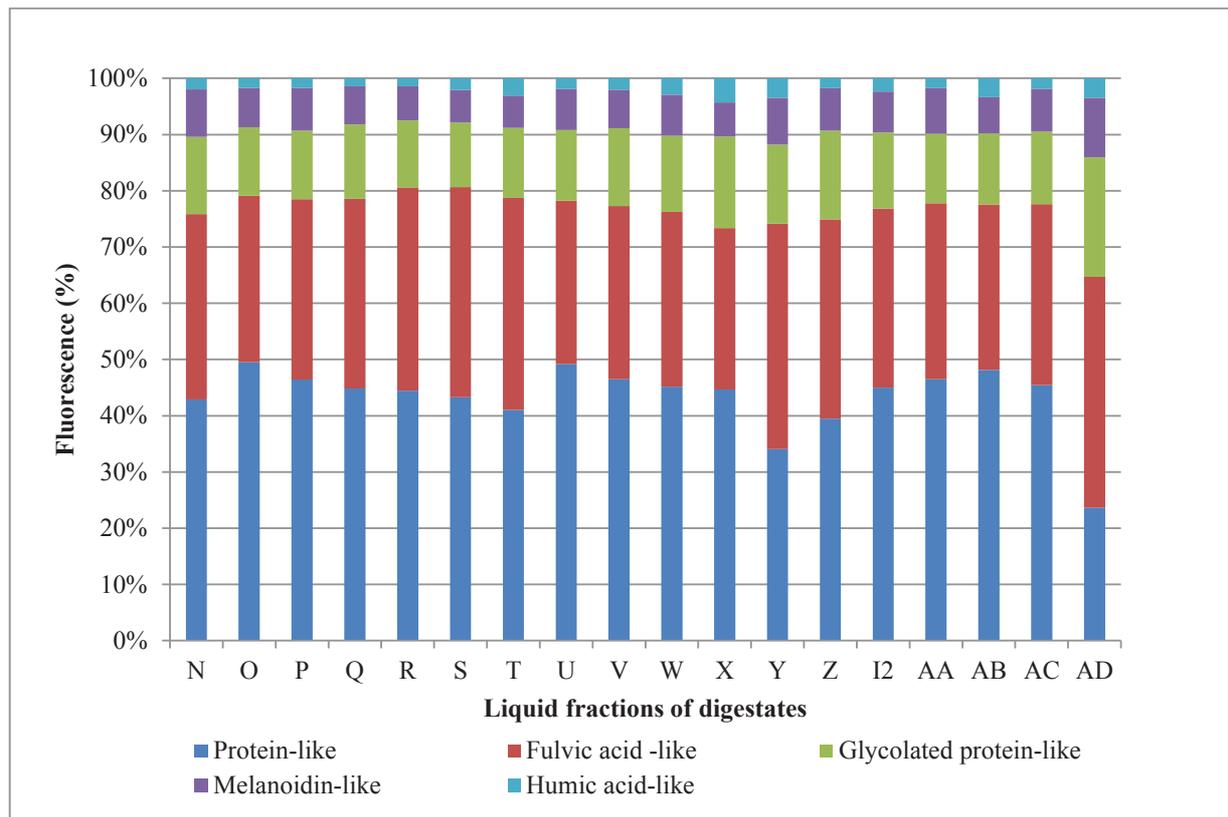


Figure 22: 3D fluorescence spectroscopy of liquid fraction of 18 digestates

Protein-like compounds ranged from 34 to 50% except AD with 24% which was close to previously found from 37 to 54%. Fulvic acid-like compounds ranged from 29 to 41% which

was slightly higher than the range from 27 to 36% in previous characterization. Glycolated protein-like compounds ranged from 11 to 16% except AD with 21%. The range was similar as previously found from 11 to 15%. Melanoidin-like ranged from 6 to 8% except AD with 11% and the range was in the range from 6 to 10% in previous characterization. Humic acid-like compounds ranged from 1 to 3% which was in the range from 1 to 4% as previously found.

#### 4.1.7. Biodegradability

Table 22 shows the biodegradability of liquid fraction of 18 digestates.

Table 22: BOD<sub>5</sub>, BOD<sub>21</sub>, biodegradability in 5 days (BOD<sub>5</sub>/COD) and biodegradability in 21 days (BOD<sub>21</sub>/COD)

Plant	BOD <sub>5</sub> (g/L)	BOD <sub>21</sub> (g/L)	BOD <sub>5</sub> /COD	BOD <sub>21</sub> /COD
N	14 ± 1	43 ± 5	0.16	0.48
O	0.34 ± 0.01	1.26 ± 0.09	0.03	0.10
P	4 ± 1	28 ± 3	0.06	0.44
Q	7.4 ± 0.5	18 ± 2	0.13	0.32
R	10 ± 1	21 ± 2	0.30	0.64
S	1.9 ± 0.3	3 ± 0	0.43	0.66
T	13 ± 2	28 ± 3	0.27	0.59
U	13 ± 1	31 ± 3	0.19	0.45
V	12 ± 1	28 ± 1	0.22	0.52
W	17 ± 3	36 ± 7	0.24	0.51
X	30 ± 3	54 ± 7	0.33	0.60
Y	16 ± 2	26.0 ± 0.9	0.39	0.63
Z	15 ± 1	35 ± 2	0.22	0.52
I2	9.6 ± 0.8	17.8 ± 0.2	0.33	0.62
AA	7 ± 1	18 ± 3	0.18	0.44
AB	11.4 ± 0.5	25 ± 3	0.34	0.75
AC	3.7 ± 0.4	8.0 ± 0.8	0.24	0.52
AD	0.09 ± 0.01	0.38 ± 0.06	0.11	0.45
First collection (chapter 3)	1.7 – 9.4	3.1 – 32.2	0.05 - 0.38	0.14 - 0.61

BOD<sub>5</sub> ranged from 0.09 to 30 g O<sub>2</sub>/L while BOD<sub>21</sub> ranged from 0.38 to 54 g O<sub>2</sub>/L. The ranges of BOD<sub>5</sub> and BOD<sub>21</sub> were far higher compared to previously found from 1.7 to 9.4 g O<sub>2</sub>/L and from 3.1 to 32.2 g O<sub>2</sub>/L for BOD<sub>5</sub> and BOD<sub>21</sub> respectively. This was due to high COD variation of

COD from 0.8 to 90.1 g O<sub>2</sub>/L compared to COD from 9.2 to 78 in previous characterization. The biodegradability of liquid fraction of 18 digestates in 5 days (BOD<sub>5</sub>/COD) was from 0.03 to 0.43 which is close to the range from 0.05 to 0.38 as previously found. The biodegradability in 21 days (BOD<sub>21</sub>/COD) had a high variation from 0.1 to 0.75 which was slightly higher than previous range from 0.1 to 0.6. The biodegradability of liquid fraction of 29 digestates had shown refractory or not easily biodegradable which the BOD<sub>5</sub>/COD ratio was even lower than the BOD<sub>5</sub>/COD ratio of pre-treated leachate from 0.33 to 0.45 (El-Gohary and Kamel, 2016).

#### 4.1.8. Summary of all the liquid fractions

The consideration of 18 more digestates in the second collection led an increase in the diversity of liquid fractions of digestate characteristics with broaden ranges or parameters in comparison to the first collection of 11 liquids fractions of digestates in Chapter 3. Table 23 shows the range of characteristics of the total 29 digestates obtained from co-digestion plants as well as the digestate characteristics obtained from an anaerobic digestion plant treating waste activated sludge (WAS).

Table 23: Range of co-digestion digestates in comparison to WAS digestate

	Units	Co-digestion digestates	WAS digestate	Average
<b>Raw digestates</b>				
TS	g TS/kg	14 - 283	30.46 ± 0.03	77 ± 55
VS	g VS/kg	5 - 115	17.6 ± 0.4	45 ± 25
MS	g MS/kg	9 - 185	12.8 ± 0.1	32 ± 34
VS/TS	%	35 - 78	58	61 ± 10
<b>Solid fractions of digestates</b>				
TS	g TS/kg	99 – 440 <sup>(a)</sup>	276 ± 4	303 ± 174
VS	g VS/kg	88 – 272 <sup>(b)</sup>	157 ± 4	205 ± 89
MS	g MS/kg	12 to 273 <sup>(c)</sup>	119.3 ± 0.4	98 ± 101
VS/TS	%	38 - 93	57	71 ± 14
<b>Liquid fractions of digestates</b>				
TS	g TS/kg	7 – 86	2.11 ± 0.01	43 ± 25
VS	g VS/kg	3 - 52	1.15 ± 0.05	26 ± 16
MS	g MS/kg	4 – 38	1.0 ± 0.1	17 ± 10
VS/TS	%	35 - 75	55	58 ± 9
0 to 50 µm	%	19 - 93	59	59 ± 20
50 to 100 µm	%	2 - 41	16	14 ± 8
100 to 500 µm	%	0 - 45	20	23 ± 13
500 to 1000 µm	%	0 - 19	5	4 ± 5
1000 to 2000 µm	%	0 - 11	0	1 ± 2
Mean size	µm	24 - 388	101	108 ± 82

Median size	µm	7 - 199	40	52 ± 41
Total COD	g/L	4 - 90	1.7 ± 0.0	42 ± 26
COD (suspended particles)	%	60 to 96 (2.6 to 82.7 g O <sub>2</sub> /L)	82 (1.4 g O <sub>2</sub> /L)	80 ± 18 (36 ± 24 g O <sub>2</sub> /L)
COD (colloids)	%	0.9 to 27 (0.2 to 9.9 g O <sub>2</sub> /L)	6 (0.1 g O <sub>2</sub> /L)	8 ± 6 (3 ± 3 g O <sub>2</sub> /L)
COD (dissolved matter)	%	2 to 24 (0.48 to 8.6 g O <sub>2</sub> /L)	12 (0.2 g O <sub>2</sub> /L)	12 ± 17 (3 ± 2 g O <sub>2</sub> /L)
TKN concentration	g N/L	1.3 - 8.3	1.05 ± 0.01	5 ± 2
TKN (suspended particles)	%	11 to 67 <sup>(d)</sup> (0.5 to 4.0 g N/L)	18 (0.2 g/L)	40 ± 15 (2 ± 1)
TKN (colloids)	%	0.2 to 19 (0.005 to 0.9 g N/L)	17 (0.19 g/L)	8 ± 4 (0.3 ± 0.2)
TKN (dissolved matter)	%	21 to 95 (0.6 to 5.2 g N/L)	65 (0.7g/L)	52 ± 16 (2 ± 1)
NH <sub>4</sub> <sup>+</sup>	g/L	0.5 - 4.7	0.63 ± 0.01	1.9 ± 0.9
NH <sub>4</sub> <sup>+</sup> /TKN (<1kDa)	%	62 - 100	92	82 ± 11
NH <sub>4</sub> <sup>+</sup> /Total TKN	%	15 - 75	60	43 ± 13
Norg (<1kDa)	g/L	0.1 - 1.1	0.05	0.4 ± 0.3
Norg/TKN (<1kDa)	%	0 - 38	8	18 ± 10
K <sup>+</sup>	g/L	0.5 - 7.1	0.1083 ± 0.0005	3 ± 2
Cl <sup>-</sup>	g/L	0.5 - 4.4	0.08 ± 0.01	2 ± 1
Na <sup>+</sup>	g/L	0.08 - 3.24	0.05 ± 0.01	0.8 ± 0.8
Mg <sup>+</sup>	g/L	0 - 0.3*	0.0400 ± 0.0005	0.13 ± 0.09*
SO <sub>4</sub> <sup>2-</sup>	g/L	0 - 0.9	0.032 ± 0.001	0.2 ± 0.2
Ca <sup>+</sup>	g/L	0.1 - 0.4*	0.021 ± 0.002	0.2 ± 0.1*
PO <sub>4</sub> <sup>3-</sup>	g/L	0 - 2.1	-	0.5 ± 0.4
pH		7.6 - 9.1	8	8.1 ± 0.3
Total alkalinity	g/L CaCO <sub>3</sub>	7.4 - 31.4 <sup>(e)</sup>	3.70 ± 0.01	18 ± 8
IC	g/L	0.5 - 4.4	0.51 ± 0.02	2 ± 1
Dissolved TOC	g/L	0.1 - 3.2	0.28 ± 0.02	1.1 ± 0.6
COD/TOC (<1kDa)	gO <sub>2</sub> /gC	0.6 - 3.3	0.8	3 ± 2
C/N (<1kDa)	-	0.5 - 3.8	0.4	1.7 ± 0.7
Turbidity	NTU	1409 - 67,067 <sup>(f)</sup>	947	26,459 ± 19,299
Conductivity	mS/cm	12 - 42.9	5	28 ± 9
SUVA <sub>254</sub>	L/mg.m	0.004 - 5.4	0.5	2 ± 1
Protein-like	%	24-54	41	45 ± 6
Fulvic-acid like	%	27-41	36	32 ± 4
Glycolated protein-like	%	11-21	14	13 ± 2
Melanoidin-like	%	6-11	8	7 ± 1
Humic acid-like	%	1-4	2	2 ± 1
BOD <sub>5</sub>	g O <sub>2</sub> /L	0.1 - 30.1	0.4 ± 0.1	8 ± 6
BOD <sub>5</sub> /COD	-	0.03 - 0.43	0.24	0.2 ± 0.1
BOD <sub>21</sub>	g O <sub>2</sub> /L	0.4 - 54.2	1 ± 0.2	19 ± 13
BOD <sub>21</sub> /COD	-	0.1 - 0.8	0.55	0.5 ± 0.2
BOD <sub>5</sub> (<1.2µm)	g O <sub>2</sub> /L	0 - 4*	0.0383 ± 0.0005	1 ± 1*
BOD <sub>5</sub> /COD (<1.2µm)	-	0 - 0.3*	0.12	0.12 ± 0.09*
BOD <sub>21</sub> (<1.2µm)	g O <sub>2</sub> /L	0.1 - 9*	0.12 ± 0.01	3 ± 3*
BOD <sub>21</sub> /COD (<1.2µm)	-	0.1 - 0.7*	0.39	0.4 ± 0.2*

a) except for T and AD with 894 and 880 respectively

(b) except T and AD with 538 and 426 respectively

(c) except T and AD with 356 and 454 respectively

(d) except for AD with 0

(e) except AD with 2.3

(f) except AD with 12

\*Range from first collection in Chapter 3

Whereas the characteristics of WAS raw digestate and solid fraction of digestate are in the range of co-digestion digestates, the liquid fractions of digestates from co-digestion plants presents very high concentrations of residual compounds than the liquid fraction of digestate from WAS. This point is particularly remarkable for TS, VS, MS, COD, TKN and  $\text{NH}_4^+$  concentrations, these latter are also associated to higher alkalinity, conductivity and turbidity in co-digestion digestates. These variations of residual compounds presence in liquid fractions of digestates could be due to the origins of the substrates or depending on the operating parameters or the type of solid-liquid separation performed. Section 4.2 will discuss the statistical analysis using principal component analysis (PCA) performed on the total 29 liquid fractions of digestates from co-digestion plants with 1 liquid fraction of digestate from WAS. The relationships between the characteristics (chemical, physical and biological) of liquid fractions of digestates in relation to the substrates, operating parameters (loading rate, type of reactor, hydraulic retention time, temperature), biogas production and type of solid-liquid separation will be explained in Section 4.2. It is worth noting that all CSTR reactors were operated in mesophilic condition while plug flow reactors (from plants I, J, I2) were operated in thermophilic condition.

## **4.2. Statistical analysis of the composition of liquid fractions of digestates**

### **4.2.1. Preliminary definition and classification of parameters**

In order to perform the statistical analysis of the digestates, a preliminary definition of groups of parameters was designed, this classification allows not having only one digestate in one group. The substrates at the input (Table 9) were grouped as sewage sludge (SS), manure (Mnr), energy crops (EnCr), crop residues (CrR), cereal residues (Cer), fats, oil and grease (FOG) and agro-food wastes (AFW) as shown in Table 24.

Table 24: Category of groups based on the type of substrates at the input

Category of groups	Substrates at the input
Sewage sludge (SS)	Sludge, waste activated sludge, solid sludge, liquid sludge, wastewater, cheese plant sludge, contents from septic tanks (and garbage)
Manure (Mnr)	All types of animal manure and slurry
Energy crops (EnCr)	Energy crops, corn silage, catch crop, grass, grass silage, sorghum silage, energy crop silage, rye, barley, whole grain plants
Crop residues (CrR)	Crop residues, sweetcorn cobs, corn withers, apple pomace, tomato leaves, sweetcorn cobs
Cereal residues (Cer)	Cereal residues, crushed grain
Fats, oil and grease (FOG)	Fats, oil, grease
Agro-food wastes (AFW)	Food wastes, fruits and vegetables, bio-wastes, municipal bio-wastes, blood, pet food, slaughterhouse wastes, mixture of cream milk, glycerin, milk industry residues, cattle feed residues, whey, glucose

The separation efficiency and separation index of all the types of solid-liquid separation for all digestates were calculated. The separation efficiency gives the proportion in solid concentrations whereas the separation index takes into account the individual flows. The separation efficiency or removal efficiency (R) was calculated using Equation (14) (Hjorth et al., 2010).

$$R = 1 - \frac{[TS]_{liq}}{[TS]_{raw}} \quad (14)$$

The calculation of Separation index (SI) was based in Equation (15) as described by Moller et al (Møller et al., 2000). SI calculation took into account the mass balance of raw digestate, solid and liquid fractions of digestates as described in

ANNEX 1.

$$SI = \frac{M_{solid} \times TS_{solid}}{M_{raw} \times TS_{raw}} \quad (15)$$

The impact of solid-liquid separation on digestate composition according to its separation efficiency and separation index will be further explained in detailed in Section 4.2.4.

#### **4.2.2. Multivariate analysis (PCA)**

An initial global PCA was performed on all the variables from all the digestates; using the calculations conditions and hypothesis described in chapter 2. The scree plot represented in Figure 23 displays the percentage of explained variance for each component of the PCA. The first component only explains 32% of variance whereas the cumulated percentage of the three main components include less than 60% of the variability (dimension 2: 16.4%, dimension 3: 10.4%). This statement indicates the multivariate analysis of data cannot build strong components that should decorrelate the variables. One assumption could be the lack of variability in some parameters, particularly the kind of anaerobic processes (mainly mesophilic CSTR reactors vs. thermophilic plug flow reactors, methane potentials in stabilized conditions) and the low ranges of some characterizations (such as pH, alkalinity, etc.).

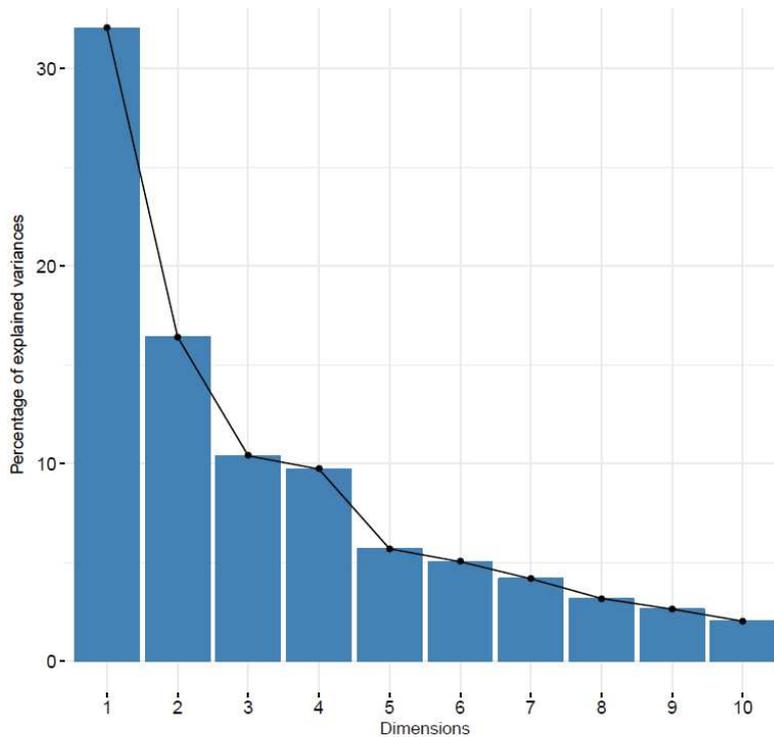


Figure 23: Scree plot for the global PCA

As a result, the representation of the digestate-individuals obtained from the PCA (Figure 24) according to the three-first components does not highlight clear groups of digestates. However digestates with sludge in the feed (WAS, H, S and E) seem to form a group different from the rest of the digestates. Also one digestate, AD, appears different from the rest of digestates and has a high impact in the construction of component 1. Digestates originated from the same plant, with same feedstocks collected at two different periods were close (I and I2).

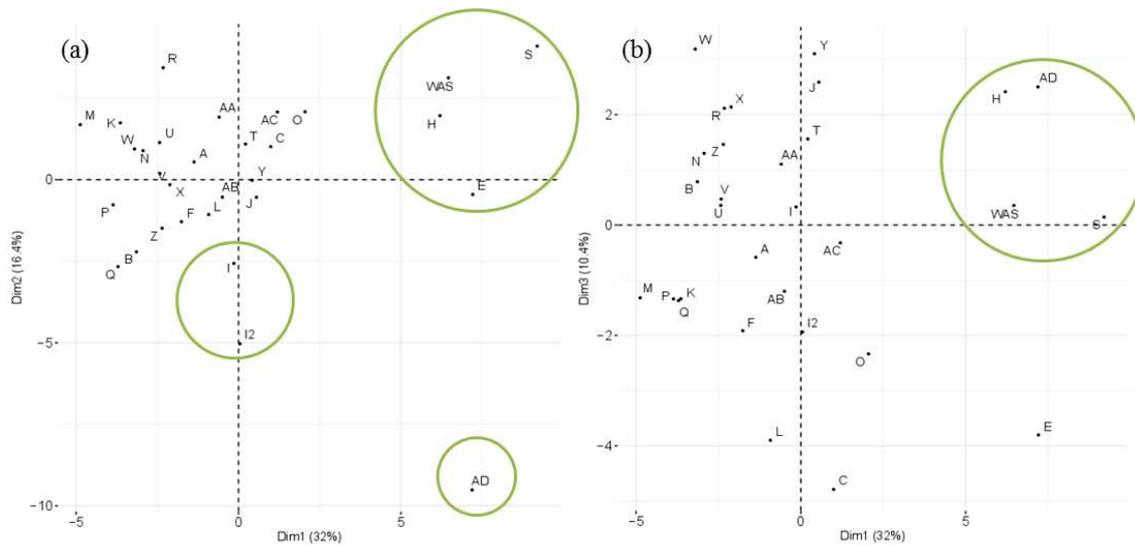


Figure 24: Biplot of digestates (a) components 1-2, b) components 1-3)

While it is hard to identify the group of liquid fractions of digestates, hierarchical clustering of all liquid fractions of digestates (Figure 25) displays a better classification of the digestates according to the PCA analysis. Figure 25 demonstrates that liquid fractions of digestates are separated into two main groups. The first group observed was mainly from sewage sludge with co-digestion with other substrates such as FOG, AFW and manure (pig). The second group observed was mainly based from agricultural and industrial feedstock. However, due to variation of the type of solid-liquid separation and feedstock compositions, it remains still unclear due to the impact of solid-liquid separation, feedstock composition, and operating parameters. Therefore, the evaluation on the impact of solid-liquid separation, feedstock composition and operating parameters will be discussed in Sections 4.2.4, 4.2.5 and 4.2.6, respectively.

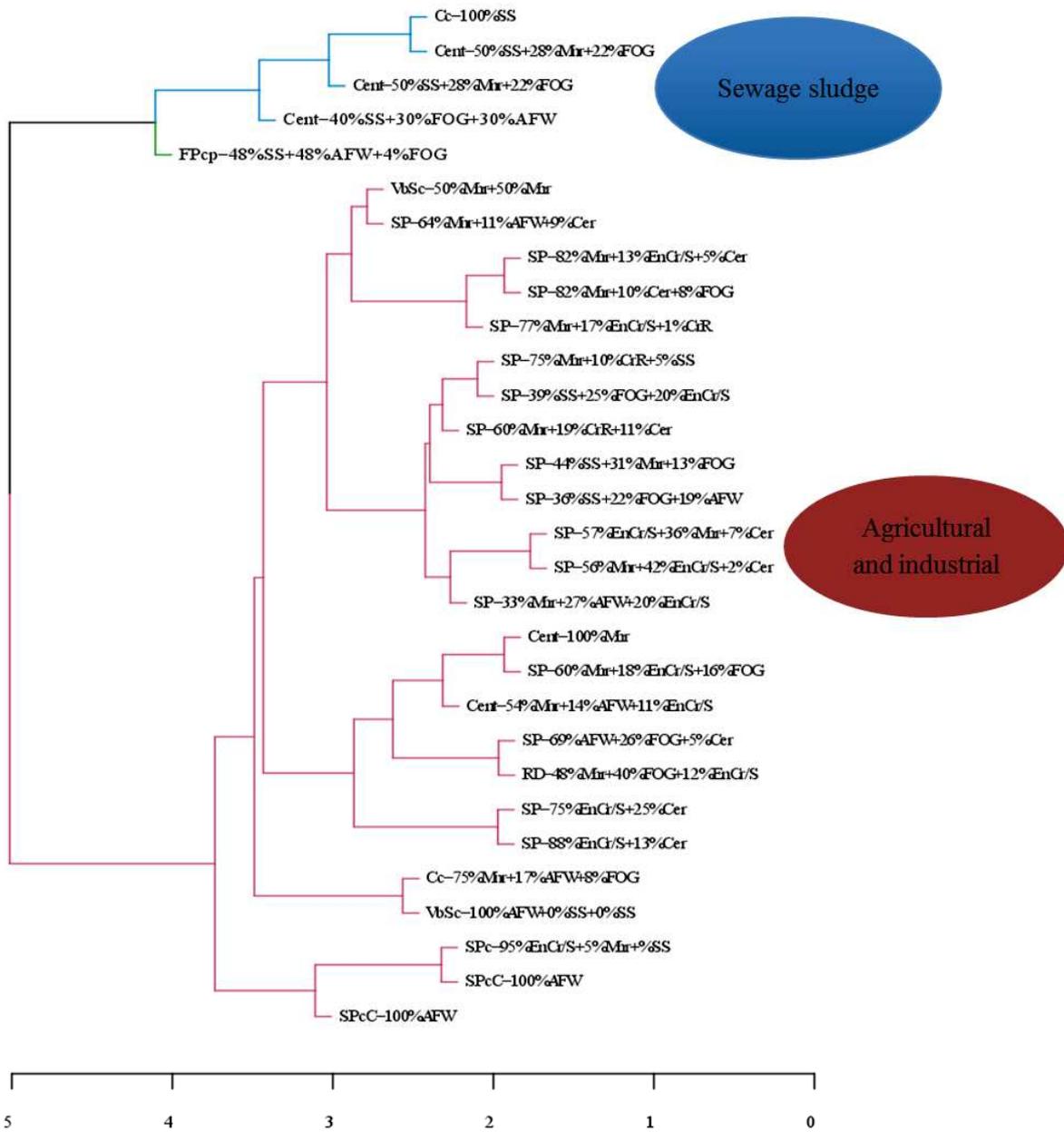


Figure 25: Hierarchical clustering of the digestates

The result of PCA, representing the variables, in the three-first components is presented in Figure 26.



