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Dynamical reduction of metabolic networks by singular perturbation theory : application to microalgae

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THÈSE DE DOCTORAT

Réduction dynamique de réseaux métaboliques par la théorie des perturbations singulières : Application aux microalgues

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Signal et des Images

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Réduction dynamique de réseaux métaboliques par la théorie des perturbations singulières : application aux microalgues.

Résumé

Les lipides des microalgues et les glucides de cyanobactéries peuvent être transformés en biodiesel et en bioéthanol, respectivement. L'amélioration de la production de ces molécules doit prendre en compte les entrées périodiques (principalement la lumière) forçant le réseau métabolique de ces organismes photosynthétiques. Il est donc nécessaire de tenir compte de la dynamique du réseau métabolique en réduisant sa dimension pour assurer la maniabilité mathématique. Le but de ce travail est de concevoir une approche originale pour réduire les réseaux métaboliques dynamiques tout en conservant la dynamique de base. Cette méthode est basée sur une séparation en échelles de temps. Pour une classe de modèles de réseaux métaboliques décrits par des ODE, la dynamique des systèmes réduits est calculée à l'aide du théorème de Tikhonov pour les systèmes singulièrement perturbés. Cette approximation quasi-stationnaire coïncide avec la dynamique du réseau d'origine, avec une erreur bornée. L'approche est d'abord développée pour les systèmes de réaction pouvant être linéarisés autour d'un point de travail et forcés par des entrées continues. Ensuite, une généralisation de cette méthode est donnée pour les réseaux à réactions rapides de cinétiques de Michaelis-Menten et tout type de cinétiques lentes, prenant également en compte un nombre fini d'entrées continues externes. La méthode de réduction met en évidence une relation entre la grandeur de la concentration des métabolites et la gamme des vitesses de réaction : les métabolites consommés par les réactions rapides ont une concentration inférieure d'un ordre de grandeur à celle des métabolites consommés à faible vitesse. Cette propriété est satisfaite pour les métabolites à dynamique rapide ne se trouvant pas dans un piège de flux, concept introduit dans ce travail. Le système réduit peut être calibré avec des données expérimentales à l'aide d'une procédure d'identification dédiée basée sur la minimisation. L'approche est illustrée par un réseau métabolique de microalgues autotrophes, comprenant le métabolisme central et représentant la dynamique des glucides et des lipides. Cette approche permet de bien ajuster les données expérimentales de Lacour et al. (2012) avec la microalgue *Tisochrysis lutea*. Enfin, un schéma visant à optimiser la production de molécules cibles est proposé en utilisant le système réduit.

Mots clés : Modélisation métabolique, réduction des réseaux métaboliques, systèmes dynamiques, théorie des perturbations singulières, microalgues.

Dynamical Reduction of Metabolic Networks by Singular Perturbation Theory: Application to Microalgae.

Abstract

Lipids from microalgae and carbohydrates from cyanobacteria can be transformed into biodiesel and bioethanol, respectively. Enhancing the production of these molecules must account for the periodic inputs (mainly light) forcing the metabolic network of these photosynthetic organisms. It is therefore necessary to account for the dynamics of the metabolic network, while reducing its dimension to ensure mathematical tractability. The aim of this work is to design an original approach to reduce dynamic metabolic networks while keeping the core dynamics. This method is based on time-scale separation. For a class of metabolic network models described by ODE, the dynamics of the reduced systems are computed using the theorem of Tikhonov for singularly perturbed systems. This Quasi Steady State Approximation accurately coincides with the original network dynamics, with a bounded error. The approach is first developed for reaction systems that can be linearized around a working point and that are forced by external continuous inputs. Then, a generalization of this method is given for networks with fast reactions of Michaelis-Menten kinetics and any type of slow kinetics, also considering a finite number of external continuous inputs. The reduction method highlights a relation between the concentration magnitude of the metabolites and the range of the reaction rates: the metabolites that are consumed by fast reactions have concentration one order of magnitude lower than metabolites consumed at slow rates. This property is satisfied for metabolites with fast dynamics that are not in a flux trap, a concept introduced in this work. The reduced system can be calibrated with experimental data using a dedicated identification procedure based on minimization. The approach is illustrated with an autotrophic microalgae metabolic network, including the core metabolism and representing the carbohydrates and lipids dynamics. The approach efficiently fits the experimental data from Lacour et al. (2012) with the microalgae *Tisochrysis lutea*. Finally, a scheme to optimize the production of target molecules is proposed using the reduced system.

Keywords: Metabolic Modeling, Reduction of Metabolic Networks, Dynamical Systems, Singular Perturbation Theory, Microalgae.

Reducción dinámica de redes metabólicas por medio de la teoría de perturbaciones singulares: aplicación a microalgas.

Resumen

Lípidos de microalgas y carbohidratos de cianobacterias se pueden transformar en biodiesel y bioetanol, respectivamente. La mejora de la producción de estas moléculas debe tener en cuenta las entradas periódicas (principalmente lumínicas) que influyen el metabolismo de estos organismos fotosintéticos. Por lo tanto, es necesario tener en cuenta la dinámica de su red metabólica, mientras se reduce su dimensión para poder hacer un análisis matemáticamente. El objetivo de este trabajo es diseñar un enfoque original para reducir redes metabólicas dinámicas mientras se mantiene su dinámica central. Este método se basa en la separación de escalas de tiempo. Para una clase de modelos de redes metabólicas descritas por EDO, la dinámica de los sistemas reducidos se calcula utilizando el teorema de Tikhonov para sistemas singularmente perturbados. Esta aproximación de estado quasi-estacionario coincide de manera precisa con la dinámica de la red original, además de tener un error acotado. El enfoque se desarrolla primero para sistemas de reacciones que pueden linealizarse alrededor de un punto de trabajo y que son forzados por entradas externas que son continuas. Luego, se proporciona una generalización de este método para redes con reacciones rápidas de cinética tipo Michaelis-Menten y cualquier tipo de cinética lenta, considerando también un número finito de entradas externas y continuas. El método de reducción resalta una relación entre la magnitud de la concentración de los metabolitos y el rango de las tasas de reacción: los metabolitos que son consumidos por reacciones rápidas tienen una concentración de un orden de magnitud menor que los metabolitos consumidos a tasas bajas. Esta propiedad se cumple para los metabolitos con dinámica rápida que no están en una trampa para flujo, un concepto introducido en este trabajo. El sistema reducido se puede calibrar con datos experimentales mediante un procedimiento de identificación, desarrollado para este caso particular, basado en la minimización. El enfoque se ilustra con una red metabólica de microalgas autótrofas, que incluye el metabolismo central y representa la dinámica de los carbohidratos y los lípidos. El enfoque se ajusta de manera eficiente a los datos experimentales de Lacour et al. (2012) con la microalga *Tisochrysis lutea*. Finalmente, se propone un esquema para optimizar la producción de moléculas objetivo utilizando el sistema reducido.

Palabras clave: Modelización metabólica, reducción de redes metabólicas, sistemas dinámicos, teoría de perturbaciones singulares, microalgas.

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**Dynamical Reduction of Metabolic
Networks by Singular Perturbation
Theory: Application to Microalgae**

Claudia López Zazueta

PhD thesis

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Introduction

Microalgae are unicellular algae, whose size ranges in the order of micrometers (μm). They are photosynthetic organisms that grow mainly in aqueous solutions. The major source of energy in microalgae is synthesized from light. These microorganism are also capable of converting carbon dioxide (CO_2) into biomass.

Microalgae are considered as a raw material with potential to produce biofuels. Some species are capable to accumulate lipids that can be turned into biodiesel [5]. In order to optimize the production of biofuels, the metabolism of microalgae has been extensively studied.

In the last decades, the genomes of some microalgae have been sequenced. The availability of genetic information has provided important bases for the development of metabolic network models [53, 38, 48].

Most of the approaches for metabolic modeling using ODE consider intracellular metabolites as constituents almost without dynamics. The common assumption is that intracellular metabolites are not accumulative metabolites and have a very rapid turnover compared to macromolecules [70], implying that they are at constant concentration. Then, the Steady State Assumption is generally applied to intracellular metabolites. However, steady state hypotheses are not suitable for all metabolisms, specially for those submitted to fluctuating external conditions, e.g. microalgae metabolism affected by the flux of light.

Several approaches have been proposed to model metabolic networks including dynamics [4, 51], but in general, their mathematical bases are not rigorously justified. In some cases, the Quasi Steady State Assumption (QSSA) is used without mathematical justification to reduce metabolic systems, which can lead to inaccurate reduced models [11, 22].

The theory of singularly perturbed systems has been used to justify the Quasi Steady State Assumption on metabolic networks [21, 22, 61, 65, 76]. Singular perturbation methods have been applied under different approaches and always assume that biological systems have several time-scales.

The objective of this work is to introduce a reduction method for metabolic networks, whose mathematical validity can be justified using the theory of singular perturbations. For this purpose, an ODE dynamical model of a

metabolic network is considered, including different time-scales. Then, the theorem of Tikhonov for singularly perturbed systems is used to obtain a Quasi Steady State Reduction, i.e. a reduced system that relies on the QSSA.

This method allows to establish a relation between reaction rate constants and metabolite concentrations. Moreover, the reduced dynamical system obtained offers a simple framework for the estimation of parameters using experimental data. Finally, the reduced dynamical model has a tractable mathematical complexity and can be used to derive optimization strategies.

In Chapter 1, the principal characteristics of microalgae and some basics for metabolic modeling are explained. In Chapter 2, the most significant mathematical definitions and results used in this work are established. In Chapter 3, the reduction and calibration method for metabolic networks is developed for linearized metabolic systems forced by continuous inputs. Additionally, an application to microalgae metabolisms is included.

In Chapter 4, the method is extended for a class of nonlinear metabolic systems with enzymatic reactions and continuous inputs. Finally, in Chapter 5 the method for nonlinear metabolic system is applied to the autotrophic microalgae metabolic network proposed by Yang et al. [79]. The reduced model obtained is calibrated with the experimental data of Lacour et al. [40]. Also, in Chapter 6, some optimization strategies to increase metabolite production are proposed using the reduced system, particularly, to improve lipids production in microalgae.

CHAPTER 1

Microalgae and Metabolic Modeling

1.1. The Microalgae

The existence of cyanobacteria and microalgae in the oceans dates back to more than three billion years ago. They are considered to be the first producers of oxygen (O_2) on Earth. These unicellular microorganisms have allowed the emergence of plant and animal life. They are at the basis of the trophic network, directly eaten by zooplankton and indirectly contributing to the biomass in the higher trophic levels [1].

Microalgae are photosynthetic organisms that use light energy to convert carbon sources, as CO_2 , into biomass. Together with the terrestrial plants, they are responsible in the constant transformation of the atmosphere, through the carbon dioxide (CO_2) fixation and the O_2 emission.

Microalgae can be found from oceans, lakes and rivers, to more extreme environments such as thermal waters, caves, ice, acid or hyper saline water, walls, tree trunks, on immersed structures and even in human. They can live in symbiosis with other organisms (e.g. bacteria or fungi). Their adaptation and survival capacities are result of their morphology and ability to synthesize different varieties of metabolites [71].

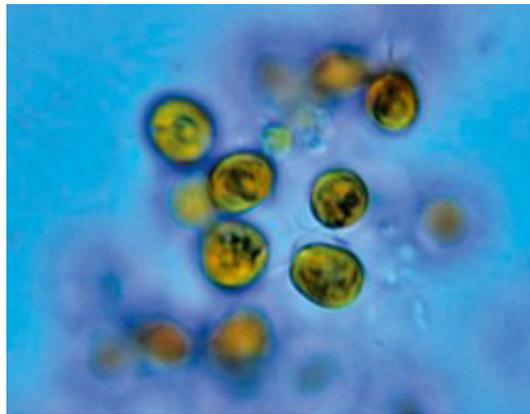


Figure 1.1. Culture of phytoplankton *Tisochrysis lutea* (©Ifremer/Raymond Kaas)

As an eukaryote cell, microalgae have a nucleus, a plasma membrane and organelles such as chloroplast and mitochondria. They synthesize O_2 and primary organic metabolites as carbohydrates, lipids and proteins.

Microalgae are mainly photoautotrophic, i.e. they use light as a source of energy and inorganic CO_2 or bicarbonate as a source of carbon. But they can also be heterotrophic or mixotrophic. In heterotrophic growth, they consume organic carbon (e.g. acetate or glucose) and can grow in the absence of light. This substitutes the CO_2 fixation of the autotrophic metabolism in microalgae, which is supported by the photosynthesis. Mixotrophic microalgae assimilate both types of carbon sources, CO_2 and organic carbon at the same time [58].

Microalgae are used in diverse areas such as the pharmaceutical industry, agro-industry, environment and renewable energy. Some of their principal applications are for [71]:

- Nutrition: source of nutrients for animal feed or as a human food source. They are used in the manufacture of natural colorants, gelling agents or sweetener.
- Pharmaceutical: microalgae synthesize vitamins and natural antioxidants. They can produce polysaccharides that allow to synthesize antioxidants, antiviral, antitumor and anticoagulant agents. They are a source of bioactive molecules and toxins used in the development of medicines for the treatment of cancer.
- Cosmetics: microalgae have antioxidant properties used in the manufacture of sun creams, pigments, hair care and anti-wrinkle products.
- Energy: production of biofuels and biohydrogen, only competitive in cases with strong biomass productivity, simple mechanical harvesting and relative low production costs.
- Environment: wastewater treatment and CO_2 sequestration for reducing greenhouse emissions. They are used to ensure nitrogen fixation in agriculture, and for surface strengthening in arid regions.
- Life-support-systems: to regenerate a breathable atmosphere (O_2 supply), water recycling, waste treatment and food provision, for example, during space missions.

1.1.1. Cultures of microalgae. Environmental, physical and biological factors affect the physiology and metabolism of microalgae. Some of the most important factors are described below, however, they can differ depending on the species [71]:



Figure 1.2. Fitoplancton Marino, a Spanish company that produces about two tons of dry microalgae matter each year (<http://ec.europa.eu>).

- **Light:** essential for the metabolism of photosynthetic microalgae, its supply can be natural through solar energy or artificial using fluorescent tubes, LED or fiber optics. Inadequate light distribution can limit productivity and growth. Microalgae can present photoinhibition (loss of photosynthetic activity) as a consequence of an excessive light exposure.
- **Temperature:** microalgae are strongly influenced by temperature, with an optimal temperature related to their natural environment. The rates of biomass growth, lipids and starch accumulation increase with higher temperatures up to a certain value [74]. Augmentation in temperature also affects the CO₂ fixation.
- **pH:** essentially depends on the concentration of CO₂ in the culture medium. A high supply of CO₂ or sulfur monoxide (SO) can produce acidification of the environment, inhibiting the growth of microalgae. In general, algae growth is optimal with a pH close to neutrality.
- **Carbon dioxide:** CO₂ is the source of inorganic carbon for phototrophic microalgae. Microalgae can assimilate CO₂ to grow, while producing oxygen and secondary metabolites. The concentration of CO₂ dissolved in the medium must be sufficient for optimal growth [74].
- **Nitrogen (N):** it is an essential nutrient for algae growth and is involved in the major metabolic pathways of microalgae. The nitrogen source may be organic (urea) or inorganic (nitrate NO₃⁻, ammonium NH₄⁺). An increase in the concentration of nitrogen in the culture, to a certain extent, leads to more significant cellular, protein and chlorophyll productivity. A deficiency of this element induces accumulation of lipid reserves (polyunsaturated fatty acids, see Figure 1.3 and Figure 1.4) and a limitation of photosynthetic and cellular activities.

- Phosphorus (P): microalgae are able to use inorganic as well as organic forms of phosphorus. It is involved in several metabolic pathways and cellular regulations. Phosphorus deficiency influences the photosynthetic activity, the accumulation of lipids reserves (see Figure 1.3) and the biomass productivity. Phosphorus may form precipitates with metal ions and, therefore, has to be supplied in excess into the medium.
- Microelements: several organic and inorganic microelements are necessary for the growth of microalgae and a deficiency in one of these may lead to reduced algae growth. Lack of sulfur (S) induces an inhibition of protein synthesis and photosynthetic activity. Sulfur limitation induces starch and lipid synthesis [74] (see Figure 1.3). Iron deficiency causes inhibition of protein and lipid synthesis. Iron also acts as a catalyst in the synthesis of chlorophyll. Magnesium is essential to nitrogenase activity and a deficiency can affect the photosynthetic mechanism of microalgae. Molybdenum may influence nitrogen assimilation at cellular level.
- Medium salinity: increment of salinity in the medium may inhibit photosynthetic activity, while changes of salinity induces osmotic and ionic stresses, leading to the formation of precipitates, increase in lipid content, higher concentration in carotenoids for some species (e.g. *Dunaliella*) and inhibition of growth.
- Agitation: the homogeneity of the culture medium is crucial to ensure optimal biomass productivity and high CO₂ fixation. Low agitation leads to difficulties in accessing nutrients and light to cells, to biomass settling and stagnant zones adverse for culture conditions, since it can lead to toxic product accumulation and cell death. On the other hand, excessive agitation causes physiological and metabolic deficiencies as well as structural damage.
- Gas-liquid mass transfer: good quality of gas-liquid mass transfer provides maximum amounts of CO₂ for fixation and regulation of pH through CO₂ dissolution in the liquid. This transfer depends on the diffusivity of CO₂ in both phases, the flow of gas and hydrodynamics.

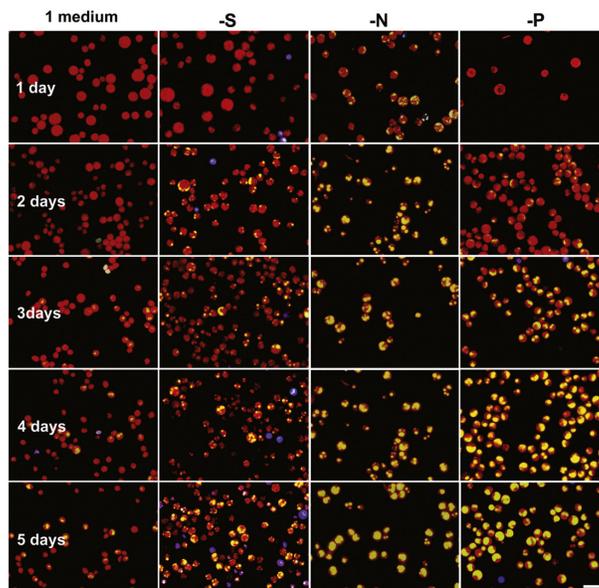


Figure 1.3. Lipid accumulation during batch cultivation of microalgae *Parachlorella kessleri*. Figure taken from [74]. Lipid bodies (yellow), chloroplast (red). Cultures were grown in complete mineral medium (1 medium), or in absence of nitrogen (-N), phosphorus (-P) or sulfur (-S). Scale bar represents 10 μm .

1.1.2. Microalgae for the Production of Biofuels. The environmental impact of human activities has turned attention to renewable energies. For example, to reduce fossil fuels usage and CO_2 emissions to the atmosphere, the generation of biofuels has been promoted in the last decades [42]. But research in biofuels still needs to be carried out, to raise their yields and reduce commercialization costs.

Microalgae is considered the most promising source of biofuel production. Biodiesel from microalgae is classified as a third generation biofuel. It presents several advantages over fossil fuels, first generation biofuels (derived from food crops) and second generation biofuels (from waste and non-food biomass).

Compared to first and second generation biofuels, microalgae biofuel represents an alternative to reduce the competition with food production, excessive water consumption and soil deterioration. Microalgae are more effective than terrestrial species for biodiesel synthesis. They present an important biomass productivity, high photosynthetic activity and large lipid storage potential up to 20-50% dry weight [71].

In conclusion, biofuels produced from microalgae are a source of energy with potential. Remarkable advantages in the culture of these microorganisms are [5, 42, 58]:

- Reduced CO₂ emissions compared to emissions from petroleum diesel [13]. As autotrophic microorganisms, they can mitigate CO₂ from human activities and produce neutral lipids (mainly triacylglycerols [TAG]) or carbohydrates, which can be transformed into biodiesel and bioethanol, respectively.
- They have a fast growth rate, which implies that productivity of microalgae biofuel is higher than those of vascular plants.
- They have the capacity to grow in salt water as well as wastewater, this means that the production of microalgae biofuels do not necessarily compete with agricultural food for land or fresh water.
- The use of microalgae for environmental processes, such as wastewater treatment, fertilization of soils, biofuels, and phytoremediation of toxic wastes.

Nevertheless, microalgae based biofuels production is an emerging technology that must be optimized [13, 46, 63]. The study of microalgae metabolic dynamics is crucial to improve microalgae biofuel production. For example, exploiting the mechanisms which drive lipids accumulation, TAG quota can be raised in a culture. Unfortunately, lipids metabolism of microalgae is rather unknown and it diverges from bacteria and plants [4]. However, metabolic modeling is a tool that can give insights to deduce optimal conditions for lipid accumulation in microalgae.

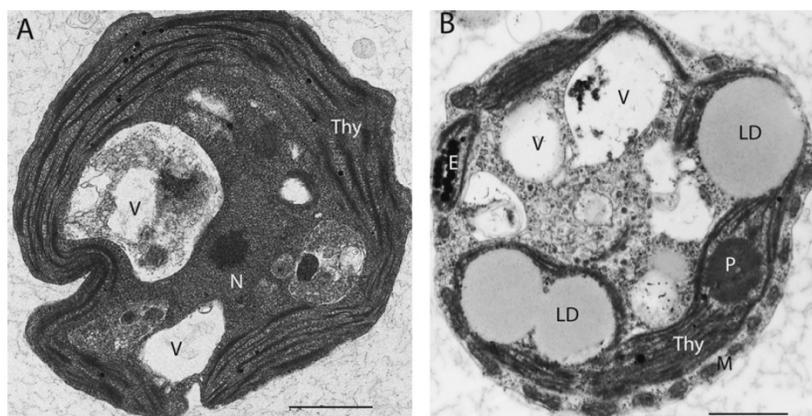


Figure 1.4. Lipid droplets in microalgae *Chlamydomonas reinhardtii*. Figure taken from Fan et al. (2011) [17]. Electron micrographs of the mutant cells grown in complete medium (A) or cells shift to medium lacking nitrogen for two days (B). In (B) it is shown the presence of lipid droplets in the chloroplast and the cytosol in a single cell. Scales bars represents 2 μm . Abbreviations: E, eyespot; LD, lipid droplets; M, mitochondria; N, nucleus; P, pyrenoid; Thy, thylakoid membranes; V, vacuoles.

1.2. Metabolic Network Modeling

Biological systems are hierarchical. One type of hierarchy is with respect to time-scales. At the faster time-scales there are elementary metabolic reactions catalyzed by enzymes. Genetic regulations in evolution are on the slower time-scales. On the other hand, there is a hierarchy of material structures, ranging from low-molecular compounds to organisms and populations. Metabolic modeling only takes into account the cell and its substructures [24].

A metabolic network is defined as a set of chemical reactions, where the metabolites acting in reactions are represented by “nodes” and the reactions by “arrows” between the nodes. In metabolic networks, chemical reactions are processes where some metabolites are consumed and transformed into others. We say that a metabolite is a substrate if it is depleted during a reaction in order to produce others metabolites, which are called products [2]. Given a metabolic network, the objective of metabolic modeling is to define a mathematical model that describes the dynamics of its network of chemical reactions.

Reactions can have more than one substrate and several products. First order reactions are reactions where only one substrate and one product are considered. For example, the reaction



is a first order reaction where A is the substrate and B the product.

To establish a system of ordinary equations which describes the concentration of metabolites A and B during the reaction $R1$, the rate of the reaction has to be taken into account. Rates of reactions can evolve during the time, but in this work we consider them as constants. Then, we denote the constant rate of reaction $R1$ by a positive number k_1 and

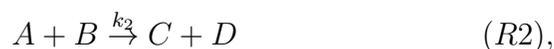


Hence, an ODE representing how A is consumed to produce B at rate k_1 (assuming a first order reaction) is

$$\begin{aligned} \frac{dA}{dt} &= -k_1 \cdot A \\ \frac{dB}{dt} &= k_1 \cdot A, \end{aligned}$$

where the variables A and B denote the molar concentrations of the respective metabolites [2].

Reactions with more than two substrates induce nonlinear systems of equations. For example, the reaction



have two substrates and two products. Following the mass action law [75], the system of equations describing the concentration dynamics of each metabolite is

$$\begin{aligned} \frac{dA}{dt} &= -k_2 \cdot A \cdot B \\ \frac{dB}{dt} &= -k_2 \cdot A \cdot B \\ \frac{dC}{dt} &= k_2 \cdot A \cdot B \\ \frac{dD}{dt} &= k_2 \cdot A \cdot B. \end{aligned}$$

Notice that when a metabolic network has exclusively first order reactions, the definition of metabolic network coincides with the definition of directed graph in Mathematics. Indeed, for a first order reaction we can identify the substrate as the origin node, the product with the final node and the reaction with a directed edge from the substrate to the product. But when there is more than one substrate or more than one product, one single “edge” might rely several origin nodes to several final nodes. For instance, reaction R2 can be graphically represented as

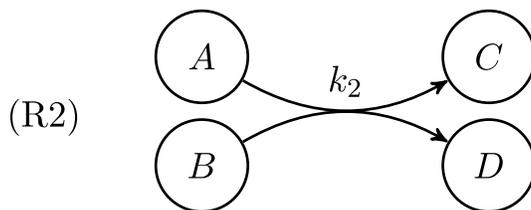


Figure 1.5. Metabolic reaction with two substrates (A and B) and two products (C and D). Its constant rate of reaction is k_2 .

1.2.1. Stoichiometric Models. Mathematical modeling in biochemistry has for principal objective the construction of kinetics models. These models are expected to predict the system dynamics on the basis of the reaction network topology and the kinetic parameters.

So far, the methods to analyze kinetics models have been mainly algebraic. These methods, for example, allow to detect conservation relations

and biochemical routes (elementary modes of fluxes) [26]. In this context, a capital concept is the stoichiometric matrix, defined below:

DEFINITION 1.1 (Stoichiometric Matrix). Consider a metabolic network with m metabolites X_1, X_2, \dots, X_m , and r reactions $R1, R2, \dots, Rr$. Then, its stoichiometric matrix is defined as the matrix N of size $m \times r$, whose entry n_{ij} is the stoichiometric factor of metabolite X_i in reaction Rj multiplied by 1 if the metabolite is produced after the reaction (i.e. if X_i is a product) or by -1 if the metabolite is consumed during the reaction (i.e. if X_i a substrate).

EXAMPLE 1.1. Suppose that we have a network with two reactions:



Then, its stoichiometric matrix is

$$N = \begin{matrix} & \begin{matrix} R1 & R2 \end{matrix} \\ \begin{matrix} X_1 \\ X_2 \\ X_3 \\ X_4 \\ X_5 \end{matrix} & \begin{pmatrix} -2 & 0 \\ -1 & 5 \\ 1 & -2 \\ 3 & 0 \\ 0 & -1 \end{pmatrix} \end{matrix} \in M_{5 \times 2}. \quad (1.1)$$

To study the dynamics of metabolite concentrations, the constant rates of reactions have to be also taken into account. These are used to define the kinetics of the network. For example, if reactions $R1$ and $R2$ have rates k_1 and k_2 , respectively, then we write



and their vector of kinetics (given by a mass action law) is

$$V(X) = \begin{pmatrix} \nu_1 \\ \nu_2 \end{pmatrix} = \begin{pmatrix} k_1 \cdot X_1 \cdot X_2 \\ k_2 \cdot X_5 \cdot X_3 \end{pmatrix}.$$

For intracellular metabolic reactions, the most common representation of metabolite dynamics is given by the ODE

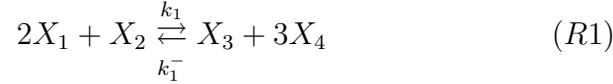
$$\frac{dX}{dt} = N \cdot V(X) - \mu \cdot X,$$

where N is the stoichiometric matrix of the reaction network, $V(X)$ the vector of kinetics and μ the rate of growth dilution [60].

For the example, the ODE of the network with reactions R1 and R2 in Example 1.1 is

$$\begin{aligned}\frac{dX_1}{dt} &= -2 \cdot k_1 \cdot X_1 \cdot X_2 - \mu \cdot X_1 \\ \frac{dX_2}{dt} &= -k_1 \cdot X_1 \cdot X_2 + 5 \cdot k_2 \cdot X_5 \cdot X_3 - \mu \cdot X_2 \\ \frac{dX_3}{dt} &= k_1 \cdot X_1 \cdot X_2 - 2 \cdot k_2 \cdot X_5 \cdot X_3 - \mu \cdot X_3 \\ \frac{dX_4}{dt} &= 3 \cdot k_1 \cdot X_1 \cdot X_2 - \mu \cdot X_4 \\ \frac{dX_5}{dt} &= -k_2 \cdot X_5 \cdot X_3 - \mu \cdot X_5.\end{aligned}$$

NOTE 1.1. When there are reversible reactions, we have to consider the rates of the reverse reactions in the kinetics. For example, if we suppose that R1 is reversible



assuming a mass action law, the kinetics vector is

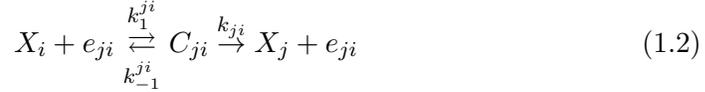
$$V(X) = \begin{pmatrix} \nu_1 \\ \nu_2 \end{pmatrix} = \begin{pmatrix} k_1 \cdot X_1 \cdot X_2 - k_1^- \cdot X_3 \cdot X_4 \\ k_2 \cdot X_5 \cdot X_3 \end{pmatrix},$$

and its stoichiometric matrix is the same defined in (1.1). Moreover, its ODE is

$$\begin{aligned}\frac{dX_1}{dt} &= -2 \cdot k_1 \cdot X_1 \cdot X_2 + 2 \cdot k_1^- \cdot X_3 \cdot X_4 - \mu \cdot X_1 \\ \frac{dX_2}{dt} &= -k_1 \cdot X_1 \cdot X_2 + k_1^- \cdot X_3 \cdot X_4 + 5 \cdot k_2 \cdot X_5 \cdot X_3 - \mu \cdot X_2 \\ \frac{dX_3}{dt} &= k_1 \cdot X_1 \cdot X_2 - k_1^- \cdot X_3 \cdot X_4 - 2 \cdot k_2 \cdot X_5 \cdot X_3 - \mu \cdot X_3 \\ \frac{dX_4}{dt} &= 3 \cdot k_1 \cdot X_1 \cdot X_2 - 3 \cdot k_1^- \cdot X_3 \cdot X_4 - \mu \cdot X_4 \\ \frac{dX_5}{dt} &= -k_2 \cdot X_5 \cdot X_3 - \mu \cdot X_5.\end{aligned}$$

1.2.2. Michaelis-Menten Reaction. Since a single reaction can involve several components, the systems of equations of some specific reactions have been studied in order to reduce it. For illustration, the classical example of an enzymatic reaction, proposed by Michaelis and Menten, is presented here.

The Michaelis-Menten model considers a substrate X_i which reacts with an enzyme e_{ji} to produce a complex C_{ji} . Then, this complex is transformed into a product X_j and the enzyme e_{ji} . This enzymatic reaction is abstracted as follows:



$$\begin{aligned} \frac{dX_i}{dt} &= -k_1^{ji} \cdot e_{ji} X_i + k_{-1}^{ji} \cdot C_{ji} & X_i(0) &= x_i^0 \\ \frac{de_{ji}}{dt} &= -k_1^{ji} \cdot e_{ji} X_i + (k_{-1}^{ji} + k_{ji}) C_{ji} & e_{ji}(0) &= e_{ji}^0 \\ \frac{dC_{ji}}{dt} &= k_1^{ji} \cdot e_{ji} X_i - (k_{-1}^{ji} + k_{ji}) C_{ji} & C_{ji}(0) &= 0 \\ \frac{dX_j}{dt} &= k_{ji} \cdot C_{ji} & X_j(0) &= x_j^0. \end{aligned}$$

The Michaelis-Menten model is usually represented as the result of a Quasi Steady State Approximation [55, 67]:

$$\begin{aligned} \frac{dX_i}{dt} &= -e_{ji}^0 k_{ji} \frac{X_i}{X_i + K_{ji}} & (1.3) \\ \frac{dX_j}{dt} &= e_{ji}^0 k_{ji} \frac{X_i}{X_i + K_{ji}} \\ C_{ji} &= \frac{e_{ji}^0 \cdot X_i}{X_i + K_{ji}} \\ e_{ji} &= e_{ji}^0 - C_{ji}, \end{aligned}$$

where

$$K_{ji} := \frac{k_{-1}^{ji} + k_{ji}}{k_1^{ji}}$$

is the Michaelis-Menten constant. Indeed, this QSSR is obtained assuming that

$$\frac{de_{ji}}{dt} + \frac{dC_{ji}}{dt} = 0$$

and that the complex C_{ji} is at QSS, i.e.,

$$k_1^{ji} \cdot (e_{ji}^0 - C_{ji}) X_i - (k_{-1}^{ji} + k_{ji}) C_{ji} = 0.$$

To see more details of the proof we refer to [55].

Nevertheless, this approximation holds if the initial substrate concentration x_i^0 is sufficiently large compared with the initial enzyme concentration e_{ji}^0 [55, 67].

1.2.3. Elementary Flux Modes. Metabolic flux analysis assumes that metabolic reactions are organized into sequences called metabolic pathways or fluxes. In a living cell, thousands of independent enzyme-catalyzed reactions can occur [2], leading to a large number of fluxes. In order to identify a relative small set of fluxes from which any flux can be composed, the concept of elementary flux mode has been introduced.

Essentially, an elementary flux mode is a flux within a metabolic network that cannot be decomposed into simpler flux modes. Here we include two equivalent definitions of elementary flux mode, or elementary mode, proposed by Heinrich et al. [25] and Klamt et al. [36].

DEFINITION 1.2 (Elementary Flux Mode [25]). A flux mode M is defined as the set

$$M = \{\nu \in \mathbb{R}^r \mid \nu = \lambda\nu^*, \lambda > 0\},$$

where ν^* is a r -dimensional vector (different from the null vector) fulfilling the following two conditions:

- (C1) Steady-state condition. $N \cdot \nu^* = 0$.
- (C2) Sign restriction. If the system involves irreversible reactions, then the corresponding entries, ν^{irr} of ν^* fulfill inequality $\nu^{irr} \geq 0$.

A flux mode M with a representative ν^* is called elementary flux mode if, and only if, ν^* fulfills condition:

- (C3) Simplicity (non decomposability). For any couple of non-null vectors ν' and ν'' with the following properties:
 - (i) ν' and ν'' satisfy restrictions (C1) and (C2),
 - (ii) ν' and ν'' contain zero elements whenever ν^* does and they include at least one additional zero component each, i.e. $\text{supp}(\nu') \subsetneq \text{supp}(\nu)$ and $\text{supp}(\nu'') \subsetneq \text{supp}(\nu)$,

ν^* is not a non-negative linear combination of ν' and ν'' , i.e.,

$$\nu^* \neq \lambda_1\nu' + \lambda_2\nu'' \quad \lambda_1, \lambda_2 > 0.$$

A slightly different definition of the Elementary Flux Mode concept can be found in the work of Klamt et al. [36]. In this definition (given below), an Elementary Mode is not considered as a set but as a single vector.

DEFINITION 1.3 (Elementary modes [36]). Let N be the stoichiometric matrix of a reaction network and Irrev the index set of irreversible reactions. An elementary mode (EM) is a flux vector e fulfilling the following three conditions:

- (i) Steady State $N \cdot e = 0$.
- (ii) Reaction reversibility $e_i \geq 0$ ($\forall i \in \text{Irrev}$).
- (iii) Support-minimality: there is no vector \tilde{e} (different from the null vector) that fulfills (i) and (ii) and $\text{supp}(\tilde{e}) \subsetneq \text{supp}(e)$.

The following proposition states that Definition 1.2 and Definition 1.3 determine the same concepts.

PROPOSITION 1.1. *Suppose that ν is a representative of a elementary flux mode M according to Definition 1.2. Then, ν is an elementary mode according Definition 1.3. Reciprocally, if e is an elementary mode according Definition 1.3, then the flux mode M represented by e is an elementary flux mode by Definition 1.2.*

NOTE 1.2. For simplicity, if ν is a representative of a elementary flux mode M according to Definition 1.2, we only say that ν is and EFM according to Definition 1.2.

Proof. If we suppose that ν is an EFM according to Definition 1.2 but that it does not satisfy Definition 1.3, then there exists a non-null vector \tilde{e} such that $\text{supp}(\tilde{e}) \subsetneq \text{supp}(\nu)$ and satisfies (i) and (ii) of Definition 1.3. Hence, we can choose a number $\lambda > 0$ such that $\nu - \lambda \cdot \tilde{e}$ satisfies the reaction reversibility condition (ii) of Definition 1.3 and that $\text{supp}(\nu - \lambda \cdot \tilde{e}) \subsetneq \text{supp}(\nu)$ [e.g. $\lambda := \min\{\nu_i/\tilde{e}_i : i \in \text{supp}(\tilde{e}) \cap \text{Irrev}\}$ or $\lambda = \nu_i/\tilde{e}_i$ with any $i \in \text{supp}(\tilde{e})$ if $\text{supp}(\tilde{e}) \cap \text{Irrev} = \emptyset$]. Moreover, $N \cdot (\nu - \lambda \cdot \tilde{e}) = 0$ since the kernel of a matrix transformation is a vector subspace. But notice that $\nu = (\nu - \lambda \cdot \tilde{e}) + \lambda \cdot \tilde{e}$, in contradiction with the simplicity condition (C3) of Definition 1.2.

Reciprocally, assume that e is a EM according to Definition 1.3 and suppose that it is not a EFM according to Definition 1.2. Then, there exists a couple of vectors ν' and ν'' that satisfy $\text{supp}(\nu') \subsetneq \text{supp}(\nu)$ and $\text{supp}(\nu'') \subsetneq \text{supp}(\nu)$. This contradicts the condition (iii) of support-minimality in Definition 1.3. \square

1.2.4. Current Approaches for Metabolic Modeling. Metabolic modeling has been an efficient tool to grasp the metabolism of an organism. This approach has gained accuracy in the last decades, and turns out to be particularly efficient to improve production of target molecules, by understanding biological processes that influence the metabolism of an organism. These models are based on simplified metabolic networks, and generally include several hundreds of reactions associated to many metabolic compounds. In order to manage the large dimension of these models, some simplifying assumptions are generally necessary.

Since metabolic networks models are high dimensional complex systems, their mathematical analysis and parameter identification is a complicated

task. As a consequence, they are difficult to use for control purposes and identifying conditions to maximize productivity by a rigorous mathematical analysis is generally not possible.

Microalgae and cyanobacteria metabolic models can include thousands of reactions and metabolites [5]. Thus, metabolic modeling techniques to simplify these systems have been applied to describe microalgae and cyanobacteria metabolisms. These include techniques based on Steady State Assumptions [12, 38, 68, 79] or reduction techniques based on a Quasi Steady State Assumption [4].

Furthermore, the metabolisms of microalgae and cyanobacteria are driven by the solar flux, which supports fixation of CO_2 through Calvin cycle. Periodic fluctuation of light induces oscillations in their metabolisms, with accumulation of metabolites (especially lipids and carbohydrates). Therefore, such metabolisms are never at steady state.

However, most of the approaches dedicated to metabolism analysis assume balanced growth, i.e. Steady State Assumption (SSA), which leads to rough approximations of fluctuating systems. For instance, Flux Balance Analysis (FBA) [34, 57] is based on linear algebra to solve the equation $N \cdot V = 0$, where N is the stoichiometric matrix and V is the vector of intracellular reaction rates. Therefore, these approaches make two hypotheses. First that the derivative of each intracellular compound (per biomass unit) is zero, and second that the dilution rate is negligible.

1.2.5. Flux Balance Analysis. The principal hypothesis of FBA is that metabolic systems attain an equilibrium under any external conditions [34]. Moreover, the dilution due to growth is often omitted in the approaches that use FBA. Under these assumptions, the ODE problem is simplified and expressed as a linear algebraic equation:

$$N \cdot V(X) = 0, \tag{1.4}$$

where N is the stoichiometric matrix and $V(X)$ the vector of kinetics. Thus, FBA consists in finding elements in the kernel of the stoichiometric matrix, to have an estimation of the kinetics vector (which in that case is a constant vector).

In general, the number of reactions is larger than the number of metabolites in a metabolic network. Then, Equation (1.4) is supposed to be underdetermined. If Equation (1.4) has a non trivial solution, it has infinitely many solutions [23, 54]. Moreover, the set of solutions intersected with the set of irreversibility constrains

$$FC = \{\nu \in \mathbb{R}^r : N \cdot \nu = 0 \text{ and } \nu_i \geq 0 \quad \forall i \in \text{Irrev}\}$$

is a convex polyhedral cone called *flux cone* [37].

In order to select a vector in the *flux cone*, some constraints and optimization criteria are imposed in FBA. Then, an objective function $Z(V)$ is set with the aim of finding a unique solution [34]. These constraints are represented in a linear programming (LP) problem:

$$\begin{aligned} \max_V Z(V) \\ N \cdot V &= 0 \\ \nu_j^L &\leq \nu_j \leq \nu_j^U, \end{aligned}$$

where Z is a linear function and ν_j the j -th entry of V [15, 57]. Linear programming problems can be solved using tools as *linprog* in Matlab (<http://www.mathworks.com>), or *karmarkar* and *linpro* in Scilab (<http://www.scilab.org>).

1.2.6. Dynamical Flux Balance Analysis. Dynamical Flux Balance Analysis (DFBA) is an extension of FBA to account for dynamics [51]. This approach assumes that cells optimize an objective criterion. Then, several steady states are assumed and, for each transition from one steady state to another, FBA solution indicates an instantaneous change of the metabolic fluxes. DFBA can be formulated in a dynamic or a static approach.

1.2.6.1. *Dynamic Optimization Approach (DOA)*. In order to obtain time profiles of fluxes and metabolites levels, DOA considers the optimization problem in a complete time interval of interest. This can be translated in the following nonlinear programming (NLP) problem:

$$\max_{X(t), V(t), B(t)} w_{end} \Phi(X, V, B)|_{t=t_f} + w_{ins} \sum_{j=0}^M \int_{t_0}^{t_f} L(X, V, B) \delta(t - t_j) dt$$

$$\begin{aligned} \text{such that} \quad & \frac{dX}{dt} = N \cdot V \cdot B \\ & \frac{dB}{dt} = \mu \cdot B \\ & \mu = \sum w_i v_i \\ & t_j = t_0 + j \frac{(t_f - t_0)}{M} \quad j = 0, \dots, M \\ & c(V, X) \leq 0 \quad |\dot{V}| \leq \dot{V}_{max} \quad \forall t \in [t_0, t_f] \\ & X \geq 0 \quad B \geq 0 \quad \forall t \in [t_0, t_f] \\ & X(t_0) = X_0 \quad B(t_0) = B_0, \end{aligned}$$

where X is the vector of metabolite concentrations, B is the biomass concentration, V is the vector of metabolic fluxes per gram (DW) of the biomass,

N is the stoichiometric matrix, μ is the growth rate set as the weighted sum of reactions that synthesize the growth precursors, w_i are the amounts of the growth precursors required per gram (DW) of biomass, X_0 and B_0 are the initial conditions for the metabolite concentrations and the biomass concentration, respectively, c is a vector function of nonlinear constraints due to kinetic expressions for fluxes, t_0 and t_f are the initial and the final times, Φ is the terminal objective function, L is the instantaneous objective function, δ is the Dirac delta function, w_{ins} and w_{end} are the weights of the instantaneous and terminal objective function, respectively.

1.2.6.2. *Static Optimization Approach (SOA)*. In SOA approach the time is divided in N time intervals and the instantaneous optimization problem is solved at the beginning of each time interval, followed by integration over the interval. The optimization problem is solved using linear programming (LP) repeatedly to obtain the flux distribution at a particular time instant. In some cases, rate of change constraints are incorporated on the metabolic fluxes [51]. The LP problem is formulated as:

$$\max_{V(t)} \sum w_i v_i(t)$$

$$\begin{aligned} \text{such that } X(t + \Delta T) &\geq 0 & X(t) &\geq 0 \\ c(X(t)) \cdot V(t) &\leq 0 & & \forall t \in [t_0, t_f] \\ |V(t) - V(t - \Delta T)| &\leq \dot{V}_{max} \Delta T & & \forall t \in [t_0, t_f] \\ X(t + \Delta T) &= X(t) + N \cdot V \cdot \Delta T \\ B(t + \Delta T) &= B(t) + \mu \cdot B(t) \cdot \Delta T, \end{aligned}$$

where ΔT is the length of the time interval in consideration. At each time instant, the dynamic equations are numerically integrated assuming that fluxes are constant over the time interval. For the class of systems involving only bilinear terms with fluxes and biomass concentration, it is possible to analytically solve the dynamic equations [51].

Even though DFBA allows to obtain a dynamic model for the prediction of source molecules and biomass time profiles, some drawbacks of this approach have been observed. For instance, the SOA can lead to approximations not appropriate to solve optimization problems in a complete time interval [76].

On the other hand, with DOA, defining an optimization function can be a complex task, since the classical optimization function to maximize the production of biomass is not suitable for some cases (e.g. to represent accumulation of metabolites due to diel cycles). Moreover, DOA allows to predict dynamically metabolic fluxes to the synthesis of biomass as a single

component, but not to the storage of metabolites or to the distribution of biomass for different metabolic task, which might be essential to study the accumulation of macromolecules of interest [4] or metabolic adaptations in the case of metabolic-genetic networks [76].

1.2.7. Dynamic Reduction of Unbalanced Metabolism. One approach to reduce metabolic systems with dynamics using the Quasi Steady State Assumption is DRUM: Dynamic Reduction of Unbalanced Metabolisms [4]. This method considers subnetworks in Quasi Steady State (QSS), which are interconnected by metabolites that can accumulate and have dynamics. Then, Elementary Flux Modes (EFM, see definition in Section 1.2.3) are computed in each subnetwork to reduce them using the QSSA. As result, the dynamics of accumulative metabolites form a reduced system of Ordinary Differential Equations (ODE).

The objective with the DRUM approach is to reduce a system of the form

$$\frac{d\xi}{dt} = N \cdot V(\xi) \cdot B - D \cdot \xi + D \cdot \xi_{in},$$

where ξ represents the metabolites concentration vector composed of biomass B , N is the stoichiometric matrix, $V(\xi)$ the vector of kinetics, D the dilution in the bioreactor and ξ_{in} is a vector function representing the input.

The approach proposes to reduce the kinetics within the subnetworks using EFM. For these, the EFM of each subnetwork have to be computed. To facilitate computations, DRUM approach also omits the dilution growth (as FBA). Then, for each subnetwork SN_i , a vector α_i with new kinetics has to be defined in order to satisfy

$$E_i \cdot \alpha_i = V_i(\xi),$$

where E_i is the EFM matrix of subnetwork SN_i and $V_i(\xi)$ is the vector with the kinetics of the reactions in subnetwork SN_i . Then, the reduced system obtained is

$$\frac{d\xi}{dt} = (S_1 \cdot E_1 \dots S_k \cdot E_k) \cdot \begin{pmatrix} \alpha_1 \\ \vdots \\ \alpha_k \end{pmatrix} \cdot B - D \cdot \xi + D \cdot \xi_{in},$$

where S_i is the matrix with the columns of N associated to the reactions of subnetwork SN_i .

However, the definition of α_i is not a trivial task if we seek to obtain reduced systems with mathematical validation. The choice is left to the person who applies the approach. This can be deduced using techniques of optimization (e.g. FBA and DFBA), and it can be validated comparing with experimental data [4].

DRUM approach has provided sound results with efficient representation of accumulation of lipids and carbohydrates in microalgae submitted to light/dark cycles. Nevertheless, it relies on a series of assumptions whose mathematical bases are not rigorously established. Moreover, the QSSA is used without delimiting the “fast” and “slow” parts of the metabolic system.

1.2.8. Theory of Singularly Perturbed Systems for QSSR. Several works have already used the Theory of Singularly Perturbed Systems to reduce metabolic systems. These approaches also take into account the QSSA.

For example, Waldherr et al. [76] consider three types of molecular species: (1) extracellular nutrients and waste products, (2) intracellular metabolites and (3) macromolecules (gene products or large metabolites forming building blocks). Also, they differentiate fluxes as exchange reactions between cell and environment, metabolic reactions (conversions between metabolites) and biomass reactions (converting metabolites into macromolecules and vice versa).

For obtaining a system with two time scales, Waldherr et al. [76] suppose that the stoichiometry of metabolites in the formation of macromolecules are large. Moreover, they also assume that macromolecules formation is proportionally slower as these large stoichiometric coefficients increase. In other words, that macromolecules have slow dynamics, while former metabolites are consumed faster.

Finally, the slow part of the model in [76] correspond to the molar amount variables of macromolecules, extracellular nutrients and waste products, while intracellular metabolites constitute the fast part. Then, a reduced system of ODE accounting for the slow variables is deduced using Tikhonov’s Theorem for singularly perturbed systems (see Section 2.2 for the formulation of Tikhonov’s Theorem).

In the approach of Radulescu et al. [61], it is assumed that the production of intermediate species is not weak (i.e. reactions producing these metabolites have large kinetics). Then, they consider intermediates species with low concentration that are consumed by some reactions of considerable high rates, and that these reactions produce terminal species which have larger concentrations.

Intermediate species constitute the fast part of the metabolic system in [61], which then can be reduced using the results of Tikhonov and Fenichel for singularly perturbed systems. Also, quasi-stationary equations can be used to express intermediate species concentration as functions of the concentration of terminal species.

However, the authors of [61] admit that quasi-stationary species are generally difficult to detect. To tackle this problem, they use the quasi-stationary equations (QSSA) and conservation conditions related to a basis of the left kernel of the stoichiometric matrix.

Other approaches differentiate time scales based on parameters found in the literature. For example, Gerdtzen et al. [21] take into account the characteristic time constants associated to reactions. Then, in their model the rate constant of each kinetic reaction is defined as the inverse of its characteristic time constant.

Gerdtzen et al. [21] focus on metabolic networks where reactions can be partitioned into fast and slow reactions based on their characteristic time constants. Also, they consider a slow term, which involves dilution due to cell growth and transport of species in and out of the cell. Using singular perturbation arguments, they derive a nonlinear ODE model of slow dynamics, which do not contain large reaction rate constants.

Therefore, the approach described in [21] consists in first finding the characteristic time constants of reactions from the literature or experimentally. However, for complex reaction rates this identification is not simple. Parameters such as activity and concentration of enzymes, and the average concentration of species that have a regulatory effect on enzyme, must be considered.

In the paper of Holzhutter et al. [27], characteristic constants are described as dependence of system variables on parameters (rate constants) in steady state. Beyond the complexity of defining characteristic time constants, for large metabolic networks with hundreds of reactions, it is a difficult task to obtain the parameters associated to each kinetic.