Table 25: Summary of correlations

Parameters	Highest correlation		Intermediary correlation	
	$r > 0.7$ or $r < -0.7$	r	$0.7 > r > 0.55$ or $-0.7 < r < -0.55$	r
<b>HRT</b>	SUVA <sub>254</sub>	0.72	CST C/N	0.64 0.55
<b>Methane yield</b>			TKN colloids	0.67
<b>Cer</b>			CST	0.67
<b>EnCr</b>			SUVA <sub>254</sub>	0.67
<b>Sewage Sludge</b>	TKN colloids	0.74		
<b>AFW</b>			MS/TS raw VS/TS raw	0.70 -0.70
<b>VS/TS solids</b>	MS/TS solids Separ. Efficiency	-0.99 -0.72	Turbidity Na <sup>+</sup>	0.64 -0.60
<b>MS/TS solids</b>	Separ. Efficiency	0.74	Turbidity Na <sup>+</sup>	-0.63 0.60
<b>Separ. Efficiency</b>	COD dissolved Turbidity TKN dissolved Conductivity	0.77 -0.70 0.70 0.72	CST Alkalinity	-0.60 -0.64
<b>VS/TS raw</b>			VS/TS liquid MS/TS liquid COD total	0.63 -0.63 0.57
<b>MS/TS raw</b>	VS/TS raw	-1	VS/TS liquid MS/TS liquid COD total	-0.63 0.63 -0.57
<b>VS/TS liquid</b>	MS/TS liquid COD total COD suspended	-1 0.79 0.74	CST Turbidity C/N Na <sup>+</sup> Cl <sup>-</sup> SUVA <sub>254</sub>	0.57 0.61 0.63 0.57 0.56 0.56
<b>MS/TS liquid</b>	COD total COD suspended	-0.79 -0.74	CST Turbidity C/N Na <sup>+</sup> Cl <sup>-</sup> SUVA <sub>254</sub>	-0.57 -0.61 -0.57 0.63 0.56 -0.56
<b>CST</b>			COD suspended Turbidity Conductivity SUVA <sub>254</sub>	0.56 0.60 -0.56 0.68
<b>pH</b>			Na <sup>+</sup> Cl <sup>-</sup>	0.57 0.63
<b>Alkalinity</b>	IC TOC C/N TKN total TKN suspended TKN colloids TKN dissolved N organic dissolved NH <sub>4</sub> <sup>+</sup>	0.97 0.71 -0.71 0.97 0.81 0.71 0.94 0.8 0.93		

	Conductivity	0.91				
<b>IC</b>	TOC	0.79	COD dissolved	0.55		
	C/N	-0.75				
	TKN total	0.97				
	TKN suspended	0.74				
	TKN colloids	0.73				
	TKN dissolved	0.95				
	N organic dissolved	0.79				
	NH <sub>4</sub> <sup>+</sup>	0.94				
Conductivity	0.94					
<b>TOC dissolved</b>			C/N	-0.61		
			TKN total	0.67		
			TKN suspended	0.62		
			TKN colloids	0.66		
			TKN dissolved	0.62		
			NH <sub>4</sub> <sup>+</sup>	0.61		
			PO <sub>4</sub> <sup>3-</sup>	0.61		
			Conductivity	0.66		
		SUVA <sub>254</sub>	-0.65			
<b>COD total</b>	COD suspended	0.93	Turbidity	0.57		
			Na <sup>+</sup>	-0.64		
			Cl <sup>-</sup>	-0.67		
			BOD <sub>21</sub>	0.60		
<b>COD suspended</b>	Turbidity	0.76	Na <sup>+</sup>	-0.66		
			Cl <sup>-</sup>	-0.73		
<b>COD colloids</b>			Conductivity	-0.57		
			COD dissolved	0.66		
<b>COD dissolved</b>			Turbidity	-0.57		
			Conductivity	0.57		
<b>Turbidity</b>			C/N	0.57		
			TKN dissolved	-0.55		
			Na <sup>+</sup>	-0.62		
			Cl <sup>-</sup>	-0.69		
			Conductivity	-0.64		
<b>C/N</b>	TKN total	-0.74	TKN suspended	-0.58		
	TKN dissolved	-0.73				
	NH <sub>4</sub> <sup>+</sup>	-0.71				
	Conductivity	-0.78				
<b>TKN suspended</b>			TKN dissolved	0.62		
			N organic dissolved	0.62		
			NH <sub>4</sub> <sup>+</sup>	0.59		
			Conductivity	0.62		
<b>TKN total</b>	TKN suspended	0.73				
	TKN colloids	0.76				
	TKN dissolved	0.98				
	N organic nitrogen	0.81				
	NH <sub>4</sub> <sup>+</sup>	0.97				
	Conductivity	0.97				
<b>TKN colloids</b>	NH <sub>4</sub> <sup>+</sup>	0.74	TKN dissolved	0.69		
					Conductivity	0.71
<b>TKN dissolved</b>	N organic dissolved	0.83				
					NH <sub>4</sub> <sup>+</sup>	0.99
					Conductivity	0.97
<b>TC/TN dissolved</b>			Protein-like	0.56		
			Fulvic acid-like	-0.58		

<b>N organic dissolved</b>	NH <sub>4</sub> <sup>+</sup> Conductivity	0.74 0.84	Na <sup>+</sup>	0.65
<b>NH<sub>4</sub><sup>+</sup></b>	Conductivity	0.96		
<b>Na<sup>+</sup></b>			Cl <sup>-</sup>	0.67
<b>Cl<sup>-</sup></b>			Conductivity Protein-like Glycolated-like	0.58 -0.61 0.59
<b>Mean Size</b>	Median size	0.89		
<b>BOD<sub>5</sub></b>	BOD <sub>21</sub>	0.85		
<b>Protein-like</b>	Fulvic acid-like Glycolated-like Melanoidin-like	-0.88 -0.84 -0.72		
<b>Glycolated-like</b>	Melanoidin-like	0.71		

From the higher degrees of correlation ( $|r| > 0.7$ ), obvious correlations were observed between parameters determined by calculation, such as: VS/TS with MS/TS in all the fractions, VS/TS and MS/TS in solid fraction with separation efficiency, COD dissolved, turbidity with separation efficiency, VS/TS and MS/TS with COD total and COD suspended, alkalinity with IC and conductivity, IC with conductivity, COD total with COD suspended, correlations between all nitrogen measurements (C/N, TKN, N organic dissolved, NH<sub>4</sub><sup>+</sup>), mean and median sizes with the fractions of fluorimetry.

On the contrary, the correlation matrix highlights some specific high correlations between parameters. Particularly, the HRT is positively correlated with SUVA<sub>254</sub>, suggesting a higher humification ratio with higher HRT. This statement will be further discussed in section 4.2.6.1.

There was only one correlation between characterization and feedstock composition, i.e. TKN in colloids is positively linked to sewage sludge in the feeding and indirectly a correlation was observed between TKN dissolved and separation efficiency (most sewage sludge based digestates were separated by centrifugation). Oliveira et al (Oliveira et al., 2016) reported the high correlation between nitrogen content in the digestate and both sludge composition or conditioning parameters. The higher the proportion of sewage sludge in the feeding, the higher TKN in colloids will be found in the liquid fraction of the digestates. In order to confirm the origin of the colloidal fraction of organic matter in the liquid digestates, anaerobic digestion experiments with micro-organisms (WAS or animal feces) in the feed could be tested. On the other hand, none of the other feedstock categories showed high correlation with digestate

characterizations. This observation may be explained by the high uncertainty of the quantities reported from the full-scale plants, the lack of specific information such as VS quantities in the feeding (instead of total mass) but also by the choice of categories that might be not specific enough.

As a result of the use of coagulants and polymers in some separation techniques, a high correlation was observed between conductivity, alkalinity and separation efficiency. Some reported coagulants were metallic salts, such as ferric chloride or aluminum sulfate which react with bicarbonate to form metallic hydroxide ( $\text{Al}(\text{OH})_3$ ,  $\text{Fe}(\text{OH})_3$ ).

$\text{BOD}_5$  is well correlated ( $r=0.85$ ) with  $\text{BOD}_{21}$ . Indeed,  $\text{BOD}_{21}$  includes the value of  $\text{BOD}_5$  and then can explain the correlation between both parameters. In our experiments, the mean ratio  $\text{BOD}_5/\text{BOD}_{21}$  for the digestates was 0.43. This value is significantly different from the usual ratio observed for raw sewage (Sibil et al., 2014) in the range of 0.6-0.9 and then  $\text{BOD}_5/\text{BOD}_{21}$  could be a specific parameter for digestate characterization.

Regarding the intermediate correlation coefficients ( $0.55 < |r| < 0.7$ ), several additional links between parameters are stated. Amongst them, some parameters are more represented for their links with various parameters. Conductivity, turbidity, CST and  $\text{SUVA}_{254}$  are the most frequent parameters that could explain also a major part of the digestates composition. From a practical point of view, these measurements (except  $\text{SUVA}_{254}$ ) are easy to perform and the results are obtained in a very short time.

In relation to the composition of the feedstock, cereal residues are positively correlated to CST, and thus to the small size solids in the liquid fraction of digestates whereas energy crop residues are positively correlated to  $\text{SUVA}_{254}$  as an indicator of the aromatic content of the digestates. The lignin content of these feedstocks is the parameter mainly discussed in the literature for supporting the lower methane potential of these compounds.  $\text{SUVA}_{254}$  and CST are complementary characterizations that support the organic matter remaining in the digestates after biodegradation. Herrmann et al (Herrmann et al., 2016a) studied various crop silages, Dandikas et al (Dandikas et al., 2014) tested many varieties of grassland, both concluding to the increase of

SUVA<sub>254</sub> after anaerobic biodegradation with limited bio-methane potential (BMP) in relation to the lignin content of the feedstock.

On the contrary, the agro-food residues are negatively correlated to VS/TS in the raw digestates that could be explained by a higher biodegradability of these feedstocks leading a lower content of organic matter after anaerobic degradation.

It is worth to mention that the fat oil and grease content of the feedstock is not involved as a significant parameter to describe the composition of digestates after separation. Even if this category was present in many full-scale plants, its low proportion (less than 40%) in every plant (see Table 9), its VS content of this fraction is not known and this fraction also contains a high fraction of water, which may explain this lack of correlation. Generally, FOGs are added in low proportion in AD plants because this substrate has high methane potential, it is completely biodegradable without organic matter release after biodegradation. As presented in Figure 27, the positive impact of FOG proportion on the methane yield is shown.

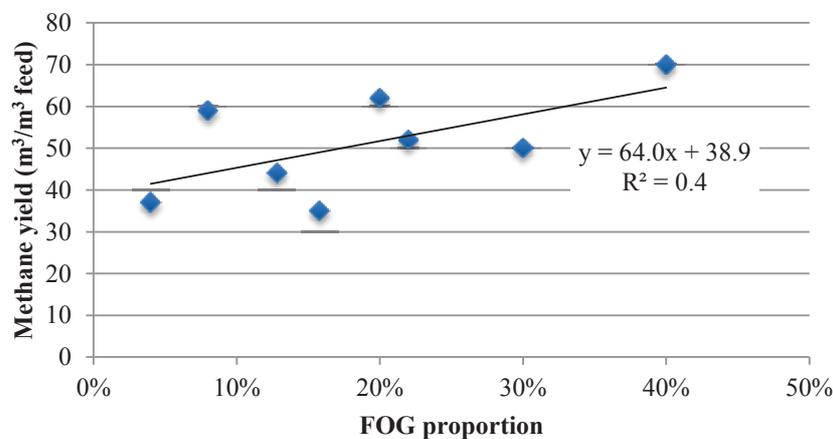


Figure 27: Methane yield (m<sup>3</sup>/m<sup>3</sup>feed) according to FOG proportion in the feedstock

#### 4.2.4. Solid-liquid separation technique impact on digestate composition

Figure 28 shows the COD concentrations of liquid fraction of 30 digestates according to the type of solid-liquid separation. It was preliminary observed that higher COD concentrations were due to separation technique from screw press, vibrating screen and rotary drum. On the contrary, lower COD concentrations were observed from separation technique from centrifuge and separation technique with addition of coagulant, flocculant or polymer.

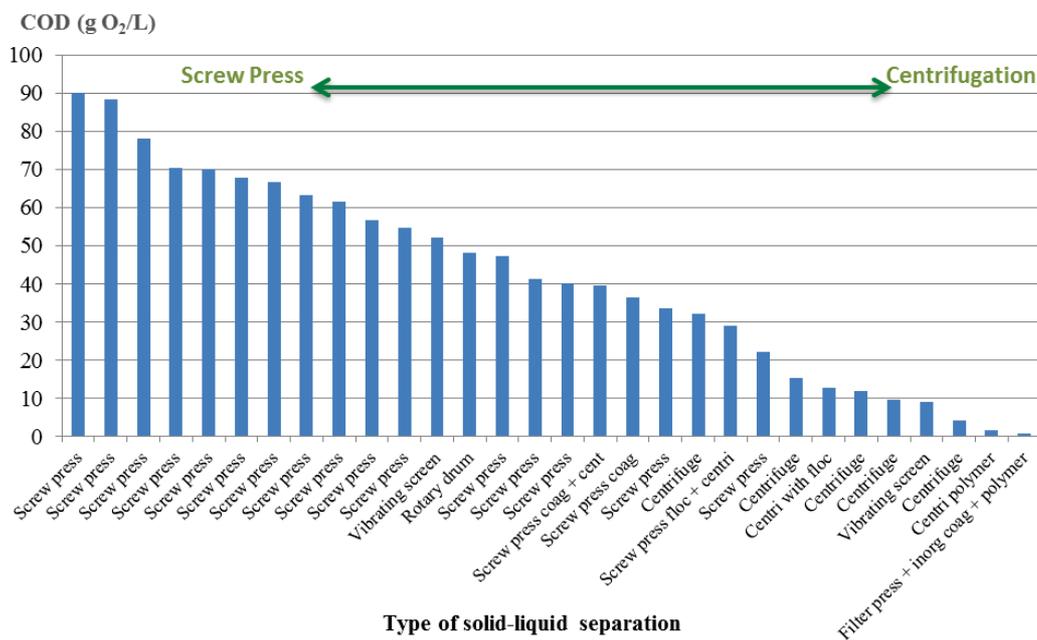


Figure 28: COD (g O<sub>2</sub>/L) of liquid fraction of 30 digestates according to type of solid-liquid separation

In order to understand better the impact of solid-liquid separation on liquid fraction of digestate, the separation efficiency and separation index were plotted as shown in Figure 29.

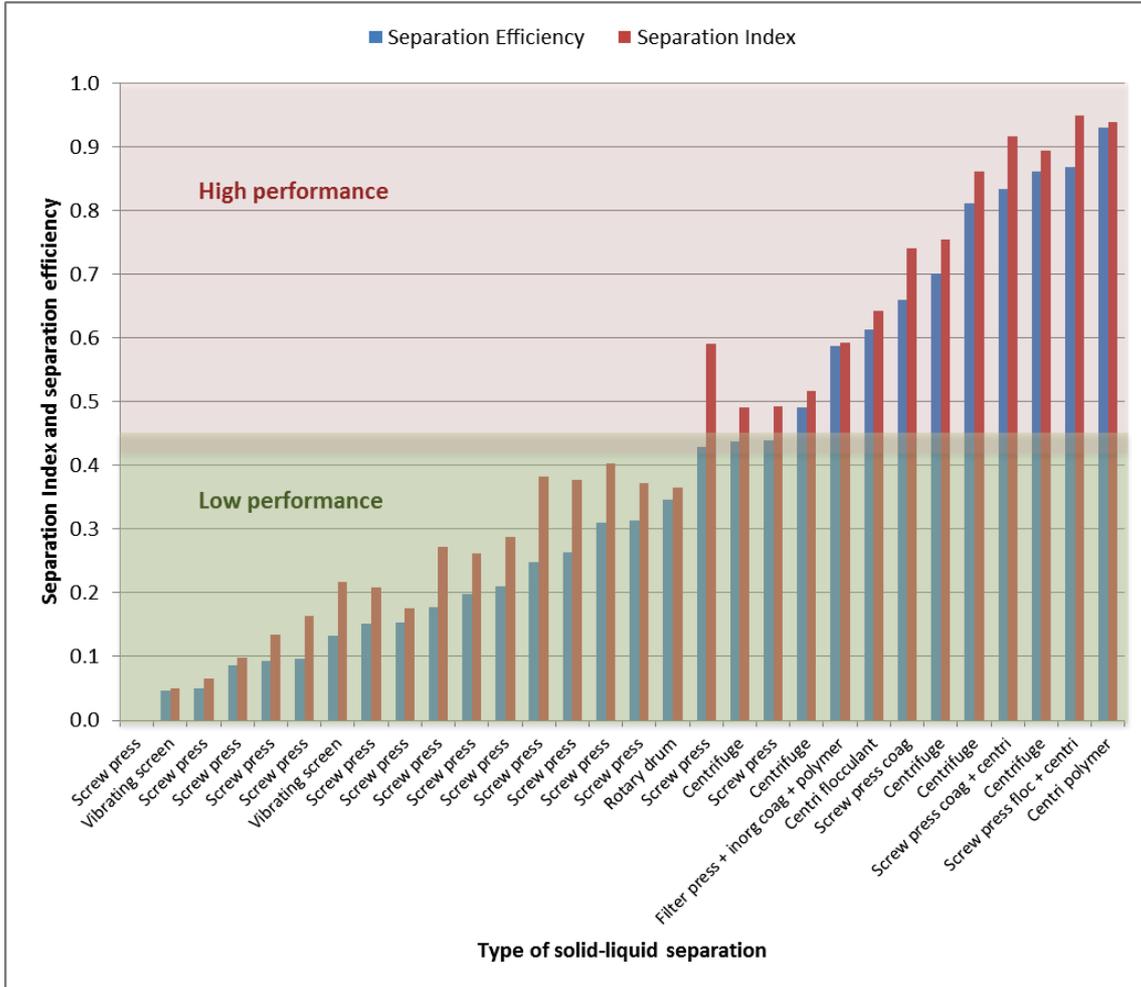


Figure 29: Separation efficiency and separation index according to the type of solid-liquid separation

It was observed that screw press, vibrating screen and rotary drum have low separation efficiency and separation index with  $\leq 44\%$  and  $\leq 59\%$ , respectively. On the contrary, centrifugation and other types of solid-liquid separation with coagulant, flocculant or polymer had shown high separation efficiency and separation index from 44 to 93% and from 49 to 94%, respectively. Based on this, the classification of solid-liquid separation efficiency can be divided into two groups; 1) Low separation efficiency (low performance) and 2) High separation efficiency (high performance) as shown in Table 26. Similar separation efficiency was also observed by Moller, 2002 where the separation efficiency of screw press was low with  $< 30\%$  compared to centrifuge which was higher from 33 to 69% (Møller, 2002).

Table 26: Classification of solid-liquid separation efficiency according to type of solid-liquid separation

Classification of solid-liquid separation efficiency	Low separation efficiency (low performance)	High separation efficiency (High performance)
Type of solid-liquid separation	<ol style="list-style-type: none"> <li>1. Screw press (SP)</li> <li>2. Vibrating screen (VbSc)</li> <li>3. Rotary drum (RD)</li> </ol>	<ol style="list-style-type: none"> <li>1. Centrifugation (Cent)</li> <li>2. Centrifugation with polymer (Cc)</li> <li>3. Centrifugation with flocculant (Cc)</li> <li>4. Screw press with flocculant + centrifugation (SPcC)</li> <li>5. Screw press with coagulant + centrifugation (SPcC)</li> <li>6. Filter press with inorganic coagulant and polymer (FPcp)</li> </ol>
Separation Efficiency range (%)	0-44	44-93
Separation Index range (%)	0-59	49-94

Based on two groups as described in Table 26, PCA was re-performed with label of two groups; High (high performance) and low (low performance) as shown in Figure 30. It was clearly observed that liquid fractions of digestates were separated into two groups based on separation efficiency. Even though liquid fraction of digestate R was separated by centrifugation (high performance solid-liquid separation), it was in the group with low efficiency due to its low separation efficiency and separation index of 44% and 49% respectively, which was in the range as other low performance solid-liquid separators. This could be due to the inefficiency of centrifuge used during solid-liquid separation.

For liquid fractions of digestates I, I2 and J with high separation efficiency and separation index of 83 and 92%, 87 and 95% and 66% and 74% respectively, they were however close to the low performance of solid-liquid separators. This can be due to liquid fractions of digestates I, I2 and J were from thermophilic plug flow reactors treating high-solids wastes where high amounts of residual organic matter were not digested during the process. Therefore, the remaining residual organic matters were still high even after very efficient solid-liquid separators.

On the contrary of I, I2 and J, liquid fractions of digestates T (originating from pig slurry, fats and corn silage), C (originating from fruits and vegetables), Y (originating from bio-waste, fats

and cereals) were separated by low efficiency solid-liquid separators with separation efficiency and separation index of 35 and 36%, 5 and 5%, 15 and 17%, respectively. However, T, C and Y were close to the group of high performance of solid-liquid separation. This can be due to the origins of the substrates which were easily biodegradable and produced lower residual organic matters concentrations in the liquid fraction after solid-liquid separation.

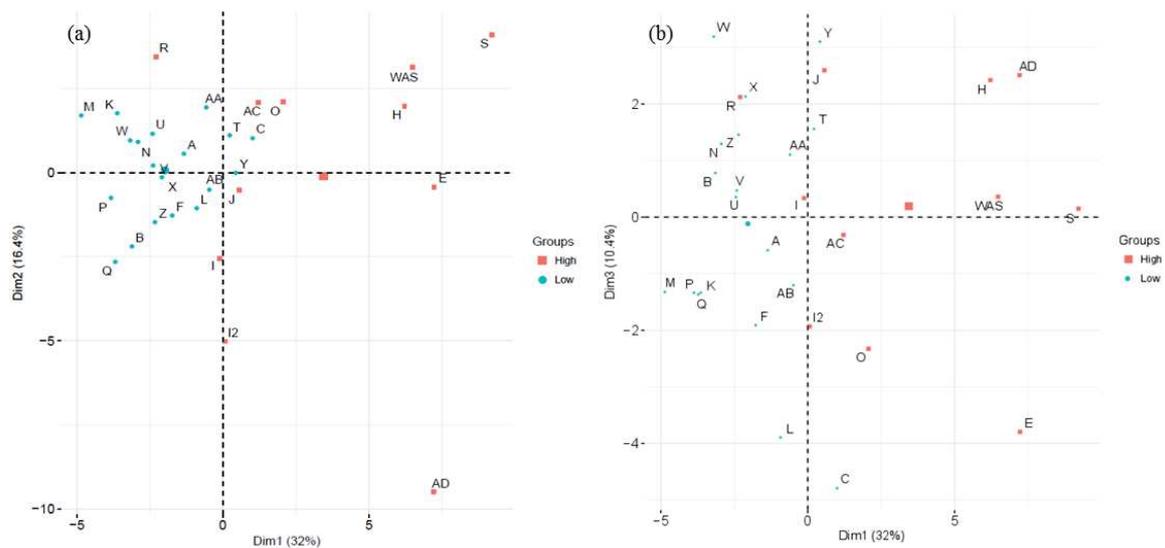


Figure 30: Individuals biplot based on separation efficiency (a) components 1-2, b) components 1-3)

R was separated by centrifugation (high performance solid-liquid separation) but was in the group with low efficiency due to its low separation efficiency of 44% with a separation index of 49% which is in the same group as low efficiency solid-liquid separators. This could be due to the inefficiency of the centrifuge used during solid-liquid separation. For samples I, I2 and J which were close to low efficiency solid-liquid separators and were from thermophilic plug flow reactors treating high-solids wastes. This can be due to the remaining high solids in plug flow reactors which were not digested during the process and later had an impact on the efficiency of solid-liquid separation. Sample T (originating from pig slurry, fats and corn silage), C (originating from fruits and vegetables), Y (originating from bio-waste, fats and cereals) were separated by low efficiency solid-liquid separators but were close to the group of high performance solid-

liquid separation. This could be due to the origins of the substrates which were easily biodegradable and produced low solids concentrations in the digestates.

#### **4.2.5. Feedstock compositions effect on digestate composition**

##### **4.2.5.1. High performance separation**

In order to evaluate the impact of feedstock compositions and to have constant variable of the type of solid-liquid separation, the hierarchical clustering was performed separately according to high and low performance of solid-liquid separation. The hierarchical clustering from the group of high efficiency of solid-liquid separation is shown in Figure 31. It is clearly observed that liquid fractions of digestates were separated into two groups where one group was mainly from sewage sludge co-digestion with other substrates such as FOG, AFW and Mnr (pig). The second group was mainly from agricultural and industrial feedstocks. This second group was clearly separated by other sub-group where the first sub-group was from liquid anaerobic digestion from CSTR while the second group was from solid-state anaerobic digestion from plug flow reactor. It was observed that in the first sub-group (from liquid anaerobic digestion), the contribution of high percentage of manure made the group separated from other main group originating from liquid anaerobic digestion which was mainly from sewage sludge.

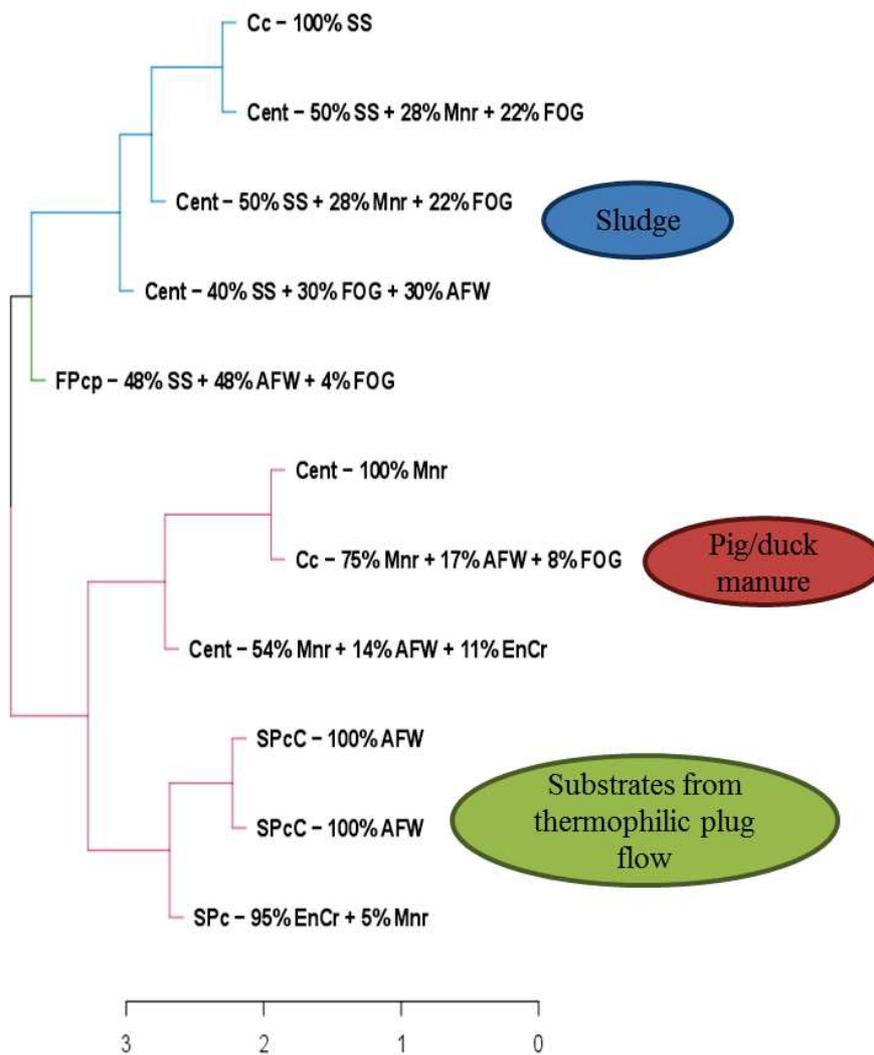


Figure 31: Clustering (high performance of solid-liquid separation)

#### 4.2.5.2. Low performance separation

The hierarchical clustering from the group of low efficiency of solid-liquid separation is shown in Figure 32.

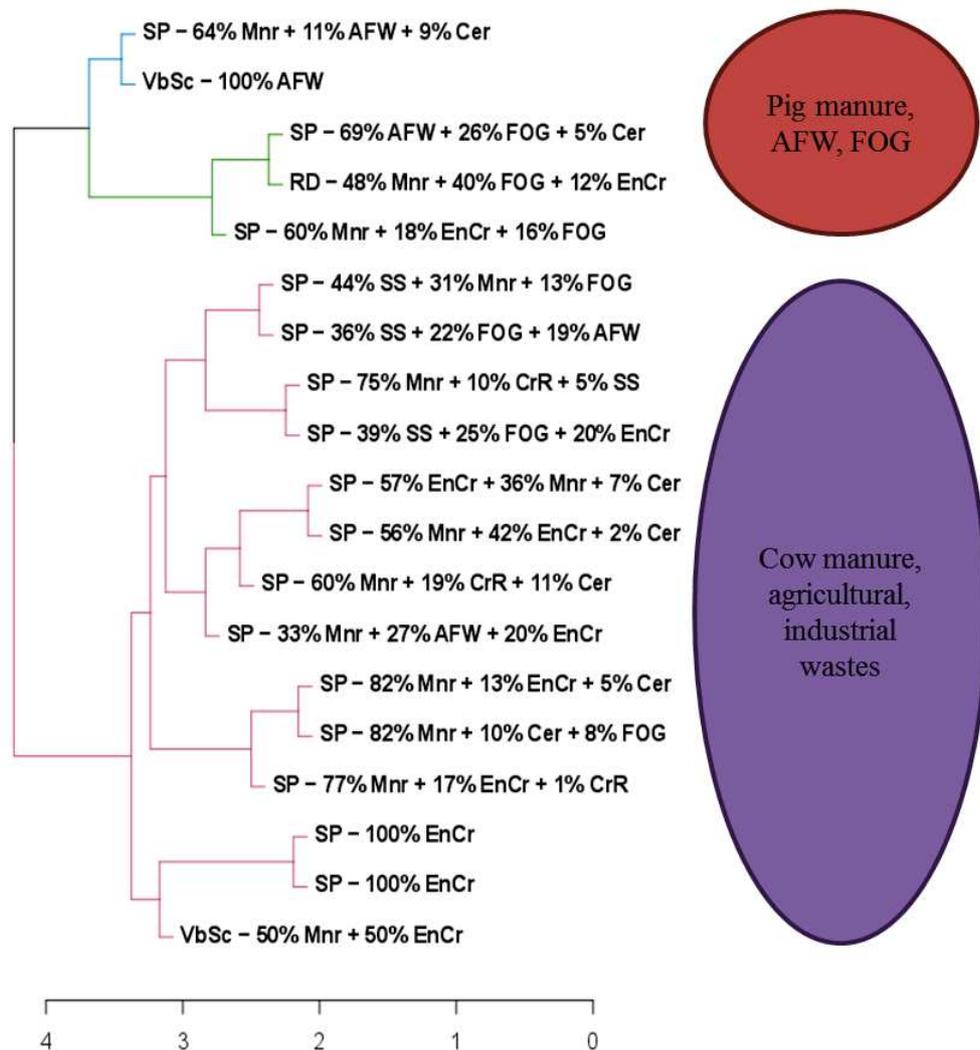


Figure 32: Clustering (low performance of solid-liquid separation)

From Figure 32, the clustering of low performance was divided into two groups. The first group was mainly originating either from Mnr, FOG or AFW. All manures in this group were from pig. The second group was from co-digestion of various agricultural and industrial wastes. In order to understand better the impact of feedstock composition to TS and COD concentrations in liquid fractions of digestates, Figure 33 was plotted for the group of low efficiency solid-liquid separation. It was observed that higher TS (Figure 33a), COD (Figure 33b) and COD dissolved (Figure 33c) concentrations in liquid fractions of digestates were observed when the percentage

of EnCr or Mnr (mostly cow manure) or both were higher. On the contrary, lower TS, COD and COD dissolved were observed from AFW, SS and Mnr (pig).

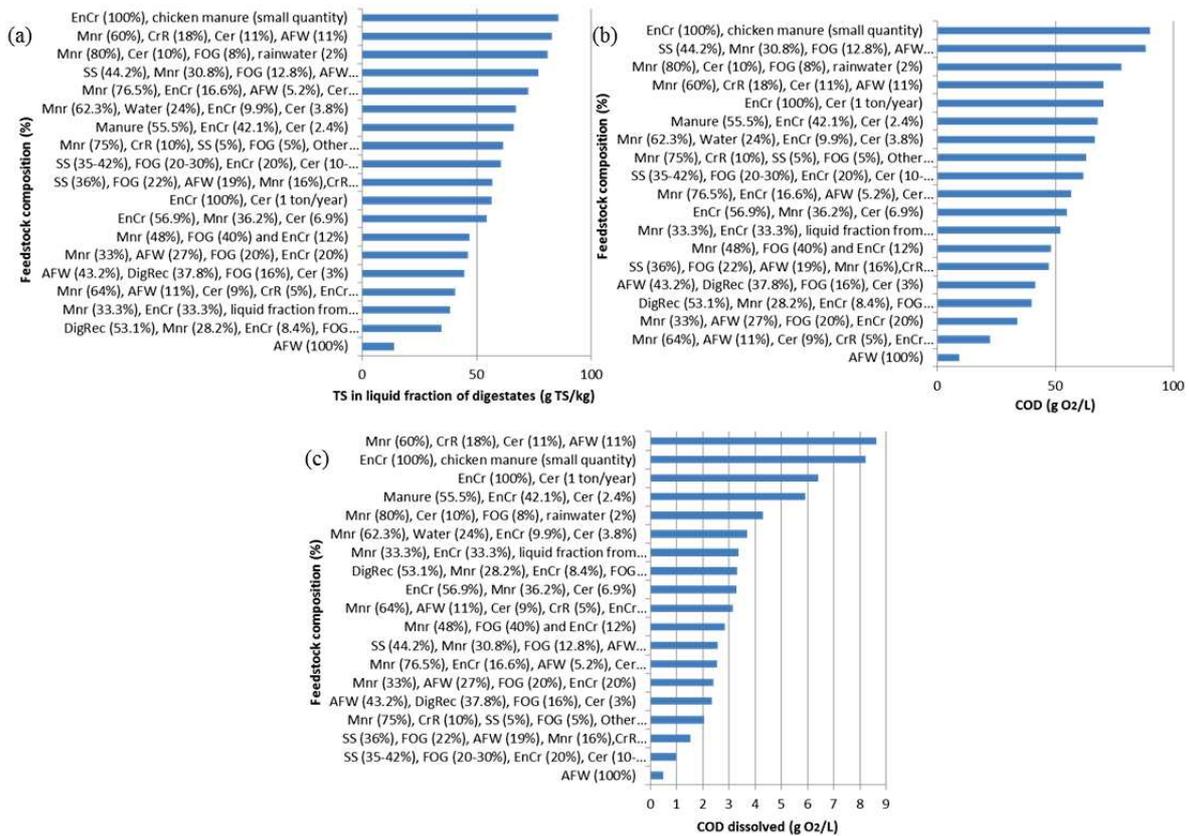


Figure 33: Feedstock composition (%) vs (a) TS (g TS/kg), (b) COD (g O<sub>2</sub>/L) and (c) COD dissolved concentrations in liquid fraction of digestates

The correlations between TS concentration and COD (in the group of low efficiency solid-liquid separation) with the percentage of energy crops (EnCr) percentage in the feedstock can be observed in Figure 34 (a) and (b) respectively. With higher percentage of energy crops in the feedstock, it was shown higher TS concentration and COD in liquid fractions of digestates with  $R^2 = 0.12$  and  $0.24$ , respectively. In addition, it was also observed that higher percentage of energy crops in the feedstock contributed to higher CST as shown in Figure 34 (c) with  $R^2 = 0.76$ .

For manure (Mnr), the correlations between TS and COD concentrations with the percentage of manure in the feedstock (%) are shown in Figure 34 (d) and (e) respectively. The percentage of

manure (Mnr) in the feedstock was observed to have correlation between TS with  $R^2 = 0.24$ . The COD correlation with manure percentage was very low which could be due to different characteristics of different types of manure.

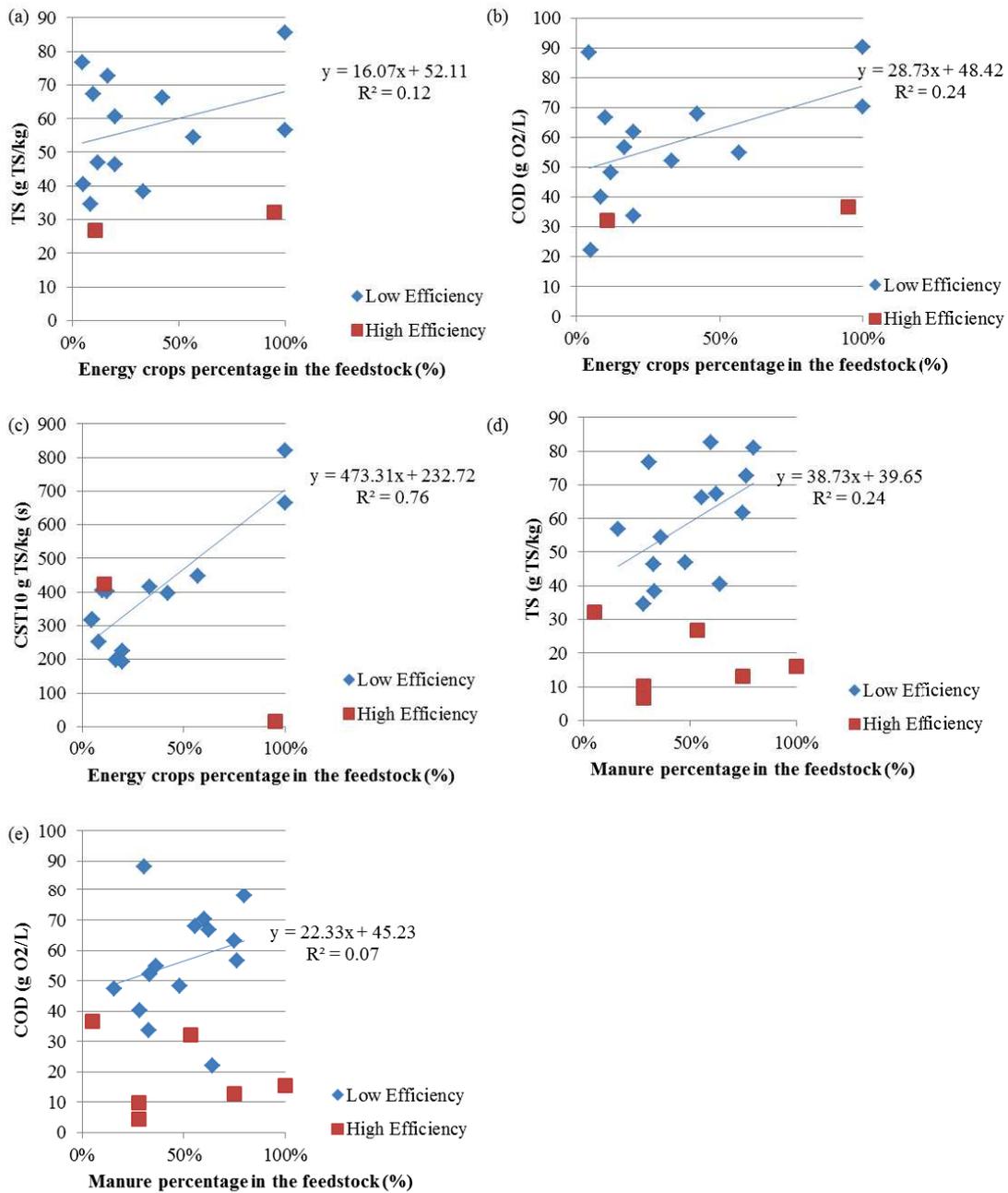


Figure 34: (a) TS concentration, (b) COD concentration and (c) CST vs energy crops percentage in the feedstock, (d) TS and (e) COD concentrations vs percentage of manure in the feedstock

However, as previously discussed according to Figure 33, higher TS, COD and COD dissolved were observed originating from cow manure which can contribute to higher TS and COD in liquid fractions of digestates. In order to refine the analysis, TS and COD correlations with cow manure percentage in the feedstock were plotted as shown in Figure 35. A strong correlation of  $R^2 = 0.64$  was observed between TS concentration and cow manure percentage in the feedstock (Figure 35a). In addition, a strong correlation,  $R^2 = 0.53$  (Figure 35b), was also observed between COD concentration and cow manure percentage. This suggested that cow manure had high contribution to high TS and COD in liquid fractions of digestates. In addition, this confirmed as previously found in Chapter 3 (see Figure 15c) that higher percentage of cow manure contributed to higher COD in liquid fraction of digestates and previously found by Ganesh et al. with increasing proportions of cow manure in the feeding increased COD in liquid fraction of digestate (Ganesh et al., 2015).

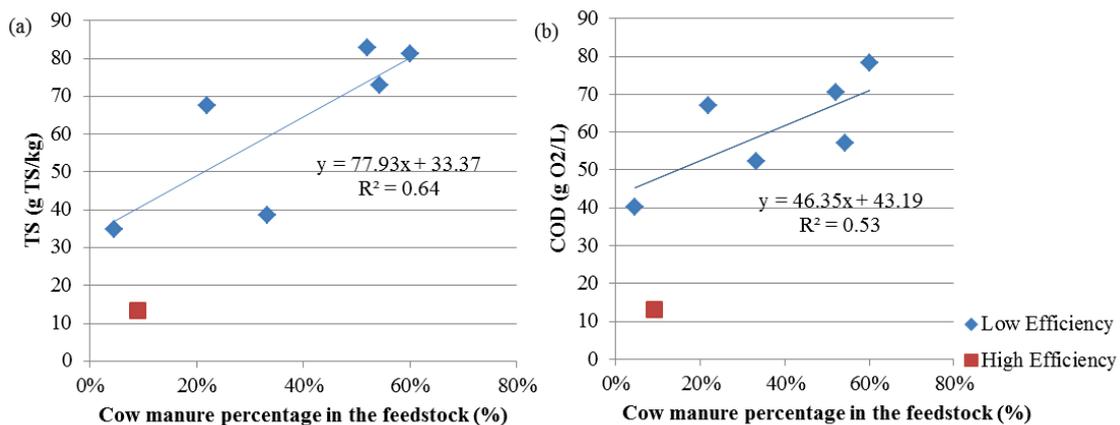


Figure 35: (a) TS and (b) COD concentrations vs cow manure percentage in the feedstock

#### 4.2.6. Anaerobic digestion operating parameters effect on digestate composition

##### 4.2.6.1. Impact of HRT on digestate characteristics

SUVA<sub>254</sub> is the parameter with highest correlation to HRT, this is represented in Figure 36. The SUVA<sub>254</sub> gives an indication about the aromatic content of the organic matter that can be correlated to the HRT applied in the reactor. With higher HRT it can be assumed that independently of the feedstock, the humification degree is increased and thus SUVA<sub>254</sub> increased

at the same time. This relationship was previously studied by Zheng et al. (Zheng et al., 2014), with various biodegradable substrates; the  $SUVA_{254}$  always increased along the degradation time. Also the authors reported differences in final  $SUVA_{254}$  depending on the substrate. As HRT also depends on the feedstock, an indirect link between  $SUVA_{254}$  and feedstock composition is assumed.

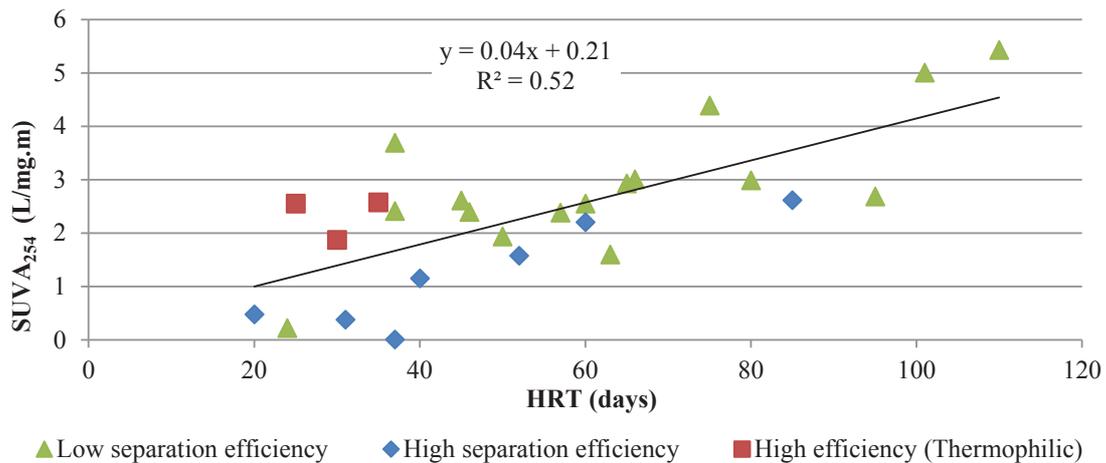


Figure 36:  $SUVA_{254}$  vs HRT (days)

The humification degree should be also represented by the fluorimetry measurements, in which humic acid-like area is described. Figure 37 aims to investigate the correlation between  $SUVA$  and the 3D fluorimetry fractions.

As represented in Figure 37, there is no correlation between fluorimetry fractions and  $SUVA$ . This was previously shown by Yang et al. (Yang et al., 2008) for organic matter in water and by Bioroza et al. (Bioroza et al., 2010) in drinking water. These two indicators represent the humification intensity from different molecules and then are not commutable.

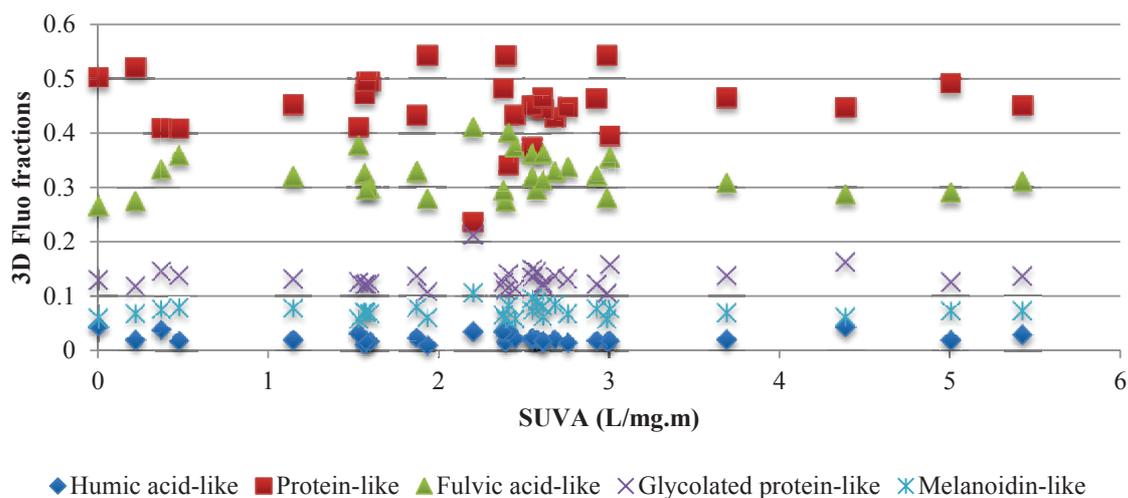


Figure 37: 3D fluo fractions vs SUVA (L/mg.m)

#### 4.2.7. Conclusion

The initial objective of having large concentration range is reached by the collection of 29 digestates, thus a good representation of various co-digestion plants is displayed. Having more substrates with Energy Crops in the second collection of digestates, the initial conclusion about the impact of manure content (Chap 3) is confirmed and better explained. According to PCA results interesting correlations were stated between HRT and SUVA, this indicator was suitable to describe the stabilization of the organic matter after biodegradation. After PCA analysis little explanation of the variability is obtained. Whereas the lab measurements were precise, reproducible, the data collected from the plants were more approximate, the sampling representatively is not ensured and feed composition not measured. The main missing information were TS and VS contents of the feedstocks. Therefore, further experiments with controlled conditions of the feed composition; reactor operating conditions and digestate composition are presented in the next chapter to investigate the impact of these parameters on the liquid fraction of the digestate.

## **5. Characterization of the residual compounds from cow manure**

As found in the previous chapter, cow manure has a high impact on the digestate composition, even if it was not possible to distinguish the relative impacts of both the feedstock composition and the kind of separation techniques (most manures were separated by screw press). The aim of the experiments which results are presented in this chapter was to try to understand the origin of the high organic matter concentrations found in some liquid fractions of digestates from full-scale plants, notably those with high share of cow manure in the feed of the digesters. For this purpose, 4 CSTR reactors were operated for 48 weeks with 3 different substrates: straw, cow dung and cow manure in order to characterize specifically the liquid fraction of digestates from each kind of substrate after liquid-solid separation. Such controlled lab-scale conditions will allow isolating the impact of the nature of the substrate. The results presented hereafter focus on both the characterization of the liquid fraction of the digestate at the end of the experiment (week 48) and its evolution with time during reactor operation. At lab-scale, separation process by screw press, vibrating screen and rotary drum are difficult to reproduce. Therefore, only centrifugation has been used in these experiments.

## **5.1. Monitoring of the reactors**

### **5.1.1. Operating conditions**

Four reactors were operated for 336 days. Reactor 1 (R1) was fed with straw, reactor 2 (R2) with cow dung and reactors 3 (R3) and 4 (R4) with cow manure. From week 1 to week 20, high solid content substrates (straw and cow manure) were diluted with tap water to feed reactors R1, R3 and R4 at a VS concentration of 15%. VS concentration of cow dung was already low at 14% hence, reactor R2 was fed at a VS concentration of 14%. During this period, the organic loading rate (OLR) was step by step increased from 0.5 to 2.5 g VS/L/d. Due to the accumulation of solids in the reactors, particularly for these fed with cow manure (R3 and R4), VS concentration fed to the reactors was reduced in the beginning of week 21 to 9% for R1 and R4 and 8% for R2. The reactors were further operated for more than 3 hydraulic retention times (HRT) to achieve steady state. For all the reactors but R3, the OLR was maintained constant at 2 g VS/L/d. For R3, feeding was stopped at the beginning of week 21 due to foaming and the reactor content was continuously stirred until end of reactor operation in the beginning of week 49 in order to observe the degradation of accumulated compounds.

### **5.1.2. pH**

Figure 38 shows the variation over time of pH during the operation of the four reactors fed with the different substrates. At the start-up of the reactors, increasing the OLR from 0.5 to 1 g VS/L/day impacted pH with decreasing pH for all the reactors as shown in Table 27. Further increase of the OLR from 1 to 2.5 had different impacts according to the type of substrate. Indeed, pH increased in R2 fed with cow dung while there was no clear effect on pH for R1 fed with straw. Reactors R3 and R4 fed with the same cow manure showed slightly different trends in the weeks 10 to 17 with a decrease in pH for reactor R3 and an increase for reactor R4.

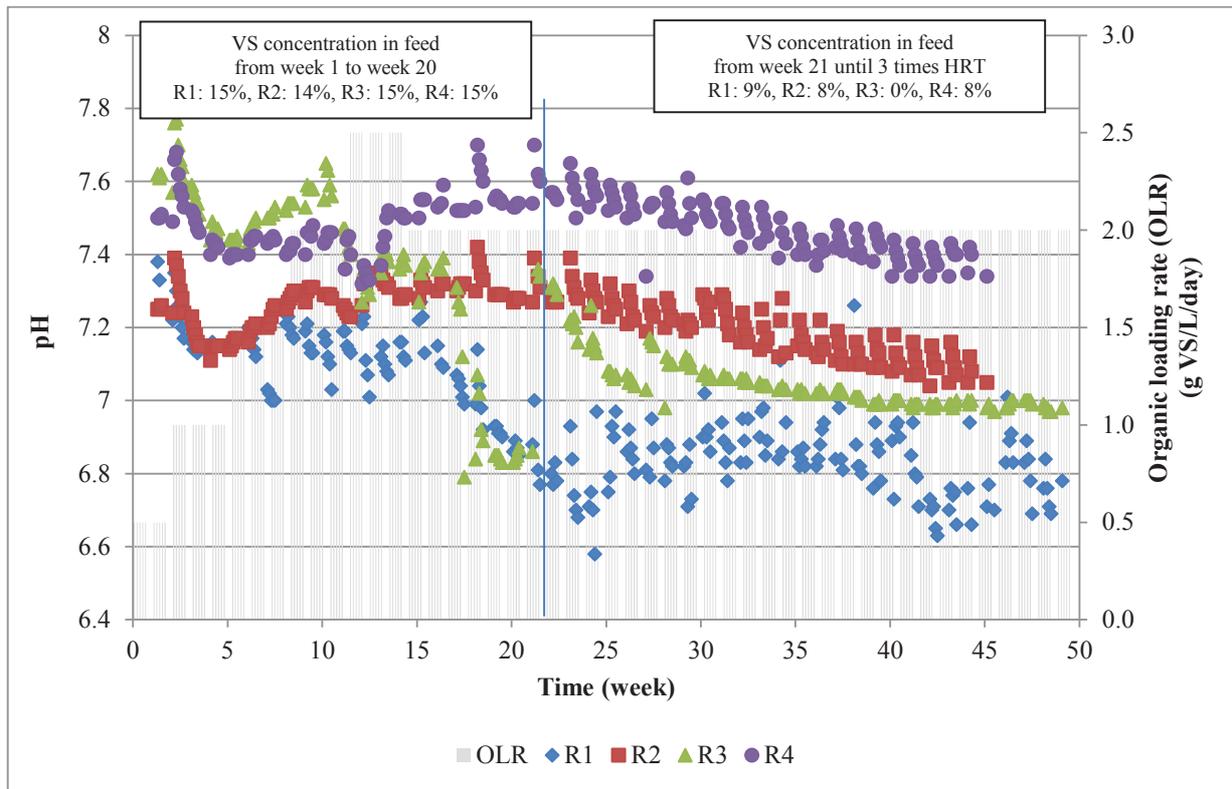


Figure 38: pH in reactor R1-straw, reactor R2-cow dung, reactor R3-cow manure, reactor R4-cow manure

During reactor operation, pH was quite stable for the 4 reactors until week 17. However, after the increase of the OLR to 2.5 g VS/L/d during 3 weeks, pH of reactors R1 and R3 became unstable even though the OLR was reduced to 2 g VS/L/d on week 16. Indeed, pH in reactor R1 was  $7.15 \pm 0.08$  until week 17 (Figure 38) but, at the end of week 17, pH started decreasing below 7 and reached 6.58 in the middle of week 24. From that date 5 mL of NaOH (32%) per week were added to reactor R1. This allowed maintaining its buffer capacity and pH above 6.7. pH was on average at  $6.86 \pm 0.09$  during the period (weeks 24 to 34). From week 34 to week 38, pH in reactor R1 was stable ( $6.9 \pm 0.1$ ) until the end of experiment ( $6.8 \pm 0.1$ ) due to different supplementations as will be explained later. Decreasing pH in a reactor fed with straw was also observed by other researchers in mono-digestion of straw which was due to lack of  $\text{NH}_4^+$  in the reactor (Peng et al., 2016).

Table 27: Average pH in reactors R1, R2, R3 and R4 with respect to different organic loading rates (OLR) during the first 20 weeks of the experiment

Week	OLR (g VS/L/day)	pH in R1	pH in R2	pH in R3	pH in R4
1-2	0.5	7.28 ± 0.06	7.30 ± 0.06	7.66 ± 0.07	7.57 ± 0.07
3-5	1	7.16 ± 0.02	7.17 ± 0.03	7.49 ± 0.06	7.44 ± 0.05
6-8	1.5	7.12 ± 0.09	7.24 ± 0.04	7.51 ± 0.02	7.43 ± 0.02
9-11	2	7.15 ± 0.05	7.28 ± 0.03	7.52 ± 0.10	7.43 ± 0.04
12-14	2.5	7.12 ± 0.06	7.31 ± 0.03	7.36 ± 0.04	7.44 ± 0.08
15-17	2	7.09 ± 0.09	7.31 ± 0.01	7.23 ± 0.21	7.54 ± 0.02
18-20	2	6.93 ± 0.08	7.31 ± 0.05	6.88 ± 0.07	7.57 ± 0.05

Acidification was also observed for reactor R3 fed with manure. Indeed, pH from Week 1 to Week 17 was  $7.47 \pm 0.12$ . However, from the end of week 17, pH started to drop from 7.12 to 6.79. pH was low at  $6.88 \pm 0.07$  from beginning of week 18 to the beginning of week 21. Moreover, an important foaming phenomenon was also observed during this period. Furthermore, this reactor started to behave very differently than reactor R4 though both reactors were fed in the same conditions. As a consequence, feeding of R3 was stopped beginning of week 21 due to foaming, decreasing pH and operational problems.

The pH in reactors R2 and R4 was much more stable than for the 2 other reactors with  $7.27 \pm 0.06$  and  $7.48 \pm 0.08$  from week 1 until week 20 and  $7.19 \pm 0.08$  and  $7.47 \pm 0.08$  from week 21 until the end, for reactors R2 and R4, respectively. After week 25, pH in reactors 2 and 4 followed a decreasing trend but always remained at high enough values.