CHAPTER 2

Mathematical Tools

In this chapter are established the principal mathematical definitions and properties used through this work. They are used in the Theorem of Tikhonov for singularly perturbed systems and in some proofs concerning the approach presented here.

2.1. The Big \mathcal{O} or Landau symbol

In Tikhonov's theorem, the Big \mathcal{O} or Landau symbol is used to characterize the difference between the solution and the approximation of a singularly perturbed system. In this work, it is also used to study the order of magnitude of parameters in metabolic systems. By means of this, for a class of metabolic networks described in the next chapters, we prove that the concentration of metabolites in the fast part of the system have concentrations one order of magnitude lower than metabolites in the slow part.

The following definitions and properties of the big \mathcal{O} or Landau symbol were mainly taken from [56].

DEFINITION 2.1. Let $\varepsilon > 0$. Two functions f and g have the same order or Landau symbol, i.e.

$$f(\varepsilon) = \mathcal{O}(g(\varepsilon)) \text{ as } \varepsilon \rightarrow 0,$$

if and only if there is a positive real number A such that

$$\lim_{\varepsilon \rightarrow 0} \frac{f(\varepsilon)}{g(\varepsilon)} = A.$$

Notice that in the limit $\varepsilon \rightarrow 0$, i.e. for $\varepsilon \in (0, 1)$, the order of magnitude can be arranged as follows:

$$\dots < \varepsilon^3 < \varepsilon^2 < \varepsilon < 1 < \varepsilon^{-1} < \varepsilon^{-2} < \varepsilon^{-3} < \dots$$

PROPERTY 2.1 (Multiplication). If $f_1 = \mathcal{O}(g_1)$ and $f_2 = \mathcal{O}(g_2)$, then $f_1 f_2 = \mathcal{O}(g_1 g_2)$. Moreover, if k is a nonzero constant (with respect to ε),

then $k \cdot g = \mathcal{O}(g)$.

PROPERTY 2.2 (Addition and subtraction). If $f_1 = \mathcal{O}(g_1)$, $f_2 = \mathcal{O}(g_2)$ and

$$\lim_{\varepsilon \rightarrow 0} \frac{g_2}{g_1} < \infty,$$

then $f_1 + f_2 = \mathcal{O}(g_1)$.

PROPERTY 2.3 (Division). If $f_1 = \mathcal{O}(g_1)$ and $f_2 = \mathcal{O}(g_2)$, then $f_2/f_1 = \mathcal{O}(g_2/g_1)$.

For functions also depending on another variable, there is the following definition of order magnitude.

DEFINITION 2.2. If $f(x, \varepsilon)$ and $g(x, \varepsilon)$ are real or complex valued functions, then

$$f(x, \varepsilon) = \mathcal{O}[g(x, \varepsilon)] \text{ as } \varepsilon \rightarrow 0$$

if there exists a positive constant $A > 0$ independent of x and ε^* such that

$$|f(x, \varepsilon)| \leq A \cdot |g(x, \varepsilon)| \text{ for all } \varepsilon \in [0, \varepsilon^*].$$

NOTE 2.1. In this work, we use a particular notation to indicate that two functions have the same order of magnitude. Indeed, if $f_1(x, \varepsilon) = \mathcal{O}(g(x, \varepsilon))$ and $f_2(x, \varepsilon) = \mathcal{O}(g(x, \varepsilon))$ as $\varepsilon \rightarrow 0$, then we denote

$$\begin{aligned} f_1(x, \varepsilon) &= \mathcal{O}(f_2(x, \varepsilon)) && \text{as } \varepsilon \rightarrow 0 \quad \text{or} \\ \mathcal{O}(f_1(x, \varepsilon)) &= \mathcal{O}(f_2(x, \varepsilon)) && \text{as } \varepsilon \rightarrow 0. \end{aligned}$$

Also, to compare the order of magnitude of two functions, we use a special notation. Suppose there exists a positive ε^* such that $g_1(x, \varepsilon) \leq g_2(x, \varepsilon)$ for all $\varepsilon \in [0, \varepsilon^*]$. Then, if $f_1(x, \varepsilon) = \mathcal{O}(g_1(x, \varepsilon))$ and $f_2(x, \varepsilon) = \mathcal{O}(g_2(x, \varepsilon))$, we write

$$\begin{aligned} f_1(x, \varepsilon) &\leq \mathcal{O}(f_2(x, \varepsilon)) && \text{as } \varepsilon \rightarrow 0 \quad \text{or} \\ \mathcal{O}(f_1(x, \varepsilon)) &\leq \mathcal{O}(f_2(x, \varepsilon)) && \text{as } \varepsilon \rightarrow 0. \end{aligned}$$

2.2. The Theorem of Tikhonov

The reduction method proposed in this work is based on time-scale separation. We suppose a system which has slow and fast dynamics, a characteristic widely recognized in biological systems. Slow-fast systems have been used in metabolic modeling, allowing to apply QSSA [62].

The QSSA represents the approximative solution of a singularly perturbed system depending on a very small parameter ε . The equation model depends on the subdivision of the flux rates into the rates of fast reactions (characterized by a factor $1/\varepsilon$) and the rates of slow reactions [66].

Singular perturbations cause a multi time-scale behavior of dynamical systems, characterized by the presence of slow and fast variations [35]. The singular perturbation model of finite-dimensional dynamic systems was extensively studied in the mathematical literature by Tikhonov and Vasil'eva [72], Levinson [43, 44], Hoppensteadt [31, 29, 30], Fenichel [19], etc. The Theorem of Tikhonov was a first result concerning approximations to the solution of a singularly perturbed system. For the following version of Tikhonov's Theorem, which is used through this work, we make reference to [39].

THEOREM 2.1 (Tikhonov's Theorem). *Let ε be a small positive scalar. Consider the system of equations*

$$\frac{dX}{dt} = f(X, Y, \varepsilon, t) \quad X(t_0) = X_0, \quad X \in \mathbb{R}^n, \quad (2.1)$$

$$\varepsilon \frac{dY}{dt} = g(X, Y, \varepsilon, t) \quad Y(t_0) = Y_0, \quad Y \in \mathbb{R}^m, \quad (2.2)$$

where f and g are continuously differentiable functions of their arguments X , Y , ε and t , in a domain of interest. The differential equation (2.2) degenerates into the algebraic equation

$$0 = g(\bar{X}, \bar{Y}, 0, t). \quad (2.3)$$

The model (2.1)-(2.2) is in standard form if and only if the following is satisfied:

- In the domain of interest, Equation (2.3) has $k \geq 1$ distinct (isolated) real roots

$$\bar{Y} = \varphi_i(\bar{X}, t), \quad i = 1, 2, \dots, k. \quad (2.4)$$

To obtain the i -th reduced model, substitute (2.4) into (2.1):

$$\frac{d\bar{X}}{dt} = f(\bar{X}, \varphi_i(\bar{X}, t), 0, t), \quad \bar{X}(t_0) = X_0. \quad (2.5)$$

Let $\tau = t/\varepsilon$ and $\hat{Y}(\tau) := Y(t) - \bar{Y}(t)$. The boundary layer system is defined as

$$\frac{d\hat{Y}}{d\tau} = g(X_0, \hat{Y}(\tau) + \bar{Y}(t_0), 0, t_0) \quad \hat{Y}(0) = Y_0 - \bar{Y}(t_0), \quad (2.6)$$

with X^0 and t_0 fixed parameters in the domain of interest.

Consider the next two conditions:

- C1. The equilibrium $\hat{Y}(\tau) = 0$ of (2.6) is asymptotically stable uniformly in X_0 and t_0 , and $Y_0 - \bar{Y}(t_0)$ belongs to its domain of attraction.
- C2. The eigenvalues of the Jacobian $\frac{\partial g}{\partial Y}$ evaluated, for $\varepsilon = 0$, along $\bar{X}(t)$ and $\bar{Y}(t)$, have real parts smaller than a fixed negative number. This is denoted as

$$\operatorname{Re}\lambda\left\{\frac{\partial g}{\partial Y}\right\} \leq -c < 0.$$

If there exists a solution \bar{X} of Equation (2.5) and conditions C1-C2 are satisfied, then the approximation

$$\begin{aligned} X(t) &= \bar{X}(t) + \mathcal{O}(\varepsilon) \\ Y(t) &= \bar{Y}(t) + \hat{Y}(\tau) + \mathcal{O}(\varepsilon) \end{aligned}$$

is valid for all $t \in [t_0, T]$, and there exists $t_1 \geq t_0$ such that

$$Y(t) = \bar{Y}(t) + \mathcal{O}(\varepsilon)$$

is valid for all $t \in [t_1, T]$.

The Theorem of Tikhonov provides an approximation to the solution of a singularly perturbed system, under some stability conditions. This approximation is characterized by an error of order $\mathcal{O}(\varepsilon)$, where ε is a small positive number. By definition of Big \mathcal{O} or Landau symbols, this implies that the error is bounded and decreases as $\varepsilon \rightarrow 0$.

2.3. Gershgorin Discs Theorem

In order to prove the stability conditions of Tikhonov's theorem, we use some properties of matrix theory for linear systems of equations. Particularly, we use that an equilibrium point of a linear ODE is stable if, and only if, the eigenvalues of the Jacobian matrix (evaluated in this point) have negative real part.

A description of bounded regions of the complex plane containing the eigenvalues $\lambda_1, \lambda_2, \dots, \lambda_n$ of a matrix $A \in \mathbb{C}^{n \times n}$ is given by the Gershgorin Theorem.

THEOREM 2.2 (Gershgorin Discs Theorem). *Let $A = (a_{ij}) \in \mathbb{C}^{n \times n}$, and let*

$$R_i(A) := \sum_{\substack{j=1 \\ j \neq i}}^n |a_{ij}|, \quad 1 \leq i \leq n$$

denote the deleted absolute row sums of A . Then, all the eigenvalues of A are located in the union of n discs

$$\bigcup_{i=1}^n \{z \in \mathbb{C} : |z - a_{ii}| \leq R_i(A)\}.$$

Furthermore, if a union of k of these discs forms a connected region that is disjoint from all the remaining $n - k$ discs, then there are precisely k eigenvalues of A in this region.

The proof of the Gershgorin Discs Theorem can be found in [32, 41]. Now we prove a proposition, which is a direct consequence of this theorem. For this, we introduce the following definitions.

DEFINITION 2.3 (Row Diagonally Dominant Matrix). Let $A = (a_{ij}) \in \mathbb{R}^{n \times n}$. We say that A is a row diagonally dominant matrix if

$$R_i(A) := \sum_{\substack{j=1 \\ j \neq i}}^n |a_{ij}| \leq |a_{ii}| \quad \forall i = 1, 2, \dots, n.$$

DEFINITION 2.4 (Strictly Row Diagonally Dominant Matrix). Let $A = (a_{ij}) \in \mathbb{R}^{n \times n}$. We say that A is a strictly row diagonally dominant matrix if

$$R_i(A) := \sum_{\substack{j=1 \\ j \neq i}}^n |a_{ij}| < |a_{ii}| \quad \forall i = 1, 2, \dots, n.$$

PROPOSITION 2.1. *Let $A = (a_{ij}) \in \mathbb{R}^{n \times n}$ be a strictly row diagonally dominant matrix. Suppose that $a_{ii} < 0$ for every $i = 1, 2, \dots, n$. Then, A is a nonsingular matrix and all its eigenvalues have negative real part. In other words, A is a stable matrix.*

Proof. Suppose that A is singular. Then, one of this eigenvalues is equal to zero. By the Gershgorin Discs Theorem, this implies that

$$0 \in \bigcup_{i=1}^n \{z \in \mathbb{C} : |z - a_{ii}| \leq R_i(A)\}.$$

Hence, there exists $i \in \{1, 2, \dots, n\}$ such that

$$|a_{ii}| \leq R_i(A),$$

which contradicts that A is a strictly row diagonally dominant matrix. We conclude that A is a nonsingular matrix.

On the other hand, by the Gershgorin Discs Theorem, any λ eigenvalue of A satisfies

$$\lambda \in \bigcup_{i=1}^n \{z \in \mathbb{C} : |z - a_{ii}| \leq R_i(A)\}.$$

Then, there exists $i \in \{1, 2, \dots, n\}$ such that

$$|\lambda - a_{ii}| < |a_{ii}|,$$

because A is strictly row diagonally dominant. But this is equivalent to

$$-2\operatorname{Re}(\lambda)a_{ii} < -\operatorname{Re}(\lambda)^2 - \operatorname{Im}(\lambda)^2 < 0,$$

because $\lambda \neq 0$. Since $a_{ii} < 0$, it follows that $\operatorname{Re}(\lambda) < 0$. Thus, every eigenvalue of A has negative real part. □

An analogous result to Proposition 2.1 for strictly column diagonally dominant matrices is also valid, as proved in the following in Corollary 2.1.

DEFINITION 2.5 (Column Diagonally Dominant). Let $A = (a_{ij}) \in \mathbb{R}^{n \times n}$. We say that A is a column diagonally dominant matrix if

$$C_i(A) := \sum_{\substack{j=1 \\ j \neq i}}^n |a_{ji}| \leq |a_{ii}| \quad \forall i = 1, 2, \dots, n.$$

DEFINITION 2.6 (Strictly Column Diagonally Dominant). Let $A = (a_{ij}) \in \mathbb{R}^{n \times n}$. We say that A is a strictly column diagonally dominant matrix if

$$C_i(A) := \sum_{\substack{j=1 \\ j \neq i}}^n |a_{ji}| < |a_{ii}| \quad \forall i = 1, 2, \dots, n.$$

COROLLARY 2.1. *Let $A = (a_{ij}) \in \mathbb{R}^{n \times n}$ be a strictly column diagonally dominant matrix. Suppose that $a_{ii} < 0$ for every $i = 1, 2, \dots, n$. Then, A is a nonsingular matrix and all its eigenvalues have negative real part. In other words, A is a stable matrix.*

Proof. Notice that A^T is a strictly row diagonally dominant matrix and recall that the determinant of a matrix is equal to the determinant of its transpose [41]. By Proposition 2.1,

$$\det(A) = \det(A^T) \neq 0.$$

Moreover, since

$$\det(A - \lambda \cdot I) = \det(A^T - \lambda \cdot I),$$

we conclude that all the eigenvalues of A have negative real part. □

2.4. Nonsingular M-Matrices

We also study the order of magnitude of the parameters in the reduced system obtained after Tikhonov's theorem. For the class of metabolic networks studied in this work, these parameters are basically obtained from the inverse of the Jacobian matrix of the fast subsystem. Therefore, we analyze the determinant and minors of this Jacobian.

Let A be a finite matrix with nonpositive off-diagonal and nonnegative diagonal entries, that is,

$$A = \begin{pmatrix} a_{11} & -a_{12} & \dots & -a_{1n} \\ -a_{21} & a_{22} & \dots & -a_{2n} \\ \vdots & \vdots & & \vdots \\ -a_{n1} & -a_{n2} & \dots & a_{nn}, \end{pmatrix}$$

where $a_{ij} \geq 0$ for every $i, j \in \{1, 2, \dots, n\}$. Then A can be expressed as

$$A = sI - B, \quad s > 0, \quad B \geq 0. \quad (2.7)$$

DEFINITION 2.7 (Spectral Radius). Let B be a square matrix. The spectral radius of B is defined as

$$\rho(B) = \max_{\lambda \in \sigma(B)} |\lambda|,$$

where $\sigma(B)$ is the spectrum of B , i.e. the set of distinct eigenvalues of B [54].

DEFINITION 2.8 (M-matrix). A matrix A of the form (2.7) is called an M-matrix if $s \geq \rho(B)$.

THEOREM 2.3. *Let*

$$Z^{n \times n} := \{A = (a_{ij}) \in \mathbb{R}^{n \times n} : a_{ij} \leq 0 \text{ if } i \neq j\}.$$

If $A \in Z^{n \times n}$, *then the following conditions are equivalent:*

- *A is a nonsingular M-matrix.*
- *All of the principal minors of A are positive.*
- *A is positive stable, that is, the real part of each eigenvalue of A is positive.*
- *A is inverse-positive, that is, A^{-1} exists and $A^{-1} \geq 0$.*

For the proof of Theorem 2.3 we make reference to [8].

DEFINITION 2.9 (Metzler matrix). A matrix $A \in \mathbb{R}^{n \times n}$ is a Metzler matrix if its off-diagonal elements are nonnegative, i.e. $a_{ij} \geq 0$ for all $i \neq j$ [18].

Notice that if A is a M -matrix, particularly $-A$ is a Metzler matrix. From Corollary 2.1 and Theorem 2.3, we deduce a proposition similar to Proposition 2.3, but for Metzler matrices with negative diagonal entries and that are also strictly column diagonally dominant matrices. This proposition is used through this work.

PROPOSITION 2.2. *Consider a strictly column diagonally dominant matrix*

$$A = \begin{pmatrix} -a_{11} & a_{12} & \dots & a_{1n} \\ a_{21} & -a_{22} & \dots & a_{2n} \\ \vdots & \vdots & & \vdots \\ a_{n1} & a_{n2} & \dots & -a_{nn} \end{pmatrix},$$

where a_{ij} are nonnegative. Then

- *A is a nonsingular matrix.*
- *All of the principal minors of A have sign equal to $(-1)^{n-1}$.*
- *A is stable, that is, the real part of each eigenvalue of A is negative.*
- *A is inverse-negative, that is, A^{-1} exists and $A^{-1} \leq 0$.*

Proof. It follows from Corollary 2.1 that A is a nonsingular stable matrix. Then, $\det(-A) = (-1)^n \det(A) \neq 0$ and $-A$ is positively stable. Indeed,

$$\begin{aligned} \det(-A - \lambda \cdot I) &= (-1)^n \det[A - (-\lambda) \cdot I] \\ &= (-1)^n \cdot [\lambda_1 - (-\lambda)] \cdot [\lambda_2 - (-\lambda)] \cdots [\lambda_n - (-\lambda)], \\ &= (-\lambda_1 - \lambda) \cdot (-\lambda_2 - \lambda) \cdots (-\lambda_n - \lambda), \end{aligned}$$

where $\lambda_1, \lambda_2, \dots, \lambda_n$ are the eigenvalues of A . Then, the eigenvalues of $-A$ are $-\lambda_1, -\lambda_2, \dots, -\lambda_n$ and they have positive real part, since $Re(\lambda_i) < 0$ for every $i = 1, 2, \dots, n$. Hence, $-A$ is a positive stable matrix.

Moreover, $-A \in Z^{n \times n}$. Thus, according to Theorem 2.3, all of the principal minors of $-A$ are positive and $(-A)^{-1} \geq 0$. Now observe that

$$A^{-1} = -(-A^{-1}) \leq 0.$$

In other words, A is inverse-negative. This implies that each principal minor of A has sign opposite to the determinant of A sign, i.e. $sgn(A_{ii}) = -sgn[\det(A)]$.

On the other hand $-A$ is inverse-positive and all of its principal minors are positive. This implies $\det(-A) > 0$. Then, $\det(A) = (-1)^n \det(-A)$ is positive if n is even and *negative* if n is odd. Therefore, the principal minors satisfy

$$sgn(A_{ii}) = (-1)^{n-1}.$$

□

2.5. Left Kernel Stoichiometry Condition

The laws of Physics and Chemistry have to be taken into account when modeling cells. Therefore, the law of mass conservation has to be satisfied. For metabolic systems at steady state, the mass conservation law is usually represented as

$$N \cdot V = 0,$$

where N is the stoichiometric matrix and V a constant vector of kinetics (e.g. in FBA [34]).

For a closed system with dynamics (not at steady state), we can assume that the sum of all metabolite concentrations is constant at any time instant. Then, if a closed metabolic system has as ODE

$$\frac{dX}{dt} = K \cdot f(X),$$

with K a square matrix, the mass conservation law is satisfied if the sum of the entries of each column is equal to zero. For a matrix K with nonpositive diagonal entries and nonnegative off-diagonal entries, this means that K is column diagonally dominant (see Definition 2.5).

Moreover, adding the dilution factor in a linear metabolic system (which is no longer closed but its kinetics still satisfy the mass conservation law)

$$\frac{dX}{dt} = K \cdot X - \mu \cdot X,$$

leads to a Jacobian matrix $(K - \mu \cdot Id)$ that is strictly column diagonally dominant (see Definition 2.6).

In Section 2.4 we prove some properties about strictly diagonally dominant square matrices. But in general, stoichiometric matrices are not square nor diagonally dominant matrices. Here we show a matrix transformation to associate a strictly diagonally dominant matrix to a stoichiometric matrix. To make this transformation, the left kernel of the stoichiometric has to contain a vector whose entries are all positive. In this case, the left kernel provides a set of conservation laws.

NOTE 2.2. Several authors have already proposed the left kernel of the stoichiometric matrix as a set of conservation relations [9, 14, 20, 61, 69]. But the idea of the stoichiometric matrix association to a strictly column diagonally dominant matrix is original.

Suppose a system of equations for a reaction network

$$\frac{dX}{dt} = N \cdot V(X) - \mu \cdot X, \quad (2.8)$$

where $N \in Mat_{m \times r}$ is the stoichiometric matrix, $V(X)$ the kinetics vector and μ the growth rate.

ASSUMPTION 2.1. There exists a positive vector

$$\beta := (\beta_1 \beta_2 \dots \beta_m), \quad \beta_i > 0 \quad \forall i,$$

in the left kernel of N . In other words,

$$\beta \cdot N = 0.$$

If Equation (2.8) is a linear system, it is possible to define a matrix $\mathcal{K} \in Mat_{r \times m}$ of constant reactions rates such that

$$N \cdot \mathcal{K} \cdot X = N \cdot V(X).$$

In this case, the Jacobian of (2.8) is $(N \cdot \mathcal{K} - \mu \cdot Id)$, with Id the identity matrix. Moreover, β is also in the left kernel of $N \cdot \mathcal{K}$ and $N \cdot \mathcal{K}$ is a square matrix with nonpositive diagonally entries and nonnegative off-diagonal entries.

PROPOSITION 2.3. Let $A \in Mat_{m \times m}$ be a matrix such that

$$\begin{aligned} a_{ii} &\leq 0 & \forall i = 1, \dots, m & \quad \text{and} \\ a_{ji} &\geq 0 & \forall i \neq j. \end{aligned}$$

Suppose there exists a positive vector β

$$\beta := (\beta_1 \beta_2 \dots \beta_m), \quad \beta_i > 0 \quad \forall i,$$

in the left kernel of A and consider the matrix

$$\text{diag}(\beta) := \begin{pmatrix} \beta_1 & 0 & \dots & 0 \\ 0 & \beta_2 & \dots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \dots & \beta_m \end{pmatrix} \in \text{Mat}_{m \times m}.$$

Then,

$$\text{diag}(\beta) \cdot A \cdot \text{diag}(\beta)^{-1}$$

is a column diagonally dominant matrix with nonpositive diagonal entries and nonnegative off-diagonal entries.

Proof. We have

$$\text{diag}(\beta) \cdot A \cdot \text{diag}(\beta)^{-1} = \begin{pmatrix} a_{11} & \frac{\beta_1}{\beta_2} a_{12} & \dots & \frac{\beta_1}{\beta_m} a_{1m} \\ \frac{\beta_2}{\beta_1} a_{21} & a_{22} & \dots & \frac{\beta_2}{\beta_m} a_{2m} \\ \vdots & \vdots & \ddots & \vdots \\ \frac{\beta_m}{\beta_1} a_{m1} & \frac{\beta_m}{\beta_2} a_{m2} & \dots & a_{mm} \end{pmatrix}.$$

Then, all the diagonal entries of A are nonpositive and all the off-diagonal entries are nonnegative. Moreover,

$$\sum_{i=1}^m \frac{\beta_i}{\beta_j} a_{ij} = 0 \quad \forall j = 1, 2, \dots, m,$$

because β is in the left kernel of A . We conclude that

$$|a_{jj}| = \sum_{\substack{i=1 \\ i \neq j}}^m \frac{\beta_i}{\beta_j} a_{ij} \quad \forall j = 1, 2, \dots, m, \quad (2.9)$$

□

Finally, as a consequence of Proposition 2.3, for any positive number $\mu > 0$,

$$\text{diag}(\beta) \cdot N \cdot \mathcal{K} \cdot \text{diag}(\beta)^{-1} - \mu \cdot Id$$

is a strictly column diagonally dominant matrix.

Let us define a system as

$$\frac{dX}{dt} = \left[\text{diag}(\beta) \cdot N \cdot \mathcal{K} \cdot \text{diag}(\beta)^{-1} - \mu \cdot Id \right] \cdot X, \quad (2.10)$$

whose Jacobian is a strictly column diagonally dominant matrix. Notice that

$$\text{diag}(\beta) \cdot N \cdot \mathcal{K} \cdot \text{diag}(\beta)^{-1} - \mu \cdot Id = \text{diag}(\beta) \cdot [N \cdot \mathcal{K} - \mu \cdot Id] \cdot \text{diag}(\beta)^{-1}.$$

Therefore, the Jacobian of system (2.8) (with linear kinetics) and the Jacobian of (2.10) are similar matrices.

NOTE 2.3. Hereafter, we consider stoichiometric matrices that have a positive vector in the left kernel. But also notice that this condition can be relaxed when the purpose is to use strictly diagonally dominant matrices only for a part of the system, e.g. a part where kinetics are linearized. In this case, the left kernel condition is restricted to a submatrix of the stoichiometric matrix. Moreover, instead of imposing an equality as in (2.9), we can seek to satisfy an inequality of the form

$$\sum_{\substack{i=1 \\ i \neq j}}^m \frac{\beta_i}{\beta_j} a_{ij} \leq |a_{jj}| \quad \forall j = 1, 2, \dots, m.$$

CHAPTER 3

Linearized Dynamic Metabolic Networks

Abstract: Here we propose a mathematical approach to reduce high dimensional linearized metabolic models, which relies on time scale separation and the Quasi Steady State Assumption. Contrary to the Flux Balance Analysis assumption that the whole system reaches an equilibrium, our reduced model depends on a small system of differential equations which represents the slow variables dynamics. Moreover, we prove that the concentration of metabolites in Quasi Steady State is one order of magnitude lower than the concentration of metabolites with slow dynamics (under some flux conditions). Also, we propose a minimization strategy to estimate the reduced system parameters. The reduction of a toy network with the method presented here is compared with other approaches. Finally, our reduction technique is applied to an autotrophic microalgae metabolic network.

3.1. Reduction Through Quasi Steady State Approximation

The main objective of our work is to provide mathematical foundations for the reduction of metabolic networks to dynamical models of low dimension. To achieve this objective, in a first stage we simplify the approach assuming linear kinetics for the whole metabolism. However, our method can be applied to any metabolic network whose kinetics can be locally linearized.

In this chapter, we compute an exact reduced model for any network in the class of systems addressed, whose metabolism is forced by a continuous input. In addition, we propose an algorithm to estimate the parameters of the reduced system.

In the method presented in this work we conserve the factor of dilution, which improves the precision of the approximation and preserves qualitative (stability) features of the original system.

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We discuss the accuracy of FBA and DRUM applied to the class of dynamic systems studied in this work. Both approaches rely on a series of hypotheses, including SSA and QSSA, respectively, and omission of the dilution due to growth.

In Section 3.2, we present the class of metabolic systems under study, which consists of metabolic networks with slow and fast first order reactions forced by a continuous input. We also write the system of Ordinary Differential Equations corresponding to any metabolic network of this class, and we formulate it as a slow-fast system. Then, we verify the conditions for applying the Theorem of Tikhonov for singularly perturbed systems [31, 39, 72] to obtain a reduced system. Using this approximation, we demonstrate that the concentration of each metabolite in Quasi Steady State (QSS) is one order of magnitude lower than the concentration of any metabolite with slow dynamics, subject to some flux constraints.

In Section 3.4, we introduce a method based on minimization for the calibration of the reduced system. In addition, when data of a metabolite involved in fast reactions is available, we can compute the corresponding parameters for its estimation in QSS.

In Section 3.5 we apply the approach to a toy metabolic network. The toy model includes a periodic input and reflects standard bricks in metabolic networks: combination of reversible and non-reversible reactions, with chains and cycles. We then compare the reduced model with FBA and the DRUM method.

Finally, we apply our method to an autotrophic microalgae metabolic network. We use the simplified network of Yang et al.[79] for this purpose. The reduction is carried out considering the macromolecules as the elements with the highest concentration. Using the data from Lacour et al.[40], we calibrated the reduced model. The simulations of the reduced model accurately fits the experimental data.

To conclude, in Section 3.7 we draw some conclusions and provide perspective for a rigorous slow-fast analysis of a larger class of metabolic networks.

3.2. The Slow-Fast Linear Metabolic Model

3.2.1. Metabolic system hypotheses. The class of metabolic networks studied in this work consists of metabolic systems with fast and slow first order reactions, which are forced by a continuous input. To describe the elements of this class, we focus on a general network assuming that one internal component (X_m) is consumed at low rate (see Figure 3.1). We consider then two subnetworks, before and after this component. The first subnetwork

we show a metabolic network example with nonlinear enzymatic reactions, whose kinetics are linearized around a functional point.

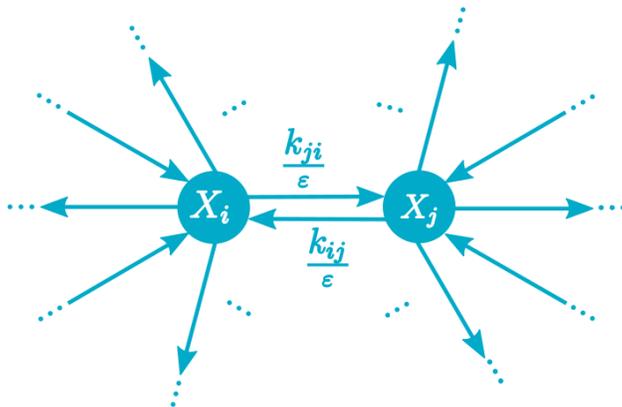


Figure 3.2. Single reactant-single product hypothesis: fast reaction k_{ji}/ϵ just consumes one metabolite (X_i) and produces another (X_j). This conditions leads to a linear system of equations.

Henceforth, we focus on a metabolic network with linear kinetics and a continuous positive input. In order to obtain some conclusions about the accumulation of metabolites, we make some assumptions about the fluxes in the network.

ASSUMPTION 3.1. We assume that a connector metabolite is not the origin of any fast reaction, nor the origin of an output with large rate.

On the other hand, for detecting when the input flux leads to the accumulation of metabolites which have fast outflows, we introduce the next definitions. For these, we consider that our metabolic network model (Figure 3.1) can be represented with a directed graph, where the metabolites are the vertex and the reactions are the directed edges (arrows). Only inputs and outputs are allowed to be edges with one vertex: final or initial vertex, respectively (the dilution due to growth is not taken into account for the graph).

In order to define a flux in a metabolic network, we recall the definition of directed path in a directed graph. We make reference to Balakrishnan et al. [3] and Benner et al. [7] for the following definition.

DEFINITION 3.1 (Directed path). Consider a directed graph G with set of vertices V and set of (directed) edges E . An edge $(v_i, v_f) \in E$ has initial vertex v_i and final vertex v_f .

A directed path, (ν_1, ν_k) -path, is a subgraph of G with a set of distinct vertices $\{\nu_1, \nu_2, \dots, \nu_k\} \subset V$ and set of edges $\{(\nu_i, \nu_{i+1}) : 1 \leq i < k\} \subset E$. We say that (ν_1, ν_k) -path has initial vertex ν_1 and final vertex ν_k .

DEFINITION 3.2 (Flux). A flux from X_i to X_j is a directed path which has as initial vertex X_i and as final vertex X_j .

The following definition of *trap* is equivalent to the definition for compartmental linear systems, considering the metabolites as compartments and the arrows (reactions, inputs and outputs) as flows into and out of the compartment. A compartmental system can also be represented by a directed graph. Then, a *trap* is defined as a compartment or a set of compartments from which there are no transfers to the environment nor to compartments that are not in the trap. Indeed, it has been proved that an autonomous linear compartmental system has a trap if, and only if, its compartmental matrix is singular [33].

DEFINITION 3.3 (Trap). Consider a graph with set of vertices \mathbf{N} and a subset of this $\mathbf{T} = \{X_{k_1}, \dots, X_{k_l}\} \subset \mathbf{N}$, $n > l \geq 1$. We say that \mathbf{T} is a *trap* if

- for every vertex $X_{k_i} \in \mathbf{T}$ there is no flux from X_{k_i} to any metabolite of $\mathbf{N} \setminus \mathbf{T}$ and
- no $X_{k_i} \in \mathbf{T}$ has an output to the exterior of the graph.

In this case, we also say that $X_{k_i} \in \mathbf{T}$ is in a *trap*.

DEFINITION 3.4 (Flux trap). Consider a flux F with initial vertex X_1 and final vertex X_n in a graph with vertices $\mathbf{N} = \{X_1, \dots, X_n\}$. We say that the graph has a *trap* for the flux F if there is a subset $\mathbf{T}_F = \{X_{k_1}, \dots, X_{k_l}\} \subset \mathbf{N} \setminus \{X_1, X_n\}$, such that

- \mathbf{T}_F is a trap (as a consequence, there is no flux from any $X_{k_i} \in \mathbf{T}_F$ to X_n) and
- for every vertex $X_{k_i} \in \mathbf{T}_F$ there is a flux from X_1 to X_{k_i} .

We also say that $X_{k_i} \in \mathbf{T}_F$ is in a *flux trap*. When it is clear which is the flux F taken into account, we only say that the graph has a *flux trap*.

REMARK 3.1. Notice that in a metabolic system with linear kinetics where the dilution factor is omitted, if there is a *trap*, then the system is undetermined, i.e. its (Jacobian) matrix is singular [33]. This case results in additional complexity from a mathematical point of view and it will not be discussed in this work, because the factor of dilution is not omitted in our approach.

On the other hand, we include the concept of *flux trap*, to detect the accumulation of metabolites with fast dynamics as a consequence of an input flux. However, from a biological point of view, the presence of *traps* or *flux traps* is very unlikely, unless organisms have been modified to indefinitely accumulate a metabolite.

In the system depicted in Figure 3.1 we assume the existence of a flux from X_1 to X_n . It then has a *flux trap* if there exists in one of the subnetworks a metabolite, X_i for some $i = 2, \dots, n-1, i \neq m$, such that there is a flux from X_1 to X_i , but there is no flux from X_i to X_n . A flux going from X_1 to X_n has to pass through X_m as well. Hence, there is a *flux trap* if and only if there is a *flux trap* in one subnetwork with fast dynamics.

3.2.2. Dynamics of Metabolite Concentrations. We write the ordinary differential equations that describes the metabolite concentrations of the model in Figure 3.1 as follows:

$$\begin{aligned}
\frac{dX_1}{dt} &= I(t) - (k_{21} + \mu)X_1 & (3.1) \\
\frac{dX_2}{dt} &= k_{21}X_1 + \left(\sum_{j=3}^{m-1} \frac{k_{2j}}{\varepsilon} X_j \right) - \left(\sum_{i=3}^{m-1} \frac{k_{i2}}{\varepsilon} + \mu \right) X_2 \\
&\vdots \\
\frac{dX_m}{dt} &= \frac{k_{m,m-1}}{\varepsilon} X_{m-1} - (k_{m+1,m} + \mu) X_m \\
&\vdots \\
\frac{dX_{n-1}}{dt} &= \left(\sum_{j=m+1}^{n-2} \frac{k_{n-1,j}}{\varepsilon} X_j \right) - \left(\sum_{\substack{i=m+1 \\ i \neq n-1}}^n \frac{k_{i,n-1}}{\varepsilon} + \mu \right) X_{n-1} \\
\frac{dX_n}{dt} &= \frac{k_{n,n-1}}{\varepsilon} X_{n-1} - \mu \cdot X_n,
\end{aligned}$$

with initial conditions $X_i(0) = x_i^0$ for every $i = 1, \dots, n$.

System (3.1) is a non-homogenous linear system, continuous in a domain $[0, T] \times \overline{\mathbb{R}_+^n}$ if $I(t)$ is continuous in the interval $[0, T]$. Moreover, system (3.1) is positive if and only if its (Jacobian) matrix is a Metzler matrix and $I(t) \geq 0$ [18].

3.2.3. Parameter definition and order of magnitude. In order to distinguish the two time-scales in system (3.1), we make the following hypothesis about the order of the parameters. For this purpose, we use the Big \mathcal{O} or Landau order symbol (see Section 2.1). Although the Landau symbol is defined for functions, we can also use it to indicate the order of our parameters. For this, we consider a parameter as the constant function which takes its value.

Recall that fast reactions have rates k_{ij}/ε , while slow reactions k_{ij} . We then suppose that ε is a small positive number such that

$$k_{ij} = \mathcal{O}(1) \quad \text{as } \varepsilon \rightarrow 0 \quad \forall i, j.$$

Thus, we consider the parameters k_{ij} as constant functions that implicitly depend on ε .

Analogously, $I(t)$ is a slow input and μ is lower or equal to the magnitude of any slow reaction (an usual hypothesis [70]). Hence, we suppose

$$\begin{aligned} I(t) &= \mathcal{O}(1) & \forall t \in [0, T], \\ \mu &\leq \mathcal{O}(1). \end{aligned} \quad (3.2)$$

For integrating the factor of dilution μ without affecting the order of the parameters in the system (3.1), we define

$$\frac{k_j}{\varepsilon} := \sum_{\substack{i=2 \\ i \neq j}}^n \frac{k_{ij}}{\varepsilon} + \mu \quad (3.3)$$

The definition given in (3.3) implies

$$\sum_{\substack{i=2 \\ i \neq j}}^n k_{ij} < k_j. \quad (3.4)$$

Also, $k_j = \mathcal{O}(1)$, as a consequence of condition (3.2) for μ .

3.2.4. Fast metabolites variable rescaling. Henceforth, we consider ε fixed. In order to write the original system (3.1) as a singularly perturbed system, we define new variables for the metabolites with fast dynamics and their initial conditions as follow:

$$\begin{aligned} Y_i &:= \frac{X_i}{\varepsilon} \\ Y_i(0) = y_i^0 &:= \frac{x_i^0}{\varepsilon}. \end{aligned}$$

Hence, we represent system (3.1) as the following slow-fast system:

$$\begin{aligned}
\frac{dY_2}{dt} &= \frac{1}{\eta} \left[k_{21}X_1 + \left(\sum_{j=3}^{m-1} k_{2j}Y_j \right) - k_2Y_2 \right] \\
&\vdots \\
\frac{dY_{m-1}}{dt} &= \frac{1}{\eta} \left[\left(\sum_{j=2}^{m-2} k_{m-1,j}Y_j \right) - k_{m-1}Y_{m-1} \right] \\
\frac{dY_{m+1}}{dt} &= \frac{1}{\eta} \left[k_{m+1,m}X_m + \left(\sum_{j=m+2}^{n-1} k_{m+1,j}Y_j \right) - k_{m+1}Y_{m+1} \right] \\
&\vdots \\
\frac{dY_{n-1}}{dt} &= \frac{1}{\eta} \left[\left(\sum_{j=m+1}^{n-2} k_{n-1,j}Y_j \right) - k_{n-1}Y_{n-1} \right]
\end{aligned} \tag{3.5}$$

$$\begin{aligned}
\frac{dX_1}{dt} &= I(t) - (k_{21} + \mu)X_1 & X_1(0) &= x_1^0 \\
\frac{dX_m}{dt} &= k_{m,m-1}Y_{m-1} - (k_{m+1,m} + \mu)X_m & X_m(0) &= x_m^0 \\
\frac{dX_n}{dt} &= k_{n,n-1}Y_{n-1} - \mu \cdot X_n & X_n(0) &= x_n^0.
\end{aligned} \tag{3.6}$$

Notice that ε is a parameter that we previously fixed. On the other hand, we consider $\eta \rightarrow 0$ to apply Tikhonov's Theorem.

In the linear system (3.5) we can consider the initial conditions as any nonnegative values, since global and local asymptotic stability are equivalent.

Hereafter, we say that Equation (3.5) and Equation (3.6) are the fast and the slow part of system (3.1), respectively. Furthermore, since the dynamics of $Y_i = X_i/\varepsilon$ are fast according to (3.5), we consider metabolites $X_2, \dots, X_{m-1}, X_{m+1}, X_{n-1}$ in Quasi Steady State.

3.2.5. Model properties. Let us rewrite the fast part (3.5) under a matrix form. We define

$$K' := \begin{pmatrix} K'_1 & 0 \\ 0 & K'_2 \end{pmatrix},$$

where

$$K'_1 := \begin{pmatrix} -k_2 & k_{23} & \dots & k_{2,m-1} \\ k_{32} & -k_3 & \dots & k_{3,m-1} \\ \vdots & \vdots & & \vdots \\ k_{m-1,2} & k_{m-1,3} & \dots & -k_{m-1} \end{pmatrix},$$

$$K'_2 := \begin{pmatrix} -k_{m+1} & k_{m+1,m+2} & \dots & k_{m+1,n-1} \\ k_{m+2,m+1} & -k_{m+2} & \dots & k_{m+2,n-1} \\ \vdots & \vdots & & \vdots \\ k_{n-1,m+1} & k_{n-1,m+2} & \dots & -k_{n-1} \end{pmatrix}.$$

Then system (3.5) can be written as

$$\eta \frac{dY}{dt} = I' + K' \cdot Y, \quad (3.7)$$

with $Y = (Y_2, \dots, Y_{m-1}, Y_{m+1}, \dots, Y_{n-1})^T$ and

$$I' = (k_{21} \cdot X_1, 0, \dots, 0, \overset{(m-1)\text{-th entry}}{k_{m+1,m} \cdot X_m}, 0, \dots, 0)^T.$$

PROPERTY 3.1. For every fixed scalars X_1, X_m, X_n , system (3.7) has a stable equilibrium point.

Proof. The matrices K'_1, K'_2 are strictly column diagonally dominant as a consequence of inequality (3.4). Therefore, they are stable matrices (see Corollary 2.1) \square

REMARK 3.2. For any constant values of X_1, X_m, X_n , we obtain

$$\bar{Y} := (\bar{Y}_2, \bar{Y}_3, \dots, \bar{Y}_{m-1}, \bar{Y}_{m+1}, \dots, \bar{Y}_{n-1}),$$

the equilibrium point of system (3.5) after computing the inverses of the matrices K'_1, K'_2 . Indeed, K'_1 and K'_2 are nonsingular matrices, because they are strictly column diagonally dominant (see Corollary 2.1). Hence,

$$\begin{pmatrix} \bar{Y}_1 \\ \bar{Y}_2 \\ \vdots \\ \bar{Y}_{m-1} \end{pmatrix} = (K'_1)^{-1} \cdot \begin{pmatrix} -k_{21}X_1 \\ 0 \\ \vdots \\ 0 \end{pmatrix}, \quad (3.8)$$

$$\begin{pmatrix} \bar{Y}_{m+1} \\ \bar{Y}_{m+2} \\ \vdots \\ \bar{Y}_{n-1} \end{pmatrix} = (K'_2)^{-1} \cdot \begin{pmatrix} -k_{m+1,m}X_m \\ 0 \\ \vdots \\ 0 \end{pmatrix}.$$

The equalities in (3.8) imply that the fast variables \bar{Y}_i in QSS are linear combinations of X_1 and X_m .

3.2.6. Boundary Layer Correction. The boundary layer correction is aiming at correcting at the initial transient of the fast variables, so that the approximation is also accurate at the very first time of the simulation. For this, we consider a time variable $\tau = t/\eta$. The boundary layer correction is defined as the function $\hat{Y}(\tau) = Y(t) - \bar{Y}(t)$. Then, the boundary layer problem of system (3.5)-(3.6) is written as

$$\begin{aligned} \frac{d\hat{Y}_2}{d\tau} &= k_{21}x_1^0 + \sum_{j=3}^{m-1} k_{2j}(\hat{Y}_j + \bar{Y}_j(0)) - k_2(\hat{Y}_2 + \bar{Y}_2(0)) \\ &\vdots \\ \frac{d\hat{Y}_{m-1}}{d\tau} &= \sum_{j=2}^{m-2} k_{m-1,j}(\hat{Y}_j + \bar{Y}_j(0)) - k_{m-1}(\hat{Y}_{m-1} + \bar{Y}_{m-1}(0)) \\ \frac{d\hat{Y}_{m+1}}{d\tau} &= k_{m+1,m}x_m^0 + \sum_{j=m+2}^{n-1} k_{m+1,j}(\hat{Y}_j + \bar{Y}_j(0)) - k_{m+1}(\hat{Y}_{m+1} + \bar{Y}_{m+1}(0)) \\ &\vdots \\ \frac{d\hat{Y}_{n-1}}{d\tau} &= \sum_{j=m+1}^{n-2} k_{n-1,j}(\hat{Y}_j + \bar{Y}_j(0)) - k_{n-1}(\hat{Y}_{n-1} + \bar{Y}_{n-1}(0)) \end{aligned} \quad (3.9)$$

with initial conditions

$$\hat{Y}_i(0) = Y_i(0) - \bar{Y}_i(0) = \frac{x_i^0}{\varepsilon} - \bar{Y}_i(0).$$

For obtaining a QSSR using the Theorem of Tikhonov, the stability of the origin for the boundary layer equation (3.9) is a necessary condition [39]. Notice that the (Jacobian) matrices of system (3.5) and system (3.9) are equal. Then, as in Property 3.1, we have the following result.

PROPERTY 3.2. Consider the boundary layer system (3.9). Then its equilibrium point $\hat{Y}(\tau) = \bar{0}$ is stable.

3.2.7. Reduced system. From Equation (3.8) we obtain the expressions of \bar{Y}_{m-1} and \bar{Y}_{n-1} in terms of X_1 and X_m , respectively. We then substitute in the slow part (3.6) and we obtain a reduced system with the variables X_1, X_m, X_n . Hence, we express the QSS approximation of the original system (3.1), deduced from the Theorem of Tikhonov, as follows:

$$\begin{aligned} \bar{X}_i &:= \varepsilon \cdot \bar{Y}_i = \varepsilon \cdot c_i \cdot \bar{X}_1 & \forall i = 2, \dots, m-1, \\ \bar{X}_i &:= \varepsilon \cdot \bar{Y}_i = \varepsilon \cdot c_i \cdot \bar{X}_m & \forall i = m+1, \dots, n-1, \end{aligned} \quad (3.10)$$

$$\begin{aligned}
\frac{d\bar{X}_1}{dt} &= I(t) - (k_{21} - \mu)\bar{X}_1 \\
\frac{d\bar{X}_m}{dt} &= k_{m,m-1}c_{m-1} \cdot \bar{X}_1 - (k_{m+1,m} + \mu)\bar{X}_m \\
\frac{d\bar{X}_n}{dt} &= k_{n,n-1}c_{n-1} \cdot \bar{X}_m - \mu \cdot \bar{X}_n.
\end{aligned} \tag{3.11}$$

where c_i are parameters that satisfy $\bar{Y}_i = c_i \cdot X_1$ or $\bar{Y}_i = c_i \cdot X_m$, respectively, deduced from Equation (3.8). The initial conditions for the reduced system (3.11) are the same that for (3.6), i.e., $\bar{X}_i(0) = x_i^0$ for $i = 1, m, n$.

PROPERTY 3.3. Let (X_i) be the solution of the original system (3.1) and (\bar{X}_i) the solution of system (3.10)-(3.11). Then

$$\begin{aligned}
X_i(t) &= \bar{X}_i(t) + \mathcal{O}(\varepsilon) & \forall t \in [0, T], \\
& & i = 1, m, n,
\end{aligned}$$

and there exists $0 \leq T_0 < T$ such that

$$\begin{aligned}
X_i(t) &= \bar{X}_i(t) + \mathcal{O}(\varepsilon^2) & \forall t \in [T_0, T], \\
& & i = 2, \dots, n-1, i \neq m.
\end{aligned}$$

Additionally, if (\hat{Y}_i) is the solution of the boundary layer system (3.9) and $\hat{X}_i := \varepsilon \hat{Y}_i$ for every $i = 2, \dots, n-1, i \neq m$,

$$X_i(t) = \bar{X}_i(t) + \hat{X}_i\left(\frac{t}{\varepsilon}\right) + \mathcal{O}(\varepsilon^2) \quad \forall t \in [0, T].$$

Proof. After Properties 3.1 and 3.2, we conclude that system (3.5)-(3.6) satisfies the conditions of Tikhonov's Theorem (see Theorem 2.1). Then, for the fast variables, a simple multiplication $X_i = \varepsilon \cdot Y_i$ leads to the desired result. \square

3.3. Magnitude of Concentration

Observe that the concentration of metabolites supposed to be in QSS is expressed as a linear combination of X_1 and X_m multiplied by ε . Since ε is a very small positive number, this suggests that the concentration of the metabolites in the slow part of the system is higher.

ASSUMPTION 3.2. System (3.1), represented in Figure 3.1, has no *flux trap* for the assumed flux going from X_1 to X_n . We refer to this conditions saying that the system has no *flux traps*.

Assumption 3.2 implies that if X_i is any metabolite in one of the subnetworks with fast dynamics and it is reached by the flux, then it has one fast outflow at least.

THEOREM 3.1 (Magnitude of Concentration Theorem – Linear Version). *Suppose system (3.1) under Assumption 3.2. Hence, with the notation of system (3.1) and Property 3.3, for every $t \in [T_0, T]$,*

$$\begin{aligned} X_i(t) &= \mathcal{O}(\varepsilon \cdot X_1(t)) & i &= 2, \dots, m-1, \\ X_i(t) &= \mathcal{O}(\varepsilon \cdot X_m(t)) & i &= m+1, \dots, n-1. \end{aligned} \quad (3.12)$$

Moreover, for every $t \in [T_0, T]$ we have

$$\begin{aligned} X_i(t) &\leq \mathcal{O}(\varepsilon \cdot X_j(t)), & j &\in \{1, m, n\}, \\ & & i &= 2, \dots, n-1, i \neq m. \end{aligned} \quad (3.13)$$

Magnitude of Concentration Theorem 3.1 states that, after the initial fast transient, any metabolite in QSS has a concentration one order of magnitude lower than any metabolite in the slow part. For the Big \mathcal{O} or Landau symbol notation used in this theorem, see Note 2.1 in Section 2.1.