### 5.1.3. Volatile fatty acids (VFA) concentrations

Volatile fatty acid concentration in the reactors was measured once a week, before the start of a new 5-day feeding, that is to say 3 days after the last feeding. All the results showed no VFA in the samples indicating that there was no VFA left at the end of the week.

### 5.1.4. N-NH<sub>4</sub><sup>+</sup> concentrations

The evolution over time of the concentrations of N-NH<sub>4</sub><sup>+</sup> in reactors R1-straw, R2-cow dung, R3-cow manure and R4-cow manure are shown in Figure 39. At the beginning of week 1, N-NH<sub>4</sub><sup>+</sup> concentrations were between 0.21 and 0.24 g N- NH<sub>4</sub><sup>+</sup>/L for each of the 4 reactors.

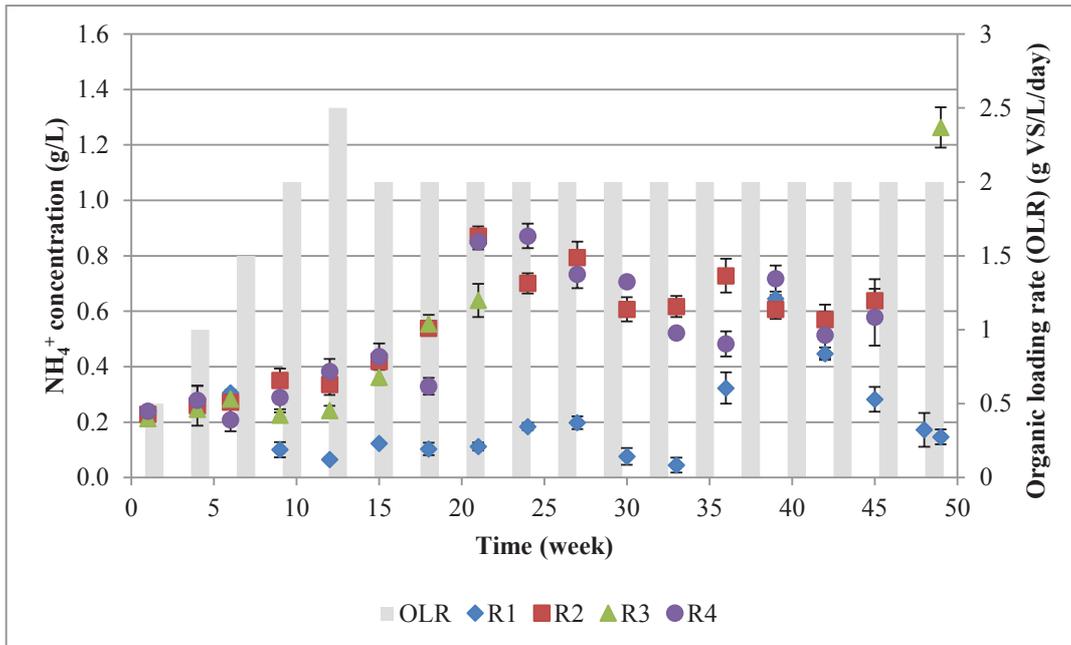


Figure 39: N-NH<sub>4</sub><sup>+</sup> concentration in reactors R1-straw, R2-cow dung, R3-cow manure and R4-cow manure

Reactor R1 which was fed with straw showed a slight increase in N-NH<sub>4</sub><sup>+</sup> concentration from beginning of reactor operation until week 6. However, the concentration decreased from 0.31 ± 0.01 g N-NH<sub>4</sub><sup>+</sup>/L in week 6 to 0.10 ± 0.03 g N-NH<sub>4</sub><sup>+</sup>/L in week 9 and remained at an average of 0.10 ± 0.02 g N-NH<sub>4</sub><sup>+</sup>/L from beginning of week 9 until beginning of week 21. Low N-NH<sub>4</sub><sup>+</sup> concentrations in R1 confirmed the inadequate total nitrogen concentration for anaerobic digestion of straw alone (Jaffar et al., 2016; Peng et al., 2016). A small and temporary increase in N-NH<sub>4</sub><sup>+</sup> content in the reactor was observed when NaOH was added to the feed to increase pH from week 24. Due to low N-NH<sub>4</sub><sup>+</sup> concentration in reactor R1 in week 33 with only 0.05 ± 0.03 g N-NH<sub>4</sub><sup>+</sup>/L, 40 mL NH<sub>4</sub>Cl (30 g/L) were added per day into reactor R1 from week 34 to week 38. Addition of NH<sub>4</sub>Cl was necessary to maintain N-NH<sub>4</sub><sup>+</sup> concentrations above 0.2 g N-NH<sub>4</sub><sup>+</sup>/L which is necessary for anaerobic digestion process (Rajagopal et al., 2013). N-NH<sub>4</sub><sup>+</sup>

concentration increased to  $0.65 \pm 0.01$  N-NH<sub>4</sub><sup>+</sup>/L in week 39. However, adding NH<sub>4</sub>Cl caused increasing chloride concentration up to  $2.83 \pm 0.05$  g/L in week 39. Therefore, addition of NH<sub>4</sub>Cl into reactor R1 was stopped. N-NH<sub>4</sub><sup>+</sup> concentration then further decreased to  $0.15 \pm 0.03$  g N-NH<sub>4</sub><sup>+</sup> /L at the end of reactor operation in week 49. From end of week 38 to the end of reactor operation, trace elements were added to supply nutrients to the microorganisms. 150 µL of Vithane (a commercial mix of trace elements) diluted 200 times (1 µL/g feeding) was added each day to reactor R1 (as well as to reactors R2 and R4).

For the reactor fed with cow dung (R2), the concentration of N-NH<sub>4</sub><sup>+</sup> increased from beginning of reactor operation until week 21. After the change in the dilution of the substrate, a slight decreasing N-NH<sub>4</sub><sup>+</sup> concentration was observed. From week 30, N-NH<sub>4</sub><sup>+</sup> concentration stabilized at an average concentration in R2 at steady state of  $0.63 \pm 0.05$  g N-NH<sub>4</sub><sup>+</sup> /L.

Reactor R4 which was fed with cow manure had a behavior quite close to that observed with cow dung alone. Indeed, a first increase in N-NH<sub>4</sub><sup>+</sup> concentration until week 21 was followed by a slight decrease of the concentration due to the change in the dilution of the substrate and finally, a stabilization at an average concentration of N-NH<sub>4</sub><sup>+</sup> at steady state of  $0.59 \pm 0.09$  g N-NH<sub>4</sub><sup>+</sup>/L.

In the operating conditions of this study, N-NH<sub>3</sub> concentration calculated based on calculation from Vidal et al, (Vidal et al., 2000) was always very low. For Reactor R1-Straw, it was below 6 mg N-NH<sub>3</sub>/L due to a low concentration in N-NH<sub>4</sub><sup>+</sup> in the reactor. For reactors R2-Cow dung and R4-Cow manure, N-NH<sub>3</sub> concentration was below 18 mg/L and 35 mg/L, respectively, because of N-NH<sub>4</sub><sup>+</sup> concentration below 0.9 g/L for both reactors and pH below 7.3 and 7.6, respectively. These N-NH<sub>3</sub> concentrations are well below the inhibition threshold (0.5 to 3 g N/L (Rajagopal et al., 2013)). The range from 0.2 to 1 g N/L has no antagonistic effect according to Rajagopal et al (Rajagopal et al., 2013).

### 5.1.5. Methane production

The methane content of the biogas was quite stable for all the reactors with an average percentage of methane of  $52 \pm 5\%$  for reactor R1-Straw,  $57 \pm 1\%$  for reactor R2-Cow dung and  $58 \pm 3\%$  and  $57 \pm 3\%$  for R3- and R4-Cow manure, respectively.

The evolution of methane yield, that is to say the volume of methane produced per g of VS fed, for reactors R1, R2, R3 and R4 is shown in Figure 40. During the first 5 weeks of the experiment, when the OLR was increased from 0.5 to 1 g VS/L/day, an increase in methane yield in all reactors was observed. However, a further increase of the organic loading rate to 1.5, 2 and then 2.5 g VS/L/day had a negative impact on methane yield for all the reactors. Hence, from week 15, the OLR was reduced to 2 and stabilized for 34 weeks at this value.

The highest methane yield was observed in reactor 1 at the beginning of the experiment, from week 3 to week 5, with an average methane production of  $328 \pm 12$  mL methane/g VS. However, this value was observed in unsteady conditions and during start-up. The decreasing pattern of methane yield from week 19 to week 24 for reactor R1 was correlated to a decreasing pH in the reactor (Figure 38) and to a lack of nitrogen (Figure 39). Despite the addition of NaOH from week 24 to week 34, methane yield in reactor 1 kept on decreasing. It significantly increased with the addition of  $\text{NH}_4\text{Cl}$  from week 34 to end of week 38 and remained fairly stable between 150 and 200 mL/g VS until the end of reactor operation in week 49. These results show that straw as single substrate has a deficiency in nitrogen for anaerobic digestion and it is necessary to bring nitrogen to the reactor either by adding chemicals or by adding a co-substrate rich in nitrogen such as pig manure for example.

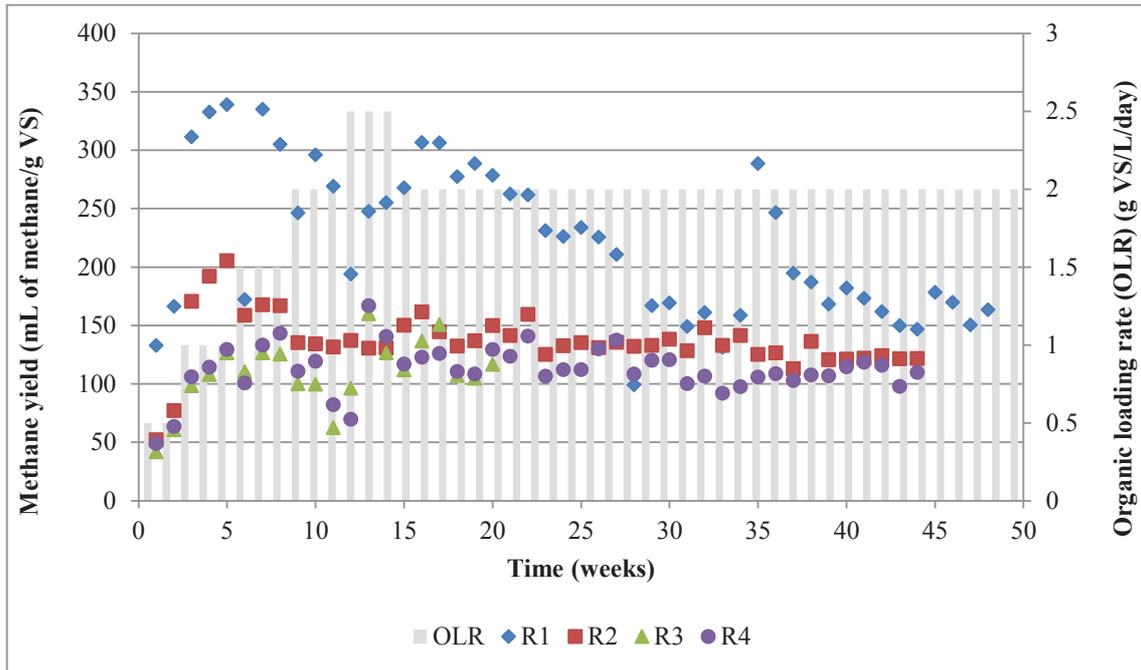


Figure 40: Methane production from R1-straw, R2-cow dung, R3-cow manure and R4-cow manure

After stabilization of the OLR at 2 g VS/L/d, the evolution of the 2 reactors fed with cow dung (R2) and cow manure (R4) was much more stable than that of R1-Straw. After 3 HRTs and at an OLR of 2 g VS/L/day, the methane yields were:  $164 \pm 12$  mL methane/g VS for the reactor R1 fed with straw,  $123 \pm 6$  mL methane/g VS for reactor R2 fed with cow dung and  $109 \pm 7$  mL methane/g VS for reactor R4 fed with cow manure. Straw led to the highest methane yield followed by cow dung and cow manure. Similar studies also observed higher methane yields from straw ( $195 \pm 3$  mL/g VS) compared to manure ( $148 \pm 41$  mL/g VS) (Møller et al., 2004). However, the methane yield ( $164 \pm 12$  mL /g VS<sub>fed</sub>) measured at an OLR of 2 gVS/L/d in R1 fed with straw was lower compared to methane potentials (or BMP) of wheat straw reported in the literature, from 293 to 378 mL/g VS (Peng et al., 2016; Sawatdeenarunat et al., 2014). However, it is still in the range of methane potentials found in other review (Frigon and Guiot, 2010) with data from 140 to 340 mL/g VS and higher than  $83 \pm 5$  mL/g VS of methane yield obtained by other researchers (Liu et al., 2015). Methane production from R2 fed with cow dung at an OLR of 2 g VS/L/d was quite stable. However, the methane yield assessed in this study ( $123 \pm 6$  mL methane/g V ) was lower in comparison to other reported methane potential of 193 mL

methane/g VS (D. Li et al., 2015a). For cow manure fed reactor (R4), methane yield obtained at steady state at an OLR of 2 g VS/L/d ( $109 \pm 7$  mL methane/g VS) was in the lower range of values reported by several authors. It is well documented in the literature that results for cattle manure can vary greatly, depending on several factors. For example, (Labatut et al., 2011) reported BMPs in the range 127-329 mL CH<sub>4</sub>/g VS with an average value of  $243 \pm 60$  mL CH<sub>4</sub>/g VS from 47 individual BMP assays performed on manure samples from six different dairy farms at various times of the year. On the other hand, (Buendía et al., 2009) reported a BMP of 84 mL/g VS. The wide range of results can be explained by the highly variable nature of cattle manure which depends on various parameters such as: breed, age, diet, management practices and also by the consequences of a possible aerobic degradation during storage of cow manure. The rather low methane yield measured in this work for cow manure, which is lower than straw and cow dung methane yields, can be explained by the fact that the sample came from quite old manure which had already undergone significant aerobic degradation during storage. This is underlined by the VS/TS ratios of only 53%, indicating a low VS fraction, compared to straw and a fresh sample of cow dung (91% and 87% VS/TS ratios, respectively) (Table 12).

For reactor R3 where feeding was stopped beginning of week 21, methane production showed a rapid decreasing pattern (Figure 41) during the weeks 21 – 25, decreasing from 7110 mL methane/week on week 20 to 5690 mL/week on week 21, until week 25 (1470 mL/week) suggesting that the slowly biodegradable fraction of the substrate which accumulated was being eliminated. From week 25, the volume of methane produced per week was quite low (20% of the value measured when the reactor was fed) and further decreased very slowly indicating that methane production was mostly due to endogenous activity and that most of the accumulated substrate had been eliminated. On week 48, that is to say after almost 6 months without feed, the anaerobic activity produced 370 mL of methane per week which represents only 5% of the methane produced per week when the reactor was fed with cow manure.

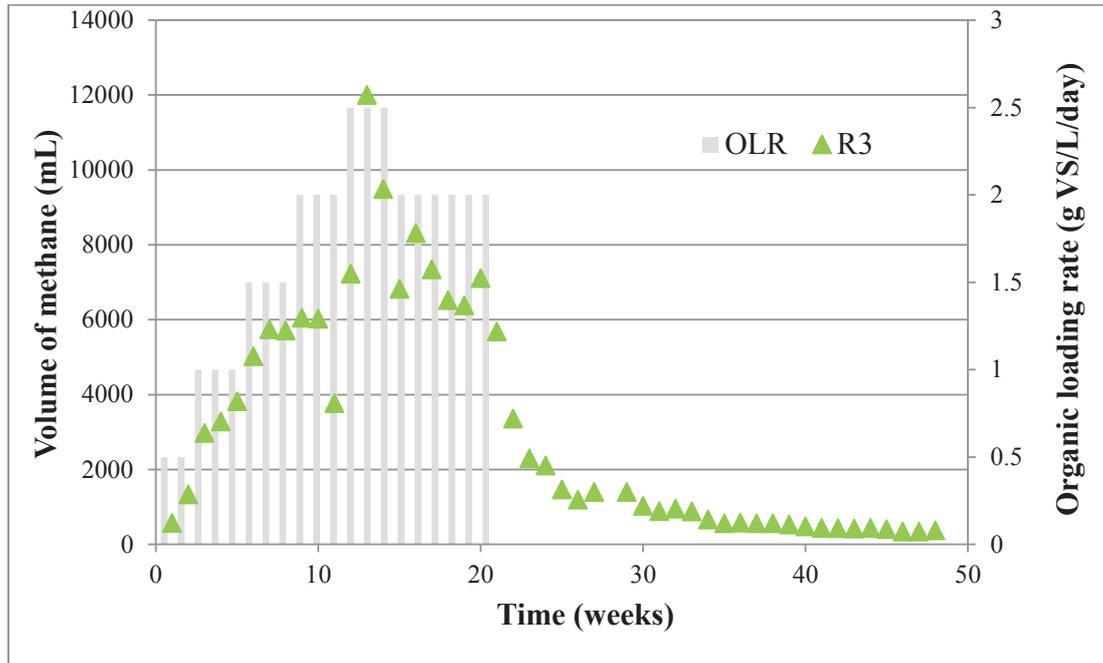


Figure 41: Volume of methane produced per week in reactor R3.

### 5.1.6. Total solids and volatile solids concentrations

Figure 42 shows the evolution over time of total solids (TS) concentrations in the reactors. Initial TS concentration in week 1 for the 4 reactors was between  $24.8 \pm 0.3$  and  $27.3 \pm 1.2$  g TS/kg. During the start-up of the reactors (up to week 20) at increasing OLRs, TS concentration of the different substrates fed was 17% for reactor R1-Straw, 18 % for reactor R2-Cow dung and 29 % for R3- and R4-Cow manure. In these conditions, TS concentrations in all reactors increased at different rates according to the substrates fed until week 21. TS concentration in the reactors was  $51.8 \pm 1.6$ ,  $94.2 \pm 0.5$ ,  $161.9 \pm 0.4$  and  $160.9 \pm 0.5$  g TS/kg for reactors R1, R2, R3 and R4, respectively. Agitation in the reactors was made by mechanical stirring but, with such high concentrations in TS in the reactors, mixing became difficult, especially for reactors R3 and R4. On week 21, it was decided to lower the concentration of the feeds by diluting more the substrates in order to decrease TS concentrations in the reactors. TS concentrations fed into each reactor was decreased from 17% to 10% for reactor R1, from 18% to 10% for reactor R2 and from 29% to 15% for reactor R4 while for R3, the feeding was stopped due an additional foaming problem. The new TS concentrations fed to reactors R1, R2 and R4 were continued for

3 times HRT. Average total solids concentrations obtained at steady state were  $67 \pm 2$ ,  $85 \pm 1$  and  $119 \pm 4$  g TS/kg for R1-straw, R2-cow dung and R4-manure, respectively. Final TS concentration in week 49 for R3 was  $148.1 \pm 0.2$  showing only 8.5% decreased after prolonged stirring in the reactor.

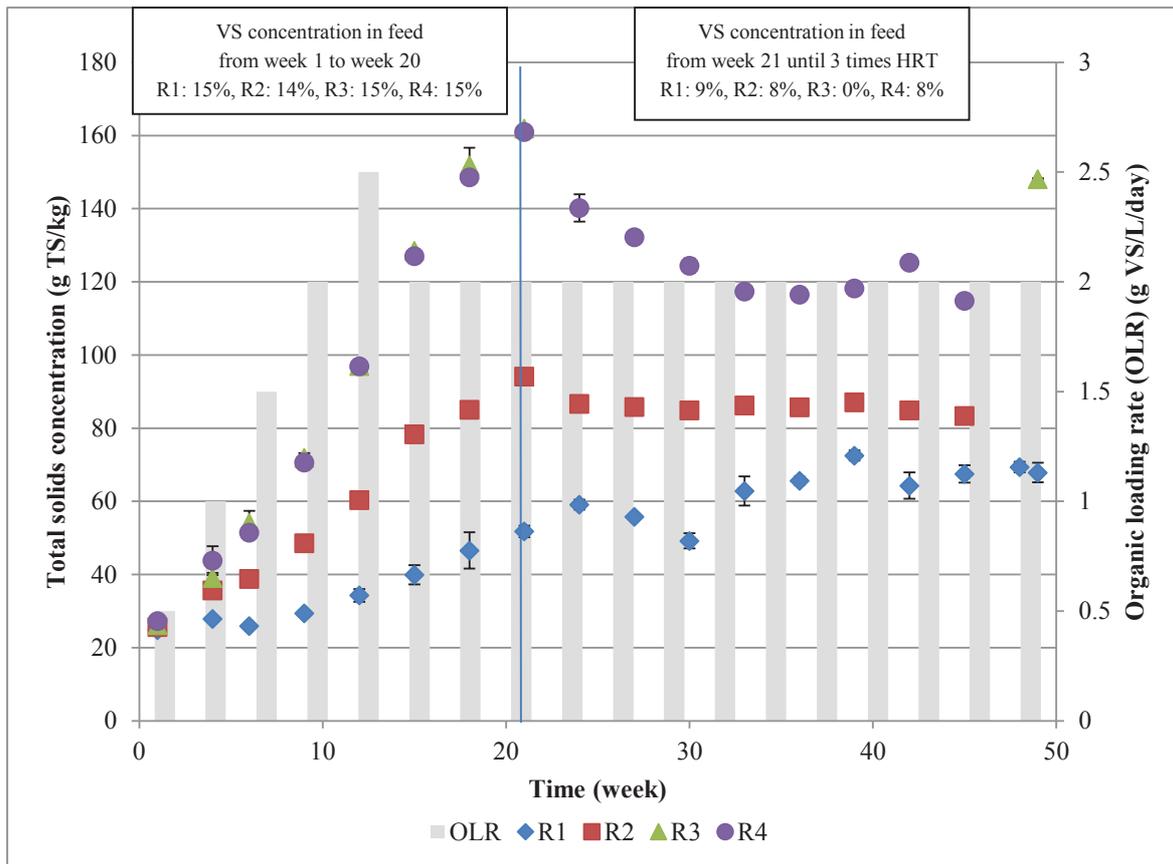


Figure 42: Total solids (TS) concentrations in reactors R1-straw, R2-cow dung, R3-cow manure and R4-cow manure

The comparison between TS concentrations in the feed and in the reactors at steady state shows that the highest TS removal was obtained with straw with 33% TS removal efficiency, followed by cow manure with 20% TS removal efficiency. Cow dung had the lowest TS removal with only 15% TS removal efficiency.

Volatile solids (VS) concentrations and their evolution over time in reactors R1, R2, R3 and R4 are shown in Figure 43. Initial VS concentration was between  $20.1 \pm 0.4$  and  $22.5 \pm 1.1$  g VS/kg

for the 4 reactors. VS concentration in the different substrates fed were 15% for reactors R1 fed with straw, R3 and R4 fed with cow manure and 14% for reactor R2 fed with cow dung. Similar to the evolutions of TS concentrations, VS concentrations in all the reactors increased until week 21. VS concentrations at the beginning of week 21, before reducing VS fed (%), were  $41 \pm 2$ ,  $69.3 \pm 0.7$ ,  $73 \pm 5$  and  $73 \pm 1$  g VS/kg for reactors R1, R2, R3 and R4 respectively. At the starting of week 21, VS concentration of the feeds was reduced and the new VS concentrations of the different substrates fed were: 9% for reactor R1 and 8% for reactors R2 and R4. Average VS concentrations at steady state were  $59 \pm 2$ ,  $68 \pm 2$  and  $71 \pm 4$  g VS/kg for R1-straw, R2-cow dung and R4-cow manure, respectively. VS removal efficiencies were 34% for straw, 15 % for cow dung and 7% cow manure. This very low value which is inconsistent with 20 % TS removal highlights the difficulty of sampling in cow manure reactors whose digestates are very inhomogeneous. It is thus more relevant to describe reactors performance in terms of biogas or methane yields rather than solids removal.

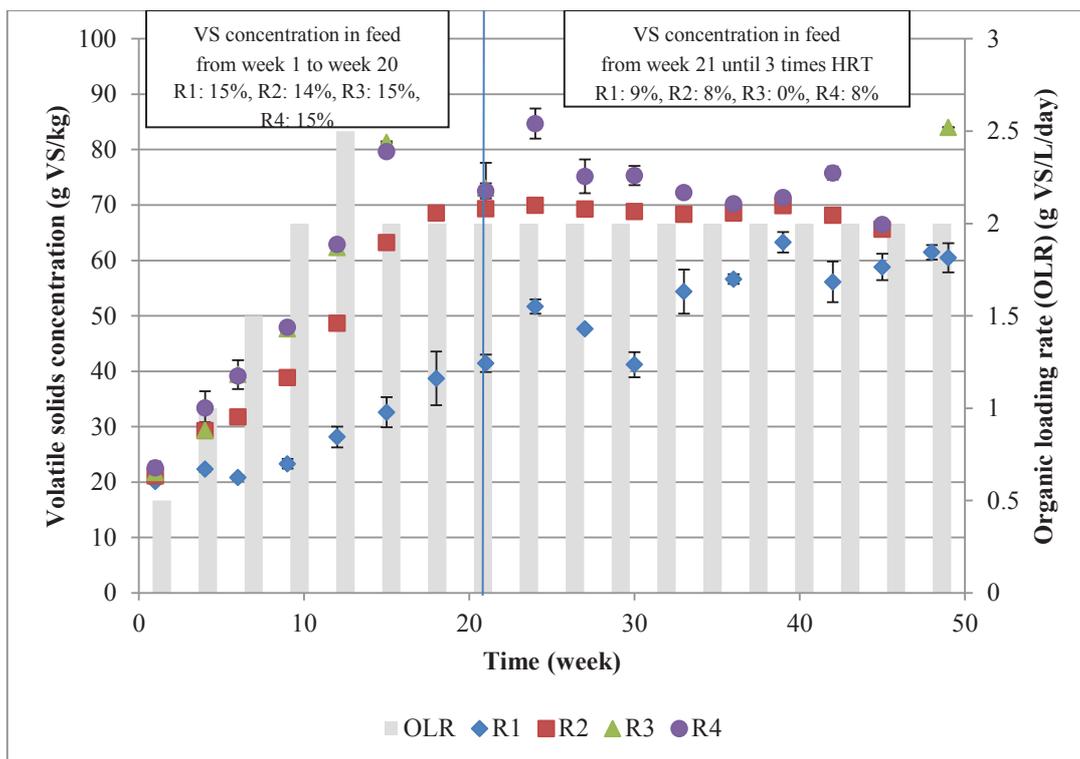


Figure 43: Volatile solids (VS) concentrations in reactors R1-straw, R2-cow dung, R3-cow manure and R4-cow manure

## 5.2. Characterization of the liquid fraction of digestates

The aim of the experiments in reactors was to produce digestate from 3 different substrates (straw, cow dung and cow manure) in order to compare the characteristics of the liquid fractions after liquid/solid separation. The reactors were operated for 48 weeks and the results presented before in this chapter show that the reactors were at steady state at the end of the experiment. The results presented hereafter focus on the characterization of the liquid fraction of the digestates produced at laboratory scale. The liquid/solid separation of the digested extracted from the reactors was made by centrifugation at 18 000 g during 20 minutes. The characterization of the liquid fraction of the final digestate when the reactor is at steady state will be presented together with its evolution with time.

### 5.2.1. Total solids and volatile solids concentrations

TS concentration in liquid fraction of digestates after centrifugation from reactors R1, R2, R3 and R4 and their evolution over time are shown in

Figure 44.