Proof of Magnitude of Concentration Theorem 3.1. To demonstrate the first affirmation we consider the equalities in (3.10) and we will show that $c_i = \mathcal{O}(1)$ if $c_i \neq 0$, for $i = 2, \dots, m-1$. Since K'_1 is a nonsingular matrix,

$$(K'_1)^{-1} = \frac{1}{\det(K'_1)} \cdot \mathcal{C},$$

where \mathcal{C} is the transpose matrix of cofactors of K'_1 [41]. We then have according to Equation (3.8)

$$\begin{aligned} \bar{Y}_i &= \frac{1}{\det(K'_1)} \mathcal{C}_{1,i-1} \cdot (-k_{21} \bar{X}_1), \\ \text{then } c_i &= \frac{1}{\det(K'_1)} \mathcal{C}_{1,i-1} \cdot (-k_{21}). \end{aligned}$$

If K'_1 has no *traps* (i.e. the subnetwork with metabolites X_2, \dots, X_{m-1} has no *traps*), then

$$\det(K'_1) = (-1)^{m-2} \cdot \mathcal{O}(k_{ij}^{m-2}),$$

as stated by Proposition A.1 of Appendix A.1. Moreover, Corollary A.2 of Appendix A.1 implies that the cofactors $\mathcal{C}_{1,i-1}$ have order

$$\mathcal{C}_{1,i-1} = (-1)^{m-1} \cdot \mathcal{O}(k_{ij}^{m-3}).$$

On the other hand, if K'_1 has a *trap* \mathbb{T} not reached by the flux, as a consequence of Proposition A.2 and Corollary A.2 of Appendix A.1,

$$\begin{aligned} \frac{\mathcal{C}_{1,i-1}}{\det(K'_1)} &= (-1) \cdot \mathcal{O}(k_{ij}^{-1}) & X_i &\notin \mathbb{T} \\ \frac{\mathcal{C}_{1,i-1}}{\det(K'_1)} &= 0 & X_i &\in \mathbb{T} \end{aligned}$$

(see Section 2.1). We conclude that

$$\frac{-k_{21} \cdot \mathcal{C}_{1,i-1}}{\det(K'_1)} = \mathcal{O}(1)$$

if $\mathcal{C}_{1,i-1} \neq 0$, for every $i = 2, \dots, m-1$. The same reasoning applies for K'_2 and the variables which are linear combinations of X_m . Hence, in accordance with (3.10),

$$\begin{aligned} \bar{X}_i &= \varepsilon \cdot c_i \cdot \bar{X}_1 = \mathcal{O}(\varepsilon \cdot \bar{X}_1) & \forall i = 2, \dots, m-1, \\ \bar{X}_i &= \varepsilon \cdot c_i \cdot \bar{X}_m = \mathcal{O}(\varepsilon \cdot \bar{X}_m) & \forall i = m+1, \dots, n-1, \end{aligned}$$

and we obtain the equalities in (3.12) from Property 3.3.

In order to verify the second affirmation, we consider the reduced system (3.11). Then, the local maximum or minimum points of \bar{X}_1 , \bar{X}_m and \bar{X}_n satisfy

$$\begin{aligned} \bar{X}_m &= \frac{k_{m,m-1} \cdot c_{m-1}}{k_{m+1,m} + \mu} \bar{X}_1 \\ \bar{X}_n &= \frac{k_{n,n-1} \cdot c_{n-1}}{\mu} \bar{X}_m. \end{aligned}$$

But $c_{m-1} = \mathcal{O}(1)$, $c_{n-1} = \mathcal{O}(1)$, and $\mathcal{O}(\mu) \leq \mathcal{O}(k_{ji})$ for all i, j as a consequence of (3.2). Thus,

$$\mathcal{O}(\bar{X}_1) = \mathcal{O}(\bar{X}_m) \leq \mathcal{O}(\bar{X}_n).$$

Finally, we obtain the equalities in (3.13) using Property 3.3. \square

3.4. Reduced model calibration

3.4.1. Calibration of the slow system. We suppose that the slow metabolite concentrations are measured at r different time instants $0 \leq t_1 < t_2 < \dots < t_r \leq T$:

$$Z_i(t_j) = X_i(t_j) + \mathcal{N}(t_j) \quad i = 1, m, n$$

where $\mathcal{N}(t_j)$ is the measurement noise. From these measurements, we first calibrate the parameters for the reduced model (3.11). The objective is to find the value for the parameter vector

$$\theta = (\theta_1, \theta_2, \theta_3, \theta_4, \theta_5)$$

such that the solution of the reduced system

$$\begin{aligned} \frac{d\bar{X}_1}{dt} &= I(t) - \theta_1 \cdot \bar{X}_1 & \bar{X}_1(0) &= x_1^0 \\ \frac{d\bar{X}_m}{dt} &= \theta_2 \cdot \bar{X}_1 - \theta_3 \cdot \bar{X}_m & \bar{X}_m(0) &= x_m^0 \\ \frac{d\bar{X}_n}{dt} &= \theta_4 \cdot \bar{X}_m - \theta_5 \cdot \bar{X}_n & \bar{X}_n(0) &= x_n^0. \end{aligned} \quad (3.14)$$

best fits the data Z_1 , Z_m and Z_n . For this, we define a cost criterion that characterizes the modeling error, e.g.:

$$F_0(\theta) = \sum_{i=1,m,n} \sum_{j=1,\dots,r} \left(Z_i(t_j) - \bar{X}_i(t_j, \theta) \right)^2.$$

The approach consists in determining the best value

$$\hat{\theta} = (\hat{\theta}_1, \hat{\theta}_2, \hat{\theta}_3, \hat{\theta}_4, \hat{\theta}_5)$$

such that

$$F_0(\hat{\theta}) = \min\{F_0(\theta) : \theta \in D\},$$

with $D \subset \mathbb{R}_+^5$ a domain for the parameters. This can be carried out by using a minimization algorithm such as the Nelder-Mead algorithm.

3.4.2. Calibration for a metabolite in QSS. After the calibration of the slow system (3.14), we can estimate the concentration dynamics of a metabolite in QSS if there are some data of this. For this purpose, we assume that a fast metabolite is measured at different time instants $0 \leq t'_1 < t'_2 < \dots < t'_{r'} \leq T$:

$$Z_i(t'_j) = X_i(t'_j) + \mathcal{N}(t'_j)$$

for some $i \in \{2, \dots, n-1\} \setminus \{m\}$.

We first use the value of the slow metabolites

$$\left(\bar{X}_i(t, \hat{\theta}) \right)_{i=1,m,n}$$

obtained from calibration of the reduced system (3.14), to estimate the parameter α_i such that

$$\begin{aligned} X_i &:= \alpha_i \cdot \bar{X}_1 & \text{if } i \in \{2, \dots, m-1\}, \\ X_i &:= \alpha_i \cdot \bar{X}_m & \text{if } i \in \{m+1, \dots, n-1\}. \end{aligned}$$

Indeed we look for value of $\hat{\alpha}_i$ that minimize the differences

$$F_1(\alpha_i) = \sum_{j=1}^{r'} \left(\alpha_i \cdot \bar{X}_1(t'_j, \hat{\theta}) - Z_i(t'_j) \right)^2$$

if $i \in \{2, \dots, m-1\}$ and

$$F_2(\alpha_i) = \sum_{j=1}^{r'} \left(\alpha_i \cdot \bar{X}_m(t'_j, \hat{\theta}) - Z_i(t'_j) \right)^2$$

if $i \in \{m+1, \dots, n-1\}$. Consequently, the least squares solution is the following [54]:

$$\hat{\alpha}_i = \frac{\sum_{j=1}^{r'} Z_i(t'_j) \cdot \bar{X}_1(t'_j, \hat{\theta})}{\sum_{j=1}^{r'} \bar{X}_1(t'_j, \hat{\theta})^2} \quad \text{if } i \in \{2, \dots, m-1\}$$

$$\hat{\alpha}_i = \frac{\sum_{j=1}^{r'} Z_i(t'_j) \cdot \bar{X}_m(t'_j, \hat{\theta})}{\sum_{j=1}^{r'} \bar{X}_m(t'_j, \hat{\theta})^2} \quad \text{if } i \in \{m+1, \dots, n-1\}.$$

In general, measurements of fast metabolites are difficult, and therefore rarely done. It is worth noting that the reduction and calibration of the model for the fast metabolites can be done for any subset of measured metabolites.

REMARK 3.3. Let us emphasize that, even if no metabolite with fast dynamics is measured, this approach provides a reduced model (3.14) which can be calibrated. If not all the accumulative metabolites are measured, the reduced model may also be calibrated. Furthermore, the dynamics of all the measured metabolites are eventually predicted by means of the reduced model of equations.

This is an important point, since the calibration of fast variables (which is indeed experimentally much more tricky) can be done on a restricted number of metabolites.

3.5. A Toy Linear Metabolic Network

We consider a toy system of enzymatic reactions mimicking the characteristics of a real metabolic network but of lower dimension. In this model we have included some recurrent features of the metabolic network of a photoautotrophic microalga cell, such as a cycle and several reversible reactions. For instance, in the chain that includes the citric acid cycle, lower glycolysis, upper glycolysis and carbohydrate synthesis.

We thus construct our example with a chain that contains one reversible reaction and a cycle. This toy Network N1 is depicted in Figure 3.3. Additionally, to represent the effect of a periodic factor on the metabolism (such as sun light for microalgae), we introduce a periodic input $I(t) = k[\cos(t \cdot \omega) + 1]$.

We suppose that slow reaction rates have order $\mathcal{O}(10^{-2})$, and fast reaction rates $\mathcal{O}(10^1)$. Then, we choose $\varepsilon := 10^{-2}/10^1 = 10^{-3}$.

For instance, we suppose the slow reaction rate $k_{21} = 0.01 \cdot \text{min}^{-1} = 10^{-2} \cdot \text{min}^{-1}$. On the other hand, we assume that the fast reaction from X_2

to X_3 has rate equal to $20 \cdot \text{min}^{-1} = 2 \times 10^1 \cdot \text{min}^{-1}$. Then, we establish

$$\frac{k_{32}}{\varepsilon} = 20 \cdot \text{min}^{-1},$$

which implies $k_{32} = 20 \cdot \varepsilon \cdot \text{min}^{-1} = 0.02 \cdot \text{min}^{-1}$. Recall that $\mathcal{O}(k_{32}) = \mathcal{O}(k_{21}) = 10^{-2}$. For the definition of the rest of parameters, see Table 3.1.

Following the method proposed in Section 3.2, we write a singularly perturbed system to apply Tikhonov's Theorem (see Figure 3.4).

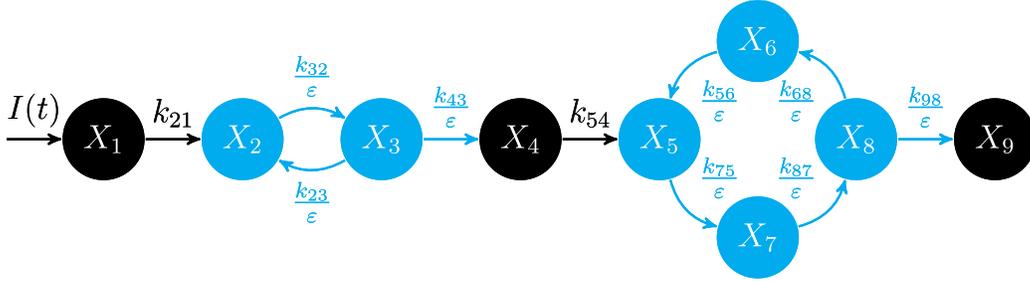


Figure 3.3. Metabolic Network N1. Metabolites X_1 , X_4 and X_9 have slow dynamics, while the others are in Quasi Steady State. Reactions from metabolites in QSS are faster than those from metabolites with slow dynamics. The input is $I(t) = k[\cos(t \cdot \omega) + 1]$.

Parameter	Value	Units	Parameter	Value	Units
ε	0.1×10^{-2}	-	k_{43}/ε	20	min^{-1}
ω	0.4×10^{-2}	-	k_{32}/ε	20	min^{-1}
μ	0.2×10^{-2}	min^{-1}	k_{68}/ε	20	min^{-1}
k	0.2×10^{-1}	min^{-1}	k_{75}/ε	10	min^{-1}
k_{21}	0.1×10^{-1}	min^{-1}	k_{23}/ε	10	min^{-1}
k_{54}	0.2×10^{-1}	min^{-1}	k_{56}/ε	10	min^{-1}
x_i^0	0.1×10^{-1}	$\mu\text{mol}/\text{m}^3$	k_{87}/ε	20	min^{-1}
			k_{98}/ε	10	min^{-1}

Table 3.1. Parameters considered for the simulation of dynamics in Network N1 (Figure 3.3). The initial conditions x_i^0 are all the same for $i = 1, \dots, 9$.

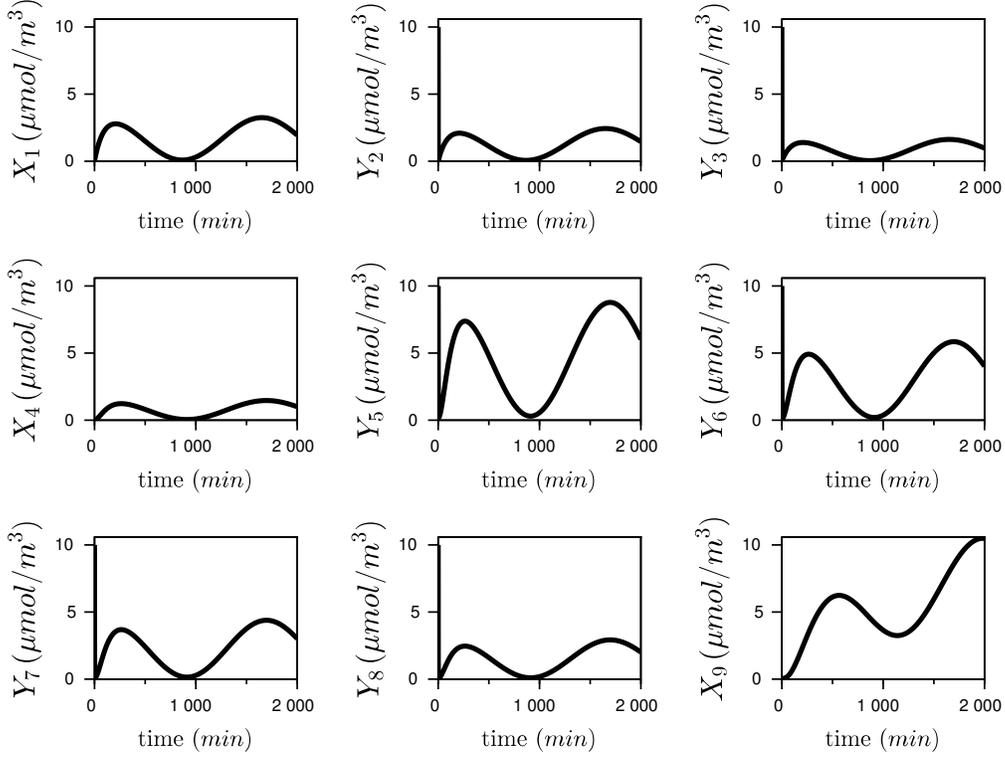


Figure 3.4. Behavior of the slow-fast system in standard form (see Equation (3.5)-(3.6)) that is obtained after the variable rescaling of Network N1 (Figure 3.3). Notice that the all the metabolite concentrations have the same order of magnitude, as a consequence of defining $Y_i = X_i/\varepsilon$ for the metabolites in the fast part. The parameters considered are specified in Table 3.1.

Moreover, we obtain an algebraic system that let us rewrite every variable of the fast part in terms of the variables of the slow part:

$$\begin{aligned}
 \bar{X}_2 &= \varepsilon \cdot \frac{k_{21} \cdot k_3}{d_1} \bar{X}_1, & \bar{X}_3 &= \varepsilon \cdot \frac{k_{21} \cdot k_{32}}{d_1} \bar{X}_1, \\
 \bar{X}_5 &= \varepsilon \cdot \frac{k_{54} \cdot k_6 \cdot k_7 \cdot k_8}{d_2} \bar{X}_4, & \bar{X}_6 &= \varepsilon \cdot \frac{k_{54} \cdot k_{68} \cdot k_{75} \cdot k_{87}}{d_2} \bar{X}_4, \\
 \bar{X}_7 &= \varepsilon \cdot \frac{k_{54} \cdot k_{75} \cdot k_6 \cdot k_8}{d_2} \bar{X}_4, & \bar{X}_8 &= \varepsilon \cdot \frac{k_{54} \cdot k_{75} \cdot k_{87} \cdot k_6}{d_2} \bar{X}_4,
 \end{aligned} \tag{3.15}$$

where $d_1 = k_3 \cdot k_2 - k_{23} \cdot k_{32}$ and $d_2 = k_5 \cdot k_6 \cdot k_7 \cdot k_8 - k_{56} \cdot k_{68} \cdot k_{75} \cdot k_{87}$.

Subsequently, we achieve a reduced system by substituting the expressions of (3.15) in the equations of the slow part:

$$\begin{aligned} \frac{d\bar{X}_1}{dt} &= k[\cos(t \cdot \omega) + 1] - (k_{21} + \mu)\bar{X}_1 & \bar{X}_1(0) &= x_1^0 \\ \frac{d\bar{X}_4}{dt} &= \frac{k_{21} \cdot k_{32}}{d_1} k_{43} \bar{X}_1 - (k_{54} + \mu)\bar{X}_4 & \bar{X}_4(0) &= x_4^0 \\ \frac{d\bar{X}_9}{dt} &= \frac{k_{54} \cdot k_{75} \cdot k_{87} \cdot k_6}{d_2} k_{98} \bar{X}_4 - \mu \bar{X}_9 & \bar{X}_9(0) &= x_9^0. \end{aligned} \quad (3.16)$$

As expected, the concentration of metabolites belonging to the fast part is one order of magnitude lower than the concentration of metabolites in the slow part (see Magnitude of Concentration Theorem 3.1, Figure 3.5 and Figure 3.6).

In Section 3.2.6, we described the boundary layer correction that can be applied to the variables in QSS. To see the effect of the boundary layer correction in this toy network, see Appendix A.3.

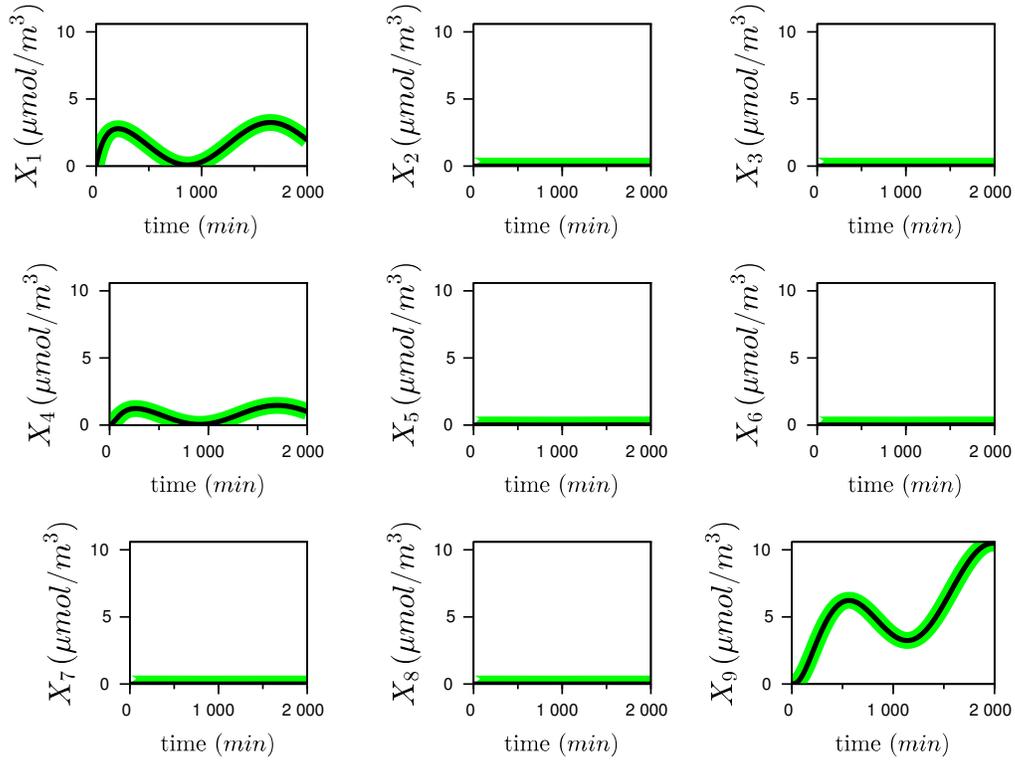


Figure 3.5. Dynamics of Network N1 (Figure 3.3). Thick green line: numerical solution of the original system (3.1); thin black line: reduced system obtained using the method developed in this work (3.15)-(3.16). The parameters considered for the simulation are specified in Table 3.1.

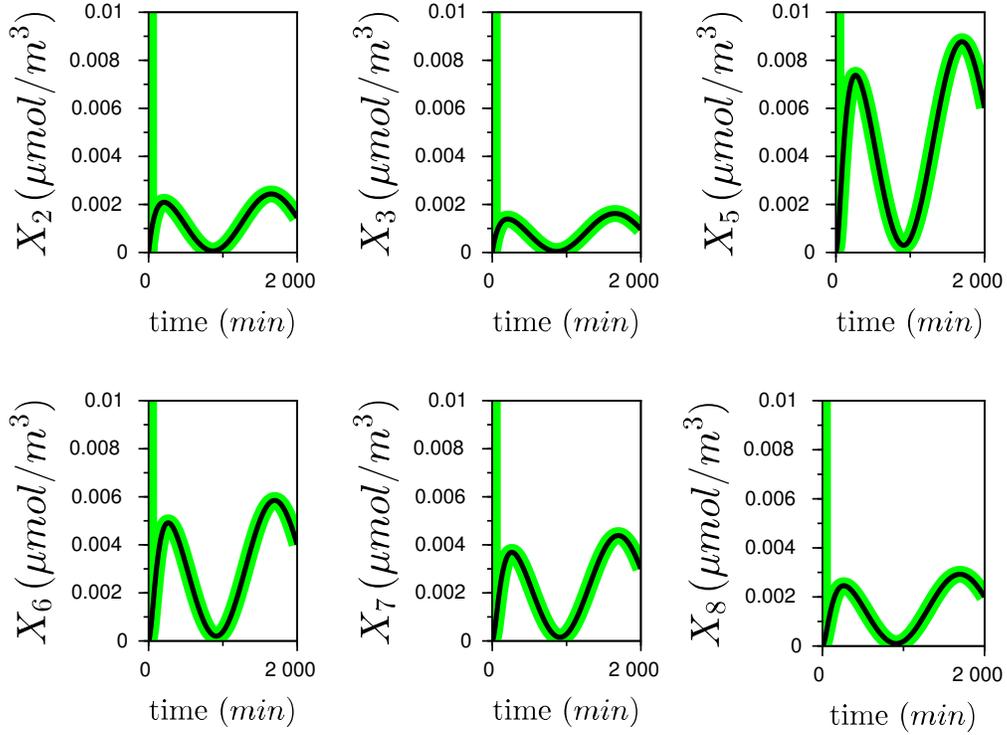


Figure 3.6. Zoom on the dynamics of metabolites in QSS (see also Figure 3.5).

3.5.1. Calibration of the slow dynamics. For this example, we consider that a few measurements of the metabolite concentrations are available. We represent it as the solution of the original system (3.1) for Network N1 plus a white noise:

$$Z_i(t_j) := X_i(t_j) + \beta(t_j) \quad j = 1, 2, \dots, r \quad (3.17)$$

where $\beta \sim \mathcal{N}(\sigma_i)$ and $\sigma_i = m(X_i) \times 10^{-1}$ for every $i = 1, \dots, n$. For simplicity we suppose that the data is obtained at the same time instants t_1, \dots, t_r for the slow and the fast parts of the system.

Following the notation in Section 3.4.1, we find the vector parameter $\hat{\theta}$ that minimizes the cost function F_0 . We used the function *fminsearch* in Scilab [6] to compute the minimum of the square differences function F_0 , with the Nelder-Mead algorithm (see Table 3.2).

i	Theoretical value θ_i	Estimated value $\hat{\theta}_i$	Error percent
1	0.12×10^{-1}	0.114×10^{-1}	4.7
2	0.10×10^{-1}	0.085×10^{-1}	15.2
3	0.22×10^{-1}	0.186×10^{-1}	15.3
4	0.20×10^{-1}	0.174×10^{-1}	12.8
5	0.20×10^{-2}	0.179×10^{-2}	10.5

Table 3.2. Original parameter (θ_i) and numerical approximation ($\hat{\theta}_i$) of the parameters for Equation (3.16), written as Equation (3.14). The parameters considered for simulation of the original dynamics in Network N1 are specified in Table 3.1.

3.5.2. Calibration of coefficients for metabolites in QSS. After estimating the parameters $\hat{\theta}_i$ as in Section 3.5.1, we consider the equalities in (3.15) for finding α_i such that

$$\begin{aligned} X_i &= \alpha_i \cdot \bar{X}_1 & i = 2, 3 \\ X_i &= \alpha_i \cdot \bar{X}_4 & i = 5, 6, 7, 8. \end{aligned} \quad (3.18)$$

We then obtain the parameters $\hat{\alpha}_i$ which resolve the linear least squares problem, as in Section 3.4.2 (see Table 3.3).

Lastly, we obtain a new approximation given by the following system:

$$\begin{aligned} \frac{d\bar{X}_1}{dt} &= k[\cos(t \cdot \omega) + 1] - \hat{\theta}_1 \bar{X}_1 & \bar{X}_1(0) &= x_1^0 \\ \frac{d\bar{X}_4}{dt} &= \hat{\theta}_2 \bar{X}_1 - \hat{\theta}_3 \bar{X}_4 & \bar{X}_4(0) &= x_4^0 \\ \frac{d\bar{X}_9}{dt} &= \hat{\theta}_4 \bar{X}_4 - \hat{\theta}_5 \bar{X}_9 & \bar{X}_9(0) &= x_9^0 \end{aligned} \quad (3.19)$$

$$\begin{aligned} \bar{X}_i &= \hat{\alpha}_i \cdot \bar{X}_1 & i &= 2, 3, \\ \bar{X}_i &= \hat{\alpha}_i \cdot \bar{X}_4 & i &= 5, 6, 7, 8. \end{aligned}$$

i	Theoretical value α_i	Estimated value $\hat{\alpha}_i$	Error percent
2	0.750×10^{-3}	0.727×10^{-3}	3.0
3	0.500×10^{-3}	0.498×10^{-3}	0.4
5	0.599×10^{-2}	0.568×10^{-2}	5.1
6	0.399×10^{-2}	0.384×10^{-2}	4.0
7	0.300×10^{-2}	0.288×10^{-2}	4.0
8	0.200×10^{-2}	0.194×10^{-2}	2.9

Table 3.3. Original parameters (α_i) and numerical approximations ($\hat{\alpha}_i$), solutions of the least square linear problems for approximating the coefficients in (3.18). The parameters considered for the simulation of the original dynamics in Network N1 are specified in Table 3.1.

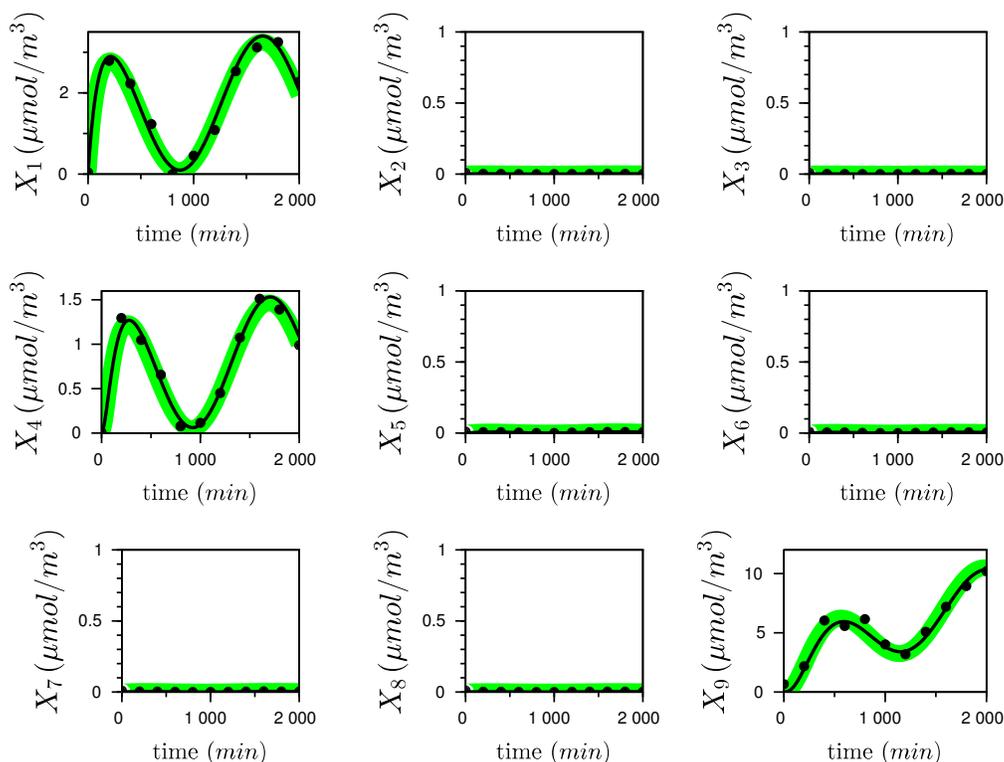


Figure 3.7. Calibrated system of toy Network N1 (Figure 3.3). Thick light line: original system (3.1); dots: supposed data with white noise (3.17); solid thin line: calibrated system (3.19) with the parameters in Table 3.2 and Table 3.3. The original parameters are specified in Table 3.1.

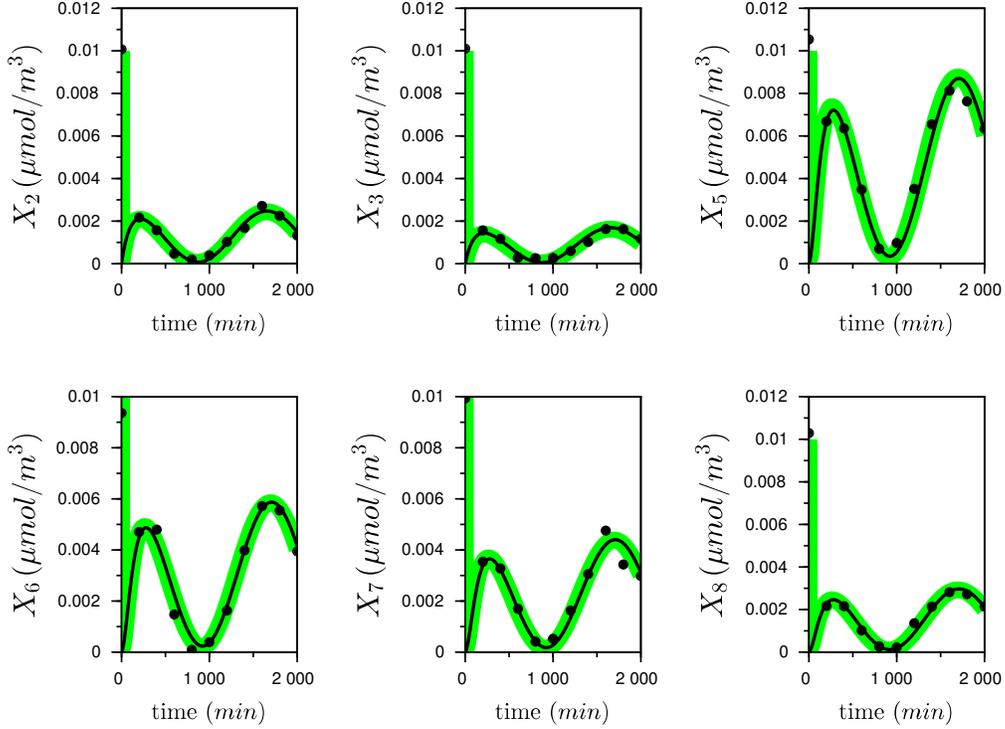


Figure 3.8. Zoom on the dynamics of metabolites in QSS (see also Figure 3.7).

3.5.3. Comparison between DRUM and FBA. The reduced system for the toy Network N1 (depicted in Figure 3.3) obtained after the DRUM[4] approach is

$$\begin{aligned}
 \frac{dX_1}{dt} &= k[\cos(t \cdot \omega) + 1] - k_{21}X_1 & X_1(0) &= x_1^0 & (3.20) \\
 \frac{dX_4}{dt} &= k_{21}X_1 - k_{54}X_4 & X_4(0) &= x_4^0 \\
 \frac{dX_9}{dt} &= k_{54}X_4 & X_9(0) &= x_9^0,
 \end{aligned}$$

(see Appendix A.4 for details). Remark that equations (3.16) and (3.20) coincide when $\mu = 0$. However, for nonzero growth rate, omitting μ can imply differences between both systems even in their qualitative behaviors (see Figure 3.9).

On the other hand, the principal hypothesis of Flux Balance Analysis is that metabolic networks reach a steady state, under any external conditions [34, 57]. From the numerical solutions depicted in Figure 3.5 and Figure 3.6, we can observe that the dynamics of the metabolic Network N1 do not reach an equilibrium in the delimited period of time. Indeed, the metabolic system

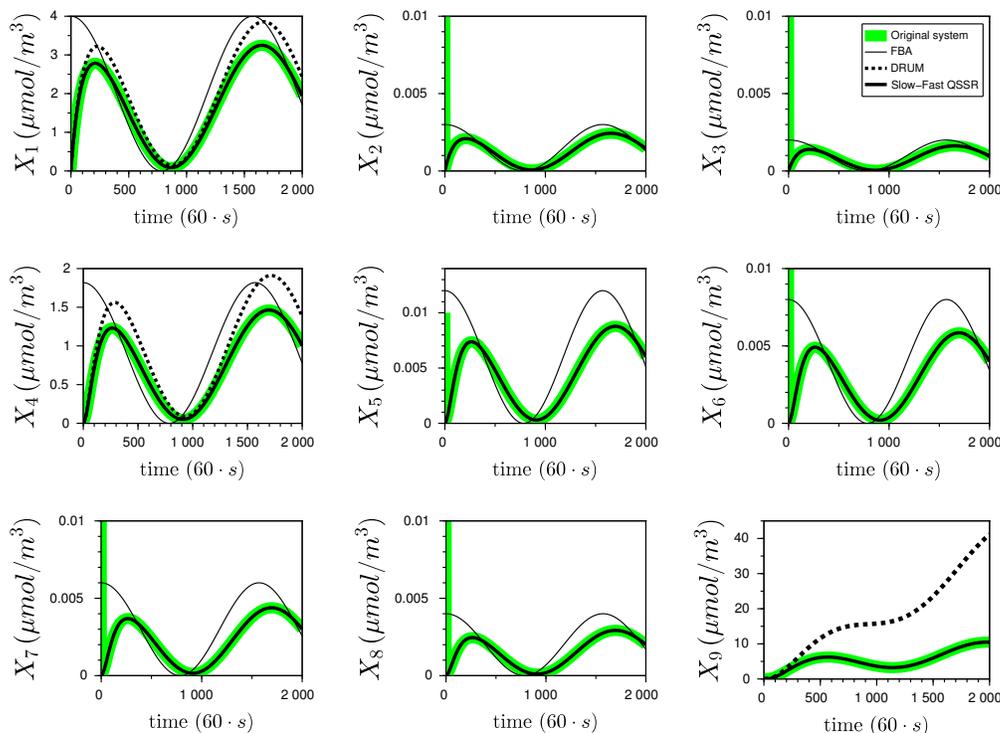


Figure 3.9. Comparison of different approaches to approximate the dynamics of Network N1 (Figure 3.3). Thick green line: graphical behavior of the original system (3.1); thick dark line: system obtained using the method proposed in this work (3.15)-(3.16); square dotted line: system obtained after DRUM (3.20); thin line: Flux Balance Analysis solution (see Appendix A.4 for details). The parameters considered are specified in Table 3.1. In this figure, for the metabolites in QSS there is no approximation from the framework Drum. For X_9 there is no approximation solution with FBA. The reduction method developed in this work gives the most accurate approximation to the original dynamics.

of N1 have no equilibrium point, because it is forced by a continuous (non constant) periodic input.

This is typically a case where FBA is a rough approximation. To illustrate this fact, we applied FBA to the toy metabolic Network N1 presented in Section 3.5 and we compare with our approach. The purpose of FBA is to resolve the algebraic equation

$$N \cdot V(X) = 0$$

for the variables X_1, \dots, X_8 , where X is the vector of metabolite concentrations, N is the stoichiometric matrix and $V(X)$ is the vector of kinetics reactions. We recall that FBA methods omit the dilution due to growth. As a consequence, the concentration of X_9 cannot be estimated from resolving

the FBA algebraic equation mentioned above. The results are explained with details in Appendix [A.4.2](#).

3.6. Reduction for Autotrophic Microalgae Metabolism

The proposed method is applied to the metabolism of autotrophic microalgae. A metabolic network taken from Yang et al. [79] was used to represent the metabolism of autotrophic microalgae. The network has 61 reactions and 59 metabolites. We assume that each enzymatic reaction can be represented by a Michaelis-Menten kinetics. Then, the metabolic system can be linearized around the working mode to end up to the structure proposed in this work (see Appendix [A.5](#) for details).

The inputs in the system are functions representing carbon dioxide (CO₂) and nitrate (NO₃) uptake rates, and a function fueled by the photon flux density (PFD), which regulates the light step in photosynthesis.

Macromolecules, such as lipids and carbohydrates, are stored during the day and reach relatively high concentration in microalgae. According to our approach, we suppose that macromolecules are in the slow part of the system (see Magnitude of Concentration Theorem 3.1). The rest of the metabolites are considered to have lower concentration, and then to be in QSS.

We assume there is no metabolite that accumulates as high as macromolecules between the inputs and the metabolites in the slow part, and that this is because the intermediate metabolites are consumed by fast reactions. A closer look at the considered metabolic network shows that the macromolecules (lipids, carbohydrates and chlorophyll particularly) are all produced directly from a metabolite in the fast part and they are not consumed after. According to the generalized reduction presented in Appendix [A.2](#), metabolites in the fast part (put into QSS) are linear combinations of the inputs.

PROPERTY 3.4. Consider a linear metabolic system of n metabolites with several inputs $I_1(t), I_2(t), \dots, I_k(t)$. Suppose that metabolites X_1, X_2, \dots, X_m are not consumed by any reaction, while $X_{m+1}, X_{m+2}, \dots, X_n$ are consumed by fast reactions. Then, applying our reduction strategy, the equations of slow metabolites are equal to linear combinations of the inputs and the term of growth dilution:

$$\frac{d\bar{X}_i}{dt} = \sum_{j=1}^k \beta_j^i I_j(t) - \mu \bar{X}_i \quad \forall i = 1, \dots, m,$$

where $\beta_j^i = 0$ if there is no flux from $I_j(t)$ to X_i .

Proof. See Appendix [A.2](#). □

Derived from Property 3.4, we can deduce the reduced system composed of the microalgae macromolecules equations:

$$\frac{dX_i}{dt} = \mathbf{b}_i \cdot L(t) + \mathbf{c}_i \cdot C_0 + \mathbf{n}_i \cdot N_0 - \mu \cdot X_i - \mathbf{e}_i,$$

$X_i(0) = x_i^0$, where X_i is the concentration of a macromolecule, $L(t)$ is the function representing the continuous evolution of light intensity, C_0 and N_0 are the controlled concentration constants of CO_2 and NO_3 , respectively, $\mu > 0$ is the dilution due to growth, \mathbf{b}_i , \mathbf{c}_i and \mathbf{n}_i are nonnegative numbers combining the parameters of the original system, as well as the real number \mathbf{e}_i that also depends on functional equilibrium points of the linearized system (see the Appendix A.5 for details). These constants will be calibrated by our approach.

We consider experimental data described in Lacour et al. [40]. The same light-day pattern has been considered as in the experiments. Photon Flux Density (PFD) at noon was $1,500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The data available only include the concentration of carbohydrate [CAR], neutral lipids [NL] and chlorophyll [CHLO]. From the simplified autotrophic microalgae metabolic network [79], we assume that Glucose-6-phosphate [G6P], Diacylglycerol [DG] and Glutamate [GLU] are the direct precursors of [CAR], [NL] and [CHLO], respectively. Also, we consider Acetyl-coenzyme A [AcCoA] as the direct precursor of [DG].

Therefore, we calibrated a system of three equations representing the concentrations of $X_1=\text{CAR}$, $X_2=\text{NL}$ and $X_3=\text{CHLO}$:

$$\begin{aligned} \frac{dX_1}{dt} &= \mathbf{b}_1 \cdot L(t) + \mathbf{c}_1 \cdot C_0 - \mu \cdot X_1 - \mathbf{e}_1, \\ \frac{dX_2}{dt} &= \mathbf{b}_2 \cdot L(t) + \mathbf{c}_2 \cdot C_0 - \mu \cdot X_2 - \mathbf{e}_2, \\ \frac{dX_3}{dt} &= \mathbf{b}_3 \cdot L(t) + \mathbf{c}_3 \cdot C_0 + \mathbf{n}_3 \cdot N_0 - \mu \cdot X_3 - \mathbf{e}_3. \end{aligned} \tag{3.21}$$

Notice that in the first two equations of (3.21) $\mathbf{n}_1 = 0$ and $\mathbf{n}_2 = 0$, because in the simplified network there is no flux from the NO_3 input to CAR nor DG (see Property 3.4).

We use the minimization method described in Section 3.4.1 and the *fminsearch* tool in Scilab (<http://www.scilab.org>), to estimate the parameters of system (3.21). The *fminsearch* tool in Scilab is based on the Nelder-Mead algorithm [6]. The numerical solution of the reduced system (3.21) is presented in Figure 3.10 and its calibrated parameters in Table 3.4. The simulations demonstrate that the reduced system accurately approximates the experimental data.

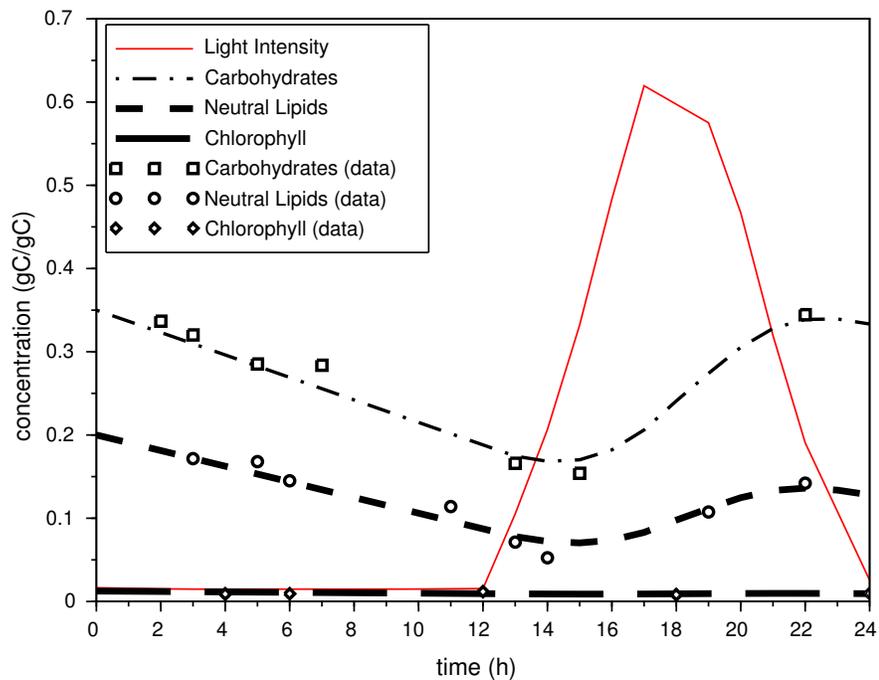


Figure 3.10. Dynamical behavior of Carbohydrates, Lipids and Chlorophyll in the metabolism of autotrophic microalgae. Experimental data was obtained from Lacour et al. [40]. The reduced model (3.21) was calibrated using the data. The parameters obtained after the calibrations are in Table 3.4. Light pattern (PFD) is also represented.

Parameter	Value	Units
\mathbf{b}_1	0.400×10^{-4}	$(\text{gC/gC}) \cdot \mu\text{mol}^{-1} \cdot \text{m}^2$
\mathbf{b}_2	0.200×10^{-4}	$(\text{gC/gC}) \cdot \mu\text{mol}^{-1} \cdot \text{m}^2$
\mathbf{b}_3	0.400×10^{-6}	$(\text{gC/gC}) \cdot \mu\text{mol}^{-1} \cdot \text{m}^2$
\mathbf{e}_1	0.147×10^{-1}	$(\text{gC/gC}) \cdot \text{s}^{-1}$
\mathbf{e}_2	0.100×10^{-1}	$(\text{gC/gC}) \cdot \text{s}^{-1}$
\mathbf{e}_3	0.300×10^{-3}	$(\text{gC/gC}) \cdot \text{s}^{-1}$
C_0	0.172×10^{-1}	gC/gC
N_0	0.287×10^{-1}	gC/gC
\mathbf{c}_1	0.100×10^{-3}	s^{-1}
\mathbf{c}_2	0.100×10^{-3}	s^{-1}
\mathbf{c}_3	0.100×10^{-3}	s^{-1}
\mathbf{n}_3	0.100×10^{-2}	s^{-1}
μ	0.100×10^{-6}	s^{-1}

Table 3.4. Parameters obtained after the calibration of system (3.21) with experimental data obtained from Lacour et al. [40]. The initial conditions of the system are $x_1^0 = 0.35 \text{ gC/gC}$, $x_2^0 = 0.2 \text{ gC/gC}$ and $x_3^0 = 0.0125 \text{ gC/gC}$.

3.7. Discussion and Conclusion

In this chapter we have reduced a metabolic network to a small number of macroscopic reactions, eliminating internal metabolites under the QSSA. We have shown that this computation is possible for a non-homogeneous linear system of n equations, accounting a continuous input. Moreover, our approach is suitable for any metabolic network whose kinetics can be locally linearized.

The method proposed in Section 3.2 incorporates the dilution factor with the coefficients of reaction rates, allowing to check the stability conditions for obtaining an accurate approximation. On the other hand, when the dilution factor is omitted for resolving a system in QSS, as most approaches do, the reduced models obtained lose accuracy, as shown for the toy network N1.

We emphasize that, writing the dynamical system into the proper canonical form of singularly perturbed systems is crucial to rigorously separate the time scales. QSSA applied to non canonical forms can lead to erroneous conclusions [11], as well as when the conditions of Tikhonov's Theorem are not fulfilled [72].

Additionally, for a system with no *flux traps*, we proved that the concentration of metabolites in QSS is very low in comparison to the accumulative metabolites (Magnitude of Concentration Theorem 3.1) as a consequence of the two time-scales. Using the reverse reasoning, we can detect slow reactions associated to accumulative metabolites and fast reactions related to metabolites with low concentration.

The Theory of Singularly Perturbed Systems is a tool which has already been used to justify the Quasi Steady State Assumption in metabolic networks [21, 22, 61, 65, 76]. However, our approach combines this tool with others and eventually it leads to an alternative approach with a different reduction strategy. Note also that we use the concepts of *trap* [33] and *flux trap*, which were, to the best of our knowledge, never considered previously for Quasi Steady State Reductions of metabolic networks. As future work, more generalized systems including nonlinear kinetics can be studied with the framework developed in this work.

CHAPTER 4

Nonlinear Dynamic Metabolic Networks

Abstract: Here we propose a rigorous approach to reduce metabolic nonlinear models. We assume that a metabolic network can be represented with Michaelis-Menten enzymatic reactions, and that it contains at least two different time-scales. We also consider a continuous (slowly) varying input in the model, such as light for microalgae, so that the system is never at steady state. Using a Quasi Steady State Reduction based on Tikhonov's Theorem, a reduced system with a characterized error is obtained. Furthermore, our analysis proves that the metabolites with slow dynamics reach higher concentrations (by one order of magnitude) than the fast metabolites. A simple example illustrates our approach and the resulting accuracy of the reduction method, also showing that it is adequate for systems with more than two time-scales.

4.1. Reduction with Dynamics in Enzymatic Reactions

Models of metabolic networks are non-linear and high dimensional systems, which makes difficult to determine their dynamical behavior and calibrate them. Here we propose a rigorous mathematical approach to reduce the dimension of a dynamical metabolic system. The reduction that we propose allows to characterize the approximation error and it is appropriate to derive model based control schemes. The idea is to keep some dynamical components of the model, that are necessary specially when dealing with microalgae and cyanobacteria.

We study a class of metabolic models of dimension n , where the enzymatic reactions rates are represented by Michaelis-Menten kinetics. The objective is to reduce this model accounting a permanently fluctuating input and including dilution of the metabolic compounds due to the growth rate. The system is not closed and never reaches a steady state. At the end, we can express a slow dynamical system of small dimension and a fast system as a

This chapter is part of the accepted version of the following article: López Zazueta, C., Bernard, O., and Gouzé, J.-L. (2018). Analytical Reduction of Nonlinear Metabolic Networks Accounting for Dynamics in Enzymatic Reactions. *Complexity Journal* [49], which has been published in final form at <https://doi.org/10.1155/2018/2342650>.

function of the variables of the slow system. The error in this reduction is small and bounded.

In Section 4.2, we introduce the class of models addressed, which contains metabolic networks composed of two (general) subnetworks of fast reactions connected by metabolites with slow dynamics. In Section 4.3, we develop a mathematical model of ODE for these metabolic systems.

In Section 4.4, after a change of variables for the metabolites with fast dynamics, the system is presented as a slow-fast system. The conditions for applying Tikhonov's Theorem for singularly perturbed systems are verified and we end-up with a reduced dynamical model, which has a bounded error.

In Section 4.5, we prove that metabolites in QSS, under some flux conditions, have concentration one order of magnitude lower than slow metabolites. Additionally, in Section 4.6, we propose an identification algorithm to estimate the parameters of the reduced system from available data.

Finally, we apply our method to a toy metabolic model in Section 4.7. This simple model is forced by a periodic input, includes standard bricks in metabolic networks and several time-scales.

4.2. Network of Enzymatic Reactions

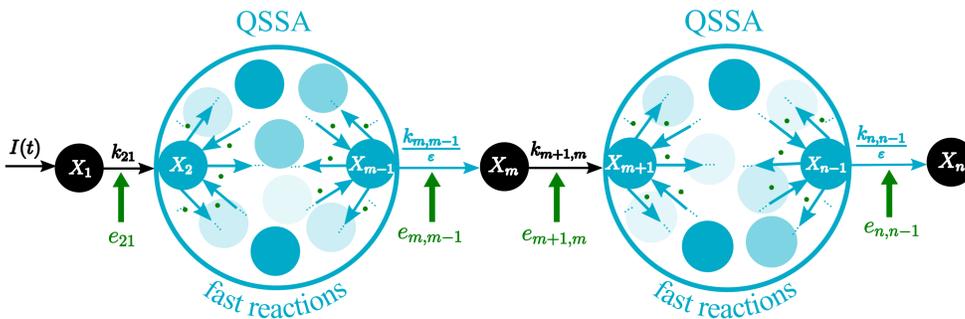


Figure 4.1. System of enzymatic reactions. An arrow from X_i to X_j represents a Michaelis-Menten reaction catalyzed by an enzyme e_{ji} , with substrate X_i , product X_j and product formation rate k_{ji} or $\frac{k_{ji}}{\epsilon}$. Fast reactions are within two subnetworks, which are interconnected by the metabolites X_1, X_m and X_n . The connector metabolites are consumed by reactions with low rates, while the metabolites in the subnetworks are consumed by fast reactions and are at Quasi Steady State.

In this section we present the class of metabolic networks studied all over the work, which are illustrated in Figure 4.1. These networks are composed of two subnetworks of fast reactions, which are interconnected by several

metabolites with slow rates of consumption. The subnetworks have an arbitrary finite number of metabolites and reactions between them.

These subnetworks are not assumed to have a specific topology. Therefore, they represent a generic case of metabolic networks. The only condition on them is that their metabolites $X_2, \dots, X_{m-1}, X_{m+1}, \dots, X_{n-1}$ are consumed by fast reactions.

The class of systems addressed in this work can be considered as a simplification or one part of a larger network. However, the results presented through this work can be extended (see Appendix B.4), allowing the study of more complex systems on the bases of this approach.

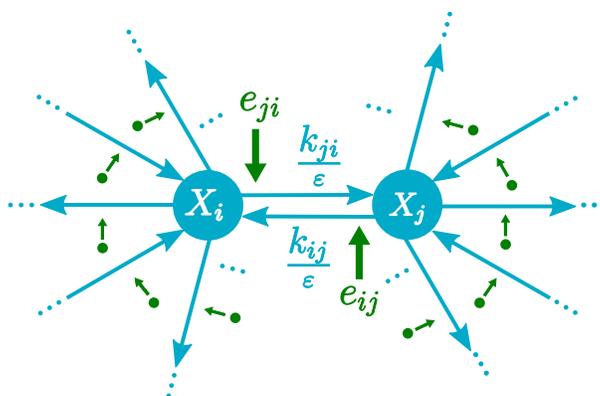


Figure 4.2. Enzymatic reactions between metabolites in QSS depicted in Figure 4.1. The metabolites inside the subnetworks are substrates or products of fast reactions catalyzed by an enzyme.

4.2.1. Summary of the methodology for reducing Slow-Fast Dynamical Metabolic models. We consider a general class of metabolic models allowing internal accumulation, represented with the network in Figure 4.1. In order to rigorously reduce this large dimensional model, our objective is to take benefit of the two-time scales and finally rewrite it in the canonical form of singularly perturbed systems. Then the Theorem of Tikhonov can be applied and a reduced system is derived with an accurate bound of the error [39].

The first challenge is to find the appropriate change of variable for the metabolites with fast dynamics, to end up with a slow-fast system

$$\begin{aligned} \frac{dX}{dt} &= F(t, X, Y, \eta) & X(0) &= x^0 \\ \frac{dY}{dt} &= \frac{1}{\eta} G(t, X, Y, \eta) & Y(0) &= y^0, \end{aligned} \quad (4.1)$$

where X is the vector of metabolites with slow dynamics, Y is the vector of metabolites with fast dynamics and η is a very small parameter. Actually,

Y results from a rescaling of fast dynamics metabolites X_{fast} in the model: $Y := X_{fast}/\eta$.

When the system is under this general form, we prove some conditions necessary to apply Tikhonov's Theorem [39] and finally we obtain a Quasi Steady State Reduction of system (4.1):

$$\frac{dX}{dt} = F(t, X, \bar{Y}, 0) \quad X(0) = x^0, \quad (4.2)$$

where \bar{Y} is a root of the equation

$$0 = G(t, X, \bar{Y}, 0).$$

If \bar{X} is a solution for (4.2), the Quasi Steady State Approximation (\bar{X}, \bar{Y}) to the solution of (4.1) satisfies

$$\begin{aligned} X &= \bar{X} + \mathcal{O}(\eta), \\ Y &= \bar{Y} + \mathcal{O}(\eta), \end{aligned}$$

after an initial fast transient. In other words, the error of the Quasi Steady State Approximation has order of magnitude η , which is supposed to be a small positive number. The reduced system differs from existing approaches, mainly because we do not neglect the metabolite dilution due to cell growth.

The mathematical validity of the Quasi Steady State Reduction (QSSR) for the class of systems considered in this work is showed from Section 4.3 to Section 4.4.

As a new striking result, this approach allows to prove that the concentration of the metabolites in Quasi Steady State is one order of magnitude lower than the metabolites with slow dynamics, i.e.,

$$\eta \cdot Y = X_{fast} \leq \eta \cdot \mathcal{O}(X).$$

The conditions under which this assertion holds are given in Section 4.5.

4.3. Considered Class of Networks

In this section, we describe the systems of the network class considered in this work. Then, in Section 4.4 and Section 4.5, we deduce a QSSR and prove some conclusions about the magnitude of metabolite concentrations (see Magnitude of Concentration Theorem 4.1) for these systems.

The results obtained in the following sections can be extended to more complex networks. For instance, considering additional slow reactions or more subnetworks of fast reactions connected by metabolites with slow dynamics (see Appendix B.4).

4.3.1. Notation. Consider the network of n enzymatic reactions depicted in Figure 4.1 and Figure 4.2, where an arrow from X_i to X_j represents an enzymatic reaction catalyzed by e_{ji} , with substrate X_i , product X_j and product formation rate k_{ji} or k_{ji}/ε . Then, every enzymatic reaction can be described with the Michaelis-Menten model (see Section 1.2.2).

However, it is necessary justify the Quasi Steady State Approximation for the Michaelis-Menten model. For this purpose, some solutions have been presented. For example, this holds if the initial substrate concentration x_i^0 is sufficiently large compared with the initial enzyme concentration e_{ji}^0 [55, 67].

We suppose that among the product formation rates there are two scales of magnitude. Reactions with large rate are within two subnetworks, which are interconnected by the metabolites X_1, X_m and X_n . We suppose that the metabolites connecting the subnetworks are consumed by reactions with low rates.

In this context, we say that a reaction is fast if its rate is large, while a reaction is slow if its rate is low. Moreover, we assume the rates of fast reactions sufficiently larger than those of the slow reactions. Then, we denote fast reactions rates by

$$\frac{k_{ji}}{\varepsilon}$$

and slow reactions rates by

$$k_{ji},$$

where ε is a small positive number.

Additionally, a continuously varying nonnegative input $I(t)$ (e.g. the CO_2 uptake in a microalgae submitted to light/dark cycles) and a growth rate $\mu > 0$, which acts as a dilution factor, are taken into account for the models.

4.3.2. Dynamical Model. According to the standard Quasi Steady State Reduction for Michaelis-Menten enzymatic reactions (described in Section 1.2.2), we write the ODE system for the model in Figure 4.1 as

$$\frac{dX_i}{dt} = F_i(t, X_1, \dots, X_n, \varepsilon, \mu) \quad X_i(0) = x_i^0, \quad (4.3)$$

where

$$F_1 := I(t) - e_0^{21} k_{21} \frac{X_1}{X_1 + K_{21}} - \mu X_1,$$

$$F_i := \sum_{j \in \{1, m, n\}} e_{ij}^0 k_{ij} \frac{X_j}{X_j + K_{ij}} + \sum_{\substack{j=2 \\ j \neq m}}^{n-1} e_{ij}^0 \frac{k_{ij}}{\varepsilon} \frac{X_j}{X_j + K_{ij}} - \sum_{j=1}^n e_{ji}^0 k_{ji} \frac{X_i}{X_i + K_{ji}} - \mu X_i,$$

for $i = m, n$, and

$$F_i := \sum_{j \in \{1, m, n\}} e_{ij}^0 k_{ij} \frac{X_j}{X_j + K_{ij}} + \sum_{\substack{j=2 \\ j \neq m}}^{n-1} e_{ij}^0 \frac{k_{ij}}{\varepsilon} \frac{X_j}{X_j + K_{ij}} - \sum_{j=1}^n e_{ji}^0 \frac{k_{ji}}{\varepsilon} \frac{X_i}{X_i + K_{ji}} - \mu X_i,$$

for every $i = 2, \dots, n-1, i \neq m$.

The variable X_i describes the i -th metabolite cell concentration, $I(t)$ is a nonnegative continuous function, ε is a small positive number, e_{ji}^0 , k_{ji} and K_{ji} are nonnegative parameters, and $\mu > 0$ is the growth rate. When there is no reaction with substrate X_i and product X_j , we define $k_{ji} = 0$, and also $k_{ii} = 0$ for every $i = 1, \dots, n$.

NOTE 4.1. In our model we can include first order (linear) reactions. In this case, instead of writing

$$e_{ji}^0 k_{ji} \cdot \frac{X_i}{X_i + K_{ji}} \quad \text{or} \quad e_{ji}^0 \frac{k_{ji}}{\varepsilon} \cdot \frac{X_i}{X_i + K_{ji}}$$

as for enzymatic reactions, we have to write

$$e_{ji}^0 k_{ji} X_i \quad \text{or} \quad e_{ji}^0 \frac{k_{ji}}{\varepsilon} X_i,$$

respectively, in Equation (4.3). For the sake of simplicity, in this work we only consider the more general case with Michaelis-Menten reactions.

In line with the QSSR of Michaelis-Menten system, we recall that $e_{ji}^0 k_{ji}$ (or $e_{ji}^0 k_{ji} / \varepsilon$ for the fast reactions) and K_{ji} are parameters related to the enzyme reaction with substrate X_i and product X_j . Indeed, e_{ji}^0 is the initial enzyme concentration, k_{ji} (or k_{ji} / ε) is the product formation rate and $K_{ji} > 0$ is the specific Michaelis-Menten constant defined as

$$K_{ji} := \frac{k_{-1}^{ji} + k_{ji}}{k_1^{ji}} \quad (4.4)$$

(see Section 1.2.2).

An important preliminary property that the dynamical system (4.3) has to obey is that, the concentration $X_i(t)$ has to remain nonnegative over the time if the initial conditions are nonnegative. In our model, this depends on the input $I(t)$. This is stated in the following Property:

PROPERTY 4.1. If the initial condition x_i^0 is nonnegative for every $i = 1, \dots, n$ and $I(t) \geq 0$ for every $t \in [0, T_1]$, then system (4.3) is positively invariant in $\overline{\mathbb{R}_+^n}$.

Proof. To verify this, we show that system (4.3) is positively invariant in $\overline{\mathbb{R}_+^n}$ if $I(t)$ is nonnegative over any interval $[0, T_1]$.

Recall that all K_{ji} is supposed to be positive and every parameter, e_{ji}^0, k_{ji}, μ , is nonnegative. Then, we have for any $i = 1, \dots, n$,

$$F_i(X_1, \dots, \underset{i\text{-th entry}}{0}, \dots, X_n, t, \varepsilon, \mu) \geq 0$$

if $X_j \geq 0$ for every $j = 1, \dots, n, j \neq i$. Therefore, system (4.3) is positively invariant in $\overline{\mathbb{R}_+^n}$. \square

4.3.3. Parameter Order of magnitude. With our notation, to represent different time-scales in the reactions, we consider ε a small positive number highlighting the difference between the parameter scale orders. We suppose that the parameters $e_{ji}^0 k_{ji}$ are of standard range, *i.e.*

$$e_{ji}^0 k_{ji} = \mathcal{O}(1) \quad \text{as } \varepsilon \rightarrow 0 \quad \forall i, j \in \{1, \dots, n\}, \quad (4.5)$$

where \mathcal{O} denotes the Big \mathcal{O} or Landau symbol. For the definition and some properties of \mathcal{O} , see Section 2.1.

Also, we suppose that the input $I(t)$ has a magnitude not larger than the slow reactions. In other words,

$$I(t) = \mathcal{O}(1)$$

The rate of growth μ is considered as a parameter smaller than any reaction rate (a standard hypothesis [70]). Here, we assume

$$\varepsilon \mu = \mathcal{O}(\varepsilon). \quad (4.6)$$

4.4. Quasi Steady State Reduction

In this section we propose a rigorous Quasi Steady State Reduction of (4.3). Its mathematical validity is proved using the Theorem of Tikhonov [39]. In other words, this theorem states that the error of this QSSR is a function of the order $\mathcal{O}(\varepsilon)$, where ε is small parameter.

We formally define the QSSR after Tikhonov's Theorem, of the metabolic network with ODE system (4.3), as the following system of dimension three:

$$\begin{aligned} \frac{d\bar{X}_1}{dt} &= I(t) - e_{21}^0 k_{21} \left(\frac{\bar{X}_1}{\bar{X}_1 + K_{21}} \right) - \mu \bar{X}_1 & (4.7) \\ \frac{d\bar{X}_m}{dt} &= \mathbf{b}_{m-1} \cdot e_{21}^0 k_{21} \frac{e_{m,m-1}^0 k_{m,m-1}}{K_{m,m-1}} \cdot \left(\frac{\bar{X}_1}{\bar{X}_1 + K_{21}} \right) \\ &\quad - e_{m+1,m}^0 k_{m+1,m} \left(\frac{\bar{X}_m}{\bar{X}_m + K_{m+1,m}} \right) - \mu \bar{X}_m \\ \frac{d\bar{X}_n}{dt} &= \mathbf{b}_{n-1} \cdot e_{m+1,m}^0 k_{m+1,m} \frac{e_{n,n-1}^0 k_{n,n-1}}{K_{n,n-1}} \cdot \left(\frac{\bar{X}_m}{\bar{X}_m + K_{m+1,m}} \right) - \mu \cdot \bar{X}_n, \end{aligned}$$

with initial conditions $\bar{X}_1(0) = x_1^0$, $\bar{X}_m(0) = x_m^0$ and $\bar{X}_n(0) = x_n^0$, and for the metabolites in QSS

$$\begin{aligned}\bar{X}_i(t) &= \varepsilon \cdot \mathbf{b}_i \cdot e_{21}^0 k_{21} \left(\frac{\bar{X}_1(t)}{\bar{X}_1(t) + K_{21}} \right) & i = 2, \dots, m-1, \\ \bar{X}_i(t) &= \varepsilon \cdot \mathbf{b}_i \cdot e_{m+1,m}^0 k_{m+1,m} \left(\frac{\bar{X}_m(t)}{\bar{X}_m(t) + K_{m+1,m}} \right) & i = m+1, \dots, n-1,\end{aligned}\quad (4.8)$$

for every $t \in [0, T_1]$. The definition of the parameters \mathbf{b}_i is given later in this section (see Proposition 4.1 and its proof).

4.4.1. Slow-Fast System. In order to write system (4.3) in the canonical form of singularly perturbed systems, we define a change of variable for the fast metabolites by

$$Y_i := \frac{X_i}{\varepsilon} \quad \forall i = 2, 3, \dots, n-1, i \neq m. \quad (4.9)$$

Let us set the initial conditions for these new variables as

$$y_i^0 := x_i^0 / \varepsilon,$$

and growth rate as

$$\tilde{\mu} = \varepsilon \mu.$$

Therefore, after the change of variable (4.9), Equation (4.3) can be rewritten as follows:

$$\begin{aligned}\frac{dX_i}{dt} &= F_i(t, X_1, \varepsilon Y_2, \dots, \varepsilon Y_{m-1}, X_m, \varepsilon Y_{m+1}, \dots, \varepsilon Y_{n-1}, X_n, \varepsilon, \mu) & (4.10) \\ & & \forall i = 1, m, n, \\ \frac{dY_i}{dt} &= \frac{1}{\varepsilon} F_i(t, X_1, \varepsilon Y_2, \dots, \varepsilon Y_{m-1}, X_m, \varepsilon Y_{m+1}, \dots, \varepsilon Y_{n-1}, X_n, \varepsilon, \tilde{\mu}) \\ & & \forall i = 2, \dots, n-1, i \neq m.\end{aligned}$$

Since ε is a very small positive number, the dynamics of Y_i are faster than those of X_i . Hence, the equations of X_i form the slow part of system (4.10), while the equations of Y_i constitute its fast part.

The previous Equation (4.10) is written with further details in the next subsection. The goal is to expose how the Quasi Steady State Reduction is obtained and validated using Tikhonov's Theorem.

4.4.2. Canonical form of singularly perturbed systems. The slow-fast system (4.10) is in the class of singularly perturbed systems of the exact

form:

$$\frac{dX_1}{dt} = I(t) - \frac{(e_{21}^0 k_{21})X_1}{X_1 + K_{21}} - \mu X_1 \quad X_1(0) = x_1^0 \quad (4.11)$$

$$\frac{dX_m}{dt} = \frac{(e_{m,m-1}^0 k_{m,m-1})Y_{m-1}}{\eta Y_{m-1} + K_{m,m-1}} - \frac{(e_{m+1,m}^0 k_{m+1,m})X_m}{X_m + K_{m+1,m}} - \mu X_m \quad X_m(0) = x_m^0$$

$$\frac{dX_n}{dt} = \frac{(e_{n,n-1}^0 k_{n,n-1})Y_{n-1}}{\eta Y_{n-1} + K_{n,n-1}} - \mu X_n \quad X_n(0) = x_n^0,$$

$$\frac{dY_2}{dt} = \frac{1}{\eta} \left[e_{21}^0 k_{21} \frac{X_1}{X_1 + K_{21}} + \left(\sum_{j=3}^{m-1} \frac{e_{2j}^0 k_{2j} \cdot Y_j}{\eta Y_j + K_{2j}} \right) - \left(\sum_{i=3}^{m-1} \frac{e_{i2}^0 k_{i2} \cdot Y_2}{\eta Y_2 + K_{i2}} \right) - \tilde{\mu} Y_2 \right] \quad (4.12)$$

⋮

$$\frac{dY_{m-1}}{dt} = \frac{1}{\eta} \left[\left(\sum_{j=2}^{m-2} \frac{e_{m-1,j}^0 k_{m-1,j} \cdot Y_j}{\eta Y_j + K_{m-1,j}} \right) - \left(\sum_{\substack{i=2 \\ i \neq m-1}}^m \frac{e_{i,m-1}^0 k_{i,m-1} \cdot Y_{m-1}}{\eta Y_{m-1} + K_{i,m-1}} \right) - \tilde{\mu} Y_{m-1} \right]$$

$$\begin{aligned} \frac{dY_{m+1}}{dt} = & \frac{1}{\eta} \left[\frac{e_{m+1,m}^0 k_{m+1,m} \cdot X_m}{X_m + K_{m+1,m}} + \left(\sum_{j=m+2}^{n-1} \frac{e_{m+1,j}^0 k_{m+1,j} \cdot Y_j}{\eta Y_j + K_{m+1,j}} \right) \right. \\ & \left. - \left(\sum_{i=m+2}^{n-1} \frac{e_{i,m+1}^0 k_{i,m+1} \cdot Y_{m+1}}{\eta Y_{m+1} + K_{i,m+1}} \right) - \tilde{\mu} Y_{m+1} \right] \end{aligned}$$

⋮

$$\frac{dY_{n-1}}{dt} = \frac{1}{\eta} \left[\left(\sum_{j=m+1}^{n-2} \frac{e_{n-1,j}^0 k_{n-1,j} \cdot Y_j}{\eta Y_j + K_{n-1,j}} \right) - \left(\sum_{\substack{i=m+1 \\ i \neq n-1}}^n \frac{e_{i,n-1}^0 k_{i,n-1} \cdot Y_{n-1}}{\eta Y_{n-1} + K_{i,n-1}} \right) - \tilde{\mu} Y_{n-1} \right],$$

with initial conditions $Y_i(0) = y_i^0$ for every $i = 2, \dots, n-1, i \neq m$.

NOTE 4.2. The Equation (4.11)-(4.12) above is a more detailed expression of (4.10). Indeed, we obtain system (4.10) when η is substituted for ε in the equations Equation (4.11)-(4.12).

An approximation to the solution of system (4.11)-(4.12) can be obtained considering the limit when $\eta \rightarrow 0$. Then, dynamics in Equation (4.12) are considered as fast and the QSSA is applied to the metabolites Y_i for every $i = 2, \dots, n-1, i \neq m$.

Hereafter, we say that Equation (4.11) is the slow part and Equation (4.12) the fast part of system (4.3).

4.4.3. Hypotheses necessary for Quasi Steady State. In the following two subsections, we check the assumptions of Tikhonov's Theorem [39]. First we demonstrate that the fast system has a single steady state (when slow variables are constants), which is not straightforward for nonlinear systems. Then we demonstrate that this steady state is asymptotically stable. Eventually, once all the conditions have been established, in Section 4.4.5 we present the result of Tikhonov's Theorem (see Section 2.2).

Consider the following algebraic system of equations, obtained from equating to 0 the terms in square brackets in (4.12) and substituting $\eta = 0$:

$$\begin{aligned}
0 &= e_{j_1}^0 k_{21} \frac{X_1}{X_1 + K_{21}} + \left(\sum_{j=3}^{m-1} e_{2j}^0 k_{2j} \frac{Y_j}{K_{2j}} \right) - \left(\sum_{i=3}^{m-1} e_{i2}^0 k_{i2} \frac{Y_2}{K_{i2}} \right) - \tilde{\mu} Y_2 \quad (4.13) \\
&\vdots \\
0 &= \left(\sum_{j=2}^{m-2} e_{m-1,j}^0 k_{m-1,j} \frac{Y_j}{K_{m-1,j}} \right) - \left(\sum_{\substack{i=2 \\ i \neq m-1}}^m e_{i,m-1}^0 k_{i,m-1} \frac{Y_{m-1}}{K_{i,m-1}} \right) - \tilde{\mu} Y_{m-1} \\
0 &= e_{m+1,m}^0 k_{m+1,m} \frac{X_m}{X_m + K_{m+1,m}} + \left(\sum_{j=m+2}^{n-1} e_{m+1,j}^0 k_{m+1,j} \frac{Y_j}{K_{m+1,j}} \right) \\
&\quad - \left(\sum_{i=m+2}^{n-1} e_{i,m+1}^0 k_{i,m+1} \frac{Y_{m+1}}{K_{i,m+1}} \right) - \tilde{\mu} Y_{m+1} \\
&\vdots \\
0 &= \left(\sum_{j=m+1}^{n-2} e_{n-1,j}^0 k_{n-1,j} \frac{Y_j}{K_{n-1,j}} \right) - \left(\sum_{\substack{i=m+1 \\ i \neq n-1}}^n e_{i,n-1}^0 k_{i,n-1} \frac{Y_{n-1}}{K_{i,n-1}} \right) - \tilde{\mu} Y_{n-1}
\end{aligned}$$

In order to apply Tikhonov's Theorem [39], we have to prove that Equation (4.13) has an isolated root for any nonnegative constant values X_1 and X_m , and that this root is asymptotically stable for the following system:

$$\begin{aligned}
\frac{dY_2}{dt} &= e_{21}^0 k_{21} \frac{X_1}{X_1 + K_{21}} + \left(\sum_{j=3}^{m-1} \frac{e_{2j}^0 k_{2j}}{K_{2j}} Y_j \right) - \left(\sum_{i=3}^{m-1} \frac{e_{i2}^0 k_{i2}}{K_{i2}} + \tilde{\mu} \right) Y_2 \quad (4.14) \\
&\vdots \\
\frac{dY_{m-1}}{dt} &= \left(\sum_{j=2}^{m-2} \frac{e_{m-1,j}^0 k_{m-1,j}}{K_{m-1,j}} Y_j \right) - \left(\sum_{\substack{i=2 \\ i \neq m-1}}^m \frac{e_{i,m-1}^0 k_{i,m-1}}{K_{i,m-1}} + \tilde{\mu} \right) Y_{m-1} \\
\frac{dY_{m+1}}{dt} &= e_{m+1,m}^0 k_{m+1,m} \frac{X_m}{X_m + K_{m+1,m}} + \left(\sum_{j=m+2}^{n-1} \frac{e_{m+1,j}^0 k_{m+1,j}}{K_{m+1,j}} Y_j \right) \\
&\quad - \left(\sum_{i=m+2}^{n-1} \frac{e_{i,m+1}^0 k_{i,m+1}}{K_{i,m+1}} + \tilde{\mu} \right) Y_{m+1} \\
&\vdots \\
\frac{dY_{n-1}}{dt} &= \left(\sum_{j=m+1}^{n-2} \frac{e_{n-1,j}^0 k_{n-1,j}}{K_{n-1,j}} Y_j \right) - \left(\sum_{\substack{i=m+1 \\ i \neq n-1}}^n \frac{e_{i,n-1}^0 k_{i,n-1}}{K_{i,n-1}} + \tilde{\mu} \right) Y_{n-1},
\end{aligned}$$

where X_1 , X_m and X_n are considered as nonnegative constants.

The purpose of finding a root of Equation (4.13) is to write the fast variables Y_i in terms of the slow variables X_i . In this case it is possible to find an analytic solution of this algebraic system, because it is a linear equation for the variables Y_i . Similarly, the asymptotical stability of this root for system (4.14) can be verified with the theory of linear systems of ODE.

PROPOSITION 4.1. *Consider X_1 and X_m as nonnegative constants values. Then system (4.14) has a single equilibrium point*

$$(\bar{Y}_i)_{i=2, \dots, n-1, i \neq m}$$

which is globally asymptotically stable. Moreover,

$$\begin{aligned}
\bar{Y}_i &= \mathbf{b}_i \cdot e_{21}^0 k_{21} \left(\frac{X_1}{X_1 + K_{21}} \right) & i = 2, \dots, m-1 \quad (4.15) \\
\bar{Y}_i &= \mathbf{b}_i \cdot e_{m+1,m}^0 k_{m+1,m} \left(\frac{X_m}{X_m + K_{m+1,m}} \right) & i = m+1, \dots, n-1,
\end{aligned}$$

where $\mathbf{b}_i \in \overline{\mathbb{R}_+}$ are nonnegative coefficients.

Proof. First notice that system (4.14) is a linear system for Y_i under the hypotheses of Proposition 4.1. Then, we just have to show that its Jacobian matrix is stable, i.e. that all its eigenvalues have negative real part [59].

The Jacobian matrix of (4.14) is

$$J = \begin{pmatrix} K_1 & 0 \\ 0 & K_2 \end{pmatrix}, \quad (4.16)$$

where

$$K_1 = \begin{pmatrix} -\sum_{i=3}^{m-1} \frac{e_{i2}^0 k_{i2}}{K_{i2}} - \tilde{\mu} & \cdots & \frac{e_{2,m-1}^0 k_{2,m-1}}{K_{2,m-1}} \\ \frac{e_{32}^0 k_{32}}{K_{32}} & \cdots & \frac{e_{3,m-1}^0 k_{3,m-1}}{K_{3,m-1}} \\ \vdots & & \vdots \\ \frac{e_{m-1,2}^0 k_{m-1,2}}{K_{m-1,2}} & \cdots & -\sum_{\substack{i=2 \\ i \neq m-1}}^m \frac{e_{i,m-1}^0 k_{i,m-1}}{K_{i,m-1}} - \tilde{\mu} \end{pmatrix}, \quad (4.17)$$

$$K_2 = \begin{pmatrix} -\sum_{i=m+2}^{n-1} \frac{e_{i,m+1}^0 k_{i,m+1}}{K_{i,m+1}} - \tilde{\mu} & \cdots & \frac{e_{m+1,n-1}^0 k_{m+1,n-1}}{K_{m+1,n-1}} \\ \frac{e_{m+2,m+1}^0 k_{m+2,m+1}}{K_{m+2,m+1}} & \cdots & \frac{e_{m+2,n-1}^0 k_{m+2,n-1}}{K_{m+2,n-1}} \\ \vdots & & \vdots \\ \frac{e_{n-1,m+1}^0 k_{n-1,m+1}}{K_{n-1,m+1}} & \cdots & -\sum_{\substack{i=m+1 \\ i \neq n-1}}^n \frac{e_{i,n-1}^0 k_{i,n-1}}{K_{i,n-1}} - \tilde{\mu} \end{pmatrix}.$$

But J is a strictly column diagonally dominant matrix, because $\tilde{\mu} > 0$. Hence, J is a stable matrix by Corollary 2.1.

The matrix form of Equation (4.13) is

$$\begin{pmatrix} K_1 & 0 \\ 0 & K_2 \end{pmatrix} \cdot \begin{pmatrix} Y_2 \\ \vdots \\ Y_{m-1} \\ Y_{m+1} \\ \vdots \\ Y_{n-1} \end{pmatrix} = - \begin{pmatrix} e_{21}^0 k_{21} \left(\frac{X_1}{X_1 + K_{21}} \right) \\ \vdots \\ 0 \\ e_{m+1,m}^0 k_{m+1,m} \left(\frac{X_m}{X_m + K_{m+1,m}} \right) \\ \vdots \\ 0 \end{pmatrix},$$

Then, the solution of the algebraic system (4.13) is

$$\begin{pmatrix} \bar{Y}_2 \\ \vdots \\ \bar{Y}_{m-1} \end{pmatrix} = (-e_{21}^0 k_{21}) \cdot (K_1)^{-1} \cdot \begin{pmatrix} \frac{X_1}{X_1 + K_{21}} \\ \vdots \\ 0 \end{pmatrix}, \quad (4.18)$$

$$\begin{pmatrix} \bar{Y}_{m+1} \\ \vdots \\ \bar{Y}_{n-1} \end{pmatrix} = (-e_{m+1,m}^0 k_{m+1,m}) \cdot (K_2)^{-1} \cdot \begin{pmatrix} \frac{X_m}{X_m + K_{m+1,m}} \\ \vdots \\ 0 \end{pmatrix}.$$

Therefore, the variables of the solution can be written as

$$\begin{aligned}\bar{Y}_i &= \mathbf{b}_i \cdot e_{21}^0 k_{21} \left(\frac{X_1}{X_1 + K_{21}} \right) & i = 2, \dots, m-1, \\ \bar{Y}_i &= \mathbf{b}_i \cdot e_{m+1,m}^0 k_{m+1,m} \left(\frac{X_m}{X_m + K_{m+1,m}} \right) & i = m+1, \dots, n-1,\end{aligned}$$

with $\mathbf{b}_i \in \mathbb{R}$. Moreover, since K_i is strictly column diagonally dominant and has negative diagonal entries, its inverse matrix is nonpositive, i.e. each entry of $(K_i)^{-1}$ is nonpositive (see Proposition 2.2). Therefore, all entries in

$$\begin{aligned}(-e_{21}^0 k_{21}) \cdot (K_1)^{-1} & \quad \text{and} \\ (-e_{m+1,m}^0 k_{m+1,m}) \cdot (K_2)^{-1}\end{aligned}$$

are nonnegative. We conclude that coefficients \mathbf{b}_i in (4.15) are nonnegative. \square

NOTE 4.3. Although Proposition 4.1 is proved for nonnegative constant values X_1 and X_m , we consider \bar{Y}_i in (4.15) also as functions of $t \in [0, T_1]$, according to Tikhonov's Theorem. Then we have the functions in (4.8), defined for the QSSR.

4.4.4. Study of the slow system. The dynamics of the slow system (metabolites which reach high concentrations) are obtained by setting $\eta = 0$ in (4.11) and substituting the fast variables Y_i for the expressions given by (4.15):

$$\begin{aligned}\frac{dX_1}{dt} &= I(t) - \frac{e_{21}^0 k_{21} \cdot X_1}{X_1 + K_{21}} - \mu X_1, \\ \frac{dX_m}{dt} &= e_{m,m-1}^0 k_{m,m-1} \frac{\bar{Y}_{m-1}}{K_{m,m-1}} - \frac{e_{m+1,m}^0 k_{m+1,m} \cdot X_m}{X_m + K_{m+1,m}} - \mu X_m, \\ \frac{dX_n}{dt} &= e_{n,n-1}^0 k_{n,n-1} \frac{\bar{Y}_{n-1}}{K_{n,n-1}} - \mu X_n.\end{aligned}$$

Then we obtain the remaining dynamical system (4.7), which provides the dynamics to the overall network.

The other variables of the metabolic network, which are the fast variables Y_i can then be reconstructed after Equation (4.8). These fast variables rely on system (4.7). Most of the variables are fast, which let us conclude that the complexity of the problem has been considerably reduced.

PROPOSITION 4.2. *If system (4.7) has nonnegative initial conditions, then it has a unique nonnegative solution $(\bar{X}_1, \bar{X}_m, \bar{X}_n)$ defined on the interval $[0, T_1]$.*

For the proof of Proposition 4.2, see Appendix B.2.

4.4.5. Tikhonov's Theorem. Propositions 4.1 and 4.2 prove that the class of systems with the form (4.11)-(4.12) satisfy the hypothesis of Tikhonov's Theorem [39]. Then, we can apply this theorem to system (4.10).

The following proposition is a consequence of Tikhonov's Theorem. The proposition states that the approximation given by the QSSR (4.7)-(4.8) has an error with order $\mathcal{O}(\varepsilon)$, after a fast initial transient for the fast variables (see Theorem 2.1).

PROPOSITION 4.3. *[Deduction of Tikhonov's Theorem] If $I(t)$ is a non-negative continuous function over $[0, T_1]$, then*

$$X_j(t) = \bar{X}_j(t) + \mathcal{O}(\varepsilon) \quad j = 1, m, n, \forall t \in [0, T_1].$$

Moreover, there exists $T_0 > 0$ such that for every $t \in [T_0, T_1]$,

$$X_i(t) = \varepsilon \left[\bar{Y}_i(t) + \mathcal{O}(\varepsilon) \right] \quad \forall i = 2, \dots, n-1, i \neq m.$$

where X_i is the solution of the original system (4.3), \bar{X}_i is the solution of (4.7) and \bar{Y}_i is the function defined in (4.8) after the algebraic Equation (4.13).

NOTE 4.4. The solution of the boundary layer problem for system (4.11)-(4.12) is similar to that of Equation (4.14). We include its demonstration in Appendix B.1.

4.5. Magnitude of Concentrations

In this section we study the magnitude of metabolite concentrations, depending on if they are associated to slow or fast reactions. They are deduced from the reduced system after Tikhonov's Theorem (4.7)-(4.8). We now show that the concentration of metabolites in QSS (that do not trap the input flux) is one order of magnitude ε lower than metabolites with slow dynamics.

In order to prove this assertion, we define the conditions under which

$$\begin{aligned} \mathbf{b}_i \cdot e_{21}^0 k_{21} &= \mathcal{O}(1), \\ \mathbf{b}_i \cdot e_{m+1,m}^0 k_{m+1,m} &= \mathcal{O}(1), \end{aligned}$$

to obtain

$$\begin{aligned} \bar{X}_i(t) &= \mathcal{O}\left(\varepsilon \cdot \frac{\bar{X}_1(t)}{\bar{X}_1(t) + K_{21}}\right) && \text{if } i \in \{2, \dots, m-1\}, \\ \bar{X}_i(t) &= \mathcal{O}\left(\varepsilon \cdot \frac{\bar{X}_m(t)}{\bar{X}_m(t) + K_{m+1,m}}\right) && \text{if } i \in \{m+1, \dots, n-1\}, \end{aligned}$$

for every $t \in [T_0, T_1]$.

4.5.1. Parameter Orders. Here we show that all off-diagonal entries of the Jacobian matrix K_i have the same order of magnitude, for both matrices defined in (4.17).

LEMMA 4.1. *Suppose that the parameters of each Michaelis-Menten enzymatic reaction (see Section 1.2.2) satisfy*

$$\mathcal{O}(k_\gamma^{j_i}) = \mathcal{O}(k_{ji}) \quad \forall i, j = 1, \dots, n, \gamma \in \{-1, 1\}. \quad (4.19)$$

Then,

$$\mathcal{O}\left(\frac{e_{ji}^0 k_{ji}}{K_{ji}}\right) = \mathcal{O}\left(\frac{e_{j'i'}^0 k_{j'i'}}{K_{j'i'}}\right) = \mathcal{O}(e_{j'i'}^0 k_{j'i'}) \quad \forall i, j, i', j' \in \{1, \dots, n\}.$$

Proof. As a consequence of (4.5),

$$\mathcal{O}(e_{ji}^0 k_{ji}) = \mathcal{O}(e_{j'i'}^0 k_{j'i'}) \quad \forall i, j, i', j' \in \{1, \dots, n\}. \quad (4.20)$$

Moreover, by the definition of the Michaelis-Menten constant (4.4) and (4.19), we have that $\mathcal{O}(K_{ji}) = 1$ and then

$$\mathcal{O}\left(\frac{e_{ji}^0 k_{ji}}{K_{ji}}\right) = \mathcal{O}(e_{ji}^0 k_{ji}) \quad \forall i, j. \quad (4.21)$$

Hence, combining (4.20) and (4.21), we have

$$\mathcal{O}\left(\frac{e_{ji}^0 k_{ji}}{K_{ji}}\right) = \mathcal{O}\left(\frac{e_{j'i'}^0 k_{j'i'}}{K_{j'i'}}\right) = \mathcal{O}(e_{j'i'}^0 k_{j'i'}) \quad \forall i, j, i', j' \in \{1, \dots, n\}.$$

□

Actually, all the entries of the matrix K_i have the same order of magnitude, as asserts the following corollary.

PROPOSITION 4.4. *Consider the matrices defined in (4.17). All the entries of K_1 and K_2 have the same order.*

Proof. According to Lemma 4.1, for the sums in the diagonal of the matrices we have

$$\sum_{\substack{i=2 \\ i \neq j}}^n \frac{e_{ij}^0 k_{ij}}{K_{ij}} = \mathcal{O}(e_{ji}^0 k_{ji}).$$

Moreover, $\tilde{\mu} = \mathcal{O}(\varepsilon)$. Then,

$$\sum_{\substack{i=2 \\ i \neq j}}^n \frac{e_{ij}^0 k_{ij}}{K_{ij}} + \tilde{\mu} = \mathcal{O}(e_{ji}^0 k_{ji}).$$

For the off-diagonal entries consider (4.21). Therefore, all the entries of K_1 and K_2 have order $\mathcal{O}(e_{ji}^0 k_{ji})$. □

4.5.2. A Theorem for Magnitude of Concentrations. In order to prove that a metabolite in QSS does not reach high concentrations, we have to suppose that it is not in a trap for the input flux. The definition of trap was introduced in [33], and we formally adapt it to the class of models considered in this work (see Appendix B.3.1). Then, we define a *flux trap*, which is a trap reached by the flux.

ASSUMPTION 4.1. There exists F a *flux* from X_1 to X_n in the system of enzymatic reactions (4.3) (depicted in Figure 4.1). Moreover, we define \mathcal{I}_{T_F} as the set of indices such that X_i is in a *flux trap* if and only if $i \in \mathcal{I}_{T_F}$.

Notice that the presence of the flux F from X_1 to X_n implies

$$\{1, m, n\} \cap \mathcal{I}_{T_F} = \emptyset.$$

Then, *flux traps* are only possible within the subnetworks in QSS. Also, $\mathcal{I}_{T_F} = \emptyset$ if there is no *flux trap*.

The following lemma sets down the order of magnitude of the parameters in (4.8), for the metabolites which are not in a *flux trap*. These parameters are used for writing the expression of fast metabolites in the QSSR.

LEMMA 4.2. *Suppose the system of enzymatic reactions (4.3) under Assumption 4.1. Consider the parameters \mathbf{b}_i of Equation (4.8), obtained in Section 4.4. Then, if $\mathbf{b}_i \neq 0$, it holds*

$$\begin{aligned} \mathbf{b}_i \cdot e_{21}^0 k_{21} &= \mathcal{O}(1) & \text{if } i \in \{2, \dots, m-1\} \setminus \mathcal{I}_{T_F}, \\ \mathbf{b}_i \cdot e_{m+1,m}^0 k_{m+1,m} &= \mathcal{O}(1) & \text{if } i \in \{m+1, \dots, n-1\} \setminus \mathcal{I}_{T_F}. \end{aligned} \quad (4.22)$$

Proof. From the results stated in Appendix B.3, particularly Theorem B.1, we have for $\mathbf{b}_i \neq 0$,

$$\begin{aligned} \mathbf{b}_i \cdot \frac{e_{21}^0 k_{21}}{K_{21}} &= \mathcal{O}(1) & \text{if } i \in \{2, \dots, m-1\} \setminus \mathcal{I}_{T_F}, \\ \mathbf{b}_i \cdot \frac{e_{m+1,m}^0 k_{m+1,m}}{K_{m+1,m}} &= \mathcal{O}(1) & \text{if } i \in \{m+1, \dots, n-1\} \setminus \mathcal{I}_{T_F}. \end{aligned}$$

Using Equation (4.21) we conclude that, for $\mathbf{b}_i \neq 0$,

$$\begin{aligned} \mathbf{b}_i \cdot e_{21}^0 k_{21} &= \mathcal{O}(1) & \text{if } i \in \{2, \dots, m-1\} \setminus \mathcal{I}_{T_F}, \\ \mathbf{b}_i \cdot e_{m+1,m}^0 k_{m+1,m} &= \mathcal{O}(1) & \text{if } i \in \{m+1, \dots, n-1\} \setminus \mathcal{I}_{T_F}. \end{aligned}$$

□

The next theorem is a powerful conclusion obtained after the QSSR (4.7)-(4.8). The Magnitude of Concentration Theorem 4.1 states that the concentration of a metabolite in QSS, which is not in a *flux trap*, is one order of

magnitude ε lower than the concentration of a metabolite with slow dynamics. This result holds even if there is a trap or a *flux trap* in the system.

THEOREM 4.1 (Magnitude of Concentration Theorem). *Consider the system of enzymatic reactions (4.3). Under Assumption 4.1, the following inequalities hold:*

$$\begin{aligned}\bar{X}_i &\leq \mathcal{O}(\varepsilon \cdot \bar{X}_1) & \forall i \in \{2, \dots, m-1\} \setminus \mathcal{I}_{\text{TF}}, \\ \bar{X}_i &\leq \mathcal{O}(\varepsilon \cdot \bar{X}_m) & \forall i \in \{m+1, \dots, n-1\} \setminus \mathcal{I}_{\text{TF}}.\end{aligned}$$

Magnitude of Concentration Theorem 4.1 states that, after the initial fast transient, any metabolite in QSS has a concentration one order of magnitude lower than the concentration of a metabolite in the slow part. For the Big \mathcal{O} or Landau symbol notation used in this theorem, see Note 2.1 in Section 2.1.

Proof of Magnitude of Concentration Theorem 4.1. Since the reduction from Tikhonov's Theorem, we have Equation (4.8), i.e.,

$$\begin{aligned}\bar{X}_i &= \varepsilon \cdot \mathbf{b}_i \cdot e_{21}^0 k_{21} \left(\frac{\bar{X}_1}{\bar{X}_1 + K_{21}} \right) & i = 2, \dots, m-1, \\ \bar{X}_i &= \varepsilon \cdot \mathbf{b}_i \cdot e_{m+1,m}^0 k_{m+1,m} \left(\frac{\bar{X}_m}{\bar{X}_m + K_{m+1,m}} \right) & i = m+1, \dots, n-1.\end{aligned}$$

Then, as stated in Lemma 4.2, for i such that $\mathbf{b}_i \neq 0$,

$$\begin{aligned}\bar{X}_i &= \mathcal{O}\left(\varepsilon \frac{\bar{X}_1}{\bar{X}_1 + K_{21}}\right) & \text{if } i \in \{2, \dots, m-1\} \setminus \mathcal{I}_{\text{TF}}, \\ \bar{X}_i &= \mathcal{O}\left(\varepsilon \frac{\bar{X}_m}{\bar{X}_m + K_{m+1,m}}\right) & \text{if } i \in \{m+1, \dots, n-1\} \setminus \mathcal{I}_{\text{TF}}.\end{aligned}$$

But $1 \leq \mathcal{O}(\bar{X}_i + K_{i+1,i})$, because system (4.7) is positively invariant and $K_{i+1,i} = \mathcal{O}(1)$. Hence,

$$\begin{aligned}\mathcal{O}\left(\frac{\bar{X}_1}{\bar{X}_1 + K_{21}}\right) &\leq \mathcal{O}(\bar{X}_1), \\ \mathcal{O}\left(\frac{\bar{X}_m}{\bar{X}_m + K_{m+1,m}}\right) &\leq \mathcal{O}(\bar{X}_m).\end{aligned}$$

We conclude that

$$\begin{aligned}\bar{X}_i &\leq \mathcal{O}(\varepsilon \cdot \bar{X}_1) & \forall i \in \{2, \dots, m-1\} \setminus \mathcal{I}_{\text{TF}}, \\ \bar{X}_i &\leq \mathcal{O}(\varepsilon \cdot \bar{X}_m) & \forall i \in \{m+1, \dots, n-1\} \setminus \mathcal{I}_{\text{TF}}.\end{aligned}$$

□

NOTE 4.5. The approach presented in this work can be used to reduce a metabolic network which has *flux traps*, obtaining an error characterization (as established in Proposition 4.3) and the conclusion of Magnitude of Concentration Theorem 4.1. But, in agreement with the Magnitude of Concentration Theorem 4.1, the magnitude of concentration of metabolites in *flux traps* cannot be bounded by the concentration of metabolites in the slow part of the system. This fact can be inferred from the proof of Magnitude of Concentration Theorem 4.1 (see Appendix B.3.2.)

The presence of a *flux trap* leads to accumulation, without reuse, of compounds in the metabolic network. However, accumulation of some compounds to large concentrations often results to cell death. For example, the accumulation of lactate has been recognized as one cause of cell death [64, 52].

4.6. Reduced Model Calibration

Now that we have described the way to synthesize the initial model of the metabolic network into a small dynamical system (for accumulating metabolites) and a set of algebraic equations, we explain how to calibrate this reduced model from experimental data. Of course, we assume that the initial stoichiometric coefficients are known, but the parameters associated to reaction rates are unknown.

Here we propose a method to estimate the parameters of the reduced system. In a first stage we identify the parameters of the reduced dynamical system representing the metabolites with higher concentration (4.7). The identification method is based on the minimization of a cost function, computing the error model with respect to experimental data.

Furthermore, if data of any metabolite in QSS is available, we can also estimate the respective parameters in (4.8), to write its concentration as a linear combination of the slow metabolites.

4.6.1. Calibration of the slow dynamics. Let us consider some experimental data of the metabolites in the slow part of the system (4.11), denoted by

$$Z_i(t_j) = X_i(t_j) + \beta_i(t_j) \quad i = 1, m, n, j = 1, \dots, r, \quad (4.23)$$

where X_i is the solution of the original system (4.3) and β_i represents an error of measurements. In order to estimate the parameters of the reduced

system (4.7), we rewrite it as

$$\begin{aligned}\frac{d\bar{X}_1}{dt} &= I(t) - \frac{\theta_1 \bar{X}_1}{\bar{X}_1 + \theta_2} - \theta_3 \cdot \bar{X}_1 & \bar{X}_1(0) &= Z_1(t_1) \\ \frac{d\bar{X}_m}{dt} &= \frac{\theta_4 \bar{X}_1}{\bar{X}_1 + \theta_2} - \frac{\theta_5 \bar{X}_m}{\bar{X}_m + \theta_6} - \theta_3 \bar{X}_m & \bar{X}_m(0) &= Z_m(t_1) \\ \frac{d\bar{X}_n}{dt} &= \frac{\theta_7 \bar{X}_m}{\bar{X}_m + \theta_6} - \theta_3 \cdot \bar{X}_n & \bar{X}_n(0) &= Z_n(t_1),\end{aligned}\quad (4.24)$$

Let $\theta = (\theta_1, \theta_2, \theta_3, \theta_4, \theta_5)$ and define a cost function $\mathcal{F}(\theta)$. This cost function has to measure the error between the solution of (4.24) and the data Z_1, Z_m, Z_n , for every value θ in a domain $D \subset \mathbb{R}^7$. For example, we can define \mathcal{F} as

$$\mathcal{F}(\theta) = \sum_{i \in \{1, m, n\}} \sum_{j=1}^r \left| \bar{X}_i(\theta, t_j) - Z_i(t_j) \right|^2. \quad (4.25)$$

Then, we have to find $\hat{\theta} = (\hat{\theta}_1, \hat{\theta}_2, \hat{\theta}_3, \hat{\theta}_4, \hat{\theta}_5)$ such that

$$\mathcal{F}(\hat{\theta}) = \min\{F(\theta) : \theta \in D\}.$$

NOTE 4.6. For obtaining the vector of parameters $\hat{\theta}$ to calibrate (4.24), it is not necessary to have data of any metabolite in QSS, X_i with $i = 2, \dots, n-1, i \neq m$. Only the data (4.23) of the metabolites in the slow part, X_1, X_m, X_n , is used.

4.6.2. Fast dynamics parameters. In some (rare) cases, measurements of some fast metabolites can be available. Generally, these data are only obtained at quasi steady state after the initial transient and for a subset of the metabolic compounds.

Supposing that we have experimental data of the metabolites in QSS after the initial fast transient,

$$\begin{aligned}Z_i(t'_j) &= X_i(t'_j) + \mathcal{N}(t'_j) & i &= 2, \dots, n-1, i \neq m, \\ T_0 &\leq t'_1 < \dots < t'_r,\end{aligned}\quad (4.26)$$

and that we have obtained $\hat{\theta}$ after calibrating (4.24), we can estimate the parameters in (4.8). As a matter of fact, in line with the reduced system (4.7)-(4.8) and the calibrated system (4.24), for the metabolites in QSS we have

$$\begin{aligned}\bar{X}_i &= \alpha_i \cdot \frac{\bar{X}_1}{\bar{X}_1 + \hat{\theta}_2} & i &= 2, \dots, m-1 \\ \bar{X}_i &= \alpha_i \cdot \frac{\bar{X}_m}{\bar{X}_m + \hat{\theta}_6} & i &= m+1, \dots, n-1,\end{aligned}\quad (4.27)$$

where α_i are the parameters to be estimated.

Here, we can explicitly resolve the linear least square problem. The least squares solution that minimize the difference between the data Z_i and the expressions in (4.27) is the following [54]:

$$\hat{\alpha}_i = \frac{\sum_{j=1}^{r'} \frac{Z_i(t'_j) \bar{X}_1(\hat{\theta}, t'_j)}{\bar{X}_1(\hat{\theta}, t'_j) + \hat{\theta}_2}}{\sum_{j=1}^{r'} \left(\frac{\bar{X}_1(\hat{\theta}, t'_j)}{\bar{X}_1(\hat{\theta}, t'_j) + \hat{\theta}_2} \right)^2} \quad \forall i = 2, \dots, m-1$$

$$\hat{\alpha}_i = \frac{\sum_{j=1}^{r'} \frac{Z_i(t'_j) \bar{X}_m(\hat{\theta}, t'_j)}{\bar{X}_m(\hat{\theta}, t'_j) + \hat{\theta}_6}}{\sum_{j=1}^{r'} \left(\frac{\bar{X}_m(\hat{\theta}, t'_j)}{\bar{X}_m(\hat{\theta}, t'_j) + \hat{\theta}_6} \right)^2} \quad \forall i = m+1, \dots, n-1.$$

Indeed, we look for values of $\hat{\alpha}_i$ that minimize the differences

$$L_i(\alpha) = \sum_{j=1}^{r'} \left(\alpha \frac{\bar{X}_1(t'_j, \hat{\theta})}{\bar{X}_1(t'_j, \hat{\theta}) + \hat{\theta}_2} - Z_i(t'_j) \right)^2 \quad i = 2, \dots, m-1$$

$$L_i(\alpha) = \sum_{j=1}^{r'} \left(\alpha \frac{\bar{X}_m(t'_j, \hat{\theta})}{\bar{X}_m(t'_j, \hat{\theta}) + \hat{\theta}_6} - Z_i(t'_j) \right)^2 \quad i = m+1, \dots, n-1.$$

NOTE 4.7. To obtain the parameter $\hat{\alpha}_i$, we only need the data Z_i (of the corresponding metabolites in QSS, X_i) and the calibrated system (4.24) with $\hat{\theta}$.