TS concentration in liquid fraction of digestates at initial stage after one week was  $1.1 \pm 0.7$ ,  $2.3 \pm 1.7$ ,  $6.0 \pm 0.7$  and  $6.7 \pm 1.8$  g TS/kg for reactors R1, R2, R3 and R4 respectively. Figure 8 suggests that TS concentrations in the liquid fractions followed quite a similar evolution than that of TS in the reactors. Indeed, during the first phase of continuous feeding at increasing OLRs, TS concentrations in the liquid fractions increased regularly and reached maximum values of  $16.6 \pm 0.8$ ,  $29.1 \pm 0.3$ ,  $75.1 \pm 0.4$  and  $75.7 \pm 0.2$  g TS/kg for reactors R1, R2, R3 and R4, respectively, on week 21 (before reducing concentration of the feeds). To compare the evolution of TS concentrations in the reactors and in the liquid fractions after centrifugation, the curves  $TS_{\text{liquid fraction}}$  versus  $TS_{\text{reactor}}$  are plotted in Figure 45 for the 3 substrates.

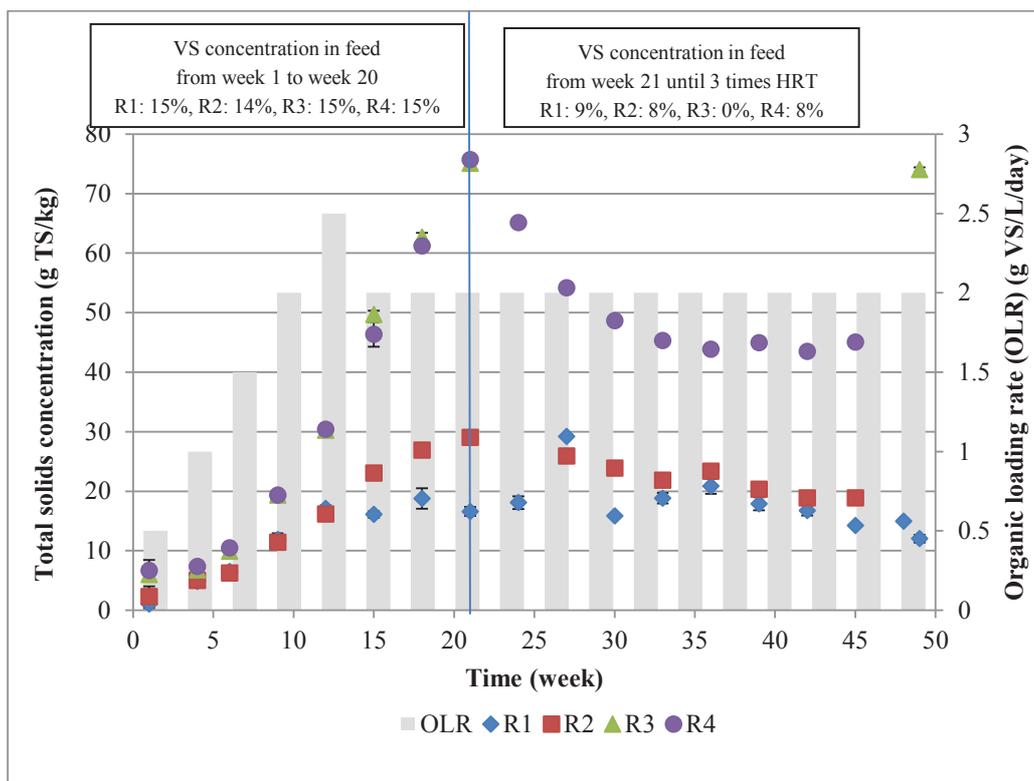


Figure 44: Total solids (TS) concentrations in liquid fraction of digestates after centrifugation from R1-straw, R2-cow dung, R3-cow manure and R4-cow manure reactors

The curves highlight two different behaviors (Figure 45). For cow dung and cow manure as substrates, TS concentration in the liquid fraction after centrifugation was fairly proportional to the TS concentration of the digestate for both substrates. Furthermore, a similar correlation was found for TS concentrations in the reactor below 100 g/L suggesting that the digestates from cow dung and cow manure behave similarly with respect to centrifugation.

On the other hand, a completely different behavior was observed with straw as substrate. TS concentration in the liquid fraction was fairly constant (between 15 and 20 g TS/kg) for TS concentrations in the reactor in the range 35 to 80 g TS/kg (week 12 to 48). This observation shows that there was no major influence of the TS concentration in the reactor on the quality of the supernatant after centrifugation for TS concentrations above 35 g TS/kg. This result suggests that the separation efficiency improved with time indicating that more particle and colloids are separated by centrifugation, probably linked to a change in their nature. Indeed, after 12 weeks, that is to say at a TS concentration of 35 g TS/kg, TS concentration in the liquid fraction was half

of that of the digestate while at the end of the experiment, TS concentration in the liquid fraction was 4.6 times lower than that of the digestate, indicating a high solid/liquid separation efficiency.

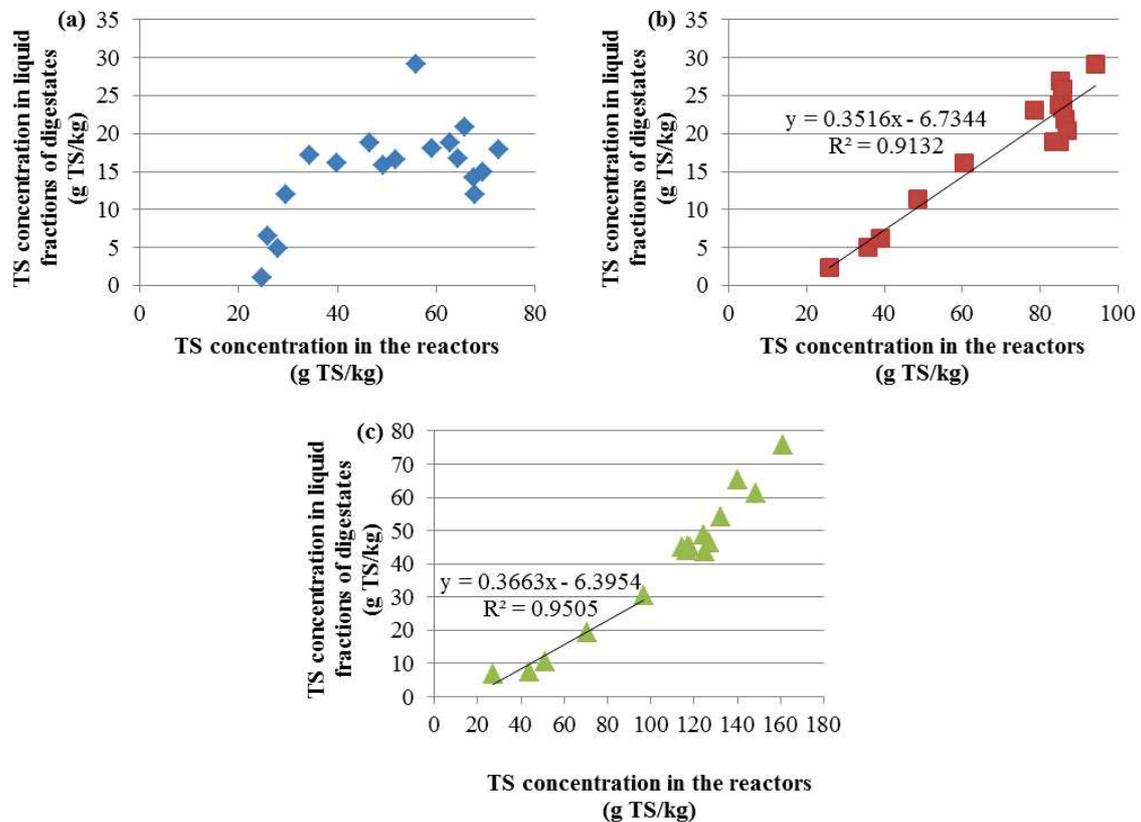


Figure 45: Evolution of TS concentration in the liquid fraction of digestates after centrifugation versus TS concentration in the reactors for R1-straw, R2-cow dung and R4-cow manure

After 3 times HRTs with the new TS concentration in feeds, the highest TS concentration in liquid fraction of digestates was found with the digestate from the cow manure fed reactor (R4) with  $44.5 \pm 0.7$  g TS/kg followed by that from cow dung and straw fed reactors with  $19.4 \pm 0.7$  and  $14.5 \pm 1.7$  g TS/kg, respectively, for reactors R2 and R1. Thus, the TS concentrations of the liquid fraction of digestates were quite close for straw and cow dung but much higher for cow manure which might be due to the use of old manure, as shown previously.

VS concentrations in the liquid fraction of digestates and their evolution during the experiment are shown in Figure 46. The global behavior of VS concentration in the liquid fraction after

centrifugation of the digestate follows the same pattern than that of TS concentration previously described in

Figure 44. At steady state, that is to say on week 48, average VS concentration in liquid fraction of digestates was the highest for reactor R4 fed with cow manure with  $30.0 \pm 0.3$  g VS/kg ( $67 \pm 1$  % VS/TS) followed by reactor R2 fed with cow dung with  $14.9 \pm 0.5$  g VS/kg ( $77 \pm 0$  % VS/TS) and reactor R1 fed with straw with  $10.2 \pm 1.5$  g VS/kg ( $70 \pm 3$  % VS/TS). The comparison of VS concentrations in the liquid fractions with the VS concentrations in the reactors show the same behavior than for TS concentrations and separation efficiency was in the same range than the values reported for TS concentrations.

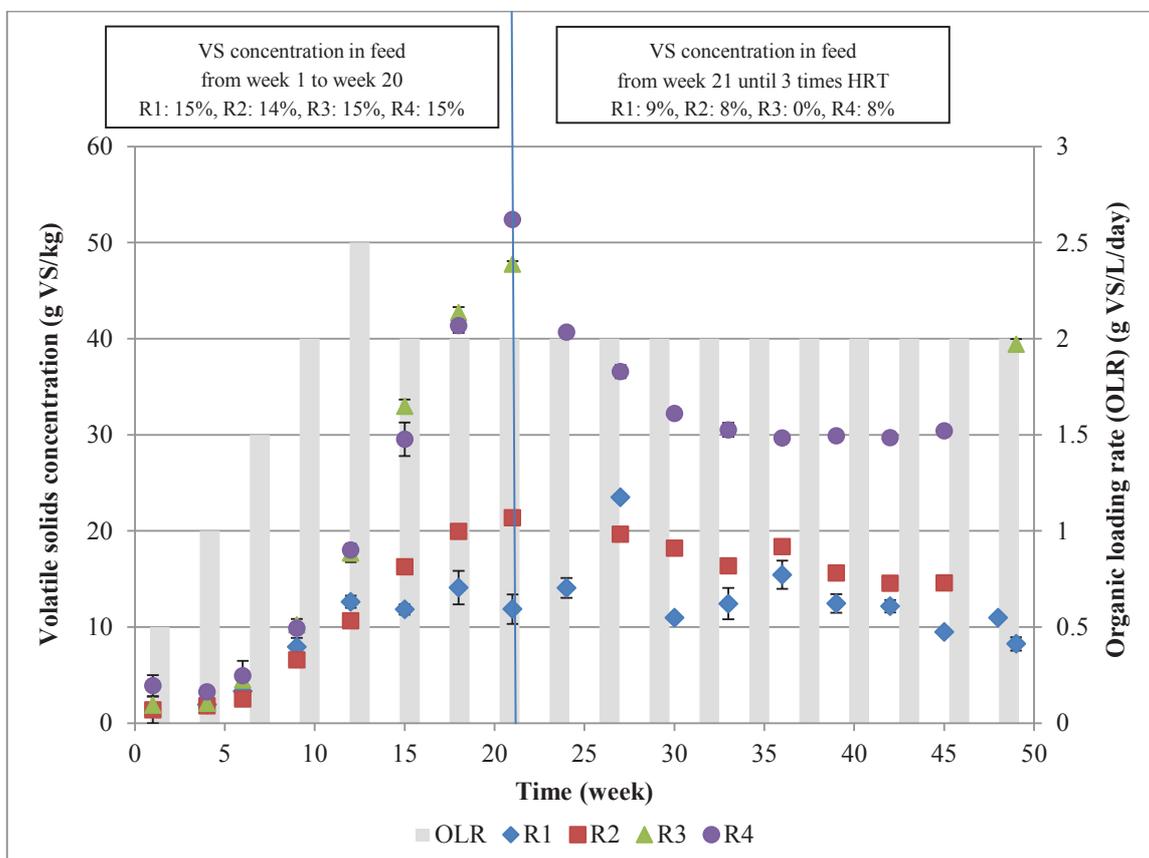


Figure 46: Volatile solids (VS) concentrations in liquid fraction of digestates from R1-straw, R2-cow dung, R3-cow manure and R4-cow manure reactors

Prolonged stirring without feeding for 28 weeks did not affect significantly liquid/solid separation of the digestate from reactor R3-Cow manure. Indeed, TS concentration at the end of

the experiment ( $74.1 \pm 0.4$  g TS/kg) was very close the value on week 21, when the feeding was stopped and there was only a slight reduction of 17.6 % in VS concentration in liquid fraction of digestate.

### 5.2.2. Chemical Oxygen Demand (COD)

The evolution of COD in liquid fraction of digestates after centrifugation is shown in Figure 47 for reactors R1, R2, R3 and R4. Initial COD in liquid fractions of digestate were  $0.67 \pm 0.10$ ,  $0.77 \pm 0.11$ ,  $0.80 \pm 0.16$  and  $0.79 \pm 0.04$  g O<sub>2</sub>/L for reactors R1, R2, R3 and R4, respectively.

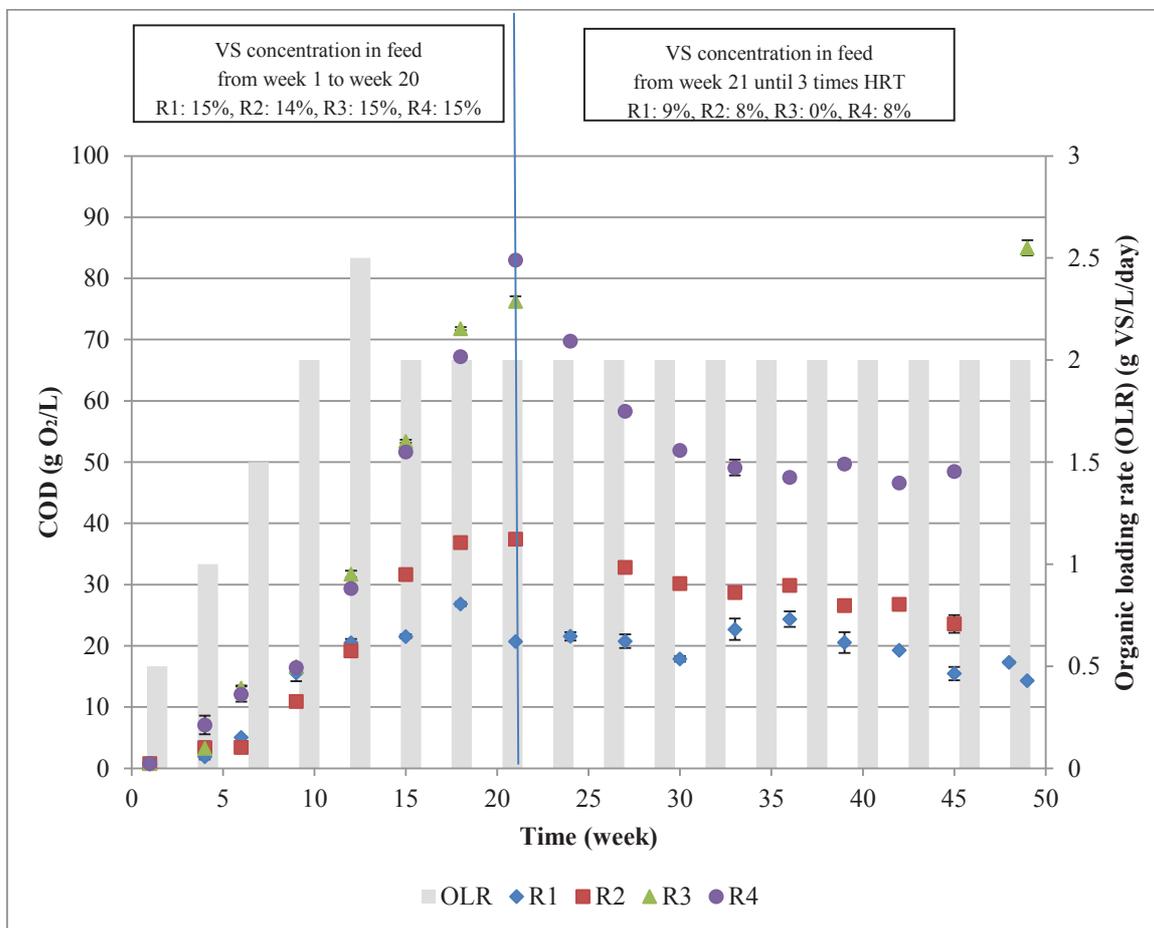
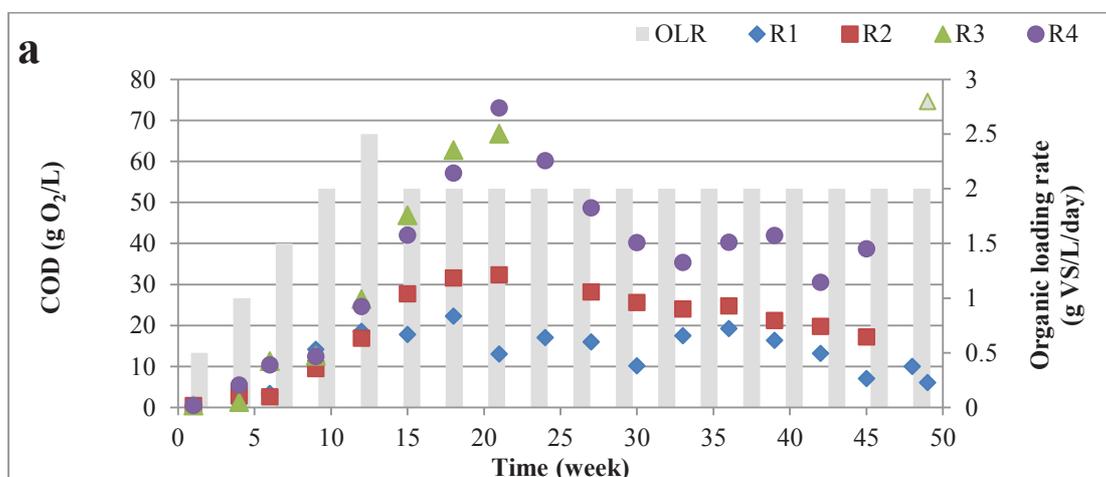


Figure 47: COD in liquid fraction of digestates after centrifugation for reactors R1-straw, R2-cow dung, R3-cow manure and R4-cow manure

The general evolution of COD is very similar to that of VS. Indeed, during the first phase when the reactors were fed with increasing OLRs, COD increased in the liquid fraction of all

digestates. Thus, at the beginning of week 21, COD were  $20.7 \pm 0.1$ ,  $37.4 \pm 0.2$ ,  $76.3 \pm 0.8$  and  $83.0 \pm 0.3$  g O<sub>2</sub>/L for liquid fractions of digestates from reactors R1, R2, R3 and R4, respectively. After the decrease of the VS concentration of the feed of all reactors at the beginning of week 21 (except R3 where feeding was stopped), a decrease in COD in liquid fraction of digestates was observed followed by a plateau, though a slight decrease was observed towards the end of the experiment for reactors R1-straw and R2-cow dung. At the end of the experiment, COD was 15.5, 23.6 and 48.5 g O<sub>2</sub>/L, on average, for reactors R1, R2 and R4, respectively.

Figure 48 shows the distribution of COD into 3 compartments after successive filtrations at 1.2 µm and 1 kDa: a- Suspended solids (> 1.2 µm), b- Colloids (1.2 µm to 1kDa) and c- Soluble fraction (< 1 kDa). For the liquid fraction of all digestates, COD was the highest for the compartments representing suspended particles (Figure 48-a). This compartment was quite stable for the liquid fraction from the reactor fed with cow manure but it was decreasing from week 30 till week 49 for reactors R1-Straw and R2-Cow dung. Figure 48-b shows that colloid content was very unstable for the liquid fraction from reactor R4-Cow manure and that it tended to increase for straw and cow dung. Soluble fraction (Figure 48-c) of all liquid fractions of digestates was low and quite stable with time. These results show that, though TS and VS concentrations in the reactors were very stable and the reactor considered at steady state, the behavior towards centrifugation and filtration still changed with time till the end of the experiment, showing a change in the nature of particles and colloids.



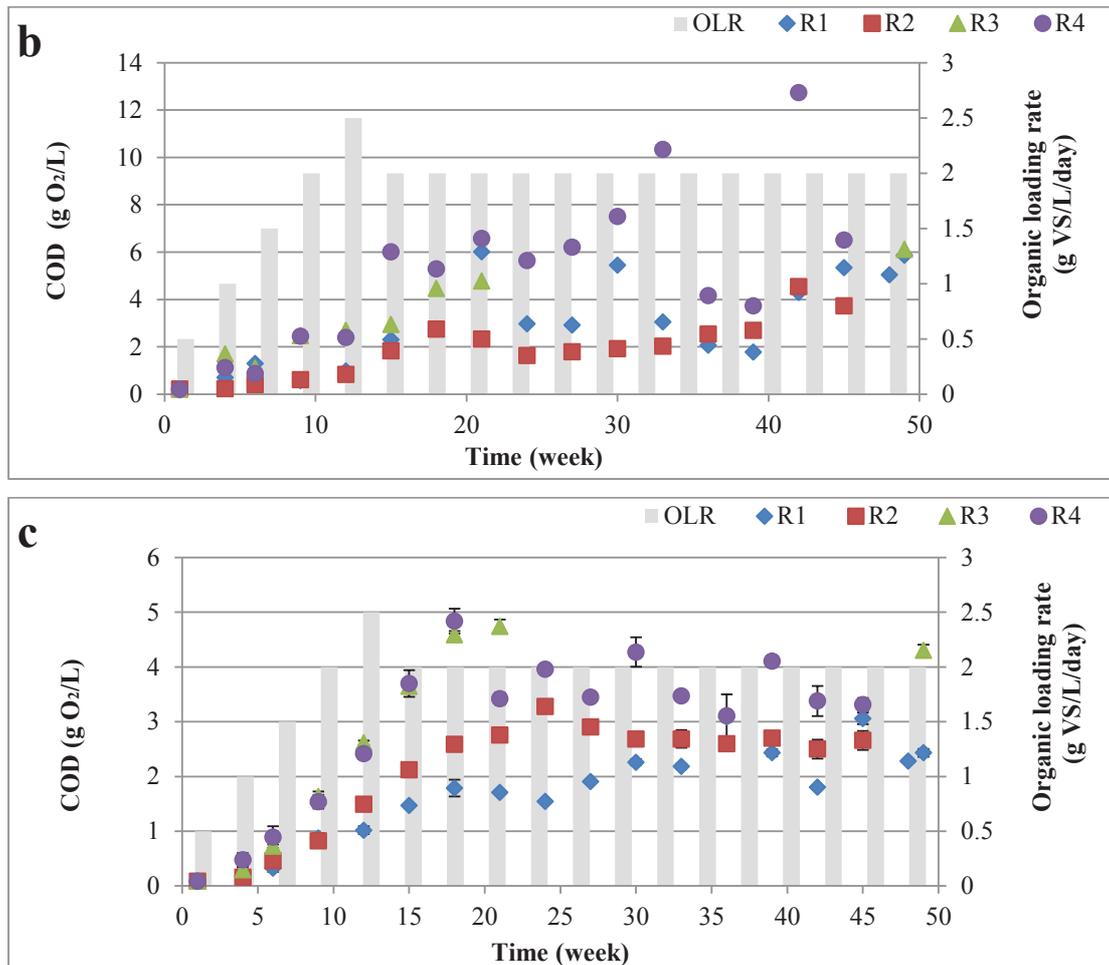


Figure 48: COD distribution in liquid fractions of digestates from reactors R1-straw, R2-cow dung, R3-cow manure and R4-cow manure: a) COD in suspended particles ( $> 1.2 \mu\text{m}$ ); b) COD in colloids ( $1.2 \mu\text{m}$  to  $1 \text{ kDa}$ ) and; c) dissolved COD ( $< 1 \text{ kDa}$ )

A summary of the characteristics of the different fractions at the end of the experiment (week 45) is presented in Table 28. As described previously, total COD of the liquid fraction of the digestate from cow manure was very high compared to total COD of the 2 other liquid fractions (straw and cow dung) and cow manure had the highest COD in all suspended particles, colloids and dissolved fractions. The organic matter distribution obtained for the liquid fraction of the digestates from cow dung and cow manure were quite close with 73-80% of suspended solids, 13-16% of colloids and the soluble part representing only a small fraction (7-11%). The distribution for the liquid fraction of the digestate from straw was quite different with a much higher proportion of soluble matter and colloids. These results suggest that cow dung can

generate a lot of suspended solids which cannot be separated by centrifugation while straw might generate quite a high quantity of colloids. It is difficult to conclude on the specific impact of straw and cow dung. This might be explained by the use of a quite old manure which had already undergone some aerobic degradation as described previously.

Table 28: Distribution in COD and % of the different compartments of the liquid fraction of digestates from reactors R1-Straw, R2-Cow dung and R4-Cow manure

<b>COD on Week 45</b>	<b>Straw (g O<sub>2</sub>/L)</b>	<b>Cow dung (g O<sub>2</sub>/L)</b>	<b>Cow manure (g O<sub>2</sub>/L)</b>
Total	15.5	23.6	48.5
Suspended particles	7.06	17.19	38.6
Colloids	5.35	3.73	6.51
Soluble	3.06	2.66	3.31
<b>% COD on Week 45</b>	<b>Straw</b>	<b>Cow dung</b>	<b>Cow manure</b>
Suspended particles	45.6	73	80
Colloids	34.6	16	13
Soluble	19.8	11	7
Total	100	100	100

For R3, final COD at the end of reactor operation in week 49 that is to say after continuous stirring without feeding for 28 weeks was  $85 \pm 1.25$  g O<sub>2</sub>/L which had shown an increase of 11.5% in COD compared to that measured in week 21. Furthermore, there was no change in the distribution of the organic matter and the SS compartment still represented 88% of total COD, even after 28 weeks of digestion. These results suggest that there was no significant degradation of COD or improvement of the liquid/solid separation by centrifugation during the 28 weeks of digestion without feeding. This shows that the COD in the liquid fraction of digestate is non-biodegradable by anaerobic digestion.

### 5.2.3. Aerobic biodegradability

BOD<sub>5</sub>, BOD<sub>21</sub>, and COD are presented in Figure 49 for the 4 liquid fractions of digestate from reactors R1-Straw, R2-Cow dung and R3- and R4-Cow manure after solid/liquid separation by centrifugation.