4.7. A Toy Enzymatic Network

In this section we apply the method developed in this work to the toy network represented in Figure 4.3. This toy network accounts for one reversible enzymatic reaction and a cycle of enzymatic reactions. Moreover, the toy network contains two subnetworks in QSS (in blue in Figure 4.3), which are interconnected by metabolites with slow rates of consumption (in black in Figure 4.3).

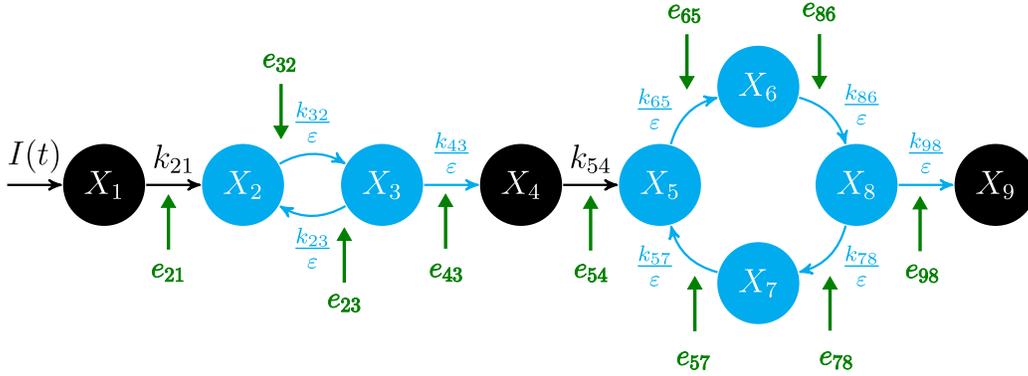


Figure 4.3. We consider that reactions represented by black arrows are slow, while reactions represented by blue arrows are fast. Metabolites in black reach high concentrations, whereas metabolites in blue have low concentration and are supposed to be in Quasi Steady State. Every reaction is catalyzed by and enzyme e_{ji} .

First we consider the ODE of the toy enzymatic network, as in Section 4.2. Then, using the time-scale separation hypothesis, we reduce this ODE with the method described in Section 4.4. Finally, we estimate the parameters of the reduced system as it is suggested in Section 4.6

All the parameters in the toy network are supposed to satisfy the conditions established in (4.5) and Section 4.5. The periodic and continuous input considered is given by

$$I(t) = k[\cos(t \cdot \omega + \pi) + 1],$$

where k is a parameter with the same order of magnitude as the slow reactions rates.

4.7.1. Reduction. We apply to the toy network our reduction scheme, as described in Section 4.4. First, to simplify the notation, we define the following parameters:

$$a_{ji} := \frac{e_{ji}^0 k_{ji}}{K_{ji}} \quad \forall i, j = 1, \dots, n,$$

$$d_1 := a_{32}(a_{43} + \tilde{\mu}) + \tilde{\mu}(a_{23} + a_{43} + \tilde{\mu}),$$

$$d_2 := (a_{65} + \tilde{\mu})(a_{86} + \tilde{\mu})(a_{57} + \tilde{\mu})(a_{78} + a_{98} + \tilde{\mu}) - (a_{65})(a_{57})(a_{86})(a_{78}).$$

Then, we obtain the following reduced system for the toy network,

$$\begin{aligned}\frac{dX_1}{dt} &= I(t) - \frac{e_{21}^0 k_{21} \cdot X_1}{X_1 + K_{21}} - \mu X_1 \\ \frac{dX_4}{dt} &= \frac{(a_{43})(a_{32})}{d_1} \cdot \frac{e_{21}^0 k_{21} \cdot X_1}{X_1 + K_{21}} - \frac{e_{54}^0 k_{54} \cdot X_4}{X_4 + K_{54}} - \mu X_4 \\ \frac{dX_9}{dt} &= \frac{(a_{98})(a_{65})(a_{86})(a_{57} + \tilde{\mu})}{d_2} \cdot \frac{e_{54}^0 k_{54} \cdot X_4}{X_4 + K_{54}} - \mu X_9,\end{aligned}\tag{4.28}$$

and the expressions for the metabolites in QSS,

$$\begin{aligned}X_2 &= \frac{\varepsilon(a_{23} + a_{43} + \tilde{\mu})}{d_1} \cdot \frac{e_{21}^0 k_{21} X_1}{X_1 + K_{21}} \\ X_3 &= \frac{\varepsilon(a_{32})}{d_1} \cdot \frac{e_{21}^0 k_{21} X_1}{X_1 + K_{21}} \\ X_5 &= \frac{\varepsilon(a_{86} + \tilde{\mu})(a_{57} + \tilde{\mu})(a_{78} + a_{98} + \tilde{\mu})}{d_2} \cdot \frac{e_{54}^0 k_{54} X_4}{X_4 + K_{54}} \\ X_6 &= \frac{\varepsilon(a_{65})(a_{57} + \tilde{\mu})(a_{78} + a_{98} + \tilde{\mu})}{d_2} \cdot \frac{e_{54}^0 k_{54} X_4}{X_4 + K_{54}} \\ X_7 &= \frac{\varepsilon(a_{65})(a_{86})(a_{78})}{d_2} \cdot \frac{e_{54}^0 k_{54} X_4}{X_4 + K_{54}} \\ X_8 &= \frac{\varepsilon(a_{65})(a_{86})(a_{57} + \tilde{\mu})}{d_2} \cdot \frac{e_{54}^0 k_{54} X_4}{X_4 + K_{54}}.\end{aligned}\tag{4.29}$$

4.7.2. Calibration of the Reduced Toy Network. We follow the procedure in Section 4.6. For simplicity we suppose that the data are measured at the same time instants t_1, \dots, t_r (we assume that 48 measurement instants are available) for the slow and the fast parts of the system.

The measurements are the variables (units $\text{g}\cdot\text{L}^{-1}$) of the original system (4.3) (for the toy network in Figure 4.3) plus a white noise:

$$Z_i(t_j) := X_i(t_j) + \beta(t_j) \quad j = 1, 2, \dots, r \tag{4.30}$$

where $\beta \sim \mathcal{N}(\sigma_i)$ and $\sigma_i = 10^{-1} \cdot \text{median}(X_i)$ for every $i = 1, \dots, n$.

As in Section 4.6, to estimate the parameters of (4.28), we use the reduced system (4.24), with $m = 4$ and $n = 9$. The cost function considered is \mathcal{F} , defined in (4.25), with $m = 4$ and $n = 9$.

The function *fminsearch* in Scilab was used for minimizing \mathcal{F} . This function is based on the Nelder-Mead algorithm to compute the unconstrained minimum of a given function [6]. For the simulations in Figure 4.4, the constant vector $\hat{\theta}$ obtained is described in Table 4.1 and $\mathcal{F}(\hat{\theta}) = 0.097$.

Note that the parameters θ_2 and θ_6 are affinity constant in Michaelis-Menten functions, whose sensitivity is low [77]. Here we have used 48 samples for parameter identification.

For illustration purpose, we suppose that the metabolites in QSS are also measured, we calculate the parameters $\hat{\alpha}$ to estimate their concentrations as explained in Section 4.6.2. Then, their concentrations are obtained according to (4.27).

We computed the numerical solution of the systems describing the dynamics in the toy network of Figure 4.3. The results are represented in Figure 4.4 and Figure 4.5. As expected, the concentrations of the metabolites in QSS are one order of magnitude ε lower than the metabolites in the slow part. Moreover, the reduced systems accurately represents all the scales of the system (which are more than two).

It is worth noting that the identification process results in a satisfying agreement between simulations of the calibrated system (4.24)-(4.27) and recorded data, as represented in Figure 4.4 and Figure 4.5.

i	Theoretical value θ_i	Initial guess	Estimated value $\hat{\theta}_i$	Units	Error percent
1	0.110	0.010	0.072	$g(L.min)^{-1}$	34.54
2	2.000	1.000	1.298	gL^{-1}	35.10
3	0.010	0.010	0.011	min^{-1}	10.00
4	0.110	0.010	0.073	$g(L.min)^{-1}$	33.63
5	0.013	0.010	0.006	$g(L.min)^{-1}$	53.85
6	2.000	1.000	2.143	gL^{-1}	7.15
7	0.013	0.010	0.016	$g(L.min)^{-1}$	23.08

Table 4.1. Parameter estimation for system (4.28), rewritten as (4.24). The estimation of this parameters only requires the slow dynamics of the toy network in Figure 4.3.

i	Theoretical value α_i	Estimated value $\hat{\alpha}_i$	Error percent
2	0.00124	0.00092	25.81
3	0.00122	0.00089	27.05
5	0.00168	0.00185	10.12
6	0.00018	0.00019	5.56
7	0.00002	0.00002	0
8	0.00017	0.00019	11.76

Table 4.2. Estimation of the parameters in (4.29), corresponding to the equalities in (4.27).

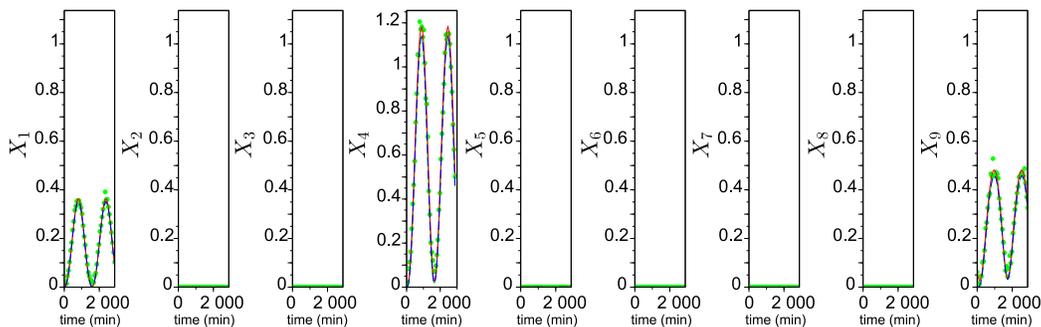


Figure 4.4. Dynamics of the toy network represented in Figure 4.3. The functions X_i represent the metabolite concentrations in units g.L^{-1} . The numerical solution of the original system (4.3) is depicted by the green line; the reduced system obtained by the method exposed in this work (4.28), by the blue dashed line; the supposed data with white noise (4.30), by green points; and the calibrated system (4.24)-(4.27) with the estimated parameters in Table 4.1 and Table 4.2, by the red line. The parameters considered are in Table 4.3 and Table 4.4. As expected, the concentrations of the metabolites in QSS are one order of magnitude ε lower than the metabolites in the slow part.

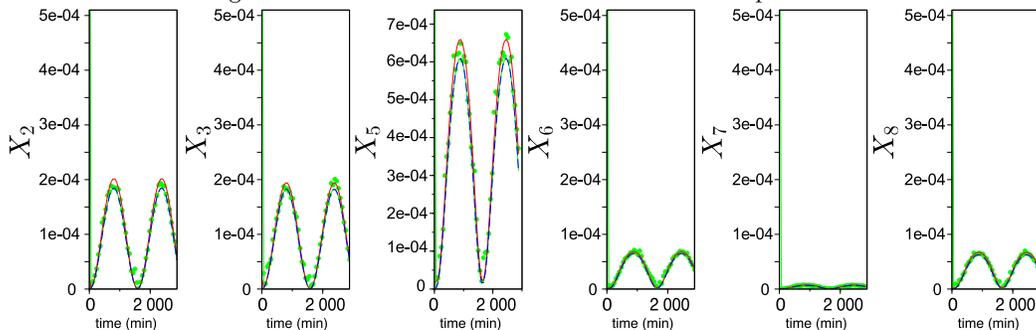


Figure 4.5. Zoom on the concentration of metabolites in QSS. In this Figure, as in Figure 4.4, it is possible to remark also different scales within each group of fast or slow metabolites.

Parameter	Value	Units
ε	0.001	–
k	0.01	$\text{g}(\text{L}\cdot\text{min})^{-1}$
μ	0.01	min^{-1}
ω	0.004	–
k_{γ}^{21}, k_{21}	1.10	min^{-1}
k_{γ}^{54}, k_{54}	0.13	min^{-1}
k_{γ}^{32}, k_{32}	1.90	min^{-1}
k_{γ}^{23}, k_{23}	0.12	min^{-1}
k_{γ}^{43}, k_{43}	1.80	min^{-1}
k_{γ}^{65}, k_{65}	0.17	min^{-1}
k_{γ}^{57}, k_{57}	1.40	min^{-1}
k_{γ}^{86}, k_{86}	1.60	min^{-1}
k_{γ}^{78}, k_{78}	0.15	min^{-1}
k_{γ}^{98}, k_{98}	1.50	min^{-1}
e_{ji}^0	0.10	$\text{g}\cdot\text{L}^{-1}$
x_i^0	0.001	$\text{g}\cdot\text{L}^{-1}$

Table 4.3. Parameters considered for the simulations in Figure 4.4. The symbol $\gamma \in \{-1, 1\}$ denotes a rate in a enzymatic reaction (see the Michaelis-Menten Equation (1.2)). The initial conditions for all the enzymes are the same, as well as the initial conditions of all the metabolites are identical, i.e. $j, i \in \{1, \dots, n\}$ in this table.

Slow rates	Value
$e_{21}^0 k_{21}$	1.1×10^{-1}
$e_{54}^0 k_{54}$	1.3×10^{-2}
Fast rates	Value
$e_{32}^0 k_{32}/\varepsilon$	1.3×10^2
$e_{23}^0 k_{23}/\varepsilon$	1.2×10^1
$e_{43}^0 k_{43}/\varepsilon$	1.8×10^2
$e_{65}^0 k_{65}/\varepsilon$	1.7×10^1
$e_{57}^0 k_{57}/\varepsilon$	1.4×10^2
$e_{86}^0 k_{86}/\varepsilon$	1.6×10^2
$e_{78}^0 k_{78}/\varepsilon$	1.5×10^1
$e_{98}^0 k_{98}/\varepsilon$	1.5×10^2

Table 4.4. Slow and fast reaction rates considered for the toy network in Figure 4.3 and the simulations in Figure 4.4. Fast reaction rates are characterized by the factor $1/\varepsilon$. All reactions rates are in units of $\text{g}(\text{L}\cdot\text{min})^{-1}$. Within each group of reaction rates (slow or fast) there are also different scales.

4.8. Discussion and Conclusions

4.8.1. Time-Scales Hypotheses. Metabolic networks can involve much more than two different time scales. Actually, our method considers the division of these in two groups of reaction rates: the kinetics slower than a certain threshold and the kinetics faster than the same threshold. Our approach eventually preserve the dynamics of the slower kinetics (keeping the different time scales). Moreover, the fastest dynamics can be approximated using the reduced system of slow dynamics.

Also, to better illustrate this important aspect, we have considered several reaction rate orders in the toy network. The reaction rates are divided into slow and fast, and each group of reactions has different scales (see Table 4.4). The simulation results illustrate the Magnitude of Concentration Theorem 4.1 (see Figure 4.4). Moreover, the reduced system accurately represents all different time scales (see Figure 4.4 and Figure 4.5).

Finally, note that it would be possible to set up a finer approximation considering several time-scales for Tikhonov's Theorem, but at the risk of higher mathematical complexity. Indeed, extended versions of Tikhonov's Theorem exist for several time-scales, using powers of ε [39, 35, 73] or even different epsilons [30]. But computations with this method highly complicates the reduction.

4.8.2. Comparison with Experimental Data. To the best of our knowledge, there are to date no example of metabolome measured at high frequency, at least for a large number of metabolites to assess the kinetics. In general, only a very limited number macromolecules (typically proteins, carbohydrates, lipids, chlorophyll,...) are recorded, specially for microalgae. However, to show that our findings are in agreement with experimental studies we considered the results of Baroukh et al. [4] for an autotrophic microalgae metabolic network.

The authors in [4] fitted parameters of a metabolic model to the set of available experimental data. We examined the reaction rates which ranged from 10^2 to 10^{-1} ($\text{h}^{-1}.\text{mM}.\text{B}^{-1}$) and compared them with the level of concentrations in the cell. Indeed, the concentration of carbohydrates has magnitude 10^2 (mM) times higher than those of the intermediate metabolites GAP, PEP and G6P (see Table 4.5 and Table 4.6). Moreover, GAP, PEP and G6P are consumed by reactions with rate order 10^1 or 10^2 ($\text{h}^{-1}.\text{mM}.\text{B}^{-1}$), while carbohydrates are consumed at rate of order 10^0 ($\text{h}^{-1}.\text{mM}.\text{B}^{-1}$). Additionally, carbohydrates are produced by a single reaction with rate of order 10^1 ($\text{h}^{-1}.\text{mM}.\text{B}^{-1}$), as well as GAP, and G6P and PEP by reactions of order 10^2 ($\text{h}^{-1}.\text{mM}.\text{B}^{-1}$). This evidences that the concentration is related to the rate of consumption, in the way predicted by the Magnitude of Concentration Theorem 4.1 in our work.

Nevertheless, we emphasize that, the reduction method proposed in this work can be used even if only some metabolites with large concentration have been measured. Indeed, such data will support the calibration of the reduced model, i.e. describing the dynamics of the slow metabolites (see Section 4.6.1).

Compound	Value	Mean value
Carbohydrates	M	$8.436 \times 10^{-1} mM$
G6P	E	$5.208 \times 10^{-3} mM$
PEP	E	$4.167 \times 10^{-3} mM$
GAP	E	$1.389 \times 10^{-3} mM$

Table 4.5. Experimental measures (M) and estimated (E) values obtained from Baroukh et al. [4], for an autotrophic microalga metabolic network. Carbon quotas of the different compounds are considered within a period of 24 hours. Light intensity values are on a interval from 0 to 1400 $\mu E \cdot m^{-2} \cdot s^{-1}$. Two different magnitudes of concentration can be distinguished among these compounds.

Compound	Production rate	Consumption rate
Carbohydrates	7.00×10^1	6.50×10^0
G6P	2.24×10^2 6.50×10^0	1.03×10^1 7.00×10^1
PEP	4.37×10^2 9.97×10^0	5.00×10^0 1.04×10^2
GAP	2.06×10^1 5.00×10^0 6.00×10^{-1}	4.47×10^2 4.37×10^2 1.88×10^1

Table 4.6. Rates are in $h^{-1} \cdot mM \cdot B^{-1}$. Typical concentrations in Table 4.5 were used to estimate the consumption rates for GAP and PEP in the lipid synthesis reaction.

4.8.3. Extensions of Results. In order to obtain reduced metabolic systems by a rigorous procedure, many extensions of the results can be obtained (see Appendix B.4). Particularly, considering more reactions between the metabolites with slow dynamics is possible (as long as these reactions are slow), without changing the equations of the fast part. Hence, modifications in the reactions between slow metabolites do not alter the equations of the metabolites in QSS, and the slow dynamics remain in the reduced system. Moreover, results analogous to the Magnitude of Concentration Theorem 4.1 still hold if the equations of the fast part are not changed (see Appendix B.4.2.2).

Furthermore, effects such as inhibition can be considered in the slow part of the system. For example, using the model of Haldane or feedback inhibition in enzyme-catalyzed subnetworks.

In addition, models with more subnetworks of fast reactions, connected by metabolites with slow dynamics, can be reduced and analyzed using the present approach.

4.8.4. Conclusions. Quasi Steady State Assumption without verifying mathematical conditions can lead to erroneous reduced systems [11, 22]. The aim of our work was to define the mathematical foundations of Quasi Steady State Reduction for metabolic networks.

We proposed a reduction for a general class of dynamical metabolic systems using time scale separation and Tikhonov's Theorem. The considered models, include Michaelis-Menten reaction rates and the possibility for slow compounds to have different kinetics. The reduction leads to a simpler model given by a small system of differential equations: regardless the initial dimension of the network, we end up with a low dimensional dynamical system, representing the dynamics of the slow variables. The dilution due to growth plays an important role and must not be neglected. It is worth noting that keeping the growth rate in the equations strongly improves approximation precision and preserves qualitative (stability) features of the original system.

We show that a metabolite in QSS, that is not in a flux trap, has a concentration one order of magnitude lower than a metabolite in the slow part of the system. This is indirectly a way to validate the hypotheses on the magnitude of the reaction kinetics.

Eventually, the calibration algorithm is very simple. It is remarkable that the reduced model can predict all the fast compounds which have been measured, regardless of the other compounds whose concentrations cannot be recorded.

This approach covers a large class of metabolic enzymatic networks (see Appendix B.4). But more work remains to be done to treat further metabolic systems. For example, networks with fast reactions that have nonlinear kinetics (different from Michaelis-Menten kinetics as presented here) can be studied in detail. Moreover, to obtain models that rigorously describe several hierarchies in metabolic networks, systems with more than two time-scales can be analyzed on the basis of the present work.

CHAPTER 5

Nonlinear Dynamical Reduction for Microalgae

Autotroph organisms require only CO_2 to supply their carbon needs, contrary to heterotroph organisms, whose carbon comes from an organic compound [2]. Microalgae are autotrophic microorganisms that can fix CO_2 and transform it in biomass [71].

The ability of some microalgae or cyanobacteria to accumulate large amount of lipids or carbohydrates, has motivated researches to domesticate this potential [78]. In particular, it became key to better understand the metabolism of autotrophic microalgae. Different studies have then set up metabolic networks for photoautotrophic microalgae [5, 38, 79], from simplified considerations of the core network up to genome-scale metabolic networks considering several compartments (e.g. chloroplast, cytosol, mitochondrion, nucleus, etc.).

Models based on these metabolic networks supported qualitative analyses of the microalgae metabolism, in particular, the autotrophic metabolism. However, quantitative studies of these models are complex, because many intracellular measurements are needed [38]. Therefore, there is a necessity for simplifying the models so that they can be calibrated with experimental data, while keeping the core dynamics. To tackle this problem, Kliphuis et al. [38] consider a metabolic network less compartmentalized and Baroukh et al. [4] propose the reduction of a compartmentalized system using the QSSA.

The approach developed in this thesis consists in reducing metabolic networks based on the QSSA. For this reduction method, the principal characteristic that defines the status of a metabolite (QSS or dynamic) is its concentration. In this framework, the Order of Magnitude Theorem presented in Chapter 3 and Chapter 4 (see Magnitude of Concentration Theorem 3.1 and Theorem 4.1), shows that the metabolites with the highest concentrations have the slowest dynamics in the metabolic system, while the metabolites with low concentration are consumed at fast rates.

Under this assumption, we first consider that macromolecules are metabolites with high concentration and, consequently, that they are involved in the slow part of the metabolic system. Also, metabolites whose concentrations

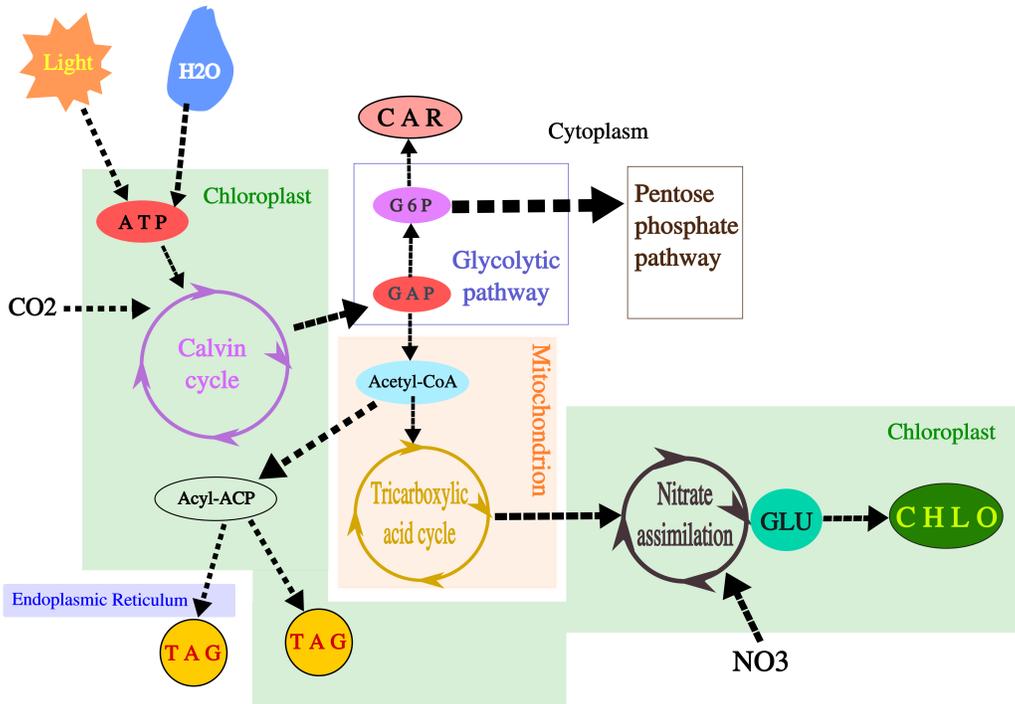


Figure 5.1. Metabolism of autotrophic microalgae.

depend on an external – possibly controlled – input (e.g. light, CO₂, nitrates, glucose, etc.) are also considered as slow variables of the system.

This classification might not be sufficient to describe important features in microalgae metabolism, as the lipids accumulation under nitrogen starvation [40]. The reason is that only considering inputs and macromolecules, we obtain that the equations of macromolecules are linear combinations of the input functions (see Property 3.4).

To consider more metabolites (variables) in our model and eventually more accurately represent complex dynamical phenomena, we consider metabolites that are between the macromolecules and the metabolites with the lowest concentration, in a scale of magnitudes of concentration. In Chapter 4, we have shown reduced systems can accurately represent dynamics with more than two time-scales using Tikhonov's Theorem (see Proposition 4.3). An example of this is presented with a toy network (see Figure 4.4 and Figure 4.5).

Unfortunately, almost nothing is known about the concentration of intracellular metabolites and the classification of their concentration range is tricky. However, metabolites at the crossroad of different metabolic pathways might have a highly dynamic concentration which tends to be accumulative (i.e. to reach high concentrations at some time periods). In some cases, these

metabolites are present in different compartments [70]. Thus, we can assume they link different subnetworks and that they have important dynamics [4].

5.1. Metabolic Network of Autotrophic Microalgae

Here we consider a simplified metabolic reaction network for autotrophic microalgae, which is detailed in Table C.2. This simplified network has 108 reactions and 80 metabolites (see Appendix C). It is based on the network proposed by Yang et al. [79]. In addition, the pathway for triacylglycerols synthesis has been detailed on the basis of the work of Liu et al. [46].

We suppose that the enzymatic fast reactions in this network can be represented by Michaelis-Menten kinetics. Then, for applying our reduction method, we have to consider for fast enzymatic reactions one substrate and one product. In some cases, we split fast reactions into several one substrate–one product enzymatic reactions (e.g. for keeping a cycle). In other cases, for a fast reaction with several metabolites, substrates and products being in a previous reaction are not taken into account. Also, metabolites that are assumed to be in abundance are not considered in some kinetics (e.g. H_2O).

Nevertheless, slow reactions can have any type of kinetics. For instance, in this model we have considered slow enzymatic reactions with two substrates (see Appendix B.4.2.3).

In a second stage, a reduced model is calibrated with the data of Lacour et al. [40] from experiments with autotrophic microalgae *Tisochrysis lutea* (*Isochrysis* sp.).

5.1.1. Neutral Lipids accumulation. Neutral lipids are mostly triacylglycerols [40], accumulated in lipid droplets [TAG-LD] [46]. We suppose two possible ways for the formation of triacylglycerol lipid droplets. The first one is the route in the chloroplast. There are evidences that lipid bodies are formed in the chloroplast in *Isochrysis* sp. and *Isochrysis galbana* [16, 45, 47]. A second route, over the endoplasmic reticulum (ER) membranes in the cytosol of microalgae, has been proposed in different works [46, 74].

5.1.2. Slow dynamics metabolites. In line with our approach, macromolecules belong to the group of slow metabolites. We consider chlorophyll [CHLO], proteins [PROT], DNA, RNA, Carbohydrates [CAR], starch [STA] and triacylglycerols in lipid droplets [TAGld] as terminal products.

Also, the metabolites derived from an external fluctuating or controlled input are also consider to be in the slow part of the system. In the work of Lacour et al. [40], the cultures of microalgae were submitted to nitrogen starvation periods and the pH was controlled by injections of CO_2 . We assume that nitrate $[\text{NO}_3]$ is the predominant form of nitrogen in the culture

[79]. Thus, we consider the absorbed photon light flux (APF), the nitrate supply (N) and carbon dioxide (CO₂) as elements in the slow part of the system.

We select the compounds whose dynamics are driven by different compartmental mechanisms and/or that are precursors in macromolecules formation. The information about the location of reactions within different organelles was taken from [79] and [46].

In the chloroplast, NADP is an electron carrier molecule [1] (energy metabolite [4]) that reacts during the light step of photosynthesis to produce ATP. Also, NADP is produced during the nitrate assimilation, when nitrite is reduced to ammonia by the action of a chloroplast-located NADPH-linked nitrite reductase [38, 58, 79]. Moreover, in the cytoplasm NADP reacts with G6P (a precursor in the synthesis of carbohydrates) to produce Ru5P. G6P connects two metabolic pathways with different metabolic functions: stocking carbon (synthesis of carbohydrates) and reducing power when it reacts with NADP within the pentose phosphate pathway [4]. For these reasons, in line with [4], NADP, NADP-cyt (NADP in the cytoplasm) and G6P are considered to be part of the slow part of the system.

Glycolysis and the tricarboxylic acid cycle (TCA cycle) provide the precursors to synthesize many important biological molecules (glutamine, amino acids, nucleotides, lipids, sugars, etc.). The mitochondrion is the place in the cell where most of the oxidation reactions occur and where most of its ATP is generated. In the mitochondrion, AcCoA is produced from PYR, which is a product of the glycolysis in the cytoplasm [1]. But the consumption of AcCoA for TAG synthesis occurs in the chloroplast. Besides, at its source in the mitochondrion, AcCoA reacts with OAA to produce ISOCIT during the TCA. The process occurs as follows. AcCoA and OAA are the substrates of a reaction which is catalyzed by the enzyme citrate synthase to produce citrate (Cit):



This reaction is strongly forward, due to the subsequent loss by hydrolysis of CoA [1]. Then, the enzyme aconitase catalyze the reaction to produce ISOCIT from Cit:



OAA is consumed and produced during the TCA cycle and it is also derived from a metabolite acting during the glycolysis: PEP. This is a significant flux through PEP that plays an important role to maintain the operation of TCA cycle [79]. Also, OAA has a potential flux from the nitrate input, via the TCA. AcCoa and OAA are both considered as part of the slow dynamics system.

Finally, glutamine (GLU) is identified as a precursor of CHLO formation. It is also precursor of many amino acids, which synthesize proteins in the cytoplasm, DNA and RNA in the nucleus. Moreover, it is also derived from an element of the TCA cycle (AKG) and it has a flux from the nitrate input. For these reasons, GLU is also considered in the slow part of the system.

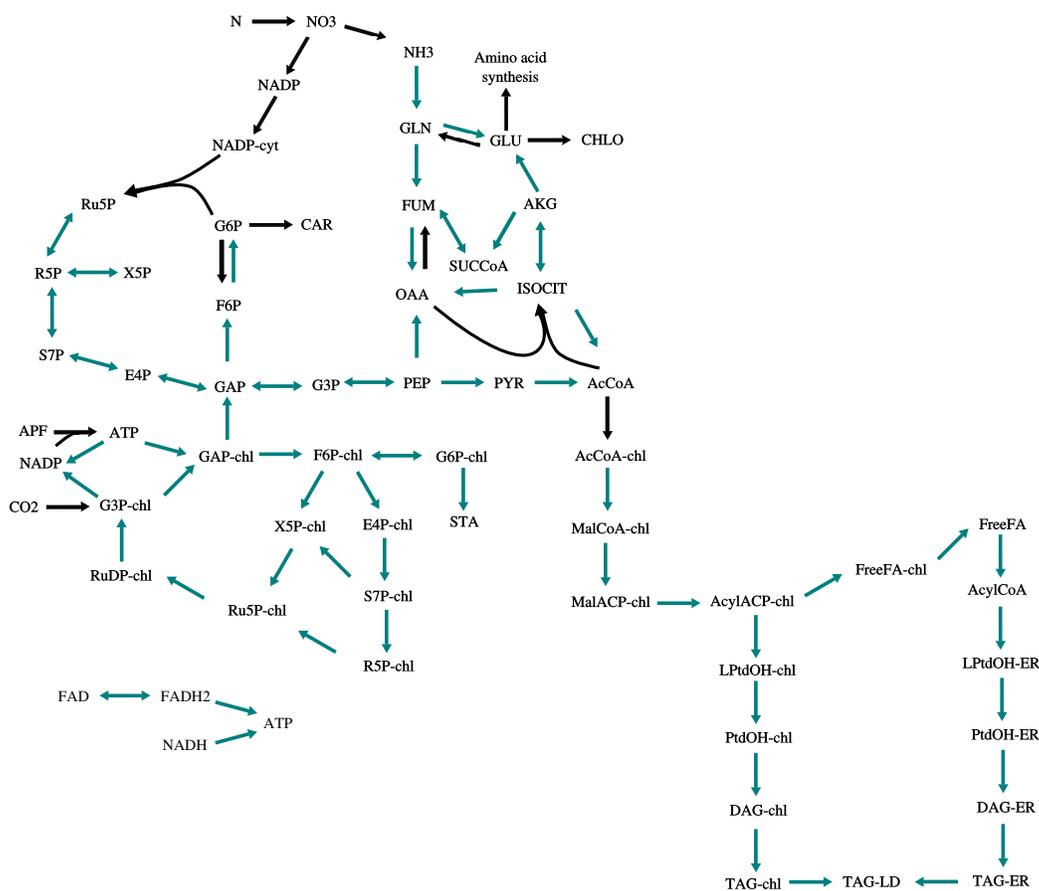


Figure 5.2. Carbohydrates, Lipid Droplets of triacylglycerol (neutral lipids) and Chlorophyll formation in autotrophic microalgae. Slow reactions are depicted by black arrows, while fast reactions by blue arrows. Abbreviations: chl, chloroplast; cyt, cytoplasm; ER, endoplasmic reticulum; LD, lipid droplets.

REMARK 5.1. We suppose that all reactions that consume the metabolites in the slow part are slow and that the rest are fast. This agrees with our approach, where metabolites in the slow part are only consumed by slow reactions, while metabolites in the fast part are consumed by at least one fast reaction.

5.2. Fluxes towards Macromolecules Synthesis

To reduce the considered metabolic network, we have to examine all fluxes between the slow metabolites. In Appendix B.4, it is explained how these fluxes determine the form of the reduced system of equations, for an enzymatic metabolic network which also accounts for any type of slow kinetics.

The objective is to find fluxes in the sense of Definition B.2 (for the nonlinear enzymatic reactions case). We computed Elementary Flux Modes (EFM) to verify the fluxes between the slow metabolites. EFM are obtained using Copasi (copasi.org). The number of obtained modes is 59.

For simplicity, we only consider the reactions for Carbohydrates, Neutral Lipids and Chlorophyll formation, since the available data of Lacour et al. [40] only recorded these macromolecules. Thus, we take into account the 27 EFM constituted by these reactions (see Table C.3).

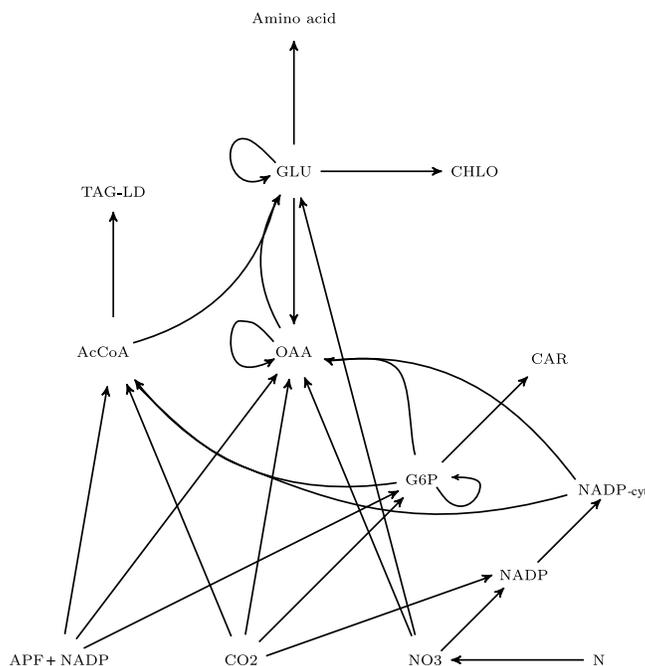


Figure 5.3. Reduced network of fluxes between slow metabolites: inputs, precursors in macromolecules formation and macromolecules (Carbohydrates, Neutral Lipids and Chlorophyll).

5.3. Reduced Dynamical Model of Autotrophic Microalgae

In this section we present a reduced ODE system of the metabolic network depicted in Figure 5.2, which accounts for Carbohydrates, Neutral Lipids and Chlorophyll formation in autotrophic microalgae.

For applying our method, a vector in the left kernel of the stoichiometric submatrix of fast reactions between metabolites in QSS (see Section 2.5 and Remark B.1) was found using the *kernel* tool in Scilab (<http://www.scilab.org>). The result is described in Appendix C.3.

We consider as inputs the light intensity $[APF(t)]$, the nitrate supply $[N(t)]$ and the constant injection of CO_2 $[a_7]$. The slow variables are

$X_1 = \text{NO}_3$,
 $X_2 = \text{CO}_2$,
 $X_3 = \text{NADP}$,
 $X_4 = \text{G6P}$,
 $X_5 = \text{AcCoA}$,
 $X_6 = \text{OAA}$,
 $X_7 = \text{GLU}$,
 $X_8 = \text{CAR}$,
 $X_9 = \text{TAG-LD}$,
 $X_{10} = \text{CHLO}$,
 $X_{11} = \text{NADP-cyt}$.

NOTE 5.1. The objective of this model is to approximate the available data from Lacour et al. [40] (Carbohydrates [CAR], Neutral Lipids [TAG-LD] and Chlorophyll [CHLO]). Thus, for simplicity, we do not consider the starch [STA], DNA, RNA and proteins [PROT] variables in this system of equations.

5.3.1. Computing the Reduced System. Our method leads to a reduced system of dynamical equations taking into account the fluxes between the slow variables and inputs. For more details see Appendix B.4. Here we apply it to the autotrophic metabolism of microalgae.

The fast variables in QSS are linear combinations of slow kinetics and inputs (see Equation (B.13) in Appendix B.4.2). Then, the expressions of fast variables in QSS have to be substituted in the equations of the slow part. To be more precise, the equation of a slow metabolite X_i has a slow kinetic $\nu(X)$ if

- X_i is a product of the slow reaction with kinetic $\nu(X)$ or

- there exists X_j a fast metabolite, such that X_j is a product of the slow reaction with kinetic $\nu(X)$ and there is a flux of fast reactions from X_j to X_i .

Analogously for the inputs, but instead of a slow kinetic there is an input function $I(t)$ (see Equation (B.12) and Note B.4).

For example, consider the equation for X_4 =G6P in the original (complete) system:

$$\begin{aligned} \frac{dX_4}{dt} = & \frac{e_{4,F6P}^0 k_{4,F6P} X[F6P]}{X[F6P] + K_{4,F6P}} - \frac{e_{F6P,4}^0 k_{F6P,4} X_4}{X_4 + K_{F6P,4}} - \frac{e_{84}^0 k_{84} X_4}{X_4 + K_{84}} \\ & - \frac{(\alpha) e_{Ru5P}^0 k_{Ru5P} X_4 X_{11}}{X_4 X_{11} + K_{Ru5P}} - \mu \cdot X_4, \end{aligned}$$

where α is a variable of mass conservation (see Appendix B.4.2.3). F6P is considered as a metabolite consumed by fast reactions, then, after the change of variable ($Y[F6P] = X[F6P]/\varepsilon$) and writing $\varepsilon = 0$, we obtain

$$\begin{aligned} \frac{dX_4}{dt} = & \frac{e_{4,F6P}^0 k_{4,F6P}}{K_{4,F6P}} Y[F6P] - \frac{e_{F6P,4}^0 k_{F6P,4} X_4}{X_4 + K_{F6P,4}} - \frac{e_{84}^0 k_{84} X_4}{X_4 + K_{84}} \\ & - \frac{(\alpha) e_{Ru5P}^0 k_{Ru5P} X_4 X_{11}}{X_4 X_{11} + K_{Ru5P}} - \mu \cdot X_4. \end{aligned}$$

But the expression of $Y[F6P]$ in QSS is a linear combination of the slow kinetics followed by a flux of fast reactions towards [F6P]:

$$Y[F6P] = (2.6)\mathbf{b}_1 \cdot k_{ATP,3} \cdot APF(t) \cdot X_3 + (2)\mathbf{b}_2 \cdot \frac{e_{G3P,2}^0 k_{G3P,2} X_2}{X_2 + K_{G3P,2}}.$$

Notice that (2.6) is the stoichiometric factor of ATP produced after the light reaction in photosynthesis (R1), and (2) is the stoichiometric factor of G3P_{chl} in the CO₂ fixation reaction (R2) (see Table C.2). We recall that parameters \mathbf{b}_1 and \mathbf{b}_2 are cofactors of the fast system Jacobian matrix, which involves several constant reaction rates and Michaelis-Menten constants (see the reduced model for the toy network in Section 4.7 as illustration, and Appendix B.4.2 for rigorous details in a general case). Then, substituting in the equation for X_4 , we have

$$\begin{aligned} \frac{dX_4}{dt} = & \frac{e_{4,F6P}^0 k_{4,F6P}}{K_{4,F6P}} \left[(2.6)\mathbf{b}_1 \cdot k_{ATP,3} \cdot APF(t) \cdot X_3 + (2)\mathbf{b}_2 \cdot \frac{e_{G3P,2}^0 k_{G3P,2} X_2}{X_2 + K_{G3P,2}} \right] \\ & - \frac{e_{F6P,4}^0 k_{F6P,4} X_4}{X_4 + K_{F6P,4}} - \frac{e_{84}^0 k_{84} X_4}{X_4 + K_{84}} - \frac{(\alpha) e_{Ru5P}^0 k_{Ru5P} X_4 X_{11}}{X_4 X_{11} + K_{Ru5P}} - \mu \cdot X_4. \end{aligned}$$

As a result of the model reduction stage, some parameters are lumped into aggregated parameters. For example, we define

$$a_{12} := \frac{e_{4,F6P}^0 k_{4,F6P}}{K_{4,F6P}} \cdot \mathbf{b}_1 \cdot k_{ATP,3}.$$

Then, the term

$$(2.6) \frac{e_{4,F6P}^0 k_{4,F6P}}{K_{4,F6P}} \cdot \mathbf{b}_1 \cdot k_{ATP,3} \cdot APF(t) \cdot X_3$$

can be more clearly rewritten as

$$(2.6) a_{12} \cdot APF(t) \cdot X_3$$

and we only estimate a parameter a_{12} for this kinetic. The aggregated set of parameters defined to simplify the notation are given in Appendix C.5.

5.3.2. Reduced System of ODE. Using the fluxes between slow variables established in Section 5.2, we deduce the following reduced system (5.1):

$$\begin{aligned}
\frac{dX_1}{dt} &= a_1 \cdot N(t) - \frac{a_2 X_1}{X_1 + a_3} - \frac{a_4 X_1}{X_1 + a_5} - \mu X_1 \\
\frac{dX_2}{dt} &= a_6(a_7 - X_2) - \frac{a_8 X_2}{X_2 + a_9} - \mu X_2 \\
\frac{dX_3}{dt} &= (2)a_{40} \cdot APF(t) \cdot X_3 + \frac{a_2 X_1}{X_1 + a_3} + \frac{(2)a_{46} X_2}{X_2 + a_9} - \frac{a_{41} X_3}{X_3 + a_{42}} - \mu X_3 \\
\frac{dX_4}{dt} &= (2.6)a_{12} \cdot APF(t) \cdot X_3 + \frac{(2)a_{13} X_2}{X_2 + a_9} - \frac{a_{14} X_4}{X_4 + a_{15}} - \frac{a_{37} X_{11} X_4}{X_{11} X_4 + a_{11}} \\
&\quad - \frac{a_{16} X_4}{X_4 + a_{17}} - \mu X_4 \\
\frac{dX_5}{dt} &= (2.6)a_{18} \cdot APF(t) \cdot X_3 + \frac{(2)a_{19} X_2}{X_2 + a_9} + \frac{a_{38} X_{11} X_4}{X_{11} X_4 + a_{11}} - \frac{a_{20} X_5}{X_5 + a_{21}} \\
&\quad - \frac{a_{22} X_5 X_6}{X_5 X_6 + a_{23}} - \mu X_5 \\
\frac{dX_6}{dt} &= (2.6)a_{24} \cdot APF(t) \cdot X_3 + \frac{a_{25} X_1}{X_1 + a_5} + \frac{(2)a_{26} X_2}{X_2 + a_9} + \frac{a_{39} X_{11} X_4}{X_{11} X_4 + a_{11}} \\
&\quad + \frac{a_{43} X_7}{X_7 + a_{44}} - \frac{a_{27} X_5 X_6}{X_5 X_6 + a_{23}} - \frac{a_{36} X_6}{X_6 + a_{28}} - \mu X_6 \\
\frac{dX_7}{dt} &= \frac{a_{29} X_1}{X_1 + a_5} + \frac{a_{30} X_5 X_6}{X_5 X_6 + a_{23}} - \frac{a_{45} X_7}{X_7 + a_{44}} - \frac{(8)a_{31} X_7}{X_7 + a_{32}} \\
&\quad - \frac{a_{33} X_7}{X_7 + a_{34}} - \mu X_7 \\
\frac{dX_8}{dt} &= \frac{a_{14} X_4}{X_4 + a_{15}} - \mu X_8 \\
\frac{dX_9}{dt} &= \frac{a_{35} X_5}{X_5 + a_{21}} - \mu X_9 \\
\frac{dX_{10}}{dt} &= \frac{a_{31} X_7}{X_7 + a_{32}} - \mu X_{10} \\
\frac{dX_{11}}{dt} &= \frac{a_{41} X_3}{X_3 + a_{42}} - \frac{a_{10} X_{11} X_4}{X_{11} X_4 + a_{11}} - \mu X_{11}.
\end{aligned} \tag{5.1}$$

The complete metabolic network for autotrophic microalgae accounts for 80 metabolites (see Appendix C.1). Thus, the dimension (80 equations) of the original ODE system is considerably reduced to the 11 equations of model (5.1), for describing Carbohydrates, Neutral Lipids and Chlorophyll formation in microalgae.

5.4. Calibrated System for *T. lutea* metabolism

Once the reduced system has been obtained, the next step is to estimate its parameters using some experimental data. We calibrated the reduced system (5.1) with the data of Lacour et al. [40], from experiments with the microalgae *Tisochrysis lutea* under nitrogen starvation.

The set of parameters, described in Table 5.1, was obtained using the calibration method described in Section 4.6. For this, the *fminsearch* minimization function based on the Nelder-Mead algorithm was used in Scilab [6]. The cost function considered was

$$F_0(a, \mu) = \sum_{i=8,9,10} \sum_{j \in T_i} [X_i(t_j, a, \mu) - Z_i(t_j)]^2, \quad (5.2)$$

where variables Z_i represent the experimental data.

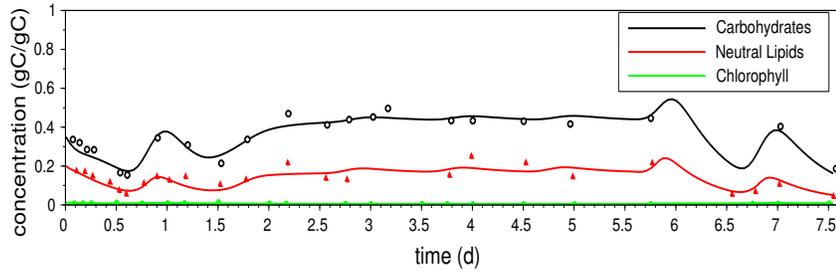


Figure 5.4. Carbohydrates, Neutral Lipids and Chlorophyll concentration. Experimental data of Lacour et al. [40] is represented: Carbohydrates by black circles, Neutral Lipids by red triangles and Chlorophyll by green points. The curves depict the numerical solution of system (5.1), with the set of parameters in Table 5.1.

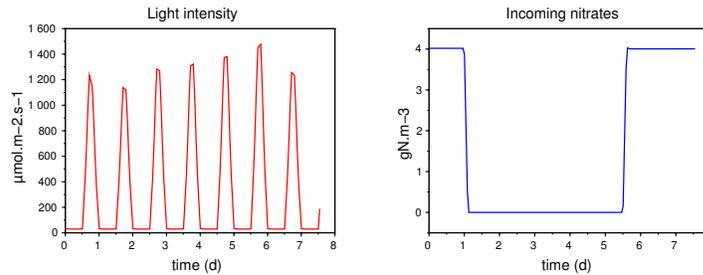


Figure 5.5. Light intensity (APF) and incoming nitrates (NO_3) for system (5.1). Data obtained from Lacour et al. [40]

Table 5.1. Parameters estimated for system (5.1) (see Figure 5.4). The parameters were obtained minimizing the cost function (5.2) with the *fminsearch* tool in Scilab [6].