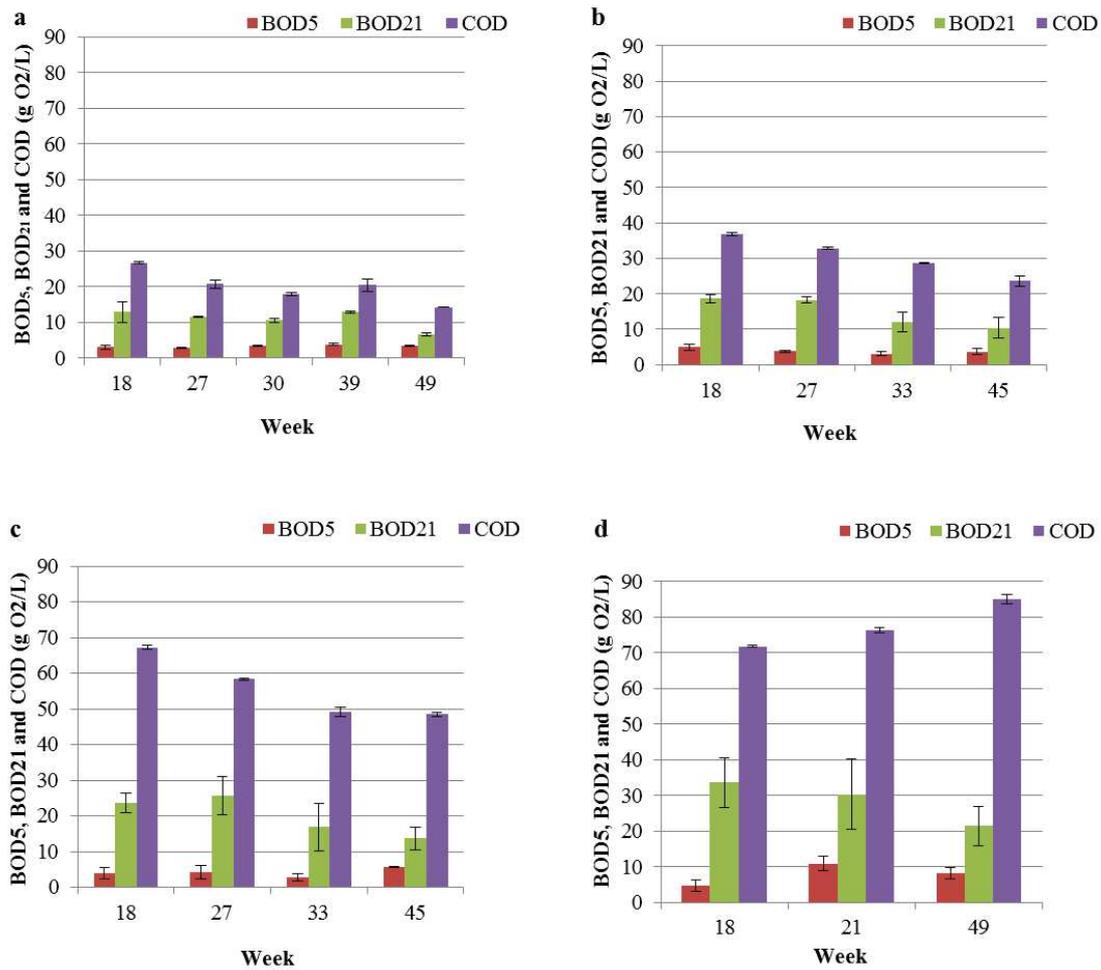


Figure 49: BOD<sub>5</sub>, BOD<sub>21</sub>, and COD of liquid fractions of digestate from reactors a- R1-straw, b- R2-cow dung, c- R4-cow manure and d- R3-cow manure

Figure 49 shows clearly that BOD<sub>5</sub> was very low for all the liquids fractions with values below 11 g/L even when COD was over 80 g/L. Furthermore, no significant evolution was observed with time as BOD<sub>5</sub> remained low compared to COD all along the experiment. The long term biodegradability (BOD<sub>21</sub>) was also quite low and BOD<sub>21</sub> generally represented less than 50% of COD. BOD<sub>21</sub> showed a tendency to decrease between weeks 27 and 49 with the lowest BOD<sub>21</sub> measured at the end of the experiment.

The ratios BOD<sub>5</sub>/COD and BOD<sub>21</sub>/COD make it possible to evaluate the biodegradability of the organic matter and the results for these 2 parameters are presented in Table 29 for the 4 liquid fractions of digestate at the end of the experiment.

Table 29: Biodegradability of liquid fraction of digestates from R1, R2, R3 and R4 at the end of reactor operation

Reactor	Substrate	BOD <sub>5</sub> /COD	BOD <sub>21</sub> /COD
R1	Straw	0,24	0,47
R2	Cow dung	0,15	0,44
R3	Cow manure	0,1	0,25
R4	Cow manure	0,12	0,28

The liquid fraction from the reactor fed with straw had the highest short term biodegradability with a ratio BOD<sub>5</sub>/COD of 0.24. The other liquid fractions had close but very low BOD<sub>5</sub>/COD ratios, in the range 0.1 – 0.15. However, all the liquid fractions of digestate had low BOD<sub>5</sub>/COD compared to the ratio value commonly found in biodegradable effluents that is to say a BOD<sub>5</sub> to COD ratio equal or above 0.5. The lowest long term biodegradability was observed in liquid fraction of digestate from cow manure with BOD<sub>21</sub>/COD of 0.28 only. Straw and cow dung had almost double ratios but which still remain quite low. The results obtained with reactor R3-cow manure in the period week 21 to week 49 show that both ratios BOD<sub>5</sub>/COD and BOD<sub>21</sub>/COD decreased even though COD increased. This result can be explained by 2 phenomena: a slight degradation of the biodegradable organic matter which accumulated in the reactor during the 28 weeks of the experiment combined to the generation of organic matter mainly non-biodegradable. At the end of the experiments, the biodegradability of the organic matter from the liquid fraction of R3-manure was similar to that of the reactor which was fed with manure in the same period (R4).

The results presented in this paragraph show that the rapidly biodegradable fraction of the organic matter present in the liquid fraction of digestates is very low and that, globally, the biodegradability of the organic matter present in the liquid fraction of digestates is low. Furthermore, the values measured with cow manure (R4) confirm the previous observations that the cow manure used in this work is old manure which had already undergone some aerobic degradation. Indeed, the ratio BOD<sub>21</sub>/COD was very low and around twice lower than that measured for the reactors fed with straw or cow dung.

The biodegradability ( $BOD_5/COD$ ) obtained in this study was in the range  $\leq 0.3$  of biodegradability ( $BOD_5/COD$ ) obtained from 24 out of 30 liquid fractions of digestates in the the previous chapter which indicated poor biodegradability.

#### 5.2.4. Spectral analysis of the liquid fraction of digestates

Organic matter complexity of the liquid fraction of digestates was investigated using both 3D fluorescence spectroscopy and UV-Visible spectrophotometry. The 3D spectra are shown in Figure 50 and the fluorescence spatialization as described by (Jimenez et al., 2014) is shown in Figure 51. Digestates were sampled at the end of the experiment from reactor R1 fed with straw, reactor R2 fed with cow dung, R3 fed with cow manure and R4 fed with cow manure.

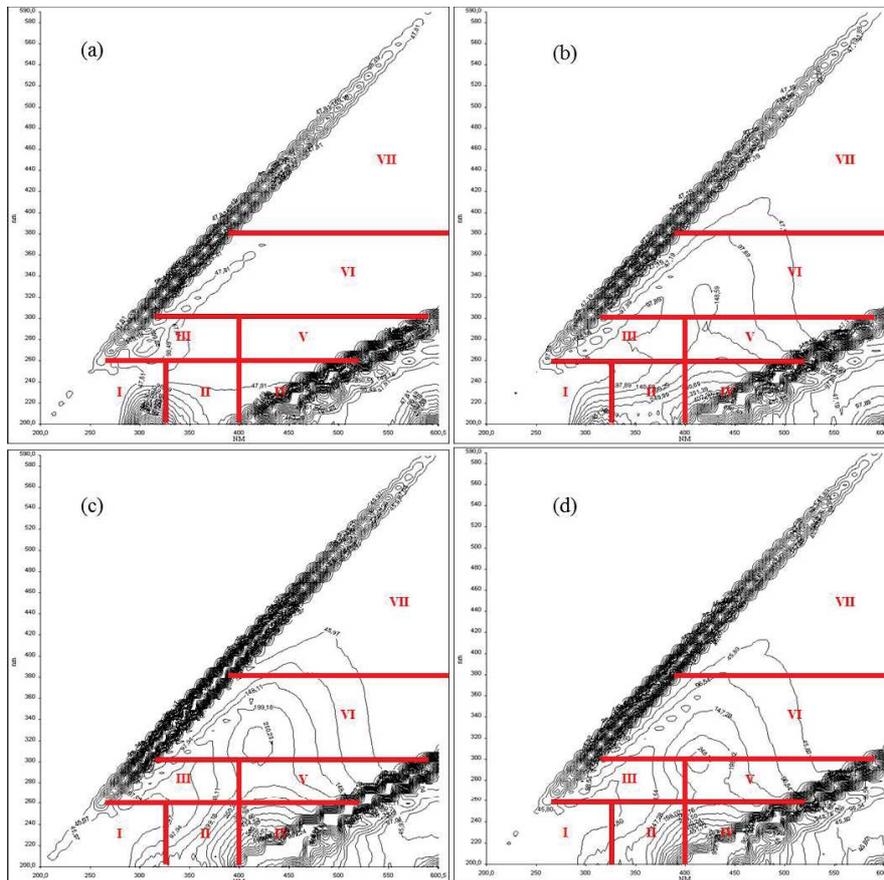


Figure 50: 3D fluorescence spectra of liquid fractions of digestates from (a) R1-straw (dilution 1/5000), (b) R2-cow dung (dilution 1/1000), (c) R3-cow manure (dilution 1/3000) and (d) R4-cow manure (dilution 1/3000)

Liquid fraction of digestate from reactor R1-Straw had the highest content of protein-like substances (Figure 51) with  $79 \pm 0.3$  % followed by reactor R2-Cow dung and reactor R4-Cow manure with  $45 \pm 0.6$  % and  $34 \pm 1.1$  %, respectively. Cow manure and cow dung mono-digestion showed the same range from 24 to 54% of protein-like as other liquids fraction of digestates from co-digestion (Chapter 4) whereas straw mono-digestion showed higher percentage of protein-like which was not degradable during the process due to its resistant properties to biodegradation (Chandra et al., 2012; Yang et al., 2015).

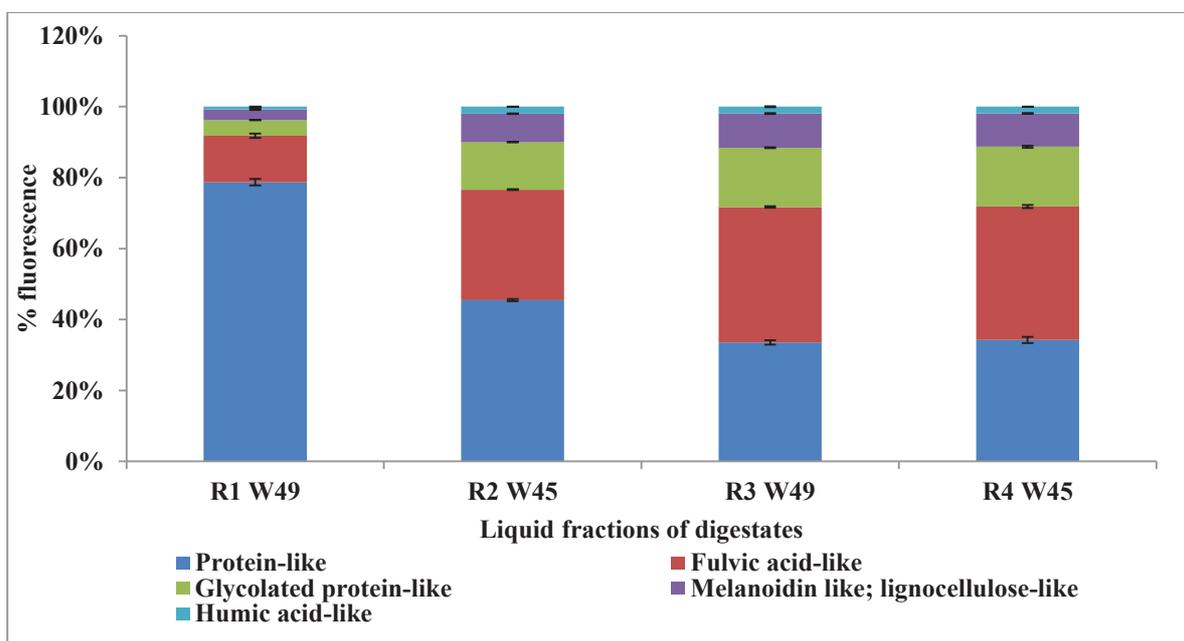


Figure 51: 3D fluorescence spatialization from 3D spectra of liquid fraction of digestates based on TOC (< 1 kDa) from R1-straw, R2-cow dung, R3-cow manure and R4-cow manure reactors

In contrast, mono-digestion of cow manure (R4) showed the highest percentage of more complex substances in the liquid fraction of digestate (66%) which is the higher range, from 46 to 76%, obtained in liquid fraction of digestate from co-digestion (Chapter 4). Mono-digestion of cow dung (R2) showed 55% of more complex substances whereas mono-digestion of straw (R1) showed the lowest value with 21%. Among the more complex compounds, liquid fraction of digestate from R4 fed with cow manure contained the highest percentage of fulvic acid-like with  $37.6 \pm 0.7$  %. The liquid fraction of digestate from R2-Cow dung had a quite close percentage ( $31.2 \pm 0.2$  %) while R1-Straw had a very low content with only  $13.1 \pm 0.1$  %. It was observed

that liquid fraction of digestate from cow manure contained a high percentage of fulvic acid-like in the upper values compared to the fulvic acid-like content (27 to 41%) obtained in liquid fraction of digestates from co-digestion in chapter 4.

The content of aromatic compounds was estimated using the SUVA ratio, measured for each reactor (Table 30).

Table 30: SUVA<sub>254</sub> (L/mg.m) of the digestates at the end of reactor operation

	Straw	Cow dung	Cow manure		
	R1W49	R2W45	R3W21	R3W49	R4W45
SUVA <sub>254</sub> (L/mg.m)	0.0	0.3	1.2	1.2	0.85

Complexity results were confirmed by SUVA<sub>254</sub> showing that R3 and R4 which were both fed with cow manure had SUVA<sub>254</sub> value of 1.2 and 0.85, respectively, which was in the range of fulvic acid from 0.6 to 3.9 L/mg.m (Zheng et al., 2014). In comparison, SUVA<sub>254</sub> for R2 was lower than 0.6 while R1-straw was 0. A slight decrease of SUVA<sub>254</sub> was only observed due to the impact of dilution as shown by R3 and R4.

Continuous stirring of R3 without feeding (between week 21 and week 49) did not show an increase of SUVA<sub>254</sub> which suggested that there was no increase of humification after continuous stirring of cow manure in the reactor. This result moderates the hypothesis made about the production of more aromatic compounds with longer degradation times, and probably explains that the correlation found in chapter 4 between SUVA<sub>254</sub> and HRT could be attributed to the indirect correlation between the HRT and the feedstock composition.

For humic acid-like compounds, all the liquid fractions of digestates had a very low content with a maximum of  $2.0 \pm 0.1$  % observed with cow dung as substrate, and a minimum of  $0.7 \pm 0.0$  % for the reactor fed with straw. Although the absence of feeding from week 21, the liquid fraction of digestate from reactor 3, showed no significant difference compared to the liquid fraction from the reactor 4 which was continuously fed with cow manure.

The results of spectral analysis show that the composition of the liquid fraction of digestates from reactors fed with cow manure and cow dung were very similar but both very different from the composition of the liquid fraction of digestate from a reactor fed only with straw. It can be concluded that the presence of complex molecules in the liquid fraction of digestates from a reactor fed with cow manure seems to be linked to cow dung. However, all the molecules described in this paragraph have a very low biodegradability, both aerobically and anaerobically, as discussed in the previous paragraph on aerobic biodegradability. Moreover, these results show that there is a major impact of the type of substrate used on anaerobic digestion on the digestate quality and complexity.

#### **5.2.5. TKN concentrations**

TKN concentration in the liquid fractions of digestates (Figure 52) presents the same pattern as  $\text{NH}_4^+$  (Figure 39). Due to no presence of nitrate and nitrite detected in liquid fraction of all digestates, TKN concentration is equal to total nitrogen concentration. Initial TKN concentration in liquid fraction of digestate from R1, R2, R3 and R4 was between 0.25 and 0.27 g N-NTK/L.

The period when the reactors were operated with increasing OLRs in the reactors had shown increasing TKN concentrations in liquid fraction of all digestates, as both  $\text{NH}_4^+$  concentration in the reactors and TS in the liquid fractions increased. The values measured in weeks 15 to 21 are difficult to interpret for reactors R2, R3 and R4 as high differences in the values can be observed for quite close weeks, though the other parameters measured had logical evolutions. This might be linked to some analytical problems with the measurement of  $\text{NH}_4^+$  during that period. For reactor R1-straw, the decrease in TKN concentration is linked to the consumption for bacterial growth.

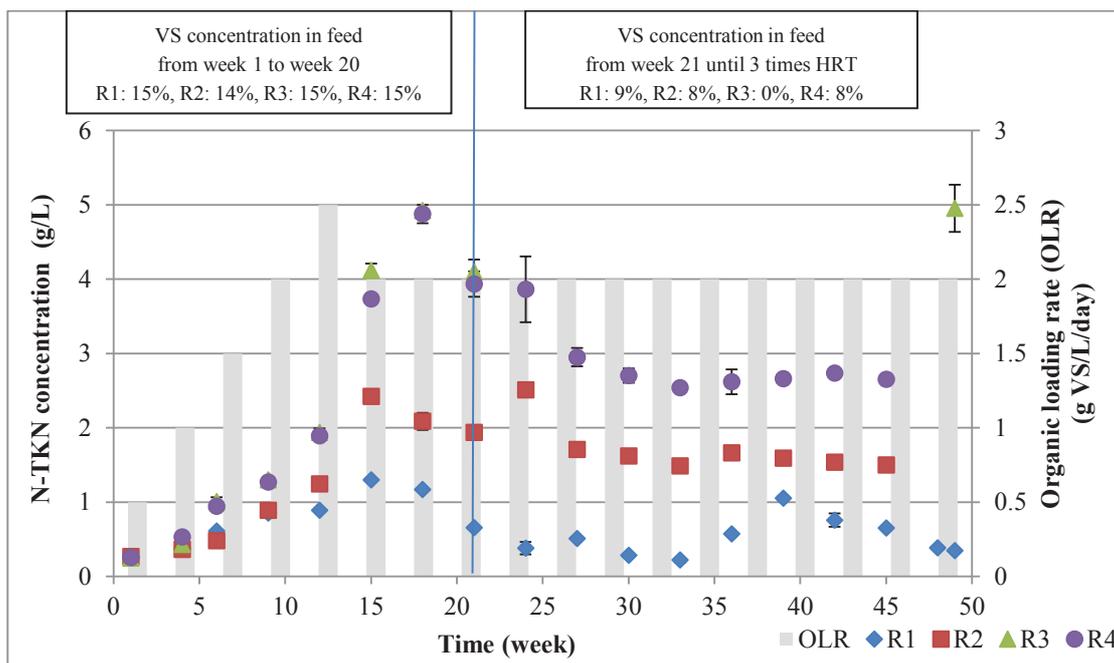


Figure 52: TKN concentrations in liquid fraction of digestates from reactors R1-straw, R2-cow dung, R3-cow manure and R4-cow manure

After week 21, when the OLR was maintained constant and the feed was diluted, TKN concentrations in the liquid fraction of reactors R2-Cow dung and R4-Cow manure reached a value around  $1.54 \pm 0.04$  and  $2.68 \pm 0.04$  g N-NTK/L, respectively. The higher TKN concentration in the liquid fraction of digestate from reactor R4-Cow manure compared to that from reactor R2-Cow dung can be mostly explained by the difference in the TS content (see Figure 44) of the 2 liquid fractions as the  $\text{NH}_4^+$  content in the 2 reactors was very close (see Figure 39). The TKN content in the liquid fraction of the digestate from reactor R1-Straw varied greatly as a consequence of the low nitrogen content of straw which requested to add  $\text{NH}_4^+$  to avoid nitrogen deficiencies and TKN concentration remained low (below 1 gN-NTK/L). This was due to low initial TKN of the straw with  $3.8 \pm 0.9$  g N/kg and its high VS concentration which required a high dilution factor for this substrate in order to maintain 8% VS in the feed. For mono-digestion of cow manure in reactor R3, continuous mixing without feeding starting at beginning week 21 until week 49 had showed to increase TKN concentration by 20.8% in its liquid fraction of digestate with final TKN concentration of  $5.0 \pm 0.3$  g N-NTK/L.

In this study, mono digestion of cow dung and cow manure showed TKN concentrations in liquid fraction of digestates in the same range as for co-digestion at industrial scale (1.3 to 6.5 g N/L, Chapter 4) or obtained from co-digestion of cattle slurry and maize (from 2.7 to 3.5 g N/L (Cavalli et al., 2016)).

### 5.2.6. Total organic carbon (TOC) and inorganic carbon (IC)

Total Organic Carbon (TOC) after filtration at 1.2  $\mu\text{m}$  (colloidal and dissolved fractions) then after filtration at 1 kDa (dissolved fraction) was measured on day 45 for the samples taken from the reactors R1, R2 and R4 as shown in Table 31. For reactor R3, the results are reported for the first week without feed (week 21) and the last week of operation (week 49).

The values measured at steady state show that dissolved TOC concentration is low for all reactors R1, R2 and R4 with an average ratio COD/TOC of 3.02 g  $\text{O}_2/\text{g C}$ , which is similar to the ratios generally measured for raw wastewater (3 g  $\text{O}_2/\text{g C}$ ).

TOC concentration from colloids follows the same trend as that of COD with the higher concentration for R4-Cow manure, followed by R1-straw and finally R2-Cow dung. However, different ratios COD/TOC have been measured with 4.61 and 4.06 g  $\text{O}_2/\text{g C}$  for the reactors fed with Straw and Cow dung, respectively, and only 2.64 g  $\text{O}_2/\text{g C}$  for reactor R4-Cow manure. These results suggest that the nature of compounds from the colloids are close for the digestate from R1-Straw and R2-Cow dung but very different for the digestate from cow manure which contains compounds with a high degree of oxidation.

Table 31: TOC of colloids and dissolved matter and IC at the end of the experiments for reactors R1-straw, R2-cow dung, R3-cow manure and R4-cow manure.

	Week	TOC of colloids (g/l)	TOC in dissolved matter fraction (g/l)	IC in dissolved matter fraction (g/l)
R1-Straw	45	1.2	1.02	0.515
R2-Cow dung	45	0.9	0.81	1
R4- Cow manure	45	2.5	1.19	1.77
R3-Cow manure	21	2.3	1.69	2.73
	49	2.2	1.6	3.04

Inorganic carbon (IC) concentrations in the liquid fraction of digestates from reactors R1, R2, and R4 are shown in Table 31. IC concentration in reactor R1-Straw is lower than for the 2 other reactors and was increased by the addition of NaOH 32%, as described previously in order to maintain the pH. For reactor R3-Cow manure results (Table 31) show that a long period without feed had no major evolution for TOC and IC concentrations.

### **5.2.7. Cations and anions concentrations**

Dissolved Potassium, Sodium and Chloride concentrations in liquid fraction of digestates and their evolution over time are shown in Figure 53.

Potassium concentration in the liquid fraction of digestates from R1-straw and R2-cow dung was low and below 1 g/L throughout the experiment indicating that the potassium concentration in both substrates was low. In contrast, potassium concentration in R4-cow manure increased dramatically during the first 20 weeks of reactor operation, then decreased when substrate dilution was increased and stabilized around 5 g/L towards the end of the experiment. Final potassium concentrations were 0.41 g/L for reactor R1-Straw (week 45), 0.37 g/L for reactor R2-Cow dung (week 45) and 5.27 g/L for reactor R4-Cow manure (week 42). The high difference in  $K^+$  concentrations between cow dung and cow manure can be explained by the presence of urine in cow manure whereas cow dung contains only feces. Indeed, about 75% of  $K^+$  is excreted by cows by urine.

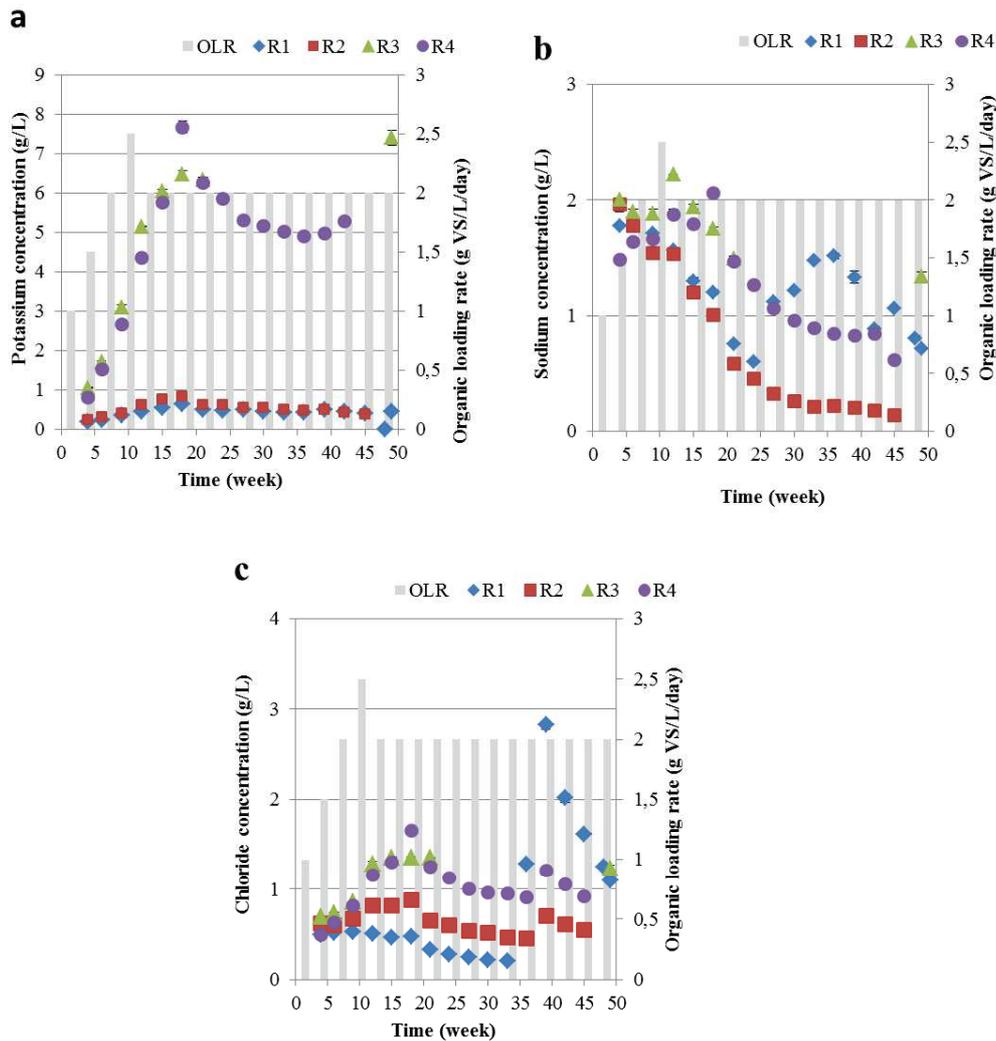


Figure 53: Evolution over time of the concentration in dissolved ions in liquid fraction of digestates from reactors R1-straw, R2-cow dung, R3-cow manure and R4-cow manure: a) Potassium; b) Sodium; c) Chloride

Initial  $\text{Na}^+$  concentration in the liquid fraction of digestates was  $1.78 \pm 0.00$ ,  $1.96 \pm 0.06$ ,  $2.00 \pm 0.01$  and  $1.49 \pm 0.18$  g/L for reactors R1, R2, R3 and R4, respectively, (see Figure 53-b). This figure shows clearly a washout of  $\text{Na}^+$  ions with time in reactors R2-Cow dung and R4-Cow manure. The liquid fraction of the digestate from R2-cow dung was shown to contain a lower  $\text{Na}^+$  concentration followed by that of cow manure with final concentrations at week 45 of  $0.17 \pm 0.03$  and  $0.71 \pm 0.09$  g/L, respectively. For R1-straw,  $\text{Na}^+$  concentration in liquid fraction of digestate decreased until week 24 but increased thereafter due to the addition of 5 mL NaOH

32% per week from week 24 to week 34 and again in week 45 due to addition of 10 mL of NaOH 32% in week 43 to maintain the pH. Final Na<sup>+</sup> concentration in liquid fraction of digestate from R1 in week 49 was  $0.72 \pm 0.00$  g/L. After the stop of feeding in R3 a small decrease in Na<sup>+</sup> concentration was observed with a final concentration of  $1.34 \pm 0.18$  g/L. Sodium is an important parameter to measure in products intended for land spreading but this parameter remained low in the liquid fraction of digestates and was below 1 g/L at steady state

The concentration of chloride (Cl<sup>-</sup>) and its evolution over time in liquid fraction of digestates from reactors R1, R2, R3 and R4 are shown in Figure 53-c. Cl<sup>-</sup> concentration at initial week 1 for R1, R2, R3 and R4 was  $0.50 \pm 0.00$ ,  $0.62 \pm 0.00$ ,  $0.69 \pm 0.01$  and  $0.50 \pm 0.01$  g/L for reactors R1, R2, R3 and R4, respectively. For reactor R1 fed with straw, continuous feeding decreased Cl<sup>-</sup> concentration in liquid fraction of digestate until lowest Cl<sup>-</sup> concentration of  $0.21 \pm 0.00$  g/L in week 33. However, Cl<sup>-</sup> concentration increased from week 36 to week 39 up to  $2.83 \pm 0.05$  g/L in week 39 due to the addition of 40 mL NH<sub>4</sub>Cl (30 g/L) in the dilution water from week 34 to week 38 as shown in Figure 39. Cl<sup>-</sup> concentration in liquid fraction of digestate in reactor R1 then continued to decrease with final concentration of  $1.09 \pm 0.02$  g/L in week 49. For reactor R2 fed with cow dung, Cl<sup>-</sup> concentration in liquid fraction of digestate was always below 1g/L and, at steady state; it was  $0.62 \pm 0.07$  g/L. For reactor R4-Cow manure, Cl<sup>-</sup> concentration in liquid fraction of digestate increased to highest concentration of  $1.66 \pm 0.02$  g/L in week 18. After decreasing VS concentration of the feed, beginning of week 21, it slightly decreased and reached a Cl<sup>-</sup> concentration at steady state of around 1 g/L. As expected, for reactor R3 fed with cow manure, Cl<sup>-</sup> concentration was fairly constant after the stop of feeding with  $1.35 \pm 0.00$  g/L in week 21 and  $1.24 \pm 0.03$  g/L in week 49.