Parameter	Value	Units	Parameter	Value	Units
$\hat{\mu}$	2.60×10^0	$\text{gC} \cdot (\text{gC} \cdot \text{d})^{-1}$	\hat{a}_1	1.46×10^{-1}	$\text{gC} \cdot (\text{gC} \cdot \text{d})^{-1} \text{gN}^{-1} \cdot \text{m}^3$
\hat{a}_2	6.33×10^0	$\text{gC} \cdot (\text{gC} \cdot \text{d})^{-1}$	\hat{a}_3	8.31×10^{-1}	$\text{gC} \cdot \text{gC}^{-1}$
\hat{a}_4	1.54×10^{-4}	$\text{gC} \cdot (\text{gC} \cdot \text{d})^{-1}$	\hat{a}_5	7.47×10^{-1}	$\text{gC} \cdot \text{gC}^{-1}$
\hat{a}_6	1.03×10^0	d^{-1}	\hat{a}_7	7.60×10^{-1}	$\text{gC} \cdot \text{gC}^{-1}$
\hat{a}_8	7.34×10^0	$\text{gC} \cdot (\text{gC} \cdot \text{d})^{-1}$	\hat{a}_9	7.44×10^{-1}	$\text{gC} \cdot \text{gC}^{-1}$
\hat{a}_{10}	7.45×10^2	$\text{gC}^2 \cdot \text{gC}^{-2} \cdot \text{d}^{-1}$	\hat{a}_{11}	1.33×10^0	$\text{gC}^2 \cdot \text{gC}^{-2}$
\hat{a}_{12}	2.22×10^{-2}	$(\text{d} \cdot \mu\text{mol})^{-1} \cdot \text{m}^2 \cdot \text{s}$	\hat{a}_{13}	1.14×10^1	$\text{gC} \cdot (\text{gC} \cdot \text{d})^{-1}$
\hat{a}_{14}	1.00×10^1	$\text{gC} \cdot (\text{gC} \cdot \text{d})^{-1}$	\hat{a}_{15}	1.39×10^0	$\text{gC} \cdot \text{gC}^{-1}$
\hat{a}_{16}	5.14×10^{-1}	$\text{gC} \cdot (\text{gC} \cdot \text{d})^{-1}$	\hat{a}_{17}	2.40×10^0	$\text{gC} \cdot \text{gC}^{-1}$
\hat{a}_{18}	5.34×10^{-2}	$(\text{d} \cdot \mu\text{mol})^{-1} \cdot \text{m}^2 \cdot \text{s}$	\hat{a}_{19}	1.66×10^1	$\text{gC} \cdot (\text{gC} \cdot \text{d})^{-1}$
\hat{a}_{20}	2.53×10^2	$\text{gC} \cdot (\text{gC} \cdot \text{d})^{-1}$	\hat{a}_{21}	8.46×10^{-1}	$\text{gC} \cdot \text{gC}^{-1}$
\hat{a}_{22}	1.78×10^4	$\text{gC}^2 \cdot \text{gC}^{-2} \cdot \text{d}^{-1}$	\hat{a}_{23}	6.53×10^{-1}	$\text{gC}^2 \cdot \text{gC}^{-2}$
\hat{a}_{24}	2.52×10^{-3}	$(\text{d} \cdot \mu\text{mol})^{-1} \cdot \text{m}^2 \cdot \text{s}$	\hat{a}_{25}	1.13×10^1	$\text{gC} \cdot (\text{gC} \cdot \text{d})^{-1}$
\hat{a}_{26}	2.07×10^0	$\text{gC} \cdot (\text{gC} \cdot \text{d})^{-1}$	\hat{a}_{27}	3.80×10^3	$\text{gC}^2 \cdot \text{gC}^{-2} \cdot \text{d}^{-1}$
\hat{a}_{28}	1.66×10^0	$\text{gC} \cdot \text{gC}^{-1}$	\hat{a}_{29}	2.19×10^0	$\text{gC} \cdot (\text{gC} \cdot \text{d})^{-1}$
\hat{a}_{30}	6.11×10^3	$\text{gC}^2 \cdot \text{gC}^{-2} \cdot \text{d}^{-1}$	\hat{a}_{31}	4.43×10^{-3}	$\text{gC} \cdot (\text{gC} \cdot \text{d})^{-1}$
\hat{a}_{32}	2.85×10^{-2}	$\text{gC} \cdot \text{gC}^{-1}$	\hat{a}_{33}	6.65×10^0	$\text{gC} \cdot (\text{gC} \cdot \text{d})^{-1}$
\hat{a}_{34}	2.73×10^{-1}	$\text{gC} \cdot \text{gC}^{-1}$	\hat{a}_{35}	9.45×10^1	$\text{gC} \cdot (\text{gC} \cdot \text{d})^{-1}$
\hat{a}_{36}	1.91×10^{-1}	$\text{gC} \cdot (\text{gC} \cdot \text{d})^{-1}$	\hat{a}_{37}	4.47×10^3	$\text{gC}^2 \cdot \text{gC}^{-2} \cdot \text{d}^{-1}$
\hat{a}_{38}	7.60×10^{-4}	$\text{gC}^2 \cdot \text{gC}^{-2} \cdot \text{d}^{-1}$	\hat{a}_{39}	3.85×10^1	$\text{gC}^2 \cdot \text{gC}^{-2} \cdot \text{d}^{-1}$
\hat{a}_{40}	3.74×10^{-4}	$(\text{d} \cdot \mu\text{mol})^{-1} \cdot \text{m}^2 \cdot \text{s}$	\hat{a}_{41}	9.56×10^0	$\text{gC} \cdot (\text{gC} \cdot \text{d})^{-1}$
\hat{a}_{42}	1.41×10^0	$\text{gC} \cdot \text{gC}^{-1}$	\hat{a}_{43}	4.44×10^{-1}	$\text{gC} \cdot (\text{gC} \cdot \text{d})^{-1}$
\hat{a}_{44}	7.68×10^{-1}	$\text{gC} \cdot \text{gC}^{-1}$	\hat{a}_{45}	8.03×10^{-1}	$\text{gC} \cdot (\text{gC} \cdot \text{d})^{-1}$
\hat{a}_{46}	1.80×10^{-1}	$\text{gC} \cdot (\text{gC} \cdot \text{d})^{-1}$			

5.5. Conclusions

In this chapter, a metabolic network for the autotrophic metabolism of microalgae has been reduced. The metabolic network is based on the reaction network presented by Yang et al. [79]. This simplified network has 108 reactions and 80 metabolites (see Appendix C.1 and Appendix C.2), meaning that the complete ODE system has 80 equations.

A reduced system of 11 variables [Equation (5.1)] and 23 kinetics was obtained (see Figure 5.3). It accounts for several inputs (light, nitrate, CO₂ supplies), the dynamics of the metabolites with slow dynamics (metabolites controlled by an input, precursors in the formation of macromolecules and macromolecules) and a factor of growth dilution.

The reduced model was calibrated using the available experimental data: light intensity and incoming nitrates (see Figure 5.5), concentration of Carbohydrates, Neutral Lipids and Chlorophyll (see Figure 5.4), obtained from Lacour et al. [40]. The calibrated model accurately describes the data of these macromolecules under nitrogen starvation (see Figure 5.4).

CHAPTER 6

Optimization of Production

This is an exploratory chapter to propose optimization strategies for the reduced system (5.1), which was obtained in the previous Chapter 5. For this purpose, we consider control parameters within kinetics and inputs of a metabolic network.

For instance, consider the ODE system describing a metabolic network

$$\frac{dX}{dt} = f(X, u, \alpha, \mu) \quad X(0) = X_0,$$

where f accounts for an input function u , the kinetics of the network, the growth rate μ , and a vector of parameters α that can be controlled (e.g. constants of enzyme concentrations or inputs). Thus, we look for the optimal values for α such that an objective criterium is optimized, e.g. maximize the concentration of a metabolite X_i .

The idea is that optimal values are at the boundary of a closed set of interest. We first explain this for linear systems that have equilibrium points. Then, control parameters are considered in the metabolic network of microalgae described in Chapter 5. Using the reduced system, we show that similar results can be obtained.

6.1. Optimization at equilibrium points

Suppose the system

$$\frac{dX}{dt} = u + A(\alpha) \cdot X - \mu X \quad X(0) = X_0, \quad (6.1)$$

with u is a constant input, μ a positive real number and

$$A(\alpha) = \begin{pmatrix} -\alpha_{11} \cdot a_{11} & \alpha_{12} \cdot a_{12} & \dots & \alpha_{1n} \cdot a_{1n} \\ \alpha_{21} \cdot a_{21} & -\alpha_{22} \cdot a_{22} & \dots & \alpha_{2n} \cdot a_{2n} \\ \vdots & \vdots & \ddots & \vdots \\ \alpha_{n1} \cdot a_{n1} & \alpha_{n2} \cdot a_{n2} & \dots & -\alpha_{nn} \cdot a_{nn} \end{pmatrix},$$

where parameters a_{ij} account for constants of reaction rates and stoichiometric factors, and parameters $\alpha_{ij} \in [0, 1]$ can be manipulated. We consider

that some of these parameters can be the same, for example, it can occur that $\alpha_{ij} = \alpha_{i'j'}$ for $i \neq i'$ and $j \neq j'$.

Assuming that $[A(\alpha) - \mu \cdot Id]$ is an invertible matrix, the equilibrium point of system (6.1) satisfies

$$X^* = [A(\alpha) - \mu \cdot Id]^{-1} \cdot (-u),$$

where Id is the identity matrix.

6.1.1. Homographic Function Approach. Suppose that the concentration of a variable X_i has to be optimized with respect to a parameter $\alpha_{i'j'}$. Let us assume that X_i^* is an homographic function with respect to $\alpha_{i'j'}$:

$$X_i^* = \frac{\alpha_{i'j'} \mathbf{B}_{11} + \mathbf{B}_{12}}{\alpha_{i'j'} \mathbf{B}_{21} + \mathbf{B}_{22}}.$$

Since homographic functions are monotone (see Appendix D), the optimal value for $\alpha_{i'j'} \in [0, 1]$ is 0 or 1.

6.1.2. Linear Programming Approach. Consider system (6.1). Suppose again that the concentration of a variable X_i has to be optimized with respect to a parameter $\alpha_{i'j'} \in [0, 1]$.

The optimization problem is nonlinear, because in the equation the factor

$$\alpha_{i'j'} \cdot X_{j'}$$

has two terms, $\alpha_{i'j'}$ and $X_{j'}$, that have to be optimized simultaneously. However, we reformulate the problem to solve it through linear programming. For this we define

$$\nu := \alpha_{i'j'} \cdot X_{j'},$$

and we compute the numerical solution of the following LP problem:

$$\max c^T \cdot \begin{pmatrix} X \\ \nu \end{pmatrix}, \quad \text{such that} \quad (6.2)$$

$$A'(\alpha) \cdot \begin{pmatrix} X \\ \nu \end{pmatrix} = -u$$

$$X \geq 0,$$

$$\nu \geq 0,$$

$$X_{j'} - \nu \geq 0,$$

where A' is a matrix such that

$$[A(\alpha) - \mu \cdot I] \cdot X = A'(\alpha) \cdot \begin{pmatrix} X \\ \nu \end{pmatrix}.$$

If

$$\begin{pmatrix} X^* \\ \nu^* \end{pmatrix}$$

is the numerical solution of the linear programming problem (6.2) and $X_{j'}^* \neq 0$, we can obtain the optimal value for $\alpha_{i'j'}$ computing

$$\alpha_{i'j'}^* = \frac{\nu^*}{X_{j'}^*}.$$

In order to lower the complexity of optimization problems, we can apply these optimization techniques to reduced ODE systems, obtained after applying the method developed in this thesis. The following example (Section 6.2) illustrates a simple case where the input is constant and the system reaches an equilibrium.

NOTE 6.1. The homographic function and the linear programming approaches can be implemented to optimize more than one control parameter, as illustrated in the example below (Section 6.2).

6.2. Optimization Strategy wit a Toy Network

In Figure 6.1 we present a toy network to solve an optimization problem. For this toy network, we suppose that two reactions can be controlled with two positive parameters α and β in the interval $[0, 1]$. The objective is to find the optimal values for α and β to increase the concentration of a final product (X_5).

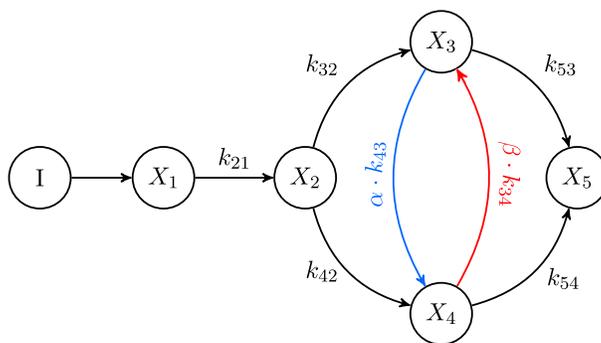


Figure 6.1. α and β are control parameters to optimize the production of X_5 .

Let us consider I as a constant input. Then, the system

$$\begin{aligned}
 \frac{dX_1}{dt} &= I - (k_{21} + \mu)X_1 \\
 \frac{dX_2}{dt} &= k_{21}X_1 - (k_{32} + k_{42} + \mu)X_2 \\
 \frac{dX_3}{dt} &= k_{32}X_2 + \beta k_{34}X_4 - (\alpha \cdot k_{43} + k_{53} + \mu)X_3 \\
 \frac{dX_4}{dt} &= k_{42}X_2 + \alpha \cdot k_{43}X_3 - (\beta \cdot k_{34} + k_{54} + \mu)X_4 \\
 \frac{dX_5}{dt} &= k_{53}X_3 + k_{54}X_4 - \mu X_5,
 \end{aligned} \tag{6.3}$$

has an equilibrium point $X^* = (X_1^*, X_2^*, X_3^*, X_4^*, X_5^*)$ (see Figure 6.2).

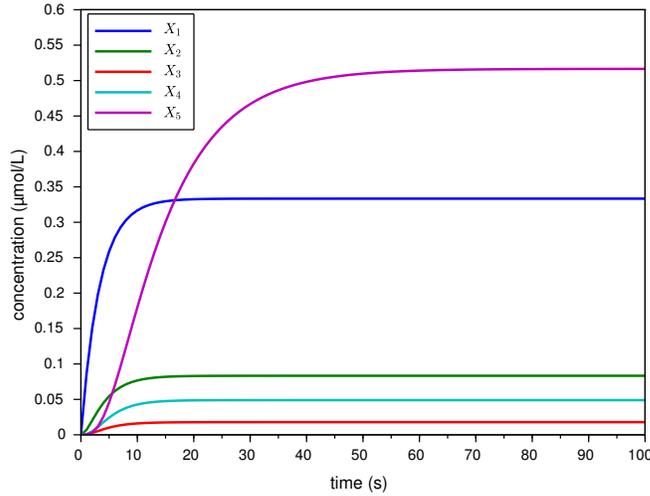


Figure 6.2. Numerical solution of system (6.3) with the parameters described in Table 6.1, $\alpha = 1$ and $\beta = 0$.

6.2.1. Homographic function. Computations show that X_5^* is an homographic function with respect to α and β :

$$X_5^*(\alpha, \beta) = \left[\frac{k_{21} \cdot I}{\mu(k_{21} + \mu)(k_{32} + k_{42} + \mu)} \right] \cdot \frac{\alpha \cdot \mathbf{a} + \beta \cdot \mathbf{b} + \mathbf{c}}{\alpha \cdot \mathbf{d} + \beta \cdot \mathbf{e} + \mathbf{f}},$$

with

$$\begin{aligned}
 \mathbf{a} &= k_{32}k_{43}k_{54} + k_{42}k_{43}k_{54} \\
 \mathbf{b} &= k_{32}k_{34}k_{53} + k_{42}k_{34}k_{53} \\
 \mathbf{c} &= k_{53}k_{32}(k_{54} + \mu) + k_{54}k_{42}(k_{53} + \mu) \\
 \mathbf{d} &= k_{43}(k_{54} + \mu) \\
 \mathbf{e} &= k_{34}(k_{53} + \mu) \\
 \mathbf{f} &= (k_{53} + \mu)(k_{54} + \mu).
 \end{aligned}$$

Defining a matrix associated to the homographic function, as described in Proposition D.1, we can deduce whether $\frac{\partial X_5^*}{\partial \alpha}$ is positive or negative, as well as for $\frac{\partial X_5^*}{\partial \beta}$. Indeed, if

$$\begin{aligned}
 \mathbf{H}_1 &:= \begin{pmatrix} \mathbf{a} & \beta \cdot \mathbf{b} + \mathbf{c} \\ \mathbf{d} & \beta \cdot \mathbf{e} + \mathbf{f} \end{pmatrix} \\
 \mathbf{H}_2 &:= \begin{pmatrix} \mathbf{b} & \alpha \cdot \mathbf{a} + \mathbf{c} \\ \mathbf{e} & \alpha \cdot \mathbf{d} + \mathbf{f} \end{pmatrix}.
 \end{aligned}$$

$$\begin{aligned}
 \frac{\partial X_5^*}{\partial \alpha} \geq 0 &\iff \det \mathbf{H}_1 \geq 0, \\
 \frac{\partial X_5^*}{\partial \beta} \geq 0 &\iff \det \mathbf{H}_2 \geq 0.
 \end{aligned}$$

Parameter	Value	Units
I	0.1	$\mu\text{mol}(\text{L}\cdot\text{s})^{-1}$
μ	0.1	s^{-1}
k_{21}	0.2	s^{-1}
k_{32}	0.3	s^{-1}
k_{42}	0.4	s^{-1}
k_{34}	0.5	s^{-1}
k_{43}	0.6	s^{-1}
k_{53}	0.7	s^{-1}
k_{54}	0.8	s^{-1}

Table 6.1. Supposed parameters for the toy network in Figure 6.1.

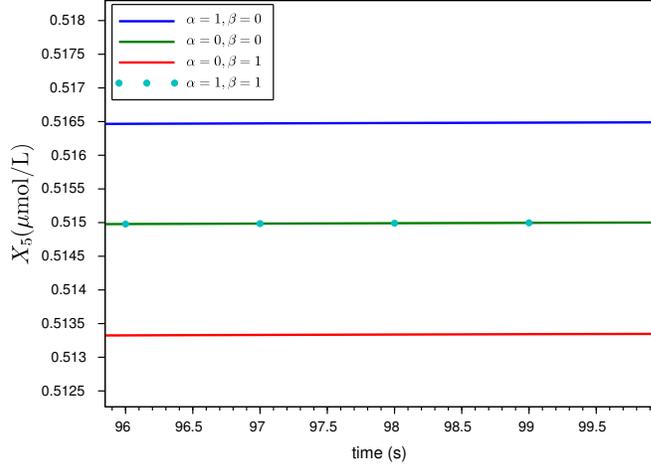


Figure 6.3. Numerical solution of X_5 in system (6.3) with the parameters described in Table 6.1 and different values for α and β . The combination $\alpha = 1, \beta = 0$ is the optimal to increase X_5 concentration.

Table 6.2. Equilibrium point of system (6.3), with $\alpha = 1$ and $\beta = 0$.

Variable	Value
X_1^*	0.3333333
X_2^*	0.0833333
X_3^*	0.0178571
X_4^*	0.0489418
X_5^*	0.5165344

Finally, recall that the objective is to increase the concentration of X_5 . For the parameter values described in Table 6.1, we obtain that the optimal values for α and β are 1 and 0, respectively (see Figure 6.3). This is in agreement Proposition D.1, which states that optimal values for homographic functions are in the boundary of a domain of interest.

6.2.2. Linear Programming. The same optimization problem can be established as a linear programming (LP) problem. This new LP problem is based on the definition of variables $\nu_1 := \alpha \cdot X_3$ and $\nu_2 := \beta \cdot X_4$, with the restrictions $0 \leq \alpha \leq 1$ and $0 \leq \beta \leq 1$. Then, it can be written as

$$\begin{aligned} \max c^T \cdot (X_1, X_2, X_3, X_4, X_5, \nu_1, \nu_2)^T, \\ c^T = (0, 0, 0, 0, 1, 0, 0) \quad \text{such that} \end{aligned} \quad (6.4)$$

$$\begin{pmatrix} -(k_{21} + \mu) & 0 & 0 & 0 & 0 & 0 & 0 \\ k_{21} & -(k_{32} + k_{42} + \mu) & 0 & 0 & 0 & 0 & 0 \\ 0 & k_{32} & -(k_{53} + \mu) & 0 & 0 & -k_{43} & k_{34} \\ 0 & k_{42} & 0 & -(k_{54} + \mu) & 0 & k_{43} & -k_{34} \\ 0 & 0 & k_{53} & k_{54} & -\mu & 0 & 0 \end{pmatrix} \cdot \begin{pmatrix} X_1 \\ X_2 \\ X_3 \\ X_4 \\ X_5 \\ \nu_1 \\ \nu_2 \end{pmatrix} = \begin{pmatrix} -I \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{pmatrix}$$

$$\begin{aligned} X_i &\geq 0 & \forall i = 1, \dots, 5, \\ \nu_1 &\geq 0 & \nu_2 \geq 0, \\ X_3 - \nu_1 &\geq 0, \\ X_4 - \nu_2 &\geq 0. \end{aligned}$$

We use the tool *karmarkar* in Scilab to solve the LP problem. Once the solution X_{opt} is obtained, α and β are deduced as $\alpha = \nu_1/X_{3opt}$ and $\beta = \nu_2/X_{4opt}$. The result is

$$\begin{aligned} \alpha' &= 0.998 \\ \beta' &= 0.001 \end{aligned}$$

which is close to the optimal solution deduced using an homographic function, which was obtained analytically. However, the analytical solution gives a better result:

$$X_5^*(1, 0) > X_{5opt},$$

with a slight difference (compare Table 6.2 and Table 6.3).

Table 6.3. Numerical solution of the linear programming problem (6.4). The optimal values obtained are $\alpha' = 0.998, \beta' = 0.001$.

Variable	Value
X_{1opt}	0.3333333
X_{2opt}	0.0833333
X_{3opt}	0.0178806
X_{4opt}	0.048921
X_{5opt}	0.5165318
ν_1	0.0178532
ν_2	0.0000328

6.3. Extensions and Discussion

A linear programming approach can avoid excessive work to compute (by hand) equilibrium points as homographic functions.

On the other hand, the linear programming approach can be extended to a type of metabolic networks with Michaelis-Menten kinetics, as the networks described in Chapter 4. For this purpose, new kinetics variables for the optimization problem can be defined as

$$v_i := \frac{X_i}{X_i + K_i}$$

$$0 \leq v_i < 1.$$

In order to set a linear problem, we have to assume that all the enzymatic reactions consuming X_i have the same Michaelis-Menten constant K_i . Also, that the dilution term is bounded and that its half-saturation coefficient is equal to K_i for X_i , i.e. the dilution term is

$$\frac{\mu X_i}{X_i + K_i}.$$

Similarly, for a kinetic variable comprising a control parameter α_j , we define

$$v_j := \frac{\alpha_j X_j}{X_j + K_j}.$$

After obtaining a solution (v_i^*, ν_i^*) of the LP problem, the optimal values for the variables X_i can be computed as

$$X_i^* := \frac{K_i \cdot v_i^*}{v_i^* - 1}.$$

where v_i^* is the numerical solution of the LP problem. Moreover, for a kinetic variable comprising a control parameter α_j , we have

$$\nu_j^* = \frac{\alpha_j X_j^*}{X_j^* + K_j},$$

where ν_j^* is the numerical solution of the LP problem. If the optimal value X_j^* is positive, we can compute

$$\alpha_j^* := \frac{\nu_j^*(X_j^* + K_j)}{X_j^*}.$$

Although it is not rigorously proved that the solution of the LP is the optimal solution, this method can be useful to improve the production of some metabolites of interest.

6.4. Lipids Production Optimization

A dynamical reduced system based on time scale separation is useful to solve optimization problems. The dimension of the problem is reduced to the number of variables with slow dynamics, which also allows to employ time discretization grids [76].

In this section we illustrate how reduced systems, obtained by the method described in this work, can be used to derive optimization strategies. For this, we suppose that some parameters in the constant reaction rates or inputs can be controlled.

For example, suppose that the initial concentration of enzymes can be regulated in a certain range. Then, in order to trace where enzyme parameters are in the reduced system, we analyze the coefficients \mathbf{b}_{ij} (see Appendix B.4.2.1, particularly Equation (B.12)).

We have already shown that \mathbf{b}_{ij} is different from zero when there is a flux, of fast reactions, from X_j to X_i (Proposition B.4). Moreover, this coefficient is proportional to the enzymes catalyzing the fast reactions in the path. The reduced system (4.28)-(4.29), of the toy network in Section 4.7, illustrates this property. Indeed, the minor to obtain \mathbf{b}_i is computed with the matrix entries that represent non-null reaction rates related to a flux (see the proof of Proposition A.3).

Here, two strategies are proposed to increase neutral lipids (TAG lipid droplets) production in microalgae per biomass unit. The first one is concerned with intracellular reactions, and the second one with the control of nitrate and CO_2 inputs.

We consider the reduced system (5.1) obtained in Chapter 5. The objective is to increase the concentration of TAG lipid droplets (X_9) manipulating some control parameters related to constant rates or inputs.

6.4.1. Strategy 1: PEP consumption. Consider the parameter $\alpha \in [0, 1]$ regulating the consumption of PEP to produce PYR or OAA (reactions R23 and R25, respectively, in Table C.2). See Figure 6.4.

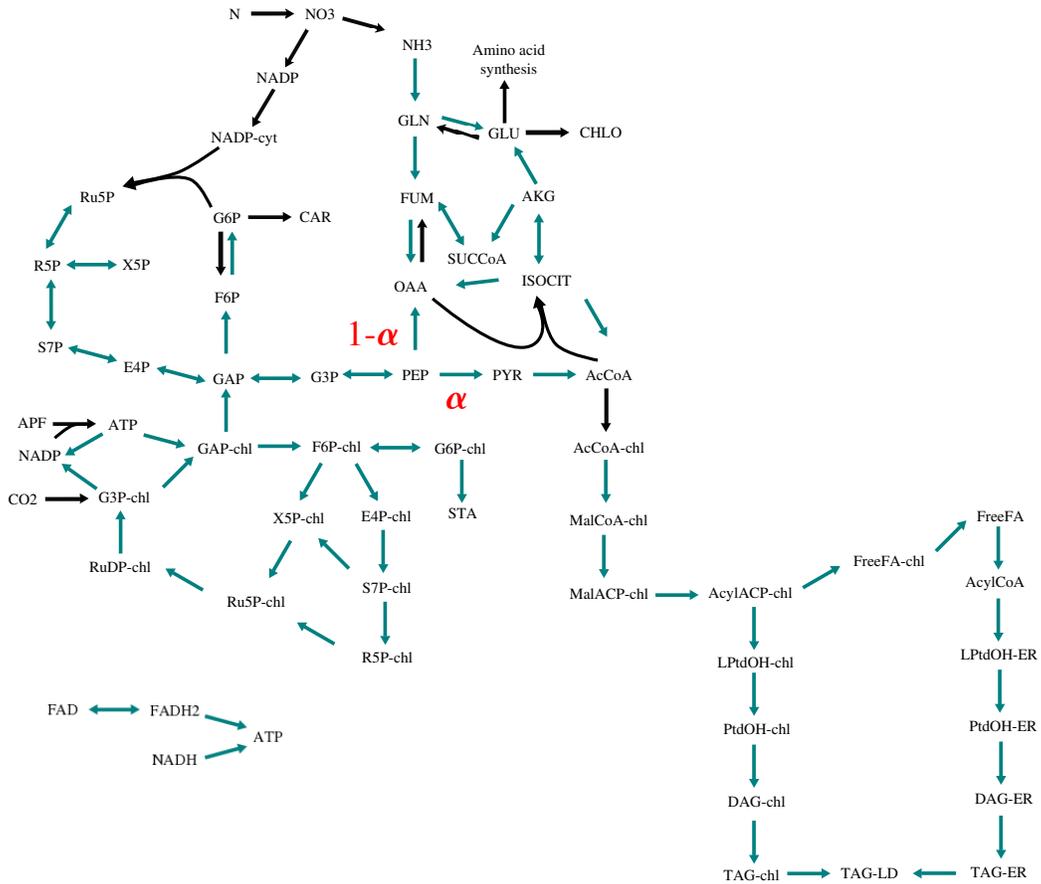


Figure 6.4. Strategy 1. Consumption of PEP distributed for PYR and OAA formation.

Consider the reduced ODE system for the network in Figure 6.4:

$$\begin{aligned}
\frac{dX_1}{dt} &= a_1 \cdot N(t) - \frac{a_2 X_1}{X_1 + a_3} - \frac{a_4 X_1}{X_1 + a_5} - \mu X_1 \\
\frac{dX_2}{dt} &= a_6(a_7 - X_2) - \frac{a_8 X_2}{X_2 + a_9} - \mu X_2 \\
\frac{dX_3}{dt} &= (2)a_{40} \cdot APF(t) \cdot X_3 + \frac{a_2 X_1}{X_1 + a_3} + \frac{(2)a_{46} X_2}{X_2 + a_9} - \frac{a_{41} X_3}{X_3 + a_{42}} - \mu X_3 \\
\frac{dX_4}{dt} &= (2.6)a_{12} \cdot APF(t) \cdot X_3 + \frac{(2)a_{13} X_2}{X_2 + a_9} - \frac{a_{14} X_4}{X_4 + a_{15}} - \frac{a_{37} X_{11} X_4}{X_{11} X_4 + a_{11}} \\
&\quad - \frac{a_{16} X_4}{X_4 + a_{17}} - \mu X_4 \\
\frac{dX_5}{dt} &= \alpha \left[(2.6)\tilde{a}_{18} \cdot APF(t) \cdot X_3 + \frac{(2)\tilde{a}_{19} X_2}{X_2 + a_9} + \frac{\tilde{a}_{38} X_{11} X_4}{X_{11} X_4 + a_{11}} \right] - \frac{a_{20} X_5}{X_5 + a_{21}} \\
&\quad - \frac{a_{22} X_5 X_6}{X_5 X_6 + a_{23}} - \mu X_5 \\
\frac{dX_6}{dt} &= \frac{a_{25} X_1}{X_1 + a_5} + (1 - \alpha) \left[(2.6)\tilde{a}_{24} \cdot APF(t) \cdot X_3 + \frac{(2)\tilde{a}_{26} X_2}{X_2 + a_9} + \frac{\tilde{a}_{39} X_{11} X_4}{X_{11} X_4 + a_{11}} \right] \\
&\quad + \frac{a_{43} X_7}{X_7 + a_{44}} - \frac{a_{27} X_5 X_6}{X_5 X_6 + a_{23}} - \frac{a_{36} X_6}{X_6 + a_{28}} - \mu X_6 \\
\frac{dX_7}{dt} &= \frac{a_{29} X_1}{X_1 + a_5} + \frac{a_{30} X_5 X_6}{X_5 X_6 + a_{23}} - \frac{a_{45} X_7}{X_7 + a_{44}} - \frac{(8)a_{31} X_7}{X_7 + a_{32}} - \frac{a_{33} X_7}{X_7 + a_{34}} - \mu X_7 \\
\frac{dX_8}{dt} &= \frac{a_{14} X_4}{X_4 + a_{15}} - \mu X_8 \\
\frac{dX_9}{dt} &= \frac{a_{35} X_5}{X_5 + a_{21}} - \mu X_9 \\
\frac{dX_{10}}{dt} &= \frac{a_{31} X_7}{X_7 + a_{32}} - \mu X_{10} \\
\frac{dX_{11}}{dt} &= \frac{a_{41} X_3}{X_3 + a_{42}} - \frac{a_{10} X_{11} X_4}{X_{11} X_4 + a_{11}} - \mu X_{11},
\end{aligned} \tag{6.5}$$

where we define $\tilde{a}_i := a_i/0.5$ in order to obtain the original reduced system (5.1) when $\alpha = 0.5$.

The strategy is to increase X_5 [AcCoA], which implies an increment in X_9 [TAG-ld] concentration. We use two facts to find when X_5 attains maximal concentration. First, we consider that, in a domain of interest (open and convex set), a local maximum (thus, the global maximum include) of X_5 is reached at time t^* such that

$$\begin{aligned}
\alpha \left[(2.6)\tilde{a}_{18} \cdot APF(t^*) \cdot X_3(t^*) + \frac{(2)\tilde{a}_{19} X_2(t^*)}{X_2(t^*) + a_9} + \frac{\tilde{a}_{38} X_{11}(t^*) X_4(t^*)}{X_{11}(t^*) X_4(t^*) + a_{11}} \right] \\
- \frac{a_{20} X_5(t^*)}{X_5(t^*) + a_{21}} - \frac{a_{22} X_5(t^*) X_6(t^*)}{X_5(t^*) X_6(t^*) + a_{23}} - \mu X_5(t^*) = 0
\end{aligned}$$

(see Equation (6.5)). We assume $\tilde{a}_{18}, \tilde{a}_{19}, \tilde{a}_{38}$ are positive values. Second, since a monotone function has its maximum and minimum values at the boundary of the domain, the optimal value for $\alpha \in [0, 1]$ is 0 or 1.

According to Figure 6.5, the optimal values to increase X_5 is $\alpha = 1$. This means that the production of PYR from PEP has to be prioritized, in contrast to the production of OAA.

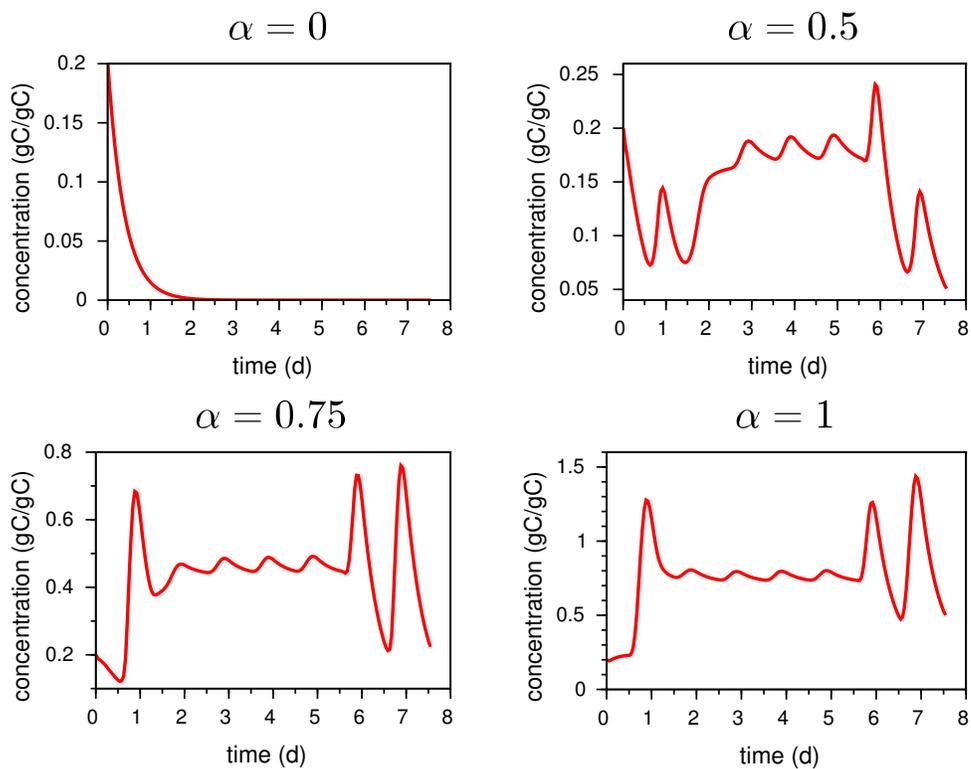


Figure 6.5. Neutral Lipids concentration (X_9) for different values of α in system (6.5). The highest concentration is reached when $\alpha = 1$. The parameters considered are in Table 5.1.

Consider the following reduced system for the network depicted in Figure 6.6:

$$\begin{aligned}
\frac{dX_1}{dt} &= \alpha \cdot \tilde{a}_1 \cdot N(t) - \frac{a_2 X_1}{X_1 + a_3} - \frac{a_4 X_1}{X_1 + a_5} - \mu X_1 & (6.6) \\
\frac{dX_2}{dt} &= a_6 (\beta \cdot \tilde{a}_7 - X_2) - \frac{a_8 X_2}{X_2 + a_9} - \mu X_2 \\
\frac{dX_3}{dt} &= (2) a_{40} \cdot APF(t) \cdot X_3 + \frac{a_2 X_1}{X_1 + a_3} + \frac{(2) a_{46} X_2}{X_2 + a_9} - \frac{a_{41} X_3}{X_3 + a_{42}} - \mu X_3 \\
\frac{dX_4}{dt} &= (2.6) a_{12} \cdot APF(t) \cdot X_3 + \frac{(2) a_{13} X_2}{X_2 + a_9} - \frac{a_{14} X_4}{X_4 + a_{15}} - \frac{a_{37} X_{11} X_4}{X_{11} X_4 + a_{11}} \\
&\quad - \frac{a_{16} X_4}{X_4 + a_{17}} - \mu X_4 \\
\frac{dX_5}{dt} &= (2.6) a_{18} \cdot APF(t) \cdot X_3 + \frac{(2) a_{19} X_2}{X_2 + a_9} + \frac{a_{38} X_{11} X_4}{X_{11} X_4 + a_{11}} - \frac{a_{20} X_5}{X_5 + a_{21}} \\
&\quad - \frac{a_{22} X_5 X_6}{X_5 X_6 + a_{23}} - \mu X_5 \\
\frac{dX_6}{dt} &= (2.6) a_{24} \cdot APF(t) \cdot X_3 + \frac{a_{25} X_1}{X_1 + a_5} + \frac{(2) a_{26} X_2}{X_2 + a_9} + \frac{a_{39} X_{11} X_4}{X_{11} X_4 + a_{11}} \\
&\quad + \frac{a_{43} X_7}{X_7 + a_{44}} - \frac{a_{27} X_5 X_6}{X_5 X_6 + a_{23}} - \frac{a_{36} X_6}{X_6 + a_{28}} - \mu X_6 \\
\frac{dX_7}{dt} &= \frac{a_{29} X_1}{X_1 + a_5} + \frac{a_{30} X_5 X_6}{X_5 X_6 + a_{23}} - \frac{a_{45} X_7}{X_7 + a_{44}} - \frac{(8) a_{31} X_7}{X_7 + a_{32}} \\
&\quad - \frac{a_{33} X_7}{X_7 + a_{34}} - \mu X_7 \\
\frac{dX_8}{dt} &= \frac{a_{14} X_4}{X_4 + a_{15}} - \mu X_8 \\
\frac{dX_9}{dt} &= \frac{a_{35} X_5}{X_5 + a_{21}} - \mu X_9 \\
\frac{dX_{10}}{dt} &= \frac{a_{31} X_7}{X_7 + a_{32}} - \mu X_{10} \\
\frac{dX_{11}}{dt} &= \frac{a_{41} X_3}{X_3 + a_{42}} - \frac{a_{10} X_{11} X_4}{X_{11} X_4 + a_{11}} - \mu X_{11}.
\end{aligned}$$

In system (6.6), the values considered are in Table 5.1 and $\tilde{a}_i := a_i/2$, to obtain as reference the calibrated system (5.1).

We first consider light [APF(t)] and nitrates [N(t)] inputs as constants. Then, the system reaches an equilibrium. To optimize the production of Neutral Lipids (X_9), we conjecture that its equilibrium is a monotone function with respect to α and β . Then in an interval $[0, 1]$, the optimal values for these parameters are on the boundary, i.e. the optimal values are 0 or 1.

This fact is illustrated in Figure 6.7, where the numerical solution of system (6.6) is depicted for different values of α and β . The optimal solution is $\alpha = 0$ and $\beta = 1$, meaning that the nitrate input should to be canceled and the CO₂ supply augmented.

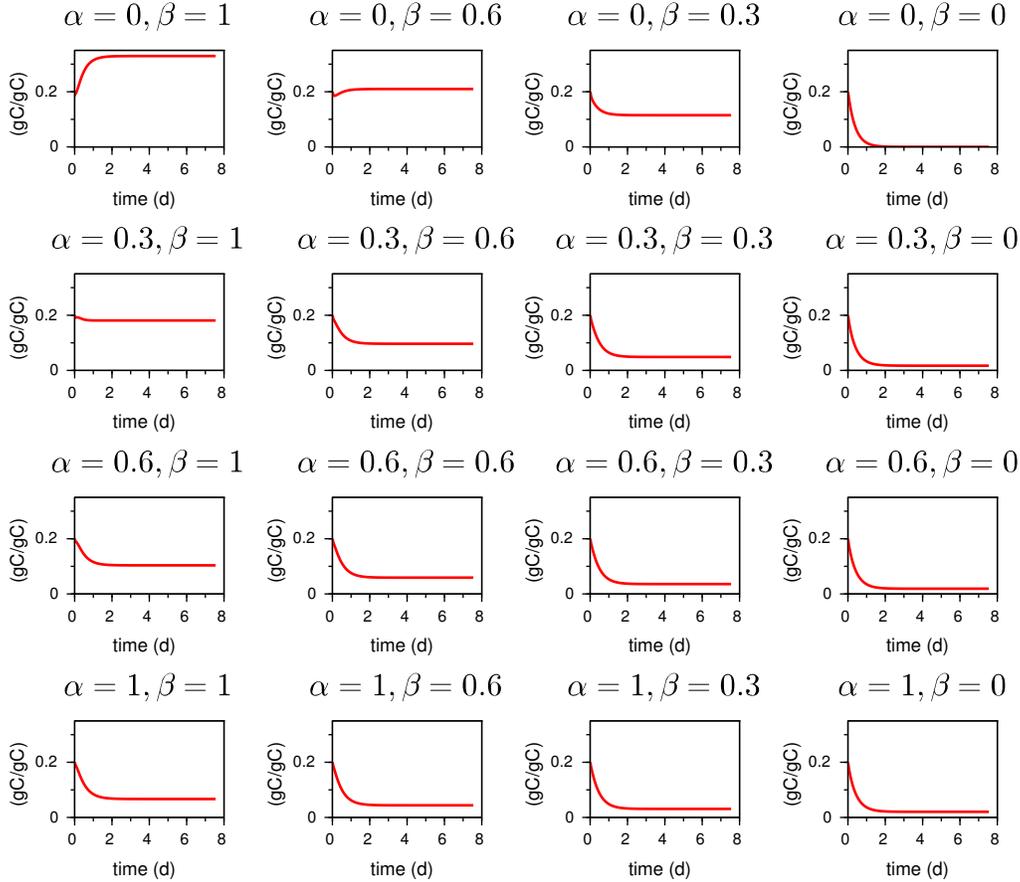


Figure 6.7. Neutral Lipids concentration (X_9) for different control parameters α and β . Constant light, CO₂ and nitrate inputs were considered for system (6.6). Light input: $APF=350.33691 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; CO₂ input: $\beta\cdot\tilde{a}_7 = \beta\cdot 3.8 \times 10^{-1}$; nitrate input: $\alpha\cdot N = \alpha\cdot 4.004 \text{gN}\cdot\text{m}^{-3}$. The set of parameters considered are in Table 5.1.

A similar result might be extended to the case where light $[APF(t)]$ and nitrate $[N(t)]$ supplies are not constants. For example, if we consider as a criterium function the sum of neutral lipids concentration at each hour and the maximum of neutral lipids concentration,

$$\sum_{t_h} X_9(t_h) + \max_t X_9(t), \quad (6.7)$$

we also obtain that the optimal solution is $\alpha = 0$ and $\beta = 1$, as in the linear case (see Figure 6.8 and Table 6.4).

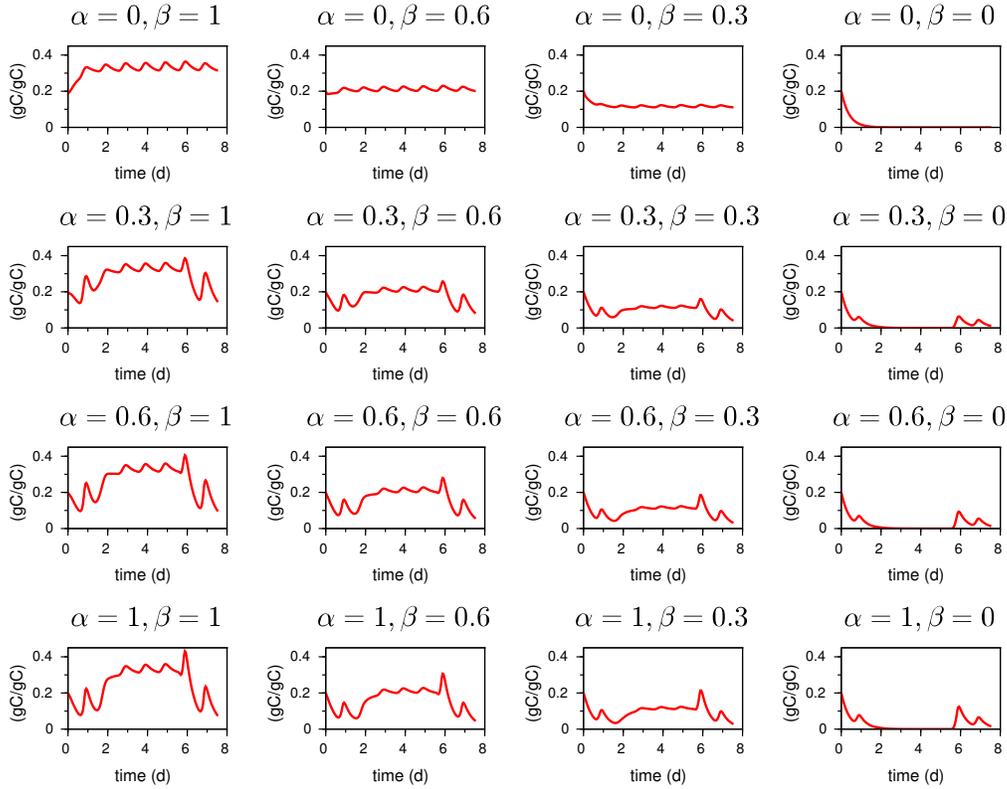


Figure 6.8. Neutral Lipids concentration (X_9), from the numerical solution of system (6.6) with no constant light and nitrate inputs. Different values of α and β in a interval $[0, 1]$ were considered. The inputs values are depicted in Figure 5.5.

Table 6.4. The criterium value (6.7) is defined as the sum of Neutral Lipids concentration at each hour and the maximum in the complete time interval. It was computed for different solutions of system (6.6), varying the parameter α and β (see Figure 6.8). The optimal values according to this criterium are $\alpha = 0$ and $\beta = 1$.

Parameters	Criterium Value (gC/gC)
$\alpha = 0, \beta = 1$	59.07
$\alpha = 0, \beta = 0.6$	38.40
$\alpha = 0, \beta = 0.3$	22.02
$\alpha = 0, \beta = 0$	2.15
$\alpha = 0.3, \beta = 1$	51.38
$\alpha = 0.3, \beta = 0.6$	32.72
$\alpha = 0.3, \beta = 0.3$	18.73
$\alpha = 0.3, \beta = 0$	4.17
$\alpha = 0.6, \beta = 1$	47.90
$\alpha = 0.6, \beta = 0.6$	30.71
$\alpha = 0.6, \beta = 0.3$	17.99
$\alpha = 0.6, \beta = 0$	4.798
$\alpha = 1, \beta = 1$	45.65
$\alpha = 1, \beta = 0.6$	29.59
$\alpha = 1, \beta = 0.3$	17.73
$\alpha = 1, \beta = 0$	5.40

In conclusion, similarly to the case of homographic functions, in the previous examples it is shown that the optimal values are reached at boundary points. However, mathematical proofs to generalize these results remain to be done. For example, to prove that the equilibrium point of a molecule of interest can be written as a monotone function with respect to some control parameters.

Conclusions

In this work, we developed a method to reduce the dimension of a dynamical metabolic system described by ODE. Contrary to nearly all existing works, the idea is to keep core dynamics of the model, that are necessary to represent the high accumulation and reuse of lipids and carbohydrates in microalgae and cyanobacteria.

To reduce a metabolic system accurately, we present a method that relies on time scale separation and the QSSA. The reduction of a system is obtained using the Theorem of Tikhonov for singularly perturbed systems, which allows to characterize the approximation error. Moreover, we provide an algorithm for a simple calibration of the reduced model.

With this approach, we can fix a threshold that divides constant reaction rates into fast and slow. In this way, we can also take into account several time-scales within the slow or the fast part, allowing to obtain reduced models that describe more complex metabolic systems.

One of the contributions of this work is a result concerning the magnitude of metabolite concentrations, deduced after the Quasi Steady State Reduction. We proved a theorem that associates time-scales with the magnitude of metabolite concentrations. Moreover, we introduce the concepts of *trap* and *flux trap* for metabolic networks, which were not before referred to for metabolic modeling.

This approach can be the preamble of further studies about nonlinear metabolic networks, leading to the characterization of more general reaction rates and metabolites magnitude of concentration. For instance, for the class of networks considered in this work, we proved that the slow variables reach the highest concentrations. However, elements in QSS with fast dynamics could also attain similar concentrations, but, as it is proven here, this only happens when these elements are in a *flux trap*.

To test and illustrate our approach, we applied it to several toy networks. As well, it was implemented in a metabolic network of autotrophic microalgae, based on the reaction network proposed by Yang et al. [79]. The reduced model was calibrated using experimental data from Lacour et al. [40]. The

data describe the microalgae *Tisochrysis lutea* submitted to light/dark conditions and nitrogen starvation. The calibrated model provides an accurate approximation of the data.

Our mathematical approach offers a significant reduction in the dimension of a dynamical metabolic system. The mathematical analysis of this reduced system becomes then tractable and it is appropriate to model based control strategies.

A next stage is the optimization of target molecules production. Using the monotonicity of homographic functions and a technique for Linear Programming, two approaches were proposed for optimization in linear metabolic systems with equilibrium points. Similar results with monotone functions might be deduced for nonlinear metabolic systems. But mathematical results remain to be obtained, particularly for the dynamical case.

APPENDIX A

Complements for Chapter 3

A.1. Supplements for the proof of Theorem 3.1

Here we present some results used for the proof of Magnitude of Concentration Theorem 3.1, the Magnitude of Concentration Theorem for the linear case. For the following demonstrations recall that all the terms $k_{ij} = \mathcal{O}(1)$ have the same order, $\mu \leq \mathcal{O}(1)$, and that ε is a very small positive number (see Subsection Section 3.2.3).

LEMMA A.1. *Consider a constant matrix $A = (a_{ij})$ of dimension $n \times n$, such that $\mathcal{O}(a_{ij}) = 1$ when $\varepsilon \rightarrow 0$ for every i, j . Suppose that A is nonsingular and let $\varepsilon\mu > 0$. Then*

$$\det(A - \varepsilon\mu \cdot I) = (-1)^n \cdot \mathcal{O}(\varepsilon\mu).$$

Proof. If $\lambda^{(1)} = 0, \lambda^{(2)}, \dots, \lambda^{(n)}$ are the eigenvalues of A , we have that

$$\begin{aligned} \det(A - \lambda \cdot I) &= (-1)^n \cdot (\lambda - \lambda^{(1)}) \cdots (\lambda - \lambda^{(n)}) \\ &= (-1)^n \cdot (\lambda)(\lambda - \lambda^{(2)}) \cdots (\lambda - \lambda^{(n)}). \end{aligned}$$

Substituting λ by $\varepsilon\mu$ in the formula above, we obtain the desired result. \square

LEMMA A.2. *Suppose that M is a column diagonal dominant matrix of size $n \times n$, such that $\det(M) \neq 0$. If every off-diagonal entry of M is nonnegative, then all the cofactors of M have the same sign equal to $(-1)^{n-1}$ and $\text{sgn}(\det(M)) = (-1)^n$.*

Proof. Since $-M$ is nonsingular and column diagonal dominant, by the Theorem of Gershgorin, $-M$ is a positive stable matrix (see Proposition 2.1). Then, according to Theorem 2.3, its inverse matrix is nonnegative (i.e. each entry of $(-M)^{-1}$ is nonnegative). But

$$-((-M)^{-1}) = (M)^{-1} = \frac{1}{\det(M)} \cdot \mathcal{C} \leq 0,$$

where

$$\mathcal{C} = \begin{pmatrix} \mathcal{C}_{11} & \mathcal{C}_{12} & \cdots & \mathcal{C}_{1n} \\ \mathcal{C}_{21} & \mathcal{C}_{22} & \cdots & \mathcal{C}_{2n} \\ \vdots & \vdots & & \vdots \\ \mathcal{C}_{n1} & \mathcal{C}_{n2} & \cdots & \mathcal{C}_{nn} \end{pmatrix}^T,$$

is the transpose matrix of cofactors of M [41]. Then

$$\frac{\mathcal{C}_{ij}}{\det(M)} \leq 0 \quad \forall i, j = 1, \dots, n,$$

which implies that all the cofactors $\mathcal{C}_{ij} = (-1)^{i+j}M_{ij}$, with M_{ij} the minor of M obtained from removing the i -th row and the j -th column, have the same sign. Moreover, since all the principal minors of $-M$ are positive, then $\det(-M) > 0$ (see Theorem 2.3). We conclude that

$$\text{sgn}(\mathcal{C}_{ij}) = (-1)^{n-1}$$

and that $\det(M) = (-1)^n \det(-M)$ is negative if n is odd and positive if n is even (see Proposition 2.2). \square

PROPOSITION A.1. *Let*

$$M_n = \begin{pmatrix} -\sum_{i=2}^n k_{i1} - k_{*1} - \varepsilon\mu & \cdots & k_{1n} \\ \vdots & \ddots & \vdots \\ k_{n1} & \cdots & -\sum_{i=1}^{n-1} k_{in} - k_{n+1,n} - \varepsilon\mu \end{pmatrix}. \quad (\text{A.1})$$

where $k_{*i} \geq 0$. Consider the directed graph $\Gamma(M_n)$ associated to M_n as a graph with n nodes X_1, \dots, X_n and an edge with origin X_i and final X_j if $k_{ji} > 0$. Suppose that $\Gamma(M_n)$ has no traps and that $k_{n+1,n} > 0$. Then,

$$\det(M_n) = (-1)^n \cdot \mathcal{O}(k_{ij}^n).$$

Proof. The matrix of the system is written as in (A.1), where $k_{*i} \geq 0$. Notice that an output from the i -th metabolite is equivalent to $k_{*i} > 0$. Here, without loss of generality, we begin supposing that the n -th has an output. Then $k_{n+1,n} > 0$.

We prove the proposition by induction over n . For $n = 2$, consider the matrix

$$M_2 = \begin{pmatrix} -k_{21} - \varepsilon\mu & k_{12} \\ k_{21} & -k_{12} - k_{32} - \varepsilon\mu \end{pmatrix} \quad (\text{A.2})$$

of a system with two metabolites and one output. The determinant of M_2 is

$$\det(M_2) = k_{21}(k_{32} + \varepsilon\mu) + \varepsilon\mu(k_{12} + k_{32} + \varepsilon\mu).$$

If $k_{21} \cdot k_{32} \neq 0$, then $\det(M_2) = \mathcal{O}(k_{ij}^2)$. We examine in which cases $k_{21} \cdot k_{32} = 0$.

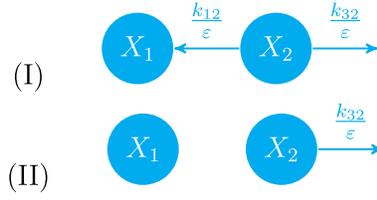


Figure A.1. Possible scenarios where $k_{21} = 0$ in a system with two metabolites and one output from X_2 . Both cases represent that X_1 is in a trap.

If $k_{32} = 0$, the system has no output, contrary to our hypothesis. On the other hand, $k_{21} = 0$ implies that X_1 is in a *trap* (see Figure A.1). We conclude that $\det(M_2) = \mathcal{O}(k_{ij}^2)$. The case in dimension $n = 2$ with more than one output is verified immediately.

We make the following *induction hypothesis*: consider a linear system of $n - 1$ metabolites with no *traps* and one output at least. If M_{n-1} is the matrix of this system, then $\det(M_{n-1}) = (-1)^{n-1} \cdot \mathcal{O}(k_{ij}^{n-1})$.

Now we prove the case of a network with n metabolites. We take into account that all the cofactors \mathcal{C}_{ij} of M_n have the same sign, as claimed by Lemma A.2. It holds

$$\det(M_n) = -k_{n+1,n}\mathcal{C}_{nn} + \left[\sum_{j=1}^{n-1} k_{jn}\mathcal{C}_{jn} - \left(\sum_{i=1}^{n-1} k_{in} + \varepsilon\mu \right) \mathcal{C}_{nn} \right], \quad (\text{A.3})$$

where $\mathcal{C}_{jn} = (-1)^{j+n}(M_n)_{jn}$ are cofactors of M_n [41].

Suppose that $k_{ni} = 0$ and $k_{*i} = 0$ for every $i \in \{1, \dots, n-1\}$. Then X_n is isolated and the rest of metabolites $\{X_1, \dots, X_{n-1}\}$ form a *trap*. Hence, $k_{ni} > 0$ or $k_{*i} > 0$ for some $i \in \{1, \dots, n-1\}$ and we can apply the hypothesis of induction to deduce that

$$\mathcal{C}_{nn} = (-1)^{n-1} \cdot \mathcal{O}(k^{n-1}).$$

On the other hand, the term in the squared brackets in (A.3) is the determinant of the matrix $(M_n + k_{n+1,n} \cdot \delta_{nn})$, where δ_{nn} is a matrix of size $n \times n$ with zero at every entry, except for in the entry nn which is equal to 1

If $k_{*i} = 0$ for every $i = 1, \dots, n-1$, then

$$\det(M_n + k_{n+1,n} \cdot \delta_{nn}) = (-1)^n \cdot \mathcal{O}(\varepsilon\mu)$$

according to Lemma A.1 and the statement of Proposition A.2 is proved. In other case, suppose $k_{*,n-1} > 0$ without loss of generality. Hence, if we develop the determinant of $(M_n + k_{n+1,n} \cdot \delta_{nn})$ by the $n - 1$ -th column and

we substitute in (A.3), we have

$$\begin{aligned} \det(M_n) = & -k_{n+1,n}\mathcal{C}_{nn} - k_{*,n-1}(M_n + k_{n+1,n} \cdot \delta_{nn})_{n-1,n-1} \quad (\text{A.4}) \\ & + \left[\sum_{\substack{j=1 \\ j \neq n-1}}^n k_{j,n-1}(M_n + k_{n+1,n} \cdot \delta_{nn})_{j,n-1} \right. \\ & \left. - \left(\sum_{i=1}^{n-2} k_{i,n-1} + \varepsilon\mu \right) (M_n + k_{n+1,n} \cdot \delta_{nn})_{n-1,n-1} \right], \end{aligned}$$

where $(M_n + k_{n+1,n} \cdot \delta_{nn})_{j,n-1}$ are cofactors of $(M_n + k_{n+1,n} \cdot \delta_{nn})$. Moreover, the matrix $(M_n + k_{n+1,n} \cdot \delta_{nn})$ satisfies the conditions of Lemma A.2. Then, all its cofactors have the same sign. Particularly, $\text{sgn}((M_n + k_{n+1,n} \cdot \delta_{nn})_{n-1,n-1}) = (-1)^{n-1}$, and then

$$\text{sgn}(-k_{n+1,n}\mathcal{C}_{nn}) = \text{sgn}(-k_{*,n-1}(M_n + k_{n+1,n} \cdot \delta_{nn})).$$

Once again, the term in square brackets in (A.4) is equal to $\det(M_n + k_{n+1,n} \cdot \delta_{nn} + k_{*,n-1} \cdot \delta_{n-1,n-1})$. We proceed as for $\det(M_n + k_{n+1,n} \cdot \delta_{nn})$ to extract the following term

$$-k_{*,n-2}(M_n + k_{n+1,n} \cdot \delta_{nn} + k_{*,n-1} \cdot \delta_{n-1,n-1})_{n-2,n-2}$$

which has the same sign as $-k_{n+1,n}\mathcal{C}_{nn}$. In n steps, we arrive to an expression of the determinant where all the terms have the same sign and one term is the determinant of a matrix whose entries by column sum $-\varepsilon\mu$. That is to say, if we define

$$\widetilde{M}_i := (M_n + k_{n+1,n} \cdot \delta_{nn} + \sum_{j=1}^{n-i} k_{*,n-j} \delta_{n-j,n-j}),$$

for every $i = 2, \dots, n$, where we define

$$\sum_{j=1}^0 k_{*,n-j} \delta_{n-j,n-j} = 0.$$

Then

$$\begin{aligned} \det(M_n) = & -k_{n+1,n}\mathcal{C}_{nn} - \sum_{i=2}^n k_{*,i-1}(\widetilde{M}_i)_{i-1,i-1} \\ & + [(-1)^n \cdot \mathcal{O}(\varepsilon\mu)], \end{aligned}$$

with $(\widetilde{M}_i)_{i-1,i-1}$ is a principal minor of \widetilde{M}_i and the term in square brackets represents

$$\det \begin{pmatrix} -\sum_{i=2}^n k_{i1} - \varepsilon\mu & k_{12} & \dots & k_{1n} \\ k_{21} & -\sum_{\substack{i=1 \\ i \neq 2}}^n k_{i2} - \varepsilon\mu & \dots & k_{2n} \\ \vdots & \vdots & \ddots & \vdots \\ k_{n1} & k_{n2} & \dots & -\sum_{i=1}^{n-1} k_{in} - \varepsilon\mu \end{pmatrix},$$

according to Lemma A.1. Moreover,

$$\text{sgn}(-k_{n+1,n}\mathcal{C}_{nn}) = \text{sgn}(-k_{*,i-1}(\widetilde{M}_i)_{i-1,i-1}) = (-1)^n,$$

for every $i = 2, \dots, n$, as a consequence of Lemma A.2. Therefore, we conclude

$$\det(M_n) = (-1)^n \cdot \mathcal{O}(k_{ij}^n).$$

□

Recall that in our model (Figure 3.1) we only suppose that there is not *flux trap*. For this reason, we analyze the determinant of the matrix associated to a system with *traps*. For instance, with the matrix M_2 defined in (A.2), if the system has a *trap*, $k_{21} = 0$ and its determinant has order $\mathcal{O}(\varepsilon\mu)$. In general, we can expect that a system with a *trap* has determinant with order $\varepsilon\mu$. This happens because a *trap* implies a block of zeros in the matrix. Indeed, remember that the j -th column of the matrix system represents the reactions whose origin is the metabolite X_j . Then, if X_j is in a *trap*, $k_{ij} = 0$ for every i with X_i out of the *trap*.

In the presence of a *trap*, the matrix of the system is reducible [41]. That is to say, after the same number of interchanges of rows than columns, the matrix of a system with a *trap* can be transformed in a square block triangular matrix (keeping the dominant diagonal structure):

$$M_n = \begin{pmatrix} M' & 0 \\ * & \mathbb{T} \end{pmatrix}, \quad (\text{A.5})$$

where M' and \mathbb{T} are square matrices that correspond to the metabolites which are not in a *trap* and the metabolites which are in a *trap*, respectively. If \mathcal{C}_{ij} is a cofactor of M_n and $\det(\mathbb{T})$ has order $\varepsilon\mu$, then the coefficients

$$\frac{k_{21}\mathcal{C}_{1i}}{\det(M') \cdot \det(\mathbb{T})}$$

can be affected by a factor of order $(\varepsilon\mu)^{-1}$ (see the proof of Magnitude of Concentration Theorem 3.1).

However, it is possible to see that when there is a *trap* which is not reached by the flux, then the determinant of the block corresponding to the *trap* is also a factor of the cofactors \mathcal{C}_{1i} , where X_i is not in that trap. This is

why we distinguish a *flux trap* of a simple *trap* (see Definitions 3.3 and 3.4), by determining if the input of interest reaches them or not.

A matrix with a *trap* that is not a *flux trap* has the following form:

$$M_n = \begin{pmatrix} M'_{r \times r} & [C_1]_{r \times s} & 0_{r \times p} \\ 0_{s \times r} & [C_2]_{s \times s} & 0_{s \times p} \\ 0_{p \times r} & [C_3]_{p \times s} & \mathbb{T}_{p \times p} \end{pmatrix}, \quad r + s + p = n, \quad (\text{A.6})$$

where \mathbb{T} represents the *trap* not reached by a specific flux, $[C_i]$ represents columns of metabolites that connect the *trap* with the rest of the system, but which are not nourished by the flux not even by the *trap*. M' is again the block corresponding to the rest of metabolites, including those with an input or an output.

Since the matrix in (A.6) is square block triangular, its determinant is the product of the determinants of the diagonal blocks [10]. Then, this determinant has a factor $\det(\mathbb{T}) = (-1)^p \cdot \mathcal{O}(\varepsilon\mu)$, as claimed by Lemma A.1.

Furthermore, the cofactors \mathcal{C}_{1j} have the factor $\det(\mathbb{T})$ for every $j = 1, \dots, r$, because the sub matrix $(M_n)_{1n}$ is also square block triangular. Then, when dividing by the determinant of M_n , this factor is neutralized. In this way we rule out having a large coefficient of order $(\varepsilon\mu)^{-1}$ for estimating the concentration of X_i , $1 \leq i \leq r$, for every metabolite that is not in a *trap*. Whereas the minors $M_{1j} = 0$ for $j > r$, as a consequence of the block of zeros in the lower left corner. In fact, the metabolites not reached by the flux are not related to this either in QSS. Therefore we have proved the following proposition.

PROPOSITION A.2. *Consider the matrix M_n in its triangular form (A.5), such that the square block \mathbb{T} corresponds to metabolites in a trap and M' to metabolites not in the trap. Then*

$$\det(M_n) = \det(M') \cdot \det(\mathbb{T}),$$

Moreover, if the trap is not reached by a specific flux, then M_n has the form (A.6) and its minors satisfy

$$(M_n)_{1j} = (M')_{1j} \cdot \det(C_2) \cdot \det(\mathbb{T}) \quad \forall j = 1, \dots, r,$$

with $(M')_{1j}$ a minor of M' , and

$$(M_n)_{1j} = 0 \quad \forall j = r + 1, \dots, n.$$

COROLLARY A.1. *If M_n has a trap, then*

$$\det(M_n) = \pm \mathcal{O}(\varepsilon\mu).$$

Proof. The square block \mathbb{T} is equal to a singular matrix minus $\varepsilon\mu \cdot I$. Then, by Lemma A.1, its determinant has order $\pm \mathcal{O}(\varepsilon\mu)$. \square

Now we consider some minors of the matrix M_n and we study the order of their determinants, as required for the proof of Magnitude of Concentration Theorem 3.1.

PROPOSITION A.3. *Let us suppose that M_n represents a system with no traps. Moreover, assume a flux from X_1 to X_n . Consider the minor of M_n resulting from removing the first line and the n -th column:*

$$(M_n)_{1n} = \det \begin{pmatrix} k_{21} & -(\sum_{\substack{i=1 \\ i \neq 2}}^n k_{i2} + \varepsilon\mu) & \dots & k_{2,n-1} \\ k_{31} & k_{32} & \dots & k_{3,n-1} \\ \vdots & \vdots & \ddots & \vdots \\ k_{n-1,1} & k_{n-1,2} & \dots & -(\sum_{\substack{i=1 \\ i \neq n-1}}^n k_{i,n-1} + \varepsilon\mu) \\ k_{n1} & k_{n2} & \dots & k_{n,n-1} \end{pmatrix} \quad (\text{A.7})$$

Then

$$0 < (M_n)_{1n} = \mathcal{O}(k_{ij}^{n-1}).$$

Proof. The demonstration is by induction over the squared matrix size. For the case of a minor with dimension two we have:

$$\begin{aligned} \det \begin{pmatrix} k_{21} & -(\sum_{\substack{i=1 \\ i \neq 2}}^3 k_{i2} + \varepsilon\mu) \\ k_{31} & k_{32} \end{pmatrix} &= k_{21}k_{32} + k_{31} \left(\sum_{\substack{i=1 \\ i \neq 2}}^3 k_{i2} + \varepsilon\mu \right) \\ &= \mathcal{O}(k_{ij}^2), \end{aligned}$$

since there is a flux from X_1 to X_3 and no *traps*. We then suppose the validity of this lemma for a minor of dimension up to $n - 2$ (*induction hypothesis*).

If we develop the determinant $(M_n)_{1n}$ by the first column, we verify that the minor resulting from striking the first column and the x -th row of the matrix in (A.7) satisfies the hypothesis of this lemma after $x - 1$ changes of columns, for $x = 1, 2, \dots, n - 2$. Hence we apply the *induction hypothesis* to these minors and we obtain that they are quantities $(-1)^{x-1} \cdot \mathcal{O}(k_{ij}^{n-2})$, where x is the number of the struck row.

Since there is no *traps* by hypothesis, the minor obtained after omitting the first column and the last row of the matrix in (A.7) has a column which is strictly diagonal dominant. We can then apply Proposition A.1 and conclude that it has order $(-1)^{n-2} \cdot \mathcal{O}(k_{ij}^{n-2})$.

Therefore, we conclude that the minor $(M_n)_{1n}$ is the sum of positive quantities of order $\mathcal{O}(k_{ij}^{n-1})$:

$$\begin{aligned} 0 < (M_n)_{1n} &= k_{21} \cdot \mathcal{O}(k_{ij}^{n-2}) + \dots \\ &\quad + (-1)^{x+1} (-1)^{x-1} k_{x1} \cdot \mathcal{O}(k_{ij}^{n-2}) + \dots \\ &\quad + (-1)^n (-1)^{n-2} k_{n1} \cdot \mathcal{O}(k_{ij}^{n-2}) \\ &= \mathcal{O}(k_{ij}^{n-1}). \end{aligned}$$

□

For the other minors we obtain a similar result. Indeed, every minor obtained from striking the first row and the x -th column can be transformed in a matrix of the form $(M_n)_{1n}$, by $n - x$ changes of rows. Therefore, the following assertion holds.

COROLLARY A.2. *When M_n has no traps, the minor $(M_n)_{1x}$ has order $(-1)^{n-x} \cdot \mathcal{O}(k_{ij}^{n-1})$, for every $x = 1, \dots, n$.*

A.2. Generalization of the QSSR: Linear Case

In this Appendix, we generalize the Quasi Steady State Reduction obtained in Section 3.2 (see Property 3.3). The following reduction is valid for any linear system of metabolic reactions, with any number of subnetworks of fast reactions and all possible reactions between metabolites. Also, the generic network can include a finite number of continuous inputs, entering at any metabolite.

A.2.1. Generic Linear System with two time-scales. As the main manuscript, we consider linear system with metabolites consumed by slow or fast reactions. Without loss of generality, we suppose that metabolites X_1, X_2, \dots, X_m are only consumed by slow reactions and that metabolites $X_{m+1}, X_{m+2}, \dots, X_n$ can be consumed by a fast reaction. Then, we consider the following system of equations:

$$\frac{dX_i}{dt} = F_i(t, X_1, \dots, X_n, \varepsilon) \quad X_i(0) = x_i^0, \quad (\text{A.8})$$

where

$$F_i := I_i(t) + \sum_{\substack{j=1 \\ j \neq i}}^m k_{ij} \cdot X_j + \sum_{j=m+1}^n \frac{k_{ij}}{\varepsilon} X_j - k_i \cdot X_i$$

for $i = 1, \dots, m$, where

$$k_i := \sum_{\substack{j=1 \\ j \neq i}}^n k_{ji} + \mu,$$

and

$$F_i := I_i(t) + \sum_{j=1}^m k_{ij} \cdot X_j + \sum_{\substack{j=m+1 \\ j \neq i}}^n \frac{k_{ij}}{\varepsilon} X_j - \frac{k_i}{\varepsilon} \cdot X_i$$

for $i = m + 1, \dots, n$, where

$$\frac{k_i}{\varepsilon} := \sum_{\substack{j=1 \\ j \neq i}}^n \frac{k_{ji}}{\varepsilon} + \mu.$$

Without loss of generality, we suppose $I_i(t) \geq 0$.

A.2.2. Canonical Form of Singularly Perturbed Systems. Equation (A.8) is a slow-fast system, where the variables X_1, X_2, \dots, X_m are in the slow part and $X_{m+1}, X_{m+2}, \dots, X_n$ are in the fast part. Indeed, making the change of variable

$$Y_i = \frac{X_i}{\varepsilon}$$

for the fast variables, we obtain

$$\frac{dX_i}{dt} = G_i(t, X_1, \dots, X_m, Y_{m+1}, \dots, Y_n, \varepsilon) \quad X_i(0) = x_i^0, \quad (\text{A.9})$$

$$\varepsilon \frac{dY_i}{dt} = G_i(t, X_1, \dots, X_m, Y_{m+1}, \dots, Y_n, \varepsilon) \quad Y_i(0) = y_i^0, \quad (\text{A.10})$$

where $y_i^0 := x_i^0/\varepsilon$,

$$G_i := I_i(t) + \sum_{\substack{j=1 \\ j \neq i}}^m k_{ij} \cdot X_j + \sum_{j=m+1}^n k_{ij} Y_j - k_i \cdot X_i$$

for $i = 1, \dots, m$, and

$$G_i := I_i(t) + \sum_{j=1}^m k_{ij} \cdot X_j + \sum_{\substack{j=m+1 \\ j \neq i}}^n k_{ij} Y_j - k_i \cdot Y_i$$

for $i = m+1, \dots, n$.

Equation (A.10) evaluated in $\varepsilon = 0$ is equivalent to algebraic equation

$$0 = G_i(t, X_1, \dots, X_m, Y_{m+1}, \dots, Y_n, 0) \quad \forall i = m+1, \dots, n,$$

which can be rewritten in the matrix form

$$\begin{aligned} & \begin{pmatrix} -k_{m+1} & k_{m+1,m+2} & \dots & k_{m+1,n} \\ k_{m+2,m+1} & -k_{m+2} & \dots & k_{m+2,n} \\ \vdots & \vdots & & \vdots \\ k_{n,m+1} & k_{n,m+2} & \dots & -k_n \end{pmatrix} \cdot \begin{pmatrix} Y_{m+1} \\ Y_{m+2} \\ \vdots \\ Y_n \end{pmatrix} \\ & = - \begin{pmatrix} I_{m+1}(t) + \sum_{j=1}^m k_{m+1,j} X_j \\ I_{m+2}(t) + \sum_{j=1}^m k_{m+2,j} X_j \\ \vdots \\ I_n(t) + \sum_{j=1}^m k_{n,j} X_j \end{pmatrix}. \end{aligned}$$

Notice that the matrix above is stable, since it is strictly column diagonally dominant [32]. Define this matrix as

$$K := \begin{pmatrix} -k_{m+1} & k_{m+1,m+2} & \dots & k_{m+1,n} \\ k_{m+2,m+1} & -k_{m+2} & \dots & k_{m+2,n} \\ \vdots & \vdots & & \vdots \\ k_{n,m+1} & k_{n,m+2} & \dots & -k_n \end{pmatrix}.$$

Then the solution to the algebraic problem is

$$\begin{pmatrix} Y_{m+1} \\ Y_{m+2} \\ \vdots \\ Y_n \end{pmatrix} = K^{-1} \cdot \begin{pmatrix} -\left(I_{m+1}(t) + k_{m+1,j} \cdot X_j\right) \\ -\left(I_{m+2}(t) + k_{m+2,j} \cdot X_j\right) \\ \vdots \\ -\left(I_n(t) + k_{n,j} \cdot X_j\right) \end{pmatrix}.$$

The entries of K^{-1} are

$$\mathbf{b}_{ij} = \frac{\mathcal{C}_{ji}}{\det K},$$

where \mathcal{C}_{ji} a cofactor of K . Thus, we write every Y_i as a linear combination of the kinetics in the slow-part and the inputs on the fast part (if they exist):

$$\frac{X_i}{\varepsilon} = Y_i = \sum_{j'=m+1}^n |\mathbf{b}_{ij'}| \cdot \left(I_{j'}(t) + \sum_{j=1}^m k_{j',j} \cdot X_j \right).$$

It follows

$$X_i = \varepsilon \cdot \left[\sum_{j'=m+1}^n |\mathbf{b}_{ij'}| \cdot \left(I_{j'}(t) + \sum_{j=1}^m k_{j',j} \cdot X_j \right) \right],$$

for $i = m + 1, \dots, n$.

A.2.3. Reduction of the Slow-Fast Linear System. To deduce the reduced system after Tikhonov's Theorem, define

$$\begin{aligned} \bar{G}_i(t, \bar{X}_1, \dots, \bar{X}_m, 0) &:= G_i(t, \bar{X}_1, \dots, \bar{X}_m, \bar{Y}_{m+1}, \dots, \bar{Y}_n, 0) = \\ I_i(t) + \sum_{\substack{j=1 \\ j \neq i}}^m k_{ij} \cdot X_j + \sum_{j=m+1}^n k_{ij} \left[\sum_{j'=m+1}^n |\mathbf{b}_{jj'}| \cdot \left(I_{j'}(t) + \sum_{i'=1}^m k_{j',i'} \cdot X_{i'} \right) \right] - k_i \cdot X_i. \end{aligned}$$

Thus, the QSSR is

$$\frac{d\bar{X}_i}{dt} = \bar{G}_i(t, \bar{X}_1, \dots, \bar{X}_m, 0) \quad \bar{X}_i(0) = x_i^0, \quad (\text{A.11})$$

for all $i = 1, \dots, m$. For the variables in QSS,

$$\bar{X}_i = \varepsilon \cdot \left[\sum_{j'=m+1}^n |\mathbf{b}_{ij'}| \cdot \left(I_{j'}(t) + \sum_{j=1}^m k_{j',j} \cdot X_j \right) \right], \quad (\text{A.12})$$

for all $i = m + 1, \dots, n$.

The following proposition is a consequence of Tikhonov's Theorem [39].

PROPOSITION A.4 (Tikhonov's Theorem). *Let (X_i) be the solution of (A.8) and (\bar{X}_i) defined by (A.11)-(A.12). If there is a solution for (A.11), then*

$$X_i(t) = \bar{X}_i(t) + \mathcal{O}(\varepsilon) \quad \forall i = 1, \dots, m, t \in [0, T_1],$$

and there exists $0 \leq T_0$ such that

$$X_i(t) = \bar{X}_i(t) + \varepsilon \cdot [\mathcal{O}(\varepsilon)] \quad \forall i = m+1, \dots, n, t \in [T_0, T_1].$$

Now we see that $|\mathbf{b}_{ij'}| = 0$ if there is not a flux from $X_{j'}$ to X_i .

PROPOSITION A.5. *Let K be the Jacobian matrix of the fast eq. (A.10) and consider metabolite $X_{j'}$. With out loss of generality suppose $1 \leq j' \leq r$ and rewrite this matrix as*

$$K = \begin{pmatrix} [K']_{r \times r} & [C_1]_{r \times s} \\ 0_{s \times r} & [C_2]_{s \times s} \end{pmatrix}, \quad r + s = n - m, \quad (\text{A.13})$$

where K' is the matrix representing the metabolites with a flux from $X_{j'}$ (including $X_{j'}$) and C_2 is the square block corresponding to metabolites not reached by any flux from $X_{j'}$. Then,

$$\det(K) = \det(K') \cdot \det(C_2).$$

Furthermore, its minors satisfy

$$(K)_{j'i} = (K')_{j'i} \cdot \det(C_2) \quad \forall i = 1, \dots, r,$$

with $(K')_{j'i}$ a minor of K' , and

$$(K)_{j'i} = 0 \quad \forall i = r+1, \dots, r+s.$$

Proof. Since K defined in (A.13) is a square block triangular matrix, its determinant is the product of the determinants of the diagonal blocks [10]. Then,

$$\det(K) = \det(K') \cdot \det(C_2).$$

For $i = 1, \dots, r$, the submatrix obtained from deleting the j' -th row and the i -th column of K is also a square block triangular matrix. Then, its determinant is

$$(K)_{j'i} = \begin{vmatrix} (K')_{j'i} & [C'_1] \\ 0 & [C_2] \end{vmatrix} = (K')_{j'i} \cdot \det(C_2) \quad \forall i = 1, \dots, r,$$

where $(K')_{j'i}$ is a minor of K' and $[C'_1]$ is the matrix C_1 without its first row. On the other hand, for $i = r+1, \dots, (r+s)$, the minor $(K)_{j'i}$ is also the determinant of a square block triangular matrix, i.e.

$$(K)_{j'i} = \begin{pmatrix} K' & C_1 \\ 0 & C_2 \end{pmatrix}_{j'i} = 0 \quad \forall i = r+1, \dots, r+s,$$

as a consequence of the block of zeros below K' . We conclude

$$(K)_{j'i} = 0 \quad \forall i = r+1, \dots, (r+s).$$

□

Proposition A.5 states that the only elements in the sum

$$\sum_{j'=m+1}^n |\mathbf{b}_{ij'}| \cdot \left(I_{j'}(t) + \sum_{j=1}^m k_{j',j} \cdot X_j \right)$$

which are different from zero are those with index j' such that there is a flux from $X_{j'}$ to X_i (i.e. when $|\mathbf{b}_{ij'}| \neq 0$). Furthermore, notice that in

$$\sum_{j=1}^m k_{j',j} \cdot X_j$$

the parameter $k_{j',j} \neq 0$ if and only if there is a reaction consuming X_j and producing $X_{j'}$, where X_j is in the slow part and $X_{j'}$ is in the fast part of the system.

A.3. Boundary layer correction for the toy model

In this section we illustrate the effects of the boundary layer correction, applied to the toy Network N1 of Section 3.5 as described in Section 3.2.6.

According to Tikhonov's Theorem [39], the solution of the original system (3.1) for the toy Network N1 (in Figure 3.5) can be approximated with an error of order $\mathcal{O}(\varepsilon)$. Indeed, in this work we prove that this approximation is valid in any time interval $[0, T]$, where the input $I(t)$ is nonnegative and continuous, if we consider the boundary layer correction \hat{X} .

The details about the boundary layer system (3.9) are in Section 3.2.6, and the approximation

$$X_i(t) = \bar{X}_i(t) + \hat{X}_i\left(\frac{t}{\varepsilon}\right) + \mathcal{O}(\varepsilon) \quad \forall t \in [0, T], \quad (\text{A.14})$$

for every $i = 2, \dots, 8, i \neq 4$, is stated in Property 3.3 at Section 3.2.7.

The boundary layer correction for the toy Network N1 of Section 3.5 is illustrated in the following Figure A.2 and Figure A.3.

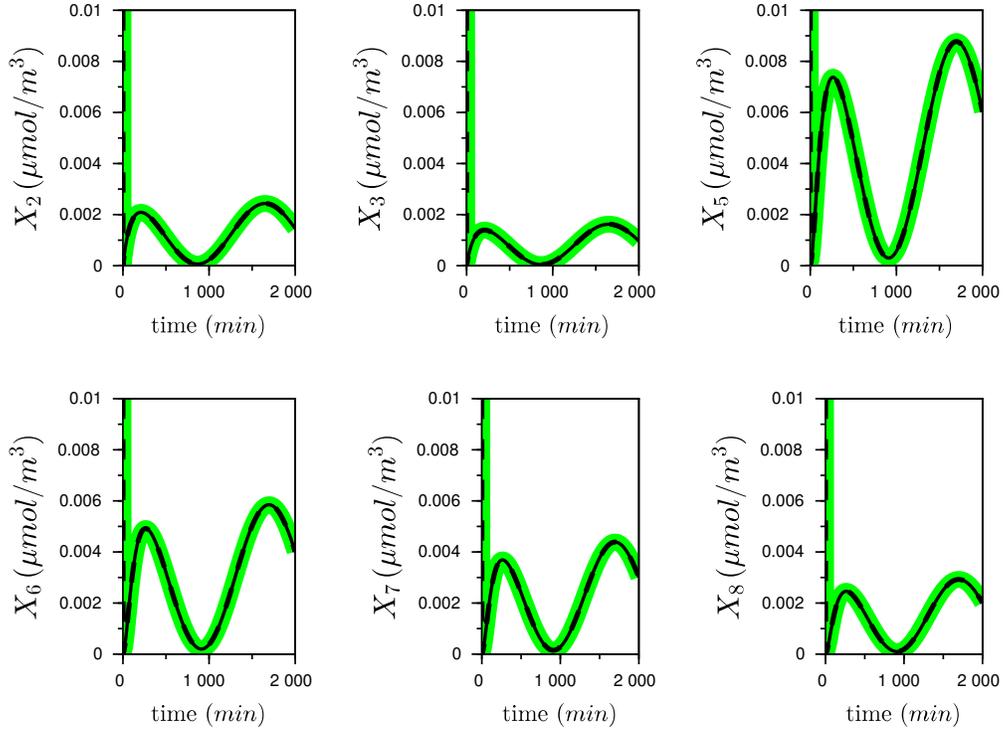


Figure A.2. Boundary layer correction applied to the toy Network N1. The boundary layer term is only added to the approximations of the metabolites in QSS. Therefore, dynamics of the slow reduced system (3.16) are the same as in Figure 3.5. Thick light line: original system (3.1); solid line: approximation obtained after the approach proposed in this work (3.15); dashed line: approximation with the boundary layer correction (A.14). The parameters considered are in Table 3.1.

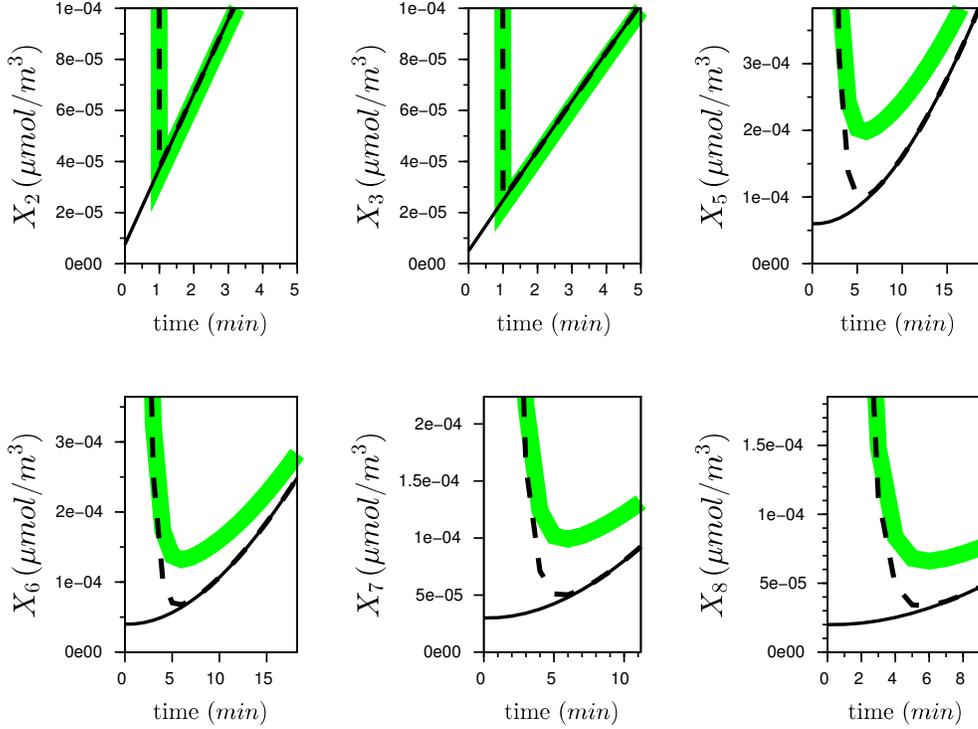


Figure A.3. Zoom on the initial fast transient of the simulations in Figure A.2. Notice that the approximation without boundary layer correction (solid line) does not have the same initial condition as the original system (thick light line), while the system with boundary layer correction (dashed line) accurately approximates the original system in the first fast transient interval.

A.4. Classical approach for the stoichiometric modeling

A.4.1. The DRUM methodology: recall and application. In this section we give the details for model reduction using DRUM, presented in Section 3.5.3, is obtained. In the DRUM approach an additional reaction for biomass synthesis is required. Here, to apply the Drum approach in a simplified framework, we have assumed that we were studying a situation where Biomass was at a constant concentration.

The formulation for the stoichiometric analysis of a metabolic system with DRUM [4] considers the original system (3.1) for Network N1 as

$$\frac{dX}{dt} = \bar{I}(t) + K \cdot V(X) \quad X(0) = \bar{x}_0, \quad (\text{A.15})$$

where X is the column vector for intracellular metabolites with entries X_1, \dots, X_9 , the initial condition $\bar{x}_0 = (x_1^0, \dots, x_9^0)^T$, the input

$$\bar{I}(t) = (k[\cos(t \cdot \omega) + 1], 0, \dots, 0)^T,$$

K is the stoichiometric matrix and $V(X)$ the vector of kinetics reactions. To be more precise,

$$K = \begin{matrix} & \nu_{21} & \nu_{32} & \nu_{43} & \nu_{54} & \nu_{56} & \nu_{75} & \nu_{68} & \nu_{87} & \nu_{98} \\ \begin{matrix} X_1 \\ X_2 \\ X_3 \\ X_4 \\ X_5 \\ X_6 \\ X_7 \\ X_8 \\ X_9 \end{matrix} & \begin{pmatrix} -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & -1 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & -1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & -1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \end{pmatrix} \end{matrix},$$

$$V(X) = \begin{pmatrix} \nu_{21} \\ \nu_{32} \\ \nu_{43} \\ \nu_{54} \\ \nu_{56} \\ \nu_{75} \\ \nu_{68} \\ \nu_{87} \\ \nu_{98} \end{pmatrix} = \begin{pmatrix} k_{21} X_1 \\ \frac{k_{32}}{\varepsilon} X_2 - \frac{k_{23}}{\varepsilon} X_3 \\ \frac{k_{43}}{\varepsilon} X_3 \\ k_{54} X_4 \\ \frac{k_{56}}{\varepsilon} X_6 \\ \frac{k_{75}}{\varepsilon} X_5 \\ \frac{k_{68}}{\varepsilon} X_8 \\ \frac{k_{87}}{\varepsilon} X_7 \\ \frac{k_{98}}{\varepsilon} X_8 \end{pmatrix}.$$

Moreover, in this approach the factor μ is neglected. For the stoichiometric analysis it is then considered the following system

$$\frac{dX}{dt} = K \cdot V(X) \quad X(0) = \bar{x}_0,$$

and the input vector $\bar{I}(t)$ is added later.

In line with the DRUM methodology [4], we consider that metabolites X_2, X_3, X_5, X_6, X_7 and X_8 are in QSS, while metabolites X_1, X_4 and X_9 can accumulate and have dynamics. This leads to the division of the metabolism in two subnetworks SN1 and SN2, as shown in Figure A.4.

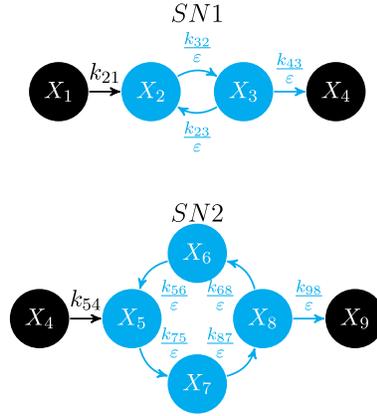


Figure A.4. Subnetworks SN1 (above) and SN2 (below) of N1. The internal metabolites X_2, X_3, X_5, X_6, X_7 and X_8 are assumed to be in Quasi Steady State. Metabolites X_1, X_4 and X_9 are supposed to be external to the subsystems in QSS.

The next step in the DRUM methodology is to summarize each subnetwork using its EFM. We compute the EFM of subnetworks SN1 and SN2 by Gauss elimination and corroborate the result with Copasi [28]. We have that the only elementary flux mode of SN1 is

$$e_1^T = \begin{pmatrix} k_{21} & \frac{k_{32}}{\epsilon} & \frac{k_{43}}{\epsilon} \\ 1 & 1 & 1 \end{pmatrix},$$

and its matrix of EFM is $E_1 := (e_1)$. Analogously, the EFM of SN2 and its matrix of EFM are, respectively

$$e_2^T = \begin{pmatrix} k_{54} & \frac{k_{56}}{\epsilon} & \frac{k_{75}}{\epsilon} & \frac{k_{68}}{\epsilon} & \frac{k_{87}}{\epsilon} & \frac{k_{98}}{\epsilon} \\ 1 & 0 & 1 & 0 & 1 & 1 \end{pmatrix},$$

$$e_3^T = \begin{pmatrix} k_{54} & \frac{k_{56}}{\epsilon} & \frac{k_{75}}{\epsilon} & \frac{k_{68}}{\epsilon} & \frac{k_{87}}{\epsilon} & \frac{k_{98}}{\epsilon} \\ 0 & 1 & 1 & 1 & 1 & 0 \end{pmatrix},$$

$$E_2 := (e_2).$$

Similar to e_1 , the mode e_2 represents an elementary flux that begins and finishes at the exterior of the system in QSS. In contrast, e_3 is a cycle in QSS, which can be omitted.

Under the QSSA, a simple algebraic computation from the EFM of the two subnetworks leads to the dynamics of the main network:

$$K \cdot V(X) = (K_1 \cdot E_1 \quad K_2 \cdot E_2) \cdot \beta, \quad (\text{A.16})$$

where K_i is the sub matrix that consists in the column vectors of K corresponding to the reactions in SNi , for $i = 1, 2$, and $\beta = (\beta_1 \quad \beta_2)^T$ is a vector

with entries satisfying

$$E_1 \cdot (\beta_1) = \begin{pmatrix} \nu_{21} \\ \nu_{32} \\ \nu_{43} \end{pmatrix}, \quad E_2 \cdot (\beta_2) = \begin{pmatrix} \nu_{54} \\ \nu_{56} \\ \nu_{75} \\ \nu_{68} \\ \nu_{87} \\ \nu_{98} \end{pmatrix},$$

where ν_{ij} is the entry of $V(X)$ corresponding to the reaction with rate k_{ij} or k_{ij}/ε , respectively. Then, we compute

$$K_1 \cdot (e_1) = \begin{matrix} & \nu_{21} & \nu_{32} & \nu_{43} \\ X_1 & -1 & 0 & 0 \\ X_2 & 1 & -1 & 0 \\ X_3 & 0 & 1 & -1 \\ X_4 & 0 & 0 & 1 \\ X_5 & 0 & 0 & 0 \\ X_6 & 0 & 0 & 0 \\ X_7 & 0 & 0 & 0 \\ X_8 & 0 & 0 & 0 \\ X_9 & 0 & 0 & 0 \end{matrix} \cdot \begin{pmatrix} 1 \\ 1 \\ 1 \end{pmatrix} = \begin{pmatrix} -1 \\ 0 \\ 0 \\ 1 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{pmatrix}$$

$$K_2 \cdot (e_2) = \begin{matrix} & \nu_{54} & \nu_{56} & \nu_{75} & \nu_{68} & \nu_{87} & \nu_{98} \\ X_1 & 0 & 0 & 0 & 0 & 0 & 0 \\ X_2 & 0 & 0 & 0 & 0 & 0 & 0 \\ X_3 & 0 & 0 & 0 & 0 & 0 & 0 \\ X_4 & -1 & 0 & 0 & 0 & 0 & 0 \\ X_5 & 1 & 1 & -1 & 0 & 0 & 0 \\ X_6 & 0 & -1 & 0 & 1 & 0 & 0 \\ X_7 & 0 & 0 & 1 & 0 & -1 & 0 \\ X_8 & 0 & 0 & 0 & -1 & 1 & -1 \\ X_9 & 0 & 0 & 0 & 0 & 0 & 1 \end{matrix} \cdot \begin{pmatrix} 1 \\ 0 \\ 1 \\ 0 \\ 1 \\ 1 \\ 1 \end{pmatrix} = \begin{pmatrix} 0 \\ 0 \\ 0 \\ -1 \\ 0 \\ 0 \\ 0 \\ 0 \\ 1 \end{pmatrix}$$

Hence, according to Equation (A.16),

$$K \cdot V(X) = \begin{pmatrix} -1 & 0 \\ 0 & 0 \\ 0 & 0 \\ 1 & -1 \\ 0 & 0 \\ 0 & 0 \\ 0 & 0 \\ 0 & 0 \\ 0 & 1 \end{pmatrix} \cdot \begin{pmatrix} \beta_1 \\ \beta_2 \end{pmatrix} = \begin{pmatrix} -\beta_1 \\ 0 \\ 0 \\ \beta_1 - \beta_2 \\ 0 \\ 0 \\ 0 \\ 0 \\ \beta_2 \end{pmatrix}$$

Substituting in Equation (A.15), we obtain

$$\frac{dX}{dt} = \bar{I}(t) + \begin{pmatrix} -\beta_1 \\ 0 \\ 0 \\ \beta_1 - \beta_2 \\ 0 \\ 0 \\ 0 \\ 0 \\ \beta_2 \end{pmatrix} \quad X(0) = \bar{x}_0.$$

Then, the DRUM approach does not take into account the equations for the metabolites in QSS and deduces the following reduced system for the

accumulative metabolites

$$\begin{aligned} \frac{dX_1}{dt} &= I(t) - \beta_1 & X_1(0) &= x_1^0 \\ \frac{dX_4}{dt} &= \beta_1 - \beta_2 & X_4(0) &= x_4^0 \\ \frac{dX_9}{dt} &= \beta_2 & X_9(0) &= x_9^0. \end{aligned} \quad (\text{A.17})$$

A crucial step in this method is to choose the entries β_1 and β_2 . This choice is arbitrary according to the DRUM method [4], but it determines the accuracy of the reduced system. Indeed, we have that β_1 has to satisfy

$$\begin{pmatrix} 1 \\ 1 \\ 1 \end{pmatrix} \cdot (\beta_1) = \begin{pmatrix} \beta_1 \\ \beta_1 \\ \beta_1 \end{pmatrix} = \begin{pmatrix} \nu_{21} \\ \nu_{32} \\ \nu_{43} \end{pmatrix} \Rightarrow \begin{aligned} \beta_1 &= \nu_{21} = k_{21}X_1 \\ \beta_1 &= \nu_{32} = \frac{k_{32}}{\varepsilon}X_2 - \frac{k_{23}}{\varepsilon}X_3 \\ \beta_1 &= \nu_{43} = \frac{k_{43}}{\varepsilon}X_3. \end{aligned}$$

Similarly, for β_2 we have

$$\begin{pmatrix} 1 \\ 0 \\ 1 \\ 0 \\ 1 \\ 1 \\ 1 \end{pmatrix} \cdot (\beta_2) = \begin{pmatrix} \beta_2 \\ 0 \\ \beta_2 \\ 0 \\ \beta_2 \\ \beta_2 \\ \beta_2 \end{pmatrix} = \begin{pmatrix} \nu_{54} \\ \nu_{56} \\ \nu_{75} \\ \nu_{68} \\ \nu_{87} \\ \nu_{98} \end{pmatrix} \Rightarrow \begin{aligned} \beta_2 &= \nu_{54} = k_{54}X_4 \\ \beta_2 &= \nu_{75} = \frac{k_{75}}{\varepsilon}X_5 \\ \beta_2 &= \nu_{87} = \frac{k_{87}}{\varepsilon}X_7 \\ \beta_2 &= \nu_{98} = \frac{k_{98}}{\varepsilon}X_8 \end{aligned}$$

From the equations above, we have to choose only one definition for β_1 and one for β_2 . The DRUM method [4] does not establish any technique for selecting these elements and the choice is left to the person who applies the method.

In this small example, it is straightforward to make the right choice for β_1 and β_2 . As matter of fact, looking at the reduced model (3.16) obtained in Section 3.5 after Tikhonov's Theorem, we can deduce that choosing

$$\begin{aligned} \beta_1 &:= \nu_{21} = k_{21}X_1, \\ \beta_2 &:= \nu_{54} = k_{54}X_4, \end{aligned} \quad (\text{A.18})$$

leads to an accurate approximation. Besides, when the terms of the metabolites in QSS appears in the reduced system after de DRUM approach, these are considered (and calibrated) as constant parameters. Therefore, the other options for β_1 and β_2 does not result in correct approximations.

Finally, substituting (A.18) in Equation (A.17), we obtain the reduced model after the DRUM approach:

$$\begin{aligned} \frac{dX_1}{dt} &= I(t) - k_{21}X_1 & X_1(0) &= x_1^0 \\ \frac{dX_4}{dt} &= k_{21}X_1 - k_{54}X_4 & X_4(0) &= x_4^0 \\ \frac{dX_9}{dt} &= k_{54}X_4 & X_9(0) &= x_9^0. \end{aligned}$$

A.4.2. Flux Balance Analysis. In this section, we applied Flux Balance Analysis to the toy model presented in Section 3.5. Because of the periodic forcing and the strong accumulation, this approach turns out to be very inaccurate.

The principal hypothesis of FBA is that all the internal metabolites of a system reach a steady state, under any external conditions [34, 57]. For our example, we consider all the metabolites X_1, \dots, X_9 as internal and we suppose that there is an input from the exterior $I(t)$.

For Flux Balance Analysis, the system of equations for the toy Network N1 of Section 3.5 is

$$\frac{dX}{dt} = N \cdot V(X), \quad (\text{A.19})$$

where X is the vector of metabolite concentrations, N is the stoichiometric matrix and $V(X)$ is the vector of kinetics reactions.