### **5.3. Water extraction of straw, cow dung and manure**

In order to help understanding the results on the composition of the liquid fraction of digestates obtained with the reactors fed with the 3 substrates, that is to say straw, cow dung and cow manure, water extraction experiments were carried out with the initial substrates used to feed the reactors. The aim of these experiments was to evaluate the quantity of compounds which can be released in water by the different substrates. The experiments consisted of diluting a given

quantity of raw substrates in water according to the conditions presented in the first 2 columns of Table 32, and maintaining the system under agitation for 1 day. The initial VS concentrations of these experiments were chosen to be the same than these in the feed of the reactors.

### 5.3.1. Chemical Oxygen Demand (COD) in supernatant

Figure 54 shows the COD in supernatant after 24 hours of water extraction. Optimal time obtained for water extraction for all substrates was 6 hours. Indeed, after 6 h, the COD extracted was constant. The lowest COD was extracted from straw with  $3.36 \pm 0.05$  g O<sub>2</sub>/L in the supernatant, followed by cow dung with  $9.6 \pm 0.1$  g O<sub>2</sub>/L and cow manure with  $22.2 \pm 2.8$  g O<sub>2</sub>/L. As a consequence, the quantity of COD extracted per g of VS added was low for straw with 33 mg COD<sub>extracted</sub>/g VS and much higher for cow dung and cow manure with 109 and 242 mg COD<sub>extracted</sub>/g VS, respectively, see (Table 32). A higher solubilization of organic matter from the substrate which biodegradability could be low, can be one of the phenomena which could explain the higher COD measured in liquid fraction of digestate from mono-digestion of manure compared to cow dung and straw, as shown in Figure 47.

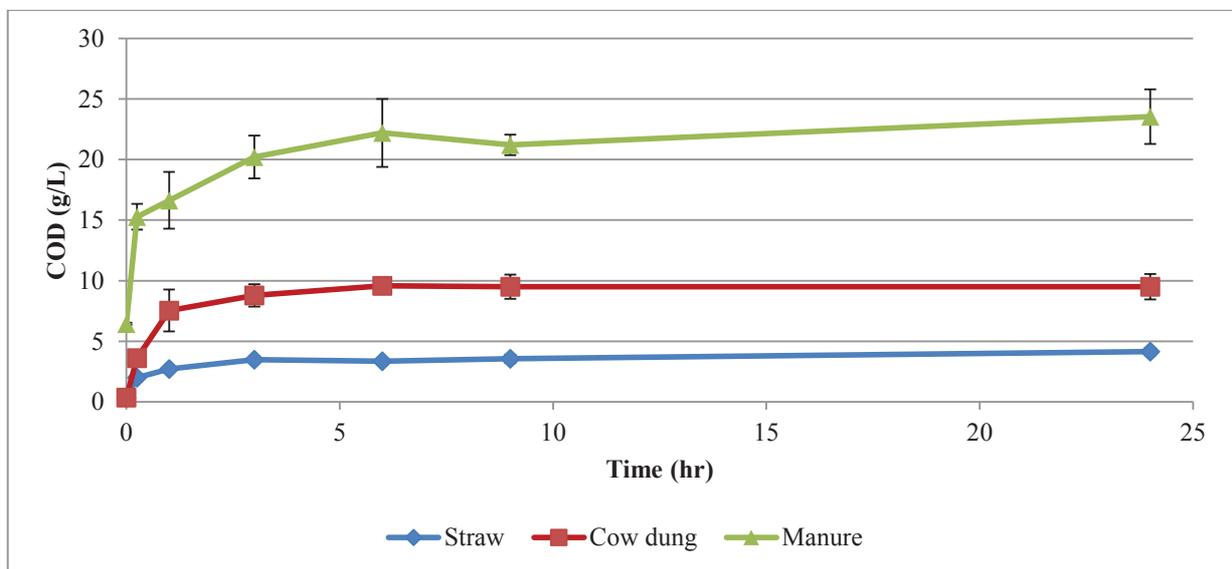


Figure 54: COD in supernatant after water extraction for straw, cow dung and cow manure

Table 32: Operating conditions and extracted COD during water extraction experiments from straw, cow dung and cow manure

	Quantity of substrate added	Volume of water added	Extracted COD after 6 h	Extracted COD/g TS <sub>added</sub>	Extracted COD/g VS <sub>added</sub>
	(g)	(mL)	(g/L)	(mg COD/g TS)	(mg COD/g VS)
<b>Straw</b>	27.9	221.1	3.36	30	33
<b>Cow Dung</b>	138.5	111.5	9.6	89	109
<b>Cow manure</b>	106.4	143.6	22.2	121	242

Figure 55 shows the distribution of the solubilized COD after successive filtrations at 1.2  $\mu\text{m}$  and 1 kDa. As previously, COD was divided into 3 fractions: suspended particle ( $> 1.2 \mu\text{m}$ ), colloids (1.2  $\mu\text{m}$  to 1 kDa) and soluble matter ( $< 1 \text{ kDa}$ ). The released organic matter had a very different profile according to the type of substrate as shown in Figure 55. Indeed, released COD was the highest for cow manure and consisted mainly of colloids with 13.6 g O<sub>2</sub>/L which represented 61% of total COD released. Cow dung mostly released suspended particles with 6.5 g O<sub>2</sub>/L that is to say 68% of total COD released by cow dung. For both cow manure and cow dung, the dissolved organic matter represented only a small fraction of released COD with 2.5 g O<sub>2</sub>/L (11% of total COD) and 0.76 g O<sub>2</sub>/L (8% of total COD), respectively. Straw released the smallest quantity of COD with a more even distribution consisting of 45% of suspended solids, 31% of dissolved matter and 24% of colloids.

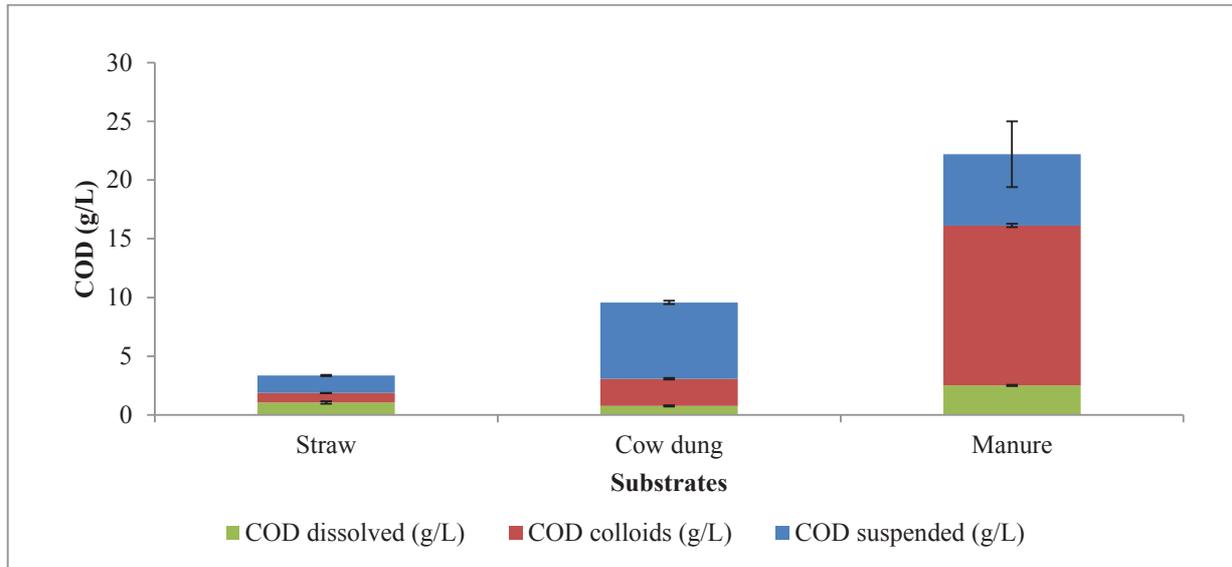


Figure 55: COD fractionation after water extraction for 6 hours for straw, cow dung and cow manure

Table 33 presents a summary of: (i) COD which could be released in the liquid supernatant from the substrate by water extraction for straw, cow dung and cow manure and its fractionation and; (ii) COD in the liquid fraction of digestates from mono-digestion of straw, cow dung and cow manure and its fractionation. From this table, 2 curves have been drawn in order to compare COD possibly released from water extraction and COD of the liquid fraction of the digestates after centrifugation (Figure 56) and to compare the distribution of the released COD for both kind of liquids (Figure 57).

Figure 56 shows that there is a correlation between COD released by water extraction and final COD of the liquid fraction of the digestates after centrifugation. This result suggests that some of the organic matter of the substrates, which can be easily solubilized, might not be eliminated during anaerobic digestion and its accumulation in the reactors might contribute significantly to the COD in the liquid fraction of the digestates after centrifugation. This phenomenon could be one of the parameters which could explain the high COD observed in the liquid fraction of the digestate from the reactor fed with cow manure.

Table 33: Summary of COD and its fractionation in liquid fraction of digestates from mono-digestion and liquid supernatant from water extraction of straw, cow dung and cow manure

	R1-Straw	Straw extraction	R2-Cow dung	Cow dung extraction	R4-Cow manure	Cow manure extraction
COD in Liquid fraction of digestate or liquid supernatant (g O <sub>2</sub> /L)	15.5	3.01	23.6	8.43	48.5	18.54
<b>COD Fractionation</b>						
COD of suspended solids (g O <sub>2</sub> /L)	7.06	1.34	17.19	5.71	38.6	5.09
COD of colloids (g O <sub>2</sub> /L)	5.35	0.76	3.73	2.02	6.51	11.36
COD of dissolved matter (g O <sub>2</sub> /L)	3.06	0.91	2.66	0.70	3.31	2.09
<b>COD Distribution</b>						
Fraction of suspended COD	45%	45%	73%	68%	80%	28%
Fraction of colloids COD	35%	25%	16%	24%	13%	61%
Fraction of Soluble COD	20%	30%	11%	8%	7%	11%

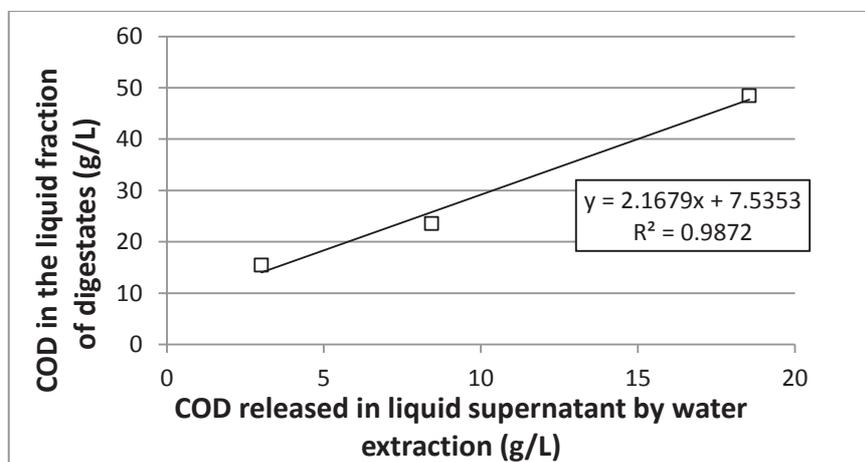


Figure 56: COD released in liquid supernatant by water extraction versus COD in the liquid fraction of digestates from mono-digestion of straw, cow dung and cow manure

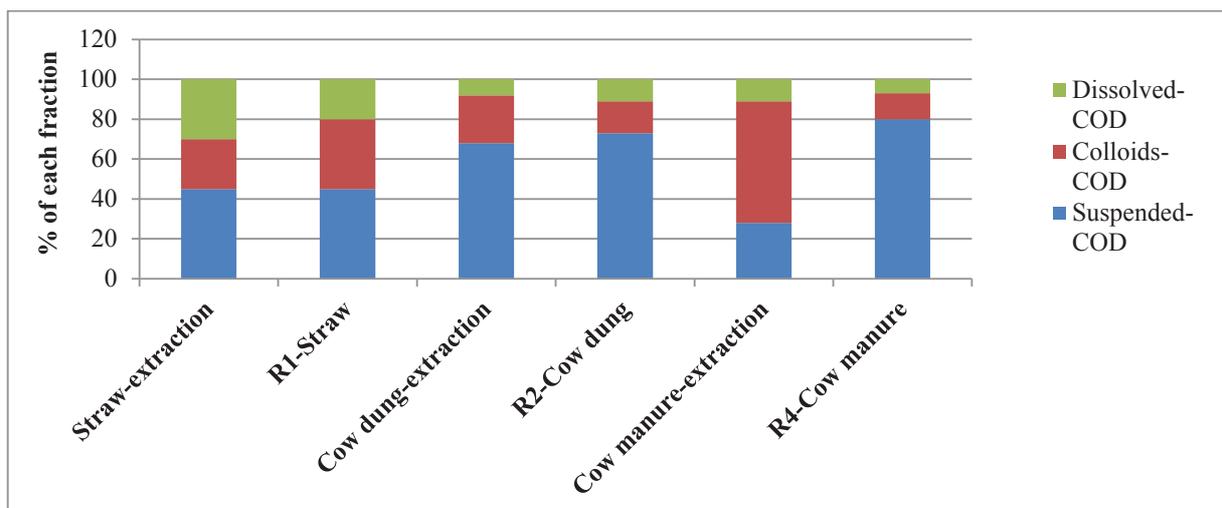


Figure 57: Distribution of COD released in the liquid supernatant of water extraction and in the liquid fraction of digestates from mono-digestion of straw, cow dung and cow manure after successive filtrations at 1.2  $\mu\text{m}$  and 1 kDa

Figure 57 represents the distribution of COD into 3 fractions after filtration at 1.2  $\mu\text{m}$  followed by filtration at 1 kDa. For straw and cow dung, distribution of COD in the liquid supernatant by water extraction and of COD in the liquid fraction of digestates was not very different. In contrast, for cow manure, the 2 liquid fractions were completely different. Indeed, in the supernatant from water extraction, colloids were the dominant form of COD, with 61%, while they represented only 13% of total COD in the liquid fraction of digestate from R4-Cow manure. For this liquid fraction, suspended solids represented 80% of total COD. This change in the distribution of the organic matter could be explained by the destructuring of the colloids in the reactor, eventually followed by anaerobic degradation, or by the agglomeration of the colloids into big particles with size greater than 1.2  $\mu\text{m}$  which cannot be separated by centrifugation.

### 5.3.2. Spectral analysis of water extracts from substrates

3D fluorescence spectra of water extraction of straw, cow dung and cow manure are shown in Figure 58 and their fluorescence spatialization is shown in Figure 59.

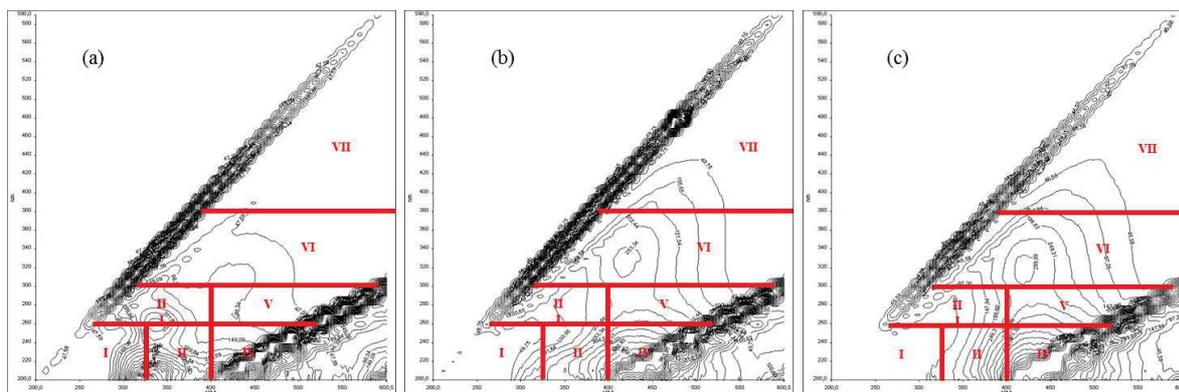


Figure 58: 3D fluorescence spectra of (a) straw (dilution 1/500), (b) cow dung (dilution 1/400) and (c) cow manure (dilution 1/1500)

The supernatant from water extraction of cow manure had the lowest fluorescence of protein-like compounds with  $25.1 \pm 0.3$  % compared to straw with the highest  $71.3 \pm 0.5$  % followed by cow dung with  $70 \pm 2$  %. In contrast, cow manure contained highest fluorescence of more complex compounds (75%) compared to cow dung (30%) and straw (29%) with  $40.54 \pm 0.03$  % of fulvic acid-like,  $20.3 \pm 0.1$  % glycolated protein-like,  $11.5 \pm 0.1$  % of melanoidin-like and  $2.6 \pm 0.1$  % humic acid-like. This can explain the high fluorescence percentage of more complex compounds of 66% after mono-digestion of cow manure as shown in Figure 59 as these compounds are already present in cow manure. In comparison to cow dung, which contained high fluorescence percentage of protein-like but low fluorescence percentage of more complex compounds (Figure 59), anaerobic digestion process increased the percentage of more complex compounds in dissolved matter (Figure 59) as shown by (Jimenez et al., 2014). This is due to the biodegradation of proteins and to recalcitrant compounds formation during anaerobic digestion. Moreover, if a comparison is made between the 3D spectra from water extraction of substrates and those from the respective digestates, the complexity observed in the substrates seems to impact the complexity of digestate. For example, the zone related to humic-substances-like analyzed for cow dung and cow manure water extractions are similar in the liquid phase spectra of their digestate. In addition, the zones 2 and 3 related to proteins-like observed on the water extraction of cow dung and wheat straw disappeared after anaerobic digestion, probably biodegraded as previously said.

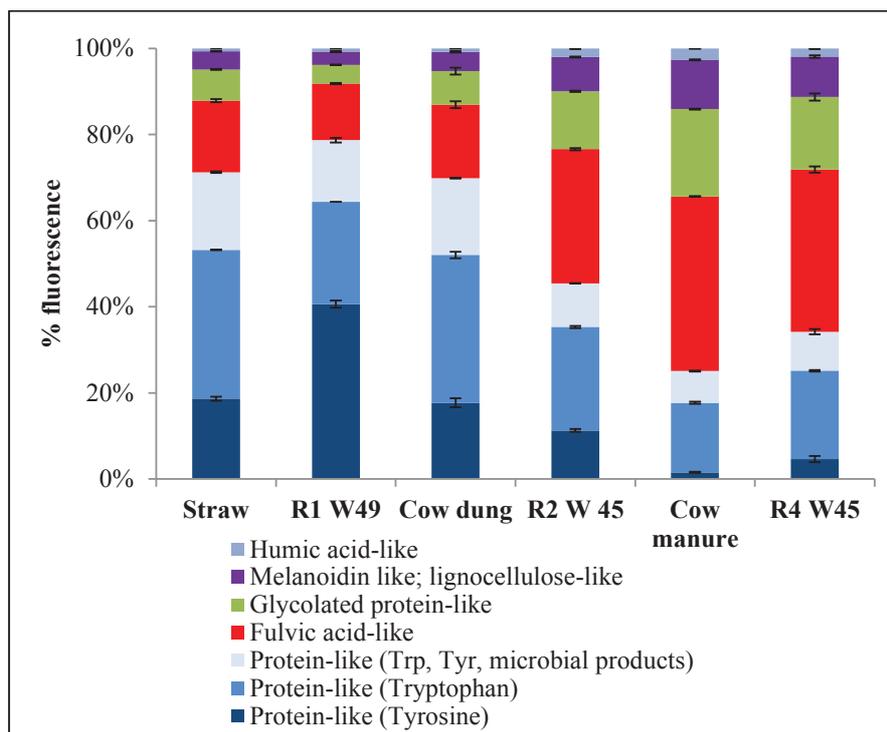


Figure 59: 3D fluorescence spatialization from 3D spectra of liquid fraction of digestates based on TOC (<1kDa)

Concerning straw, the supernatant from water extraction shows a large part of protein-like molecules as for the liquid fraction of straw digestate. If a comparison is made between these two samples on the fluorescence distribution of proteins-like zones (1 to 3) as proposed in Figure 59, zones 2 and 3 decreased and zone 1 increased. A fraction of the protein-like compounds from zones 2 and 3 was probably biodegraded and/or absorbed on the solids of the digestate. Concerning zone 1, this latter increased in the digestate. The same observation is made for cow manure (e.g. composed also of wheat straw) but not for cow dung. One hypothesis on the origin of these compounds could be the production of other molecules released during anaerobic digestion. Indeed, wheat straw is a lignocellulosic compound. During anaerobic digestion, lignin would be partly destructured and its components released in the supernatant. Phenol-like compounds are components of lignin. Moreover, these compounds are naturally fluorescent at similar excitation/emission wavelength as proteins (e.g. 210-270/300) (Tchaikovskaya et al., 2000). This could be an explanation for the increase of zone 1 after digestion of wheat straw,

phenomenon not observed with cow dung. In all cases, as proposed previously, substrate quality has an impact on the quality of the liquid fraction of digestate after solid-liquid separation.

UV-absorbance was measured at 254 nm, and the relative SUVA<sub>254</sub> calculated for each substrate after water extraction and anaerobic digestion, is shown in Table 34. It was observed that after water extraction, straw released quite low amount of soluble aromatic compounds, contrary to cow manure, which released high amount of soluble aromatic compounds. This can be due to the lignin structure of straw which is insoluble in water. In contrast, cow dung and cow manure released high amount of aromatic compounds which results from the previous microbial hydrolysis.

Table 34: SUVA and A (254 nm) after water extraction and anaerobic digestion

	Straw	Cow dung	Cow manure
SUVA <sub>254</sub> (L/mg.m) after water extraction	0.03	0.8	3.0
A <sub>254</sub> removal after AD (%)	93%	-27%	19% + 9% during starvation period

With the same concentrations as the feed to the reactor as water extraction, it was observed that straw had the highest removal of absorbance with 93%. There was only low absorbance removal of cow manure with 19% after digestion, the assumption can be made for the removal of absorbance, can be due either that the aromatic compounds were digested during the process or being adsorbed onto biomass. Contrary to the assumption of increasing of aromatic compounds and humification due to anaerobic digestion process, another 9% was removed after starvation period (28 weeks of continuous stirring without feeding in manure fed reactor). In comparison, cow dung digestate was observed with higher aromatic compounds after anaerobic digestion process. This increase can be explained by the released of aromatic compounds from the substrate or by the production of exopolymeric substances by microorganisms.

### 5.3.3. Potassium concentrations

As high concentrations of  $K^+$  were found for the liquid fraction of the digestate from the reactor fed with cow manure, this ion was measured in the supernatant from water extractions. The concentration in the liquid phase after 6 h of extractions were 0.36 g of  $K^+$ /L for R1-Straw, 0.41 g of  $K^+$ /L for R2-Cow dung and 4.16 g of  $K^+$ /L for R4-Cow manure. This corresponds to the release of 4, 5 and 45 mg of  $K^+$ /g VS added for R1-Straw, R2-Cow dung and R4-Cow manure, respectively. The results obtained in the water extraction experiments confirm that cow manure can release about 10 times more  $K^+$  in the liquid phase than straw or cow dung due to the presence of urine.

### 5.4. Discussion

Main results on residual compounds produced at steady state in the liquid fraction obtained after centrifugation of digestates from the lab-scale CSTR reactors are summarized in Table 35. It was observed that the cow manure fed reactor contained highest TS and VS concentrations in liquid fraction of digestate followed by cow dung and straw fed reactors. As a consequence, cow manure fed reactor was shown to contain highest COD in liquid fraction of digestate. This result can be explained by the kind of cow manure used which had already undergone a significant aerobic degradation, when straw and cow dung were fresh samples which were not stored. COD distribution highlighted a similar behavior between cow manure and cow dung with most of COD as suspended particles. The composition of the liquid fraction from the reactor fed with straw only suggests that this substrate might be a significant source of colloids in the liquid fractions of digestates. These results show that liquid fractions of digestate have quite high COD with a high proportion of small particles. The addition of a coagulant before centrifugation should be a way to decrease dramatically TS concentration and COD of the liquid fractions.

All liquid fractions of digestates showed very low aerobic biodegradability with the lowest from cow manure and cow dung fed reactors indicating that an aerobic post treatment for example, by an activated sludge system, cannot be envisaged, even after removal of colloids and suspended

particles, as removal efficiency will be very low. This result was due to the accumulation of complex compounds like humic substances in liquid fraction of all digestates.

In addition, cow manure fed reactor was shown to contain highest total nitrogen in liquid fraction of digestate compared to cow dung fed reactor. In contrast, straw fed reactor showed lack of total nitrogen supply and nitrogen needs to be added either by the addition of a chemical product or of a co-substrate rich in nitrogen such as pig manure. With respect to the ion concentrations, the main difference was observed with potassium with low concentration in the liquid fraction of digestates from straw and cow manure and a more than 10 times higher concentration in liquid fraction from cow manure. As cow manure contains feces and urine, the high  $K^+$  content is linked to the presence of urine in cow manure.

Table 35: Summary of the characteristics of the liquid fraction of digestates from steady-state lab-scale CSTR reactors

	R1-Straw	R2-Cow dung	R4-Cow manure
TS concentration (g/L)	14,5	19,4	44,5
VS concentration (g/L)	10,2	14,9	30
COD <sub>t</sub> (g/L)	15,5	23,6	48,5
% COD <sub>suspended</sub>	46%	73%	80%
% COD <sub>colloids</sub>	35%	16%	13%
% COD <sub>soluble</sub>	20%	11%	7%
BOD <sub>5</sub> (g/L)	3,4	3,6	5,7
BOD <sub>21</sub> (g/L)	6,7	10,4	13,7
BOD <sub>5</sub> /COD	0,24	0,15	0,12
BOD <sub>21</sub> /COD	0,44	0,25	0,28
N-TKN concentration (g/L)	N added	1,54	2,68
$K^+$ concentration (g/L)	0,41	0,37	5,27
$Na^+$ concentration (g/L)	$Na^+$ added	0,17	0,7
$Cl^-$ concentration (g/L)	$Cl^-$ added	0,62	1

The results of the characterization of the liquid fraction of digestate from the reactor fed with cow manure were compared with the composition of 4 liquid fractions of digestates from industrial-scale digesters with high cow manure share (52 to 60%) and which characterization was described in chapters 3 and 4, see Table 36.

Table 36: Comparison of the characteristics of the liquid fraction digestates from lab-scale cattle manure monodigestion and full-scale manure codigestion.