We recall that FBA methods omit the dilution due to growth. Then, the factor $\mu \cdot X$ does not appear on Equation (A.19) and we have

$$N = \begin{matrix} & I(t) & \nu_{21} & \nu_{32} & \nu_{43} & \nu_{54} & \nu_{56} & \nu_{75} & \nu_{68} & \nu_{87} & \nu_{98} \\ \begin{matrix} X_1 \\ X_2 \\ X_3 \\ X_4 \\ X_5 \\ X_6 \\ X_7 \\ X_8 \\ X_9 \end{matrix} & \begin{pmatrix} 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & -1 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & -1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 \end{pmatrix} \end{matrix},$$

$$V(X) = \begin{pmatrix} I(t) \\ \nu_{21} \\ \nu_{32} \\ \nu_{43} \\ \nu_{54} \\ \nu_{56} \\ \nu_{75} \\ \nu_{68} \\ \nu_{87} \\ \nu_{98} \end{pmatrix} = \begin{pmatrix} k[\cos(t \cdot \omega) + 1] \\ k_{21}X_1 \\ \frac{k_{32}}{\varepsilon}X_2 - \frac{k_{23}}{\varepsilon}X_3 \\ \frac{k_{43}}{\varepsilon}X_3 \\ k_{54}X_4 \\ \frac{k_{56}}{\varepsilon}X_6 \\ \frac{k_{75}}{\varepsilon}X_5 \\ \frac{k_{68}}{\varepsilon}X_8 \\ \frac{k_{87}}{\varepsilon}X_7 \\ \frac{k_{98}}{\varepsilon}X_8 \end{pmatrix}.$$

The purpose of FBA is to resolve the algebraic system

$$N \cdot V(X) = 0$$

for the variables X_1, \dots, X_8 . In our example, the algebraic system is linear and we can compute the solution as follows:

$$\begin{aligned} X_1(t) &= \frac{k[\cos(t \cdot \omega) + 1]}{k_{21}} & (A.20) \\ X_2(t) &= \varepsilon \cdot \frac{k_{21} \cdot (k_{23} + k_{43})}{d'_1} X_1(t) \\ X_3(t) &= \varepsilon \cdot \frac{k_{21} \cdot k_{32}}{d'_1} X_1(t) \\ X_4(t) &= \frac{k_{43}}{\varepsilon k_{54}} X_3(t) \\ X_5(t) &= \varepsilon \cdot \frac{k_{54} \cdot k_{56} \cdot k_{87} \cdot (k_{68} + k_{98})}{d'_2} X_4(t) \\ X_6(t) &= \varepsilon \cdot \frac{k_{54} \cdot k_{68} \cdot k_{75} \cdot k_{87}}{d'_2} X_4(t), \\ X_7(t) &= \varepsilon \cdot \frac{k_{54} \cdot k_{75} \cdot k_{56} \cdot (k_{78} + k_{98})}{d'_2} X_4(t), \\ X_8(t) &= \varepsilon \cdot \frac{k_{54} \cdot k_{75} \cdot k_{87} \cdot k_{56}}{d'_2} X_4(t), \end{aligned}$$

where $d'_1 = (k_{23} + k_{43}) \cdot k_{32} - k_{23} \cdot k_{32} = k_{43} \cdot k_{32}$ and $d'_2 = k_{75} \cdot k_{56} \cdot k_{87} \cdot (k_{68} + k_{98}) - k_{56} \cdot k_{68} \cdot k_{75} \cdot k_{87} = k_{75} \cdot k_{56} \cdot k_{87} \cdot k_{98}$.

A.5. Local Linearization for an Enzymatic System

In this section we take into consideration a network similar to N1 (Figure 3.3), but composed of nonlinear enzymatic reactions, whose substrates and products are the metabolites X_1, \dots, X_n . Then, we show how to locally linearized its metabolic system around a functional point. The ODE system of Michaelis-Menten enzymatic reactions for Network N2 (Figure A.5)

is written as follows:

$$\begin{aligned}
 \frac{dX_1}{dt} &= u(t) - \frac{e_{21}^0 k_{21} X_1}{X_1 + K_{21}} - \mu X_1 & X_1(0) &= x_1^0 \\
 \frac{dX_2}{dt} &= \frac{e_{21}^0 k_{21} X_1}{X_1 + K_{21}} + \frac{k_{23}}{\varepsilon} \frac{e_{23} X_3}{X_3 + K_{23}} - \frac{k_{32}}{\varepsilon} \frac{e_{32} X_2}{X_2 + K_{32}} - \mu X_2 & X_2(0) &= x_2^0 \\
 \frac{dX_3}{dt} &= \frac{k_{32}}{\varepsilon} \frac{e_{32} X_2}{X_2 + K_{32}} - \frac{k_{23}}{\varepsilon} \frac{e_{23} X_3}{X_3 + K_{23}} - \frac{k_{43}}{\varepsilon} \frac{e_{43} X_3}{X_3 + K_{43}} - \mu X_3 & X_3(0) &= x_3^0 \\
 \frac{dX_4}{dt} &= \frac{k_{43}}{\varepsilon} \frac{e_{43} X_3}{X_3 + K_{43}} - \frac{e_{54} k_{54} X_4}{X_4 + K_{54}} - \mu X_4 & X_4(0) &= x_4^0 \\
 \frac{dX_5}{dt} &= \frac{e_{54} k_{54} X_4}{X_4 + K_{54}} + \frac{k_{56}}{\varepsilon} \frac{e_{56} X_6}{X_6 + K_{56}} - \frac{k_{75}}{\varepsilon} \frac{e_{75} X_5}{X_5 + K_{75}} - \mu X_5 & X_5(0) &= x_5^0 \\
 \frac{dX_6}{dt} &= \frac{k_{68}}{\varepsilon} \frac{e_{68} X_8}{X_8 + K_{68}} - \frac{k_{56}}{\varepsilon} \frac{e_{56} X_6}{X_6 + K_{56}} - \mu X_6 & X_6(0) &= x_6^0 \\
 \frac{dX_7}{dt} &= \frac{k_{75}}{\varepsilon} \frac{e_{75} X_5}{X_5 + K_{75}} - \frac{k_{87}}{\varepsilon} \frac{e_{87} X_7}{X_7 + K_{87}} - \mu X_7 & X_7(0) &= x_7^0 \\
 \frac{dX_8}{dt} &= \frac{k_{87}}{\varepsilon} \frac{e_{87} X_7}{X_7 + K_{87}} - \frac{k_{68}}{\varepsilon} \frac{e_{68} X_8}{X_8 + K_{68}} - \frac{k_{98}}{\varepsilon} \frac{e_{98} X_8}{X_8 + K_{98}} - \mu X_8 & X_8(0) &= x_8^0 \\
 \frac{dX_9}{dt} &= \frac{k_{98}}{\varepsilon} \frac{e_{98} X_8}{X_8 + K_{98}} - \mu X_9 & X_9(0) &= x_9^0,
 \end{aligned}
 \tag{A.21}$$

where the input $u(t) = I(t) := k[\cos(t \cdot \omega) + 1]$, e_{ji}^0 is the initial concentration for the enzyme catalyzing the reaction with substrate X_i and product X_j , k_{ji} (or k_{ji}/ε for the fast reactions) is the product formation rate and K_{ji} is the Michaelis-Menten constant of the same enzymatic reaction.

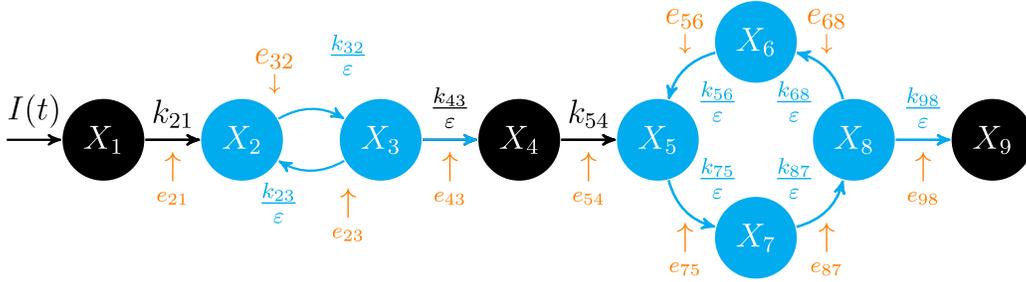


Figure A.5. Metabolic Network N2. Arrows between metabolites represent enzymatic reactions catalyzed by an enzyme e_{ji} , with substrate X_i , product X_j and product formation rate k_{ji} or k_{ji}/ε , respectively. The input $I(t) = k[\cos(t \cdot \omega) + 1]$ is a periodic continuous function.

To describe how we proceed to linearized the kinetics of N2, let us consider a non linear system:

$$\dot{x} = f(x, u), \tag{A.22}$$

and a steady state x^* associated to a constant input u^* . Therefore

$$f(x^*, u^*) = 0.$$

Now we define a linearized system associated to (A.22) around (x^*, u^*) :

$$\dot{x} = \left. \frac{\partial f}{\partial x} \right|_{x^*, u^*} (x - x^*) + \left. \frac{\partial f}{\partial u} \right|_{x^*, u^*} (u - u^*) \quad (\text{A.23})$$

In our specific example, we take

$$u^* := k \sim \text{mean}(I(t)).$$

Then, we calculate the equilibrium point of Equation (A.21) when the input is equal to this constant value. In other words, we obtain the equilibrium points for the variables of Equation (A.21), when $u(t) = u^*$, as

$$X_i^* := \lim_{t \rightarrow \infty} X_i(t) \in \overline{\mathbb{R}^+} \quad \forall i = 1, \dots, 9.$$

Hence, according to Equation (A.23), we obtain the following linearized system associated to Equation (A.21):

$$\begin{aligned} \frac{dX_1}{dt} &= k[\cos(t \cdot \omega)] - (a_{21} + \mu)(X_1 - X_1^*) & X_1(0) &= x_1^0 & (\text{A.24}) \\ \frac{dX_2}{dt} &= a_{21}(X_1 - X_1^*) + \frac{a_{23}}{\varepsilon}(X_3 - X_3^*) \\ &\quad - \left(\frac{a_{32}}{\varepsilon} + \mu \right) (X_2 - X_2^*) & X_2(0) &= x_2^0 \\ \frac{dX_3}{dt} &= \frac{a_{32}}{\varepsilon}(X_2 - X_2^*) \\ &\quad - \left(\frac{a_{23}}{\varepsilon} + \frac{a_{43}}{\varepsilon} + \mu \right) (X_3 - X_3^*) & X_3(0) &= x_3^0 \\ \frac{dX_4}{dt} &= \frac{a_{43}}{\varepsilon}(X_3 - X_3^*) - (a_{54} + \mu)(X_4 - X_4^*) & X_4(0) &= x_4^0 \\ \frac{dX_5}{dt} &= a_{54}(X_4 - X_4^*) + \frac{a_{56}}{\varepsilon}(X_6 - X_6^*) \\ &\quad - \left(\frac{a_{75}}{\varepsilon} + \mu \right) (X_5 - X_5^*) & X_5(0) &= x_5^0 \\ \frac{dX_6}{dt} &= \frac{a_{68}}{\varepsilon}(X_8 - X_8^*) - \left(\frac{a_{56}}{\varepsilon} + \mu \right) (X_6 - X_6^*) & X_6(0) &= x_6^0 \\ \frac{dX_7}{dt} &= \frac{a_{75}}{\varepsilon}(X_5 - X_5^*) - \left(\frac{a_{87}}{\varepsilon} + \mu \right) (X_7 - X_7^*) & X_7(0) &= x_7^0 \\ \frac{dX_8}{dt} &= \frac{a_{87}}{\varepsilon}(X_7 - X_7^*) \\ &\quad - \left(\frac{a_{68}}{\varepsilon} + \frac{a_{98}}{\varepsilon} + \mu \right) (X_8 - X_8^*) & X_8(0) &= x_8^0 \\ \frac{dX_9}{dt} &= \frac{a_{98}}{\varepsilon}(X_8 - X_8^*) - \mu(X_9 - X_9^*) & X_9(0) &= x_9^0, \end{aligned}$$

where

$$a_{ji} := \frac{e_{ji}k_{ji}K_{ji}}{(X_i^* + K_{ji})^2} \in [0, \infty) \quad \forall i, j \in \{1, \dots, 9\}.$$

Numerical simulations (Figure A.6 and Figure A.7) show that the linearized system (A.24) is a feasible representation of the nonlinear system (A.21).

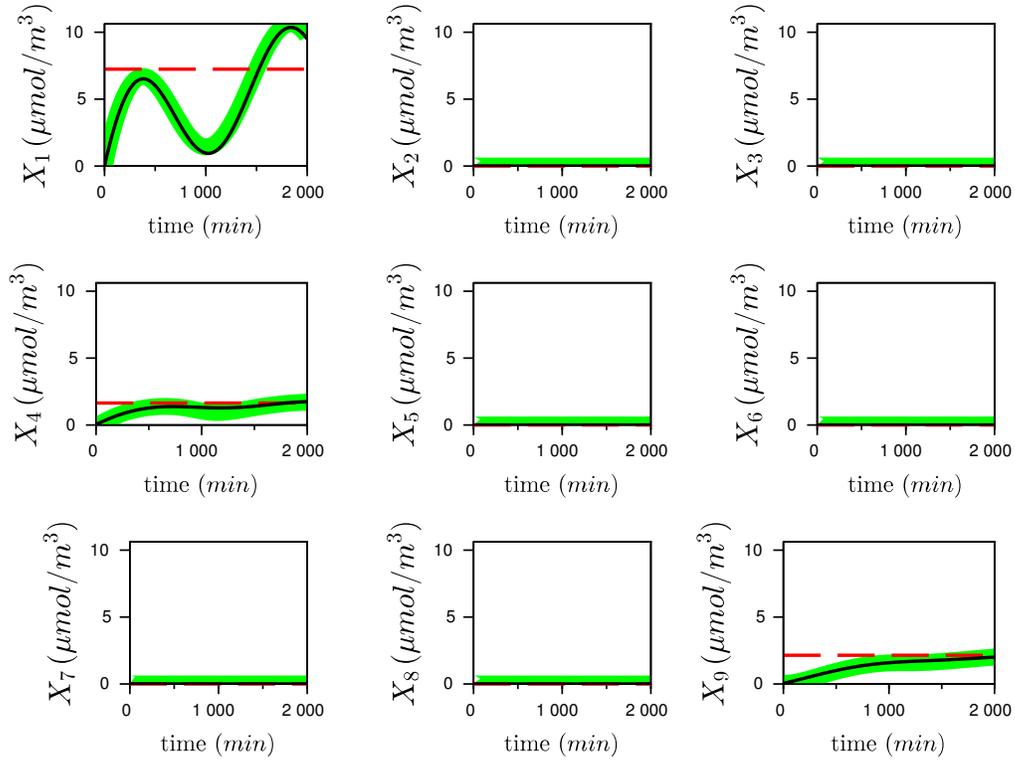


Figure A.6. Dynamics of the network with enzymatic reactions N2 (Figure A.5). Thick light solid line: nonlinear system describing the Michaelis-Menten reactions of N2 (A.21); thin dark solid line: linearized system (A.24); dashed line: value of the functional equilibrium points (X_i^*). The parameters considered for the simulation are stated in Table A.1.

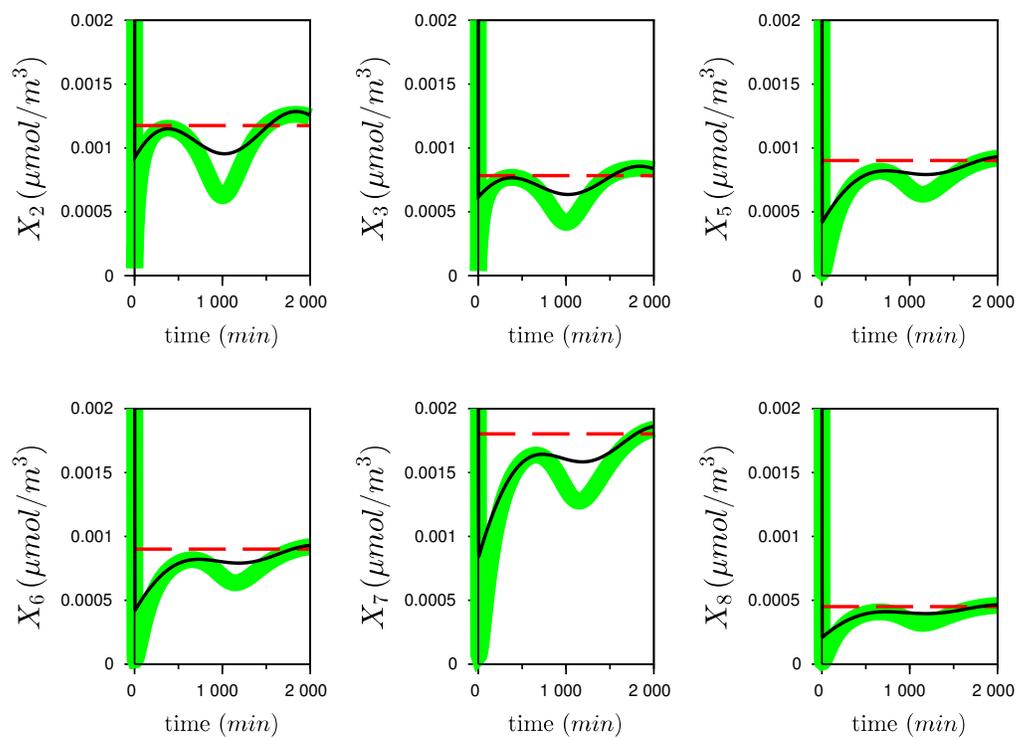


Figure A.7. Zoom on dynamics of the network with enzymatic reactions N2 (see also Figure A.6).

Parameter	Value	Units	Parameter	Value	Units
ε	0.1×10^{-2}	-	$e_{23}k_{23}/\varepsilon$	10	min^{-1}
ω	0.4×10^{-2}	-	$e_{32}k_{32}/\varepsilon$	20	min^{-1}
μ	0.2×10^{-2}	min^{-1}	$e_{43}k_{43}/\varepsilon$	20	min^{-1}
k	0.2×10^{-1}	min^{-1}	$e_{56}k_{56}/\varepsilon$	10	min^{-1}
$e_{21}k_{21}$	0.1×10^{-1}	min^{-1}	$e_{68}k_{68}/\varepsilon$	20	min^{-1}
$e_{54}k_{54}$	0.1×10^{-1}	min^{-1}	$e_{75}k_{75}/\varepsilon$	20	min^{-1}
x_i^0	0.1×10^{-1}	$\mu mol/m^3$	$e_{98}k_{98}/\varepsilon$	20	min^{-1}
X_2^*	0.1×10^{-2}	$\mu mol/m^3$	$e_{87}k_{87}/\varepsilon$	10	min^{-1}
X_3^*	0.8×10^{-3}	$\mu mol/m^3$	K_{ji}	2	$\mu mol/m^3$
X_5^*	0.9×10^{-3}	$\mu mol/m^3$	X_1^*	7.24	$\mu mol/m^3$
X_6^*	0.9×10^{-3}	$\mu mol/m^3$	X_4^*	1.64	$\mu mol/m^3$
X_7^*	0.2×10^{-2}	$\mu mol/m^3$	X_9^*	2.15	$\mu mol/m^3$
X_8^*	0.5×10^{-3}	$\mu mol/m^3$			

Table A.1. Parameters considered for the numerical simulation of systems (A.21) and (A.24), depicted in Figure A.6 and Figure A.7. The Michaelis-Menten constants K_{ji} are considered to be all the same, as well as the initial conditions x_i^0 for $i = 1, \dots, 9$.

APPENDIX B

Complements for Chapter 4

B.1. Boundary layer

A second condition related to the uniform convergence of approximations when $\eta \rightarrow 0$ has to be verified with the boundary layer of Equation (4.14) [39]. For this we define the boundary layer correction $\hat{Y}(\tau) = Y(t) - \bar{Y}(t)$, $\tau = t/\eta$, and the boundary layer problem:

$$\begin{aligned}
 \frac{d\hat{Y}_2}{d\tau} &= e_{21}^0 k_{21} \frac{x_1^0}{x_1^0 + K_{21}} + \left[\sum_{j=3}^{m-1} \frac{e_{2j}^0 k_{2j}}{K_{2j}} (\hat{Y}_j + \bar{Y}_j(0)) \right] \\
 &\quad - \left[\sum_{i=3}^{m-1} \frac{e_{i2}^0 k_{i2}}{K_{i2}} + \tilde{\mu} \right] (\hat{Y}_2 + \bar{Y}_2(0)) \\
 &\quad \vdots \\
 \frac{d\hat{Y}_{m-1}}{d\tau} &= \left[\sum_{j=2}^{m-2} \frac{e_{m-1,j}^0 k_{m-1,j}}{K_{m-1,j}} (\hat{Y}_j + \bar{Y}_j(0)) \right] \\
 &\quad - \left[\sum_{\substack{i=2 \\ i \neq m-1}}^m \frac{e_{i,m-1}^0 k_{i,m-1}}{K_{i,m-1}} + \tilde{\mu} \right] (\hat{Y}_{m-1} + \bar{Y}_{m-1}(0)) \\
 &\quad \vdots \\
 \frac{d\hat{Y}_{m+1}}{d\tau} &= e_{m+1,m}^0 k_{m+1,m} \frac{x_m^0}{x_m^0 + K_{m+1,m}} + \left[\sum_{j=m+2}^{n-1} \frac{e_{m+1,j}^0 k_{m+1,j}}{K_{m+1,j}} (\hat{Y}_j + \bar{Y}_j(0)) \right] \\
 &\quad - \left[\sum_{i=m+2}^{n-1} \frac{e_{i,m+1}^0 k_{i,m+1}}{K_{i,m+1}} + \tilde{\mu} \right] (\hat{Y}_{m+1} + \bar{Y}_{m+1}(0)) \\
 &\quad \vdots \\
 \frac{d\hat{Y}_{n-1}}{d\tau} &= \left[\sum_{j=m+1}^{n-2} \frac{e_{n-1,j}^0 k_{n-1,j}}{K_{n-1,j}} (\hat{Y}_j + \bar{Y}_j(0)) \right] \\
 &\quad - \left[\sum_{\substack{i=m+1 \\ i \neq n-1}}^n \frac{e_{i,n-1}^0 k_{i,n-1}}{K_{i,n-1}} + \tilde{\mu} \right] (\hat{Y}_{n-1} + \bar{Y}_{n-1}(0)),
 \end{aligned} \tag{B.1}$$

with initial conditions $\hat{y}_i^0(0) = y_i^0 - \bar{Y}_i(0)$ for every $i = 2, \dots, n-1, i \neq m$.

PROPOSITION B.1. *The equilibrium point $\hat{Y} = \bar{0}$ of system (B.1) is asymptotically stable.*

Proof. First notice that system (B.1) is linear, since (4.14) is linear. That $\hat{Y} = \bar{0}$ is an equilibrium point of system (B.1) is a consequence of Equation (4.18). Moreover, the Jacobian matrix of system (B.1) is the same that (4.14). Therefore, as in the proof of Proposition 4.1, we conclude that the origin is asymptotically stable for system (B.1). \square

On the other hand, the boundary layer correction \hat{Y} allows to correct the error of the approximation (4.8) at the initial fast transition. Indeed, notice that the initial condition y_i^0 in (4.12) can be different from $\bar{Y}_i(0)$ in (4.8). But

$$\bar{Y}_i(0) + \hat{Y}_i(0) = \bar{Y}_i(0) + Y_i(0) - \bar{Y}_i(0) = y_i^0$$

Moreover, the boundary layer correction \hat{Y} vanishes quickly [39] since

$$\lim_{\tau \rightarrow \infty} \hat{Y}(\tau) = \lim_{\eta \rightarrow 0} (Y(t) - \bar{Y}(t)) = 0.$$

B.2. Solution of the Slow System

Proof of Proposition 4.2. As in the proof of Proposition 4.1, we use the fact that $I(t)$ is a nonnegative continuous function on $[0, T_1]$ and all the parameters in (4.7) are nonnegative real numbers. Hence, system (4.7) is positively invariant in $\overline{\mathbb{R}_+^3}$.

Let us denote $F(t, X)$ the right hand of Equation (4.7). Then, we have that F and $\frac{\partial F}{\partial X}$ are continuous on $[0, T_1] \times \overline{\mathbb{R}_+^3}$. Moreover, $\frac{\partial F}{\partial X}$ is uniformly bounded on $[0, T_1] \times \overline{\mathbb{R}_+^3}$.

As a consequence, we can deduce from the Global Existence and Uniqueness Theorem [35] that (4.7) has a unique solution $\bar{X}(t)$ over $[0, T_1]$. \square

B.3. Supplements for the proof of Theorem 4.1

Here we prove some results used for the proof of Magnitude of Concentration Theorem 4.1, for metabolic networks with enzymatic reactions.

B.3.1. Fluxes, Traps and Flux Traps. In order to see when the metabolites in QSS do not accumulate, we have to introduce the following definitions.

DEFINITION B.1. We define a directed graph Γ related to the network in Figure 4.1, equivalent to system (4.3), as follows: the substrates and products X_i , $i = 1, \dots, n$, are the vertices of the Γ . Then, if $e_{ji}^0 k_{ji} \neq 0$ (i.e. if there is a reaction with substrate X_i and product X_j , an the initial concentration of

the enzyme catalyzing this function is positive) there is an edge with initial node X_i and final node X_j . In a similar way, we define the graph associated to a subsystem of (4.3), with metabolites $\{X_{i_1}, \dots, X_{i_r}\} \subset \{X_1, \dots, X_n\}$.

The concept of directed graph allows the following definition (see also Definition 3.1):

DEFINITION B.2 (Flux). A flux from X_i to X_j is a directed path which has as initial vertex X_i and as final vertex X_j .

Traps and *flux traps* are defined as for the linear case. See Definition 3.3 and Definition 3.4. If the graph associated to a network has a *flux*, *trap* or *flux trap*, we also say that the network has a *flux*, *trap* or *flux trap*, respectively.

B.3.2. Matrix analysis. Consider the Jacobian matrix defined in (4.16). For the sake of simplicity we denote

$$l_j := \sum_{\substack{i=2 \\ i \neq j}}^n \frac{e_{ij}^0 k_{ij}}{K_{ij}} + \tilde{\mu}$$

$$l_{ij} := \frac{e_{ij}^0 k_{ij}}{K_{ij}},$$

where $k_{ij} = 0$ if there is no reaction from X_j to X_i . Then,

$$J = K' := \begin{pmatrix} K'_1 & 0 \\ 0 & K'_2 \end{pmatrix},$$

where

$$K_1 = K'_1 := \begin{pmatrix} -l_2 & l_{23} & \dots & l_{2,m-1} \\ l_{32} & -l_3 & \dots & l_{3,m-1} \\ \vdots & \vdots & & \vdots \\ l_{m-1,2} & l_{m-1,3} & \dots & -l_{m-1} \end{pmatrix},$$

$$K_2 = K'_2 := \begin{pmatrix} -l_{m+1} & l_{m+1,m+2} & \dots & l_{m+1,n-1} \\ l_{m+2,m+1} & -l_{m+2} & \dots & l_{m+2,n-1} \\ \vdots & \vdots & & \vdots \\ l_{n-1,m+1} & l_{n-1,m+2} & \dots & -l_{n-1} \end{pmatrix}.$$

THEOREM B.1. *Suppose that the graph associated to (4.3) satisfies Assumption 4.1. Consider the expression of the metabolites in QSS (4.8) and define*

$$\begin{aligned} c_i &:= \mathbf{b}_i \cdot l_{21} & i &= 2, \dots, m-1 \\ c_i &:= \mathbf{b}_i \cdot l_{m+1,m} & i &= m+1, \dots, m-1. \end{aligned}$$

Then for every $i \in \{2, \dots, m-1, m+1, \dots, n-1\} \setminus \mathcal{I}_{\mathbb{F}_T}$,

$$c_i = \mathcal{O}(1) \quad (\text{if } c_i \neq 0).$$

We recall that $i \in \{2, \dots, m-1, m+1, \dots, n-1\} \setminus \mathcal{I}_{\mathbb{F}_T}$ means the metabolite X_i is not in a *flux trap*.

Before proving Theorem B.1, we demonstrate several propositions. The proof of Theorem B.1 is in Appendix B.3.3. For this, we have to analyze the order of the parameters

$$\begin{aligned} c_i &= \frac{1}{\det(K'_1)} \mathcal{C}_{1,i-1} \cdot (-l_{21}) & \forall i = 2, \dots, m-1, \\ c_i &= \frac{1}{\det(K'_2)} \mathcal{C}'_{1,i-m} \cdot (-l_{m+1,m}) & \forall i = m+1, \dots, n-1, \end{aligned}$$

where $\mathcal{C}_{1,i-1}$ and $\mathcal{C}'_{1,i-m}$ are the cofactors of K'_1 and K'_2 , respectively.

The goal of the following proposition is to define the order of some M' minors, as required for the Proof of Theorem B.1.

Recall that in Assumption 4.1 we only take into consideration *flux traps*. For this reason, we also analyze the determinant of the matrix associated to a system with *traps*. For instance, with the matrix M_2 defined in (A.2), if $\Gamma(M_2)$ has a *trap*, $l_{21} = 0$ and its determinant has order $\mathcal{O}(\varepsilon\mu)$.

In general, we can expect that a graph $\Gamma(M_n)$ with a *trap* has a determinant with order $\varepsilon\mu$. As a consequence, the matrix M_n is ill-conditioned. This happens because a *trap* implies a block of zeros in the matrix. Indeed, the j -th column of the matrix system represents the edges whose origin is the metabolite X_j . Then, if X_j is in a *trap*, $l_{ij} = 0$ for every i with X_i out of the *trap*.

If \mathcal{C}_{ij} is a cofactor of M_n and $\det(\mathbb{T})$ has order $\varepsilon\mu$, then the coefficients

$$\frac{\mathcal{C}_{1i}}{\det(M') \cdot \det(\mathbb{T})} \cdot (-l_{21})$$

can be affected by a factor of order $(\varepsilon\mu)^{-1}$. However, in the following proposition we prove that when there is a trap \mathbb{T} , $\det(\mathbb{T})$ is also a factor of the cofactor \mathcal{C}_{1i} if X_i is not in the trap.

PROPOSITION B.2. *Let M_n be a matrix defined as in (A.1) and \mathbb{F} a flux from X_1 to X_n . If M_n has traps (not reached by \mathbb{F}) or flux traps for \mathbb{F} , then*

M_n has the form

$$M_n = \begin{pmatrix} [M']_{r \times r} & [C_1]_{r \times s} & 0_{r \times p} & 0_{r \times q} \\ 0_{s \times r} & [C_2]_{s \times s} & 0_{s \times p} & 0_{s \times q} \\ 0_{p \times r} & [C_3]_{p \times s} & [\mathbf{T}]_{p \times p} & 0_{p \times q} \\ [*]_{q \times r} & [C_4]_{q \times s} & 0_{q \times p} & [\mathbf{T}_F]_{q \times q} \end{pmatrix}, \quad r + s + p + q = n, \quad (\text{B.2})$$

where M' is a matrix with no traps, \mathbf{T} is the square block corresponding to metabolites in traps not reached by F , \mathbf{T}_F to metabolites which are in flux traps and C_2 to metabolites that connect the traps to the rest of the network but which not have a flux from the input. Then,

$$\det(M_n) = \det(M') \cdot \det(C_2) \cdot \det(\mathbf{T}) \cdot \det(\mathbf{T}_F).$$

Furthermore, its minors satisfy

$$(M_n)_{1j} = (M')_{1j} \cdot \det(C_2) \cdot \det(\mathbf{T}) \cdot \det(\mathbf{T}_F) \quad \forall j = 1, \dots, r,$$

with $(M')_{1j}$ a minor of M' , and

$$(M_n)_{1j} = 0 \quad \forall j = r + 1, \dots, r + s + p.$$

NOTE B.1. Notice that the block $[*]_{q \times r}$ is different from zero if there is a flux from X_1 to the flux trap (\mathbf{T}_F).

Proof. Since M_n defined in (B.2) is a square block triangular matrix, its determinant is the product of the determinants of the diagonal blocks [10]. Then,

$$\begin{aligned} \det(M_n) &= \begin{vmatrix} M' & C_1 \\ 0 & C_2 \end{vmatrix} \cdot \det(\mathbf{T}) \cdot \det(\mathbf{T}_F) \\ &= \det(M') \cdot \det(C_2) \cdot \det(\mathbf{T}) \cdot \det(\mathbf{T}_F). \end{aligned}$$

For $j = 1, \dots, r$, the submatrix obtained from deleting the first row and the j -th column of M_n is also a square block triangular matrix. Then, its determinant is

$$\begin{aligned} (M_n)_{1j} &= \begin{vmatrix} (M')_{1j} & [C'_1] \\ 0 & [C_2] \end{vmatrix} \cdot \det(\mathbf{T}) \cdot \det(\mathbf{T}_F) \\ &= (M')_{1j} \cdot \det(C_2) \cdot \det(\mathbf{T}) \cdot \det(\mathbf{T}_F), \end{aligned}$$

where $(M')_{1j}$ is a minor of M' and $[C'_1]$ is the matrix C_1 without its first row. On the other hand, for $j = r + 1, \dots, (r + s + p)$, the minor $(M_n)_{1j}$ is also the determinant of a square block triangular matrix, i.e.

$$(M_n)_{1j} = \begin{pmatrix} M' & C_1 & 0 \\ 0 & C_2 & 0 \\ 0 & C_3 & \mathbf{T} \end{pmatrix}_{1j} \cdot \det(\mathbf{T}_F).$$

On the other hand, we have that the minor

$$\begin{pmatrix} M' & C_1 & 0 \\ 0 & C_2 & 0 \\ 0 & C_3 & \mathbb{T} \end{pmatrix}_{1j} = 0 \quad \forall j = r+1, \dots, (r+s+p),$$

as a consequence of the block of zeros below M' . We conclude

$$(M_n)_{ij} = 0 \quad \forall j = r+1, \dots, (r+s+p).$$

□

Finally, to analyze the minors of M' , the block of M_n corresponding to the subgraph with no traps, we refer to Proposition A.3 and Corollary A.2.

B.3.3. Proof of Theorem B.1.

Proof of Theorem B.1. Since K'_1 is a nonsingular matrix,

$$(K'_1)^{-1} = \frac{1}{\det(K'_1)} \cdot \mathcal{C},$$

where \mathcal{C} is the transpose matrix of cofactors of K'_1 [41] (i.e. $\mathcal{C}_{ji} = (-1)^{j+i}(K'_1)_{ji}$). We then have according to Equation (4.18)

$$\bar{Y}_i = \frac{1}{\det(K'_1)} \mathcal{C}_{1,i-1} \cdot (-e_{21}^0 k_{21} \frac{\bar{X}_1}{\bar{X}_1 + K_{21}})$$

Then, by definition of c_i ,

$$c_i = \frac{1}{\det(K'_1)} \mathcal{C}_{1,i-1} \cdot (-l_{21}).$$

If K'_1 has no *traps* (i.e. the subnetwork with metabolites X_2, \dots, X_{m-1} has no *traps*), then

$$\det(K'_1) = (-1)^{m-2} \cdot \mathcal{O}(l_{ij}^{m-2}),$$

as stated by Proposition A.1. Moreover, Corollary A.2 implies that the cofactors $\mathcal{C}_{1,i-1}$ have order

$$\mathcal{C}_{1,i-1} = (-1)^{m-1} \cdot \mathcal{O}(l_{ij}^{m-3}).$$

On the other hand, if K'_1 has a *trap* \mathbb{T} not reached by the flux or a *flux trap* \mathbb{T}_F , as a consequence of Corollary A.2, Propositions A.1 and B.2,

$$\begin{aligned} \frac{\mathcal{C}_{1,i-1}}{\det(K'_1)} &= (-1) \cdot \mathcal{O}(l_{ij}^{-1}) && \text{if } X_i \notin \mathbb{T}_F, \mathcal{C}_{1,i-1} \neq 0, \\ \frac{\mathcal{C}_{1,i-1}}{\det(K'_1)} &= 0 && \text{if } X_i \in \mathbb{T}. \end{aligned}$$

We conclude that

$$\frac{-l_{21} \cdot \mathcal{C}_{1,i-1}}{\det(K'_1)} = \mathcal{O}(1)$$

if $\mathcal{C}_{1,i-1} \neq 0$, for $i \in \{2, \dots, m-1\} \setminus \mathcal{I}_{\mathcal{T}_F}$. The same reasoning applies for K'_2 and the variables of the second subnetwork X_{m+1}, \dots, X_{n-1} . \square

B.4. Extension to General Metabolic Networks

In Appendix A.2, we have shown that our reduction method can be extended to metabolic networks with first order (linear) reactions and any topology. On the other hand, in Chapter 4 we consider a class of networks analogous to the linear network studied in Chapter 3, but accounting enzymatic nonlinear reactions. For this class of nonlinear networks we obtained similar results to that presented for the linear case.

First, in Appendix B.4.1 we extend the results of Chapter 4 to general Michaelis-Menten reactions networks, meaning that the topology of these networks is generalized in the sense that, given any two metabolites, these can be involved as substrate or product of an enzymatic reaction (Michaelis-Menten) or a first order reaction. This can be seen as a simpler example of the generalization in Appendix B.4.2.

In Appendix B.4.2, we prove that the nonlinear reduction method proposed in Chapter 4 can be extended to networks accounting any type of kinetics in the slow part and enzymatic of first order fast reactions. Moreover, we consider that this applied to a metabolic network with nontrivial stoichiometry.

B.4.1. Michaelis-Menten Reactions Network. Consider a network of enzymatic reactions with n metabolites. We do not suppose any restriction for its topology, meaning that given any two metabolites X_i and X_j , it can exist an enzymatic reaction which consumes X_i and produce X_j . In this case, the reaction is catalyzed by the enzyme with initial concentration e_{ji}^0 . Moreover, its Michaelis-Menten constant is denoted by K_{ji} and the product formation rate is k_{ji} if the reaction is slow and k_{ji}/ε if the reaction is fast.

For this his enzymatic network, we consider the following system of equations:

$$\frac{dX_i}{dt} = F_i(t, X_1, \dots, X_n, \varepsilon, \mu) \quad X_i(0) = x_i^0, \quad (\text{B.3})$$

where

$$F_i := I_i(t) + \sum_{\substack{j=1 \\ j \neq i}}^m e_{ij}^0 k_{ij} \frac{X_j}{X_j + K_{ij}} + \sum_{j=m+1}^n e_{ij}^0 \frac{k_{ij}}{\varepsilon} \frac{X_j}{X_j + K_{ij}} - \sum_{\substack{j=1 \\ j \neq i}}^n e_{ji}^0 k_{ji} \frac{X_i}{X_i + K_{ji}} - \mu X_i$$

for $i = 1, \dots, m$, and

$$F_i := I_i(t) + \sum_{\substack{j=1 \\ j \neq i}}^m e_{ij}^0 k_{ij} \frac{X_j}{X_j + K_{ij}} + \sum_{\substack{j=m+1 \\ j \neq i}}^n e_{ij}^0 \frac{k_{ij}}{\varepsilon} \frac{X_j}{X_j + K_{ij}} - \sum_{\substack{j=1 \\ j \neq i}}^n e_{ji}^0 \frac{k_{ji}}{\varepsilon} \frac{X_i}{X_i + K_{ji}} - \mu X_i$$

for $i = m + 1, \dots, n$.

Equation (B.3) is a slow-fast system, where the variables X_1, X_2, \dots, X_m are in the slow part and $X_{m+1}, X_{m+2}, \dots, X_n$ are in the fast part. Indeed, making the change of variable

$$Y_i = \frac{X_i}{\varepsilon}$$

for the fast variables, we obtain

$$\frac{dX_i}{dt} = G_i(t, X_1, \dots, X_m, Y_{m+1}, \dots, Y_n, \varepsilon, \mu) \quad X_i(0) = x_i^0, \quad (\text{B.4})$$

$$\varepsilon \frac{dY_i}{dt} = G_i(t, X_1, \dots, X_m, Y_{m+1}, \dots, Y_n, \varepsilon, \tilde{\mu}) \quad Y_i(0) = y_i^0, \quad (\text{B.5})$$

where $y_i^0 := x_i^0/\varepsilon$ and $\tilde{\mu} := \varepsilon \cdot \mu$ are fixed values (do not change as $\varepsilon \rightarrow 0$),

$$G_i := I_i(t) + \sum_{\substack{j=1 \\ j \neq i}}^m e_{ij}^0 k_{ij} \frac{X_j}{X_j + K_{ij}} + \sum_{j=m+1}^n e_{ij}^0 k_{ij} \frac{Y_j}{\varepsilon Y_j + K_{ij}} - \sum_{\substack{j=1 \\ j \neq i}}^n e_{ji}^0 k_{ji} \frac{X_i}{X_i + K_{ji}} - \mu X_i$$

for $i = 1, \dots, m$, and

$$G_i := I_i(t) + \sum_{j=1}^m e_{ij}^0 k_{ij} \frac{X_j}{X_j + K_{ij}} + \sum_{\substack{j=m+1 \\ j \neq i}}^n e_{ij}^0 k_{ij} \frac{Y_j}{\varepsilon Y_j + K_{ij}} - \sum_{\substack{j=1 \\ j \neq i}}^n e_{ji}^0 k_{ji} \frac{Y_i}{\varepsilon Y_i + K_{ji}} - \tilde{\mu} Y_i$$

for $i = m + 1, \dots, n$.

Evaluating the functions G_i in $\varepsilon = 0$, we have

$$G_i(t, X_1, \dots, X_m, Y_{m+1}, \dots, Y_n, 0, \mu) := I_i(t) + \sum_{\substack{j=1 \\ j \neq i}}^m e_{ij}^0 k_{ij} \frac{X_j}{X_j + K_{ij}} + \sum_{j=m+1}^n \frac{e_{ij}^0 k_{ij}}{K_{ij}} Y_j - \sum_{\substack{j=1 \\ j \neq i}}^n e_{ji}^0 k_{ji} \frac{X_i}{X_i + K_{ji}} - \mu X_i$$

for $i = 1, \dots, m$, and

$$G_i(t, X_1, \dots, X_m, Y_{m+1}, \dots, Y_n, 0, \tilde{\mu}) := I_i(t) + \sum_{j=1}^m e_{ij}^0 k_{ij} \frac{X_j}{X_j + K_{ij}} + \sum_{\substack{j=m+1 \\ j \neq i}}^n \frac{e_{ij}^0 k_{ij}}{K_{ij}} Y_j - \sum_{\substack{j=1 \\ j \neq i}}^n \frac{e_{ji}^0 k_{ji}}{K_{ji}} Y_i - \tilde{\mu} Y_i$$

for $i = m + 1, \dots, n$.

Moreover, Equation (B.5) evaluated in $\varepsilon = 0$ is equivalent to the algebraic equation

$$0 = G_i(t, X_1, \dots, X_m, Y_{m+1}, \dots, Y_n, 0, \tilde{\mu}) \quad \forall i = m+1, \dots, n,$$

which can be rewritten in the matrix form

$$K \cdot \begin{pmatrix} Y_{m+1} \\ Y_{m+2} \\ \vdots \\ Y_n \end{pmatrix} = - \begin{pmatrix} I_{m+1}(t) + \sum_{j=1}^m e_{m+1,j}^0 k_{m+1,j} \frac{X_j}{X_j + K_{m+1,j}} \\ I_{m+2}(t) + \sum_{j=1}^m e_{m+2,j}^0 k_{m+2,j} \frac{X_j}{X_j + K_{m+2,j}} \\ \vdots \\ I_n(t) + \sum_{j=1}^m e_{n,j}^0 k_{nj} \frac{X_j}{X_j + K_{nj}} \end{pmatrix}.$$

where

$$K := \begin{pmatrix} -\sum_{\substack{j=1 \\ j \neq m+1}}^n \frac{e_{j,m+1}^0 k_{j,m+1}}{K_{j,m+1}} - \tilde{\mu} & \frac{e_{m+1,m+2}^0 k_{m+1,m+2}}{K_{m+1,m+2}} & \dots & \frac{e_{m+1,n}^0 k_{m+1,n}}{K_{m+1,n}} \\ \frac{e_{m+2,m+1}^0 k_{m+2,m+1}}{K_{m+2,m+1}} & -\sum_{\substack{j=1 \\ j \neq m+2}}^n \frac{e_{j,m+2}^0 k_{j,m+2}}{K_{j,m+2}} - \tilde{\mu} & \dots & \frac{e_{m+2,n}^0 k_{m+2,n}}{K_{m+2,n}} \\ \vdots & \vdots & \ddots & \vdots \\ \frac{e_{n,m+1}^0 k_{n,m+1}}{K_{n,m+1}} & \frac{e_{n,m+2}^0 k_{n,m+2}}{K_{n,m+2}} & \dots & -\sum_{\substack{j=1 \\ j \neq n}}^n \frac{e_{j,n}^0 k_{j,n}}{K_{j,n}} - \tilde{\mu} \end{pmatrix}.$$

Notice that K is stable matrix, since it is strictly column diagonally dominant (see Corollary 2.1). Also remark that we only need the matrix K representing the fast equations to be strictly column diagonally dominant. Thus, a transformation (as described in Section 2.5) of the complete stoichiometric matrix has not to be imposed in this approach.

The solution to the algebraic problem is

$$\begin{pmatrix} Y_{m+1} \\ Y_{m+2} \\ \vdots \\ Y_n \end{pmatrix} = K^{-1} \cdot \begin{pmatrix} -\left(I_{m+1}(t) + \sum_{j=1}^m e_{m+1,j}^0 k_{m+1,j} \frac{X_j}{X_j + K_{m+1,j}} \right) \\ -\left(I_{m+2}(t) + \sum_{j=1}^m e_{m+2,j}^0 k_{m+2,j} \frac{X_j}{X_j + K_{m+2,j}} \right) \\ \vdots \\ -\left(I_n(t) + \sum_{j=1}^m e_{n,j}^0 k_{nj} \frac{X_j}{X_j + K_{nj}} \right) \end{pmatrix}.$$

B.4.1.1. Reduced System. To deduce the reduced system after Tikhonov's Theorem, define

$$\begin{aligned} \bar{G}_i(t, \bar{X}_1, \dots, \bar{X}_m, 0, \mu) &:= G_i(t, \bar{X}_1, \dots, \bar{X}_m, \bar{Y}_{m+1}, \dots, \bar{Y}_n, 0, \mu) = \\ &I_i(t) + \sum_{\substack{j=1 \\ j \neq i}}^m e_{ij}^0 k_{ij} \frac{\bar{X}_j}{\bar{X}_j + K_{ij}} - \sum_{\substack{j=1 \\ j \neq i}}^n e_{ji}^0 k_{ji} \frac{\bar{X}_i}{\bar{X}_i + K_{ji}} - \mu \bar{X}_i \\ &+ \sum_{j=m+1}^n \frac{e_{ij}^0 k_{ij}}{K_{ij}} \left[\sum_{j'=m+1}^n |\mathbf{b}_{jj'}| \cdot \left(I_{j'}(t) + \sum_{i'=1}^m e_{j',i'}^0 k_{j',i'} \frac{\bar{X}_{i'}}{\bar{X}_{i'} + K_{j',i'}} \right) \right], \end{aligned}$$

where

$$\mathbf{b}_{ij} = \frac{\mathcal{C}_{ji}}{\det K}$$

is an entry of K^{-1} and \mathcal{C}_{ji} a cofactor of K (see Appendix B.3.3)

Thus, the QSSR is

$$\frac{d\bar{X}_i}{dt} = \bar{G}_i(t, \bar{X}_1, \dots, \bar{X}_m, 0, \mu) \quad \bar{X}_i(0) = x_i^0,$$

for all $i = 1, \dots, m$. For the variables in QSS,

$$\bar{X}_i = \varepsilon \cdot \left[\sum_{j'=m+1}^n |\mathbf{b}_{ij'}| \cdot \left(I_{j'}(t) + \sum_{j=1}^m e_{j',j}^0 k_{j',j} \frac{\bar{X}_j}{\bar{X}_j + K_{j',j}} \right) \right],$$

for all $i = m+1, \dots, n$.

B.4.1.2. *Concentration Order of Magnitude.* Assuming $\mathcal{O}(e_{ij}^0 k_{ij}) = \mathcal{O}(e_{i'j'}^0 k_{i'j'}^0)$ for every i, j, i', j' , and $\mathcal{O}(K_{ij}) = 1$ for every i, j , we can prove

$$0 \leq \mathbf{b}_{ij} \cdot (-e_{ij}^0 k_{ij}) = \mathcal{O}(1) \quad \forall j = 1, \dots, m, \forall i = m+1, \dots, n,$$

if $\mathbf{b}_{ij} \neq 0$ and Y_i is not in a *flux trap* for a flux from X_j .

Thus, we write every Y_i as a linear combination of the Michaelis-Menten kinetics in the slow-part and the inputs on the fast part (if there exists):

$$\frac{\bar{X}_i}{\varepsilon} = \bar{Y}_i := \sum_{j'=m+1}^n |\mathbf{b}_{ij'}| \cdot \left(I_{j'}(t) + \sum_{j=1}^m e_{j',j}^0 k_{j',j} \frac{\bar{X}_j}{\bar{X}_j + K_{j',j}} \right).$$

Recall that $K_{j'j} = \mathcal{O}(1)$. It follows

$$\bar{X}_i \leq \varepsilon \cdot \left[\sum_{j'=m+1}^n |\mathbf{b}_{ij'}| \cdot \left(I_{j'}(t) + \sum_{j=1}^m e_{j',j}^0 k_{j',j} \cdot \bar{X}_j \right) \right],$$

for $i = m+1, \dots, n$. Then,

$$\bar{X}_i \leq \varepsilon \cdot \mathcal{O} \left(\sum_{j'=m+1}^n \left[|\mathbf{b}_{ij'}| \cdot I_{j'}(t) + \sum_{j=1}^m H_{j',j} \cdot \bar{X}_j \right] \right), \quad (\text{B.6})$$

where

$$H_{j',j} = \begin{cases} 1 & |\mathbf{b}_{ij'}| \cdot (e_{j',j}^0 k_{j',j}) \neq 0 \\ 0 & |\mathbf{b}_{ij'}| \cdot (e_{j',j}^0 k_{j',j}) = 0. \end{cases}$$

NOTE B.2. The inequality (B.6) means that concentration of the fast metabolite X_i is bounded by ε times de sum of some slow metabolites concentrations and some factors of inputs to the fast part.

If we suppose that there are not inputs to the fast part of the system, (B.6) is equivalent to

$$\bar{X}_i \leq \varepsilon \cdot \mathcal{O} \left(\sum_{j'=m+1}^n \left[\sum_{j=1}^m H_{j',j} \cdot \bar{X}_j \right] \right) \quad \forall i = m+1, \dots, n. \quad (\text{B.7})$$

NOTE B.3. The inequality (B.7) means that concentration of the fast metabolite X_i is bounded by ε times de sum of some slow metabolites concentrations.

In Appendix B.4.2, we show under which flux conditions it is satisfied

$$|\mathbf{b}_{ij'}| \cdot (e_{j',j}^0 k_{j',j}) \neq 0,$$

for a more general class of networks which contains the present one (see Proposition B.4).

B.4.2. General Slow Reactions Enzymatic Network. In this section we also consider a network of n metabolites, where slow reactions have any type of kinetics (particularly nonlinear) and fast reactions are enzymatic (Michaelis-Menten) reactions. We consider a general topology, with any restriction, which is determined by the slow kinetics and the Michaelis-Menten reactions.

Here we consider that the network has a nontrivial stoichiometry matrix. We assume that the constant rates of fast reactions between the metabolites in the fast part are already normalized, in order to have a strictly column diagonally dominant matrix. For the reactions with slow dynamics, we consider the stoichiometric coefficients denoted as $s(j)_{ij}$ or $s(i)_{ij}$, for the substrate and the product of a reaction with rate $f_{ij}(X_1, \dots, X_m)$, respectively. To conserve the sense of slow reaction, we suppose that $\mathcal{O}(s(j)_{ij} \cdot e_{ij}^0 k_{ij}) = \mathcal{O}(s(i)_{ij} \cdot e_{ij}^0 k_{ij}) = 1$ as $\varepsilon \rightarrow 0$.

Without loss of generality, for a fast reaction producing slow metabolite, we denote k_{ij} and \tilde{k}_{ij} the rate of production in the equation of the substrate and the product, respectively. This let us assume that a nontrivial stoichiometry for this reaction, i.e. $k_{ij} \neq