	R4-Cow manure	Digestate B	Digestate F	Digestate Q	Digestate U
Cow Manure content	100%	60%	52%	54%	56%
TS concentration (g/L)	44,5	80,9	82,7	72,6	66,2
VS concentration (g/L)	30	52,3	44,5	48,1	41,7
VS/TS (g/L)	0,67	0,65	0,54	0,66	0,63
COD <sub>t</sub> (g/L)	48,5	78	70,3	56,7	67,9
% COD <sub>suspended</sub>	80%	86%	81%	91%	83%
% COD <sub>colloids</sub>	13%	8%	6%	4%	8%
% COD <sub>soluble</sub>	7%	6%	12%	4%	9%
BOD <sub>5</sub> (g/L)	5,7	5,6	3,7	7,4	13
BOD <sub>21</sub> (g/L)	13,7	12,9	9,8	18,2	30,6
BOD <sub>5</sub> /COD	0,12	0,1	0,1	0,1	0,2
BOD <sub>21</sub> /COD	0,28	0,2	0,1	0,3	0,5
N-TKN concentration (g/L)	2,68	4,7	5,8	3,8	5,7
K <sup>+</sup> concentration (g/L)	5,27	4,7	4,7	3,6	6,7
Na <sup>+</sup> concentration (g/L)	0,7	0,5	1,1	0,6	0,3
Cl <sup>-</sup> concentration (g/L)	1	13	1,8	1,2	1,7

Table 36 shows that the composition of all liquid fractions was quite close and it is possible to draw the main characteristics of liquid fractions of digestate with a high percentage of cow manure:

- They all had high organic matter content made-up for more than 80% of non-separated solids and a very low fraction of soluble compounds. The difference in concentrations of TS and VS and COD between the liquid fractions from industrial plants and that from reactor R4 can be explained by a different separation technique. Indeed, the digestate from industrial plants were all separated by screw press when centrifugation was used at laboratory scale
- All liquid fractions had low aerobic biodegradability indicated by low DBO<sub>5</sub>/COD and BOD<sub>21</sub>/COD ratios.
- Furthermore, NTK and K<sup>+</sup> concentrations were quite high with values in the range 2.68 – 5.8 and 3.6 – 6.7, respectively. As explained before, cow urine plays an important role in these concentrations.

## 5.5. Conclusion

In this chapter on mono-digestion of 3 substrates, straw, cow dung and cow manure, 3 reactors were operated for 48 weeks, that is to say until steady state were achieved in all reactors, at OLR of 2 g VS/L/day and VS concentration in the feeds of 8-9%. Biogas and methane productions achieved in stable state showed that straw had the highest methane yield followed by cow dung and cow manure. pH was stable for cow dung and cow manure fed reactors whereas pH for straw fed reactor was decreasing due to lack of NH<sub>4</sub><sup>+</sup> and it was necessary to control pH. In the reactors, highest TS concentrations were observed from cow manure fed reactor followed by cow dung and straw indicating that cow manure had the lowest biodegradability. It is important to notice that the cow manure used in this work had low biodegradability and methane yield indicating that it was old manure which had already undergone some significant aerobic biodegradation during storage. For reactor R3-Cow manure, continuous mixing without feeding did not change significantly the composition of the digestate indicating that there was no major degradation of the accumulated organic matter during mixing only.

This mono digestion study showed that the type of substrate has main contribution to residual compounds produced in liquid fraction of digestates. Indeed, the results confirmed that cow

manure could generate high organic matter content in liquid fraction of digestate. However, it is difficult to assign a specific influence of straw or cow dung as both liquid fractions had a lower organic content than that from cow manure. The different results for cow manure can be attributed to the fact that it was old manure which had already undergone aerobic degradation. Furthermore, urine which is not present in cow manure or straw might have played a role, as shown for example for potassium. This was also supported from the water extraction experiments where cow manure extraction was shown to contain highest COD followed by cow dung and straw. In all the liquid fractions of all mono-digestates, the highest fraction of COD was in suspended solid fraction, but this fraction was less predominant in the case of wheat straw. In contrast, cow manure water extraction showed a highest fraction of COD in colloids fraction, which was partially biodegraded or flocculated during anaerobic digestion. Concerning fluorescent compounds extracted from the substrates, wheat straw and cow dung ones presented a lower complexity than old cow manure ones. The most significant change after anaerobic digestion was observed for cow dung with an increase in the complexity of fluorescent compounds.

## CONCLUSION AND PERSPECTIVES

Anaerobic digestion produces two main products: biogas and digestate. Biogas is valorized by the production of energy and the digestate, generally as fertilizer by land application. In the case of low solids anaerobic digestion, also called wet anaerobic digestion, which represents the highest number of digesters for solid substrates in the world, the digestate has a rather low TS concentration, generally below 12%. As a consequence and in order to reduce the costs, a solid-liquid separation is provided allowing to produce a solid fraction of digestate, with TS concentration of about 30%, more suitable for land application. During solid-liquid separation, huge volumes of effluent are produced that need to be addressed. However, the literature review revealed a significant lack of data on the liquid fraction of digestate which can be problematic for the design and sizing of treatment solutions for these effluents. Accordingly, the purpose of this thesis was to provide new and comprehensive information on the composition of the liquid fraction of digestates which will be useful for both researchers and engineers in the design of innovative treatment solutions.

In this thesis, two complementary approaches have been used for studying the liquid fraction of digestates: i) collecting digestates from full scale and ii) producing digestates at lab-scale. Each approach presents its own advantages and limitations, indeed:

- At lab-scale, separation process by screw press, vibrating screen and rotary drum is difficult to reproduce. Therefore, only centrifuge can be performed. Furthermore, operating lab-scale reactor allowed well controlled conditions though obtaining steady-state conditions requires several months and the number of lab-scale digestates cannot be multiplied.
- In full-scale, it is possible to obtain a high number of different digestates but it is very difficult to obtain thorough information from process parameters and feedstock composition. In particular, if the nature of the feedstocks is well defined, their exact proportion is more uncertain. In addition to that, feedstock such as cow manure can have different composition itself as it was pre-mixed with straw that are used for bedding. The mixing ratio of cow dung and straw (manure) for each co-digestion plants can highly vary from one to other.

Eleven liquid fractions of digestates obtained after solid-liquid separation at full-scale co-digestion plants treating solid wastes were fully characterized in detail with the measurement of

about 26 physico-chemical and biological parameters. Each liquid fraction of digestate was fractionated by successive filtrations at different sizes (100  $\mu\text{m}$ , 41  $\mu\text{m}$ , 10  $\mu\text{m}$ , 1.2  $\mu\text{m}$ , 0.45  $\mu\text{m}$ , 0.2  $\mu\text{m}$ , 100 kDa, 10 kDa and 1 kDa). From the size fractionation, the organic matter was then grouped into 3 categories: suspended particles ( $> 1,2 \mu\text{m}$ ), colloids (1.2  $\mu\text{m}$  to 1 kDa) and dissolved matter ( $< 1 \text{ kDa}$ ). In a second step, in order to have more representative data and to be able to perform statistical analysis, 18 additional liquid fractions of digestates from full-scale co-digestion plants treating solid wastes as well as 1 liquid fraction of digestate from waste activated sludge (WAS) digestion were characterized in simplified forms (after filtration at 1.2  $\mu\text{m}$  and 1 kDa) representing raw liquid, suspended particles, colloids and dissolved matter.

The results of the analytical characterization of the 29 samples showed that liquid fractions of digestates contained high concentrations of organic matter with high variations from one sample to the other with COD in the range 4.4 to 90 g  $\text{O}_2/\text{L}$ . Furthermore, suspended particles represented the more important category with 60 to 96% of the organic matter. Colloids and soluble organic matter content was rather low with 1 to 27% and 2 to 24% of total COD, respectively. BOD test performed showed that the organic matter had low biodegradability which indicates that a biological aerobic post treatment of liquid fraction of digestate might be quite inefficient. Therefore, a first physico-chemical treatment can be proposed to remove the organic matter present as particles and colloids which should have quite a high efficiency. However, the treatment of the remaining organic matter, mostly soluble but which contains complex molecules with low biodegradability might be problematic and reaching standards for discharge in the environment might be difficult. Furthermore, in the case of final treatment of the pre-treated liquid fraction in a domestic wastewater treatment plant, the consequences of the discharged organic matter on the operation of the domestic treatment plant should be evaluated. Notably, the effect on the quality of the treated effluent discharged from the domestic plant should be checked as the non-biodegradable soluble organic matter from the anaerobic plant might have a negative impact. It is clear that future research is needed in order to propose acceptable solutions, both for technical and financial aspects.

Besides, liquid fractions of digestates contained high variation of nutrients concentrations, especially total nitrogen from 1.3 to 8.3 g N/L, ammonia from 0.5 to 4.7 g N/L and potassium

from 0.5 to 7.1 g/L. The removal, recovery and reuse of nutrients have been extensively studied by many researchers but without any consideration of organic matter.

In this thesis, it was observed that the separation technique had contribution on the residual organic matter in liquid fraction of digestate. High performances of solid-liquid separation with 44 to 93% separation efficiency were observed by centrifuge and other separation systems with addition of coagulant, flocculent or polymer. On the contrary, low performance of solid-liquid separation with separation efficiency less than 44% were observed from screw press, vibrating screen and rotary drum causing higher remaining residual organic matters in liquid fraction of digestates. In addition to that, feedstocks containing high percentage of manure as well as energy crops were observed to contribute to higher residual organic compounds in liquid fraction of digestate. The percentage of cow manure in the feedstocks was observed to have a strong correlation with TS and COD concentrations. Furthermore, among the operating parameters, longer HRT was observed to increase SUVA<sub>254</sub> which was linked to fulvic acid compounds in dissolved matter. In addition to that, plug flow reactor in thermophilic condition contributed to higher residual organic compounds in liquid fraction of digestate compared to CSTR reactor in mesophilic condition. However during the digestate collection, it was not possible to get samples neither from thermophilic CSTR nor from mesophilic plug flow reactors.

In order to understand the impact of cow manure (mix of cow dung, urine and straw) on the residual compounds produced, 4 lab-scale reactors were operated for 48 weeks at OLR 2 g VS/L/day, that is to say until steady state was reached after the reactor had been operated for more than 3 hydraulic retention times. The reactors were specifically fed with straw, cow dung or cow manure. The lowest methane production and methane yield were observed in mono-digestion of cow manure followed by cow dung and straw. Furthermore, the liquid fraction from this reactor had the highest COD in suspended, colloids and dissolved matter compared to cow dung and straw. In terms of COD distribution, high COD percentage of 80% in suspended particles was observed in mono-digestion of cow manure. The liquid fraction of digestate from mono-digestion of cow manure contained the highest concentration of non-biodegradable residual organic matters compared to the 2 others. This was supported by the high percentage of more complex compounds such fulvic acid-like, glycolated protein-like, melanoidin-like and

humic acid-like which were already present in the cow manure after water extraction and after anaerobic digestion. This confirmed the impact of cow manure as feedstock which had high contribution to the residual organic matter in liquid fraction of digestates.

Long continuous mixing of another reactor fed with cow manure for 20 weeks and then operated without feeding for 28 weeks showed very low residual methane production, no significant COD decrease and no change in spectral measurements. This confirmed the extremely low anaerobic biodegradability of the residual compounds from old cow manure, but also indicated the origin of complex and aromatic molecules were mostly originated from the substrate.

Besides, liquid fraction of digestate from mono-digestion of cow manure was shown to have high residual of total nitrogen, ammonia and chloride concentrations. The urine of cow can contribute to higher potassium in cow manure which later caused higher potassium in liquid fraction of digestate.

In order to understand the impact of cow manure itself as well as straw and cow dung respectively, on the residual organic compounds produced, the extraction with water was conducted on cow manure, straw and cow dung respectively. It was observed that cow manure already contained high residual organic matters in supernatant. The water extraction showed that high COD in suspended and colloids were mainly originating from cow dung while COD in dissolved was originating from both cow dung and straw. It was observed that anaerobic digestion process increased COD in liquid fraction of digestate. However, the percentage of COD in colloids from cow manure decreased after the process.

In order to complete the reported work and to understand better the origin of residual compounds in liquid fraction of digestates, several suggestions can be proposed for future research.

In the continuation of the work presented here, research on the impact of different substrates should be strengthened by including other animal manures and energy crops, which were shown to have high contribution to residual organic matter in liquid fraction of digestate. This could be addressed by mono-digestion of these substrates at lab-scale.

In addition, it would be worth to study in lab-scale controlled conditions the impact of operating parameters of the digester. In particular, the impact of hydraulic retention time or organic loading rate and temperature on the residual organic compounds produced in suspended, colloids and dissolved should be investigated for a given substrate. Indeed in the present study, few samples were available from thermophilic digestion, additional samples should be analysed. Furthermore, a study comparing mesophilic and thermophilic conditions is also suggested at lab scale in order to have a better control of the operating conditions. It also may be interesting to study the effect of the type of reactor, i.e. high solids or wet anaerobic digestion.

Besides, research on the impact of recirculation of liquid fraction of digestate is recently getting more and more interest. Therefore, it is also important to observe the impact of recirculation on the accumulation of organic matter in liquid fraction of digestate especially their distribution in suspended, colloids and dissolved.

Physico-chemical treatment of liquid fractions of digestates should be studied in order to remove suspended solids and colloids. However, even if successful, such process would not be proposed as sole process to treat the liquid fraction of digestate because of the remaining soluble non-biodegradable organic matter. Other processes such as advanced oxidation or ozonation might be also investigated in order to design a full treatment for both suspended solids/colloids and dissolved matter. After these steps, there is a particular interest in the valorization of phosphorus from the liquid fraction of digestates. For that, struvite precipitation is the most advanced option for phosphorus valorization, after removal of suspended solids and colloids, as they are the main limiting factors for this process. Regarding land application of liquid fraction of digestates, pathogen content study should be addressed in accordance with the Regulations, which may give more interest in the study and application of thermophilic digestion.



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## ANNEX 1: Separation index calculation based on mass balance

The calculation of Separation index (SI) described by Moller et al (Møller et al., 2000) in Equation (15) is shown below:

$$SI = \frac{M_{solid} \times [TS]_{solid}}{M_{raw} \times [TS]_{raw}} \quad (15)$$

Taking into account of mass balance as shown in Equation (16) and Equation (17)

$$M_{raw} \times [TS]_{raw} = (M_{solid} \times [TS]_{solid}) + (M_{liquid} \times [TS]_{liquid}) \quad (16)$$

$$M_{raw} = M_{solid} + M_{liquid} \quad (17)$$

Where;

$M_{solid}$ : Mass of solid fraction of digestate (kg)

$[TS]_{solid}$ : TS concentration in solid fraction of digestate (g TS/kg)

$M_{raw}$ : Mass of raw digestate (kg)

$[TS]_{raw}$ : TS concentration in raw digestate (g TS/kg)

$M_{liquid}$ : Mass of liquid fraction of digestate (kg)

$[TS]_{liquid}$ : TS concentration in liquid fraction of digestate (g TS/kg)

Equation (16) was normalized by  $M_{raw}$  to obtain Equation (19) as shown below:

$$\frac{M_{raw} \times [TS]_{raw}}{M_{raw}} = \frac{M_{solid} \times [TS]_{solid}}{M_{raw}} + \frac{M_{liquid} \times [TS]_{liquid}}{M_{raw}} \quad (18)$$

$$[TS]_{raw} = \frac{M_{solid} * [TS]_{solid}}{M_{raw}} + \frac{M_{liquid} * [TS]_{liquid}}{M_{raw}} \quad (19)$$

and Equation (17) was also normalized by  $M_{raw}$  to obtain Equation (21) as shown below:

$$\frac{M_{raw}}{M_{raw}} = \frac{M_{solid}}{M_{raw}} + \frac{M_{liquid}}{M_{raw}} \quad (20)$$

$$\frac{M_{liquid}}{M_{raw}} = 1 - \frac{M_{solid}}{M_{raw}} \quad (21)$$

Substitute Equation (21) into Equation (19) to obtain Equation (25) as shown below:

$$[TS]_{raw} = \frac{M_{solid} * [TS]_{solid}}{M_{raw}} + \left(1 - \frac{M_{solid}}{M_{raw}}\right) * [TS]_{liquid} \quad (22)$$

$$[TS]_{raw} = \frac{M_{solid}}{M_{raw}} * [TS]_{solid} + [TS]_{liquid} - \frac{M_{solid}}{M_{raw}} * [TS]_{liquid} \quad (23)$$

$$[TS]_{raw} - [TS]_{liquid} = \frac{M_{solid}}{M_{raw}} * ([TS]_{solid} - [TS]_{liquid}) \quad (24)$$

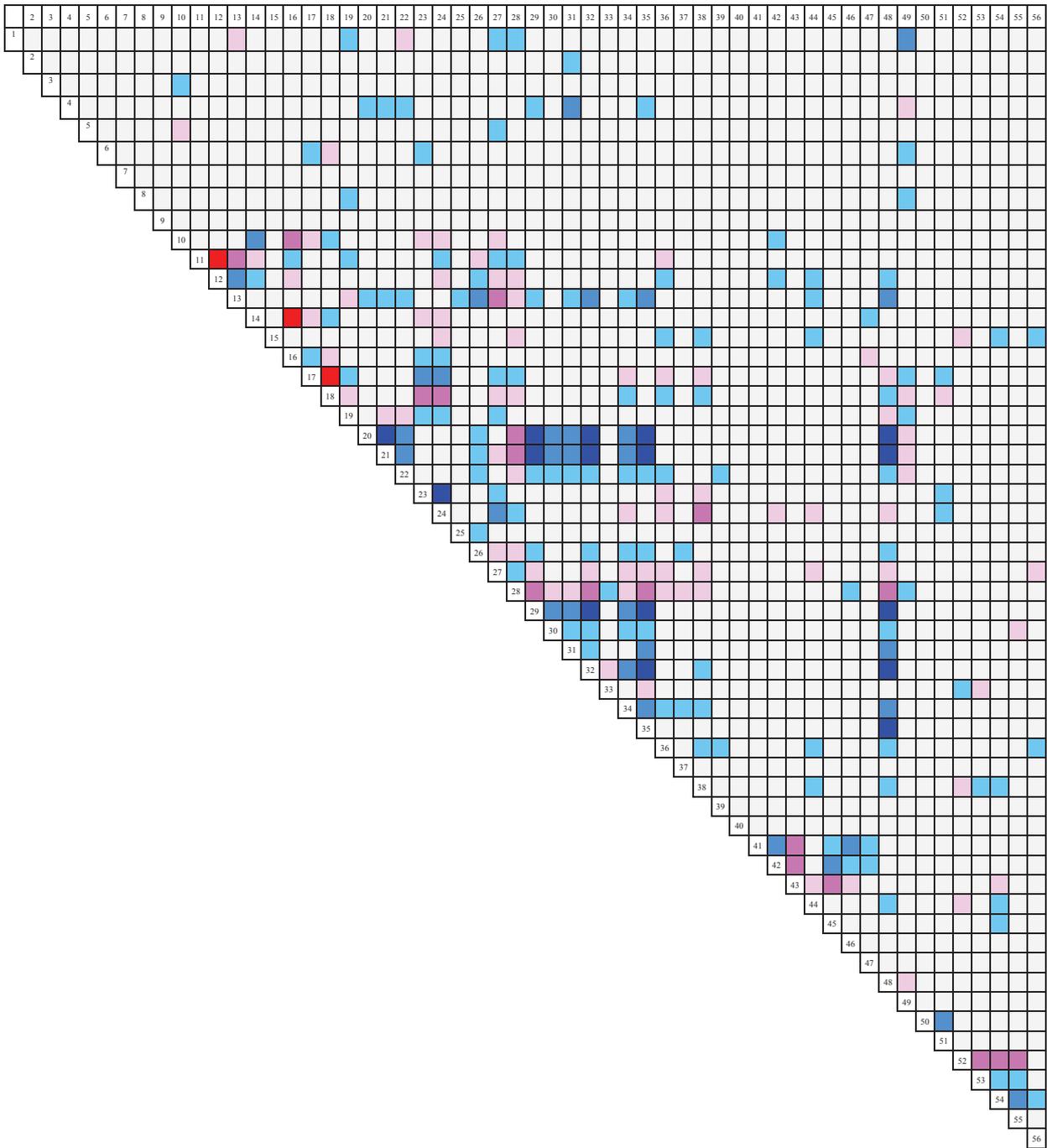
$$\frac{M_{solid}}{M_{raw}} = \frac{[TS]_{raw} - [TS]_{liquid}}{[TS]_{solid} - [TS]_{liquid}} \quad (25)$$

Substitute Equation (25) into Equation (15) to obtain Equation (26) where SI calculation was made based on Equation (26).

$$SI = \frac{[TS]_{solid}}{[TS]_{raw}} * \frac{[TS]_{raw} - [TS]_{liquid}}{[TS]_{solid} - [TS]_{liquid}} \quad (26)$$

**ANNEX 2: Correlation matrix (p-value <0.01) of all characteristics, operating parameters, methane yield and types of substrates of all 29 digestates from co-digestion plants and 1 digestate from plant treating WAS**

No	Parameters	No	Parameters	Correlation coefficient
1	HRT	29	TKN total/TS	0.9 to 1
2	Methane yield	30	TKN suspended/TS	0.7 to 0.89
3	OLR	31	TKN colloids/TS	0.47 to 0.69
4	SS	32	TKN dissolved/TS	-0.47 to 0.47
5	Mnr	33	TC/TN dissolved	-0.47 to -0.69
6	EnCr	34	N organic dissolved/TS	-0.7 to -0.89
7	CrR	35	NH <sub>4</sub> <sup>+</sup> /TS	-0.9 to -1
8	Cer	36	Na <sup>+</sup> /TS	
9	FOG	37	K <sup>+</sup> /TS	
10	AFW	38	Cl <sup>-</sup> /TS	
11	VS/TS solid	39	PO <sub>4</sub> <sup>3-</sup> /TS	
12	MS/TS solid	40	SO <sub>4</sub> <sup>2-</sup> /TS	
13	Separ. Efficiency	41	Mean size	
14	MS/TS raw	42	Median size	
15	pH	43	Size 0.375-0.50µm	
16	VS/TS raw	44	Size 0.50-100µm	
17	VS/TS liquid	45	Size X100-500µm	
18	MS/TS liquid	46	Size X500-1000µm	
19	CST	47	Size X1000-5000µm	
20	Alkalinity/TS	48	Conductivity/TS	
21	IC/TS	49	SUVA <sub>A254</sub>	
22	TOC dissolved/TS	50	BOD <sub>5</sub> /TS	
23	COD total/TS	51	BOD <sub>21</sub> /TS	
24	COD suspended/TS	52	Protein-like	
25	COD colloids/TS	53	Fulvic acid-like	
26	COD dissolved/TS	54	Glycolated protein-like	
27	Turbidity/TS	55	Melanoidin-like	
28	C/N	56	Humic acid-like	





## ABSTRACT

Agricultural and centralized biogas plants are facing a fast development, leading to the production of huge amounts of digestates. Whereas the solid fraction of digestates is used as fertilizer, the liquid fraction can be valorized by the recovery of mineral materials, although the presence of organic compounds may be problematic for the treatment and disposal of this effluent. The objectives of this thesis are: i) to characterize the liquid fraction of digestates and to provide guidelines for its further treatment, ii) to explain the variability of residual compounds in the liquid fraction of digestates in relation with substrate origin, process parameters and the type of solid-liquid separation. In a first part, eleven digestates from full scale codigestion plants were thoroughly characterized. Their liquid fractions were fractionated by nine successive filtrations, which allowed quantifying the contribution of suspended particles, coarse and fine colloids and dissolved matter on several physico-chemical and biological parameters. Organic compounds were mainly found in suspended particles ( $> 1.2 \mu\text{m}$ ) and presented low aerobic biodegradability. To enlarge the data base, eighteen more digestates from codigestion and one digestate from waste activated sludge were characterized; their liquid fractions were fractionated by two successive filtrations ( $1.2 \mu\text{m}$  and  $1 \text{kDa}$ ). Principal component analysis, hierarchical clustering analysis and correlation matrix carried out on the 30 digestates set, highlighted the major impact of the solid-liquid separation process on the composition of the liquid fraction of digestates. In the high performance separation process group, subgroups allowed separating digestates from sewage sludge, digestates from pig manure and digestates from plug-flow thermophilic processes. In the low performance separation process group, COD and total solids concentration in the liquid fraction of digestates were correlated to the percentage of energy crops and cow manure in the feed. Finally, SUVA parameter which accounts for aromatic compounds content and the stabilization of organic matter, was correlated to the retention time in digester for the whole digestates set. To understand the origin of residual compounds in the liquid fraction of digestates from co-digestion of high proportions of cattle manure, four CSTR reactors fed with wheat straw, cow dung and cow manure were operated for 48 weeks. Anaerobic digestion performances showed that cow manure had undergone some aerobic degradation during its storage at the farm. Therefore, the liquid fraction of manure digestate had the highest concentrations in organic compounds. In addition, these organic compounds had the highest complexity measured by 3D fluorimetry. The low biodegradability of organic compounds and their high percentage in suspended particles suggest the development of physico-chemical separation process such as coagulation for the treatment of the liquid fraction of digestates.

## RESUME

Le secteur de la méthanisation à la ferme ou territoriale connaît un fort développement, entraînant la production de grandes quantités de digestats. Si la fraction solide de ces digestats est généralement utilisée en tant qu'amendement organique, la fraction liquide peut être valorisée par la récupération des éléments minéraux bien que la présence de composés organiques pose des problèmes de traitement et d'élimination. Les objectifs de cette thèse sont i) de caractériser la fraction liquide des digestats et donner des premiers éléments pour leur futur traitement et ii) d'expliquer la variabilité des composés résiduels présents dans cette fraction liquide en relation avec l'origine des substrats, les paramètres du procédé de méthanisation et le type de séparation solide/liquide. Dans une première partie, nous avons caractérisé de manière détaillée onze digestats issus d'installations industrielles de co-digestion. Le fractionnement par neuf filtrations successives de la fraction liquide des digestats a permis de quantifier la contribution des matières en suspension, des colloïdes grossiers et fins et des matières dissoutes aux différents paramètres physico-chimiques et biologiques. Il a été montré la faible biodégradabilité aérobie des composés organiques dont la majeure partie se trouve dans la matière en suspension ( $> 1,2 \mu\text{m}$ ). Pour élargir la base de données, nous avons caractérisé dix-huit digestats supplémentaires et un digestat de boues de station d'épuration dont les fractions liquides ont été fractionnées par deux filtrations successives ( $1,2 \mu\text{m}$  et  $1 \text{kDa}$ ). L'analyse en composantes principales, la classification hiérarchique et la matrice de corrélation réalisées sur l'ensemble des 30 digestats ont mis en avant l'impact majeur des techniques de séparation solide/liquide sur la composition de la fraction liquide des digestats. Dans le groupe des techniques de séparation hautement performantes, des sous-groupes ont permis de distinguer les digestats à base de boues d'épuration, ceux à base de lisiers porcins et ceux issus de procédés piston thermophiles. Dans le groupe des procédés de séparation à faible performance, les teneurs en DCO et matières sèches de la fraction liquide des digestats ont été corrélées aux teneurs en cultures énergétiques et fumiers bovins dans l'alimentation. Finalement, pour l'ensemble des digestats, le paramètre SUVA, lié à la teneur en matière aromatique et à la stabilisation des composés, a été corrélé au temps de séjour des digesteurs. Pour comprendre l'origine des composés résiduels observés dans la fraction liquide des digestats issus de la co-digestion avec de fortes proportions de fumier bovin, quatre réacteurs CSTR alimentés avec respectivement de la paille de blé, de la bouse de vache et du fumier ont été conduits pendant 48 semaines. Les performances de méthanisation ont mis en évidence le caractère âgé du fumier qui avait été partiellement dégradé pendant le stockage à la ferme. Ainsi la fraction liquide du digestat du fumier a présenté les plus fortes concentrations en composés organiques qui en outre présentaient une complexité, mesurée par fluorimétrie 3D, plus élevée que les autres substrats. La fraction liquide du digestat issue de la méthanisation de la paille a révélé les plus fortes proportions de DCO dans les fractions colloïdales et dissoutes. La faible biodégradabilité des composés organiques et leur forte proportion dans les matières en suspension suggèrent le développement de procédés physico-chimiques de séparation tels que la coagulation pour le traitement de la fraction liquide des digestats.

**DISCIPLINE:** Génie des Procédés

**MOTS-CLES :** méthanisation, déchets solides, fumier bovin, séparation solide-liquide, matière organique, biodégradabilité.

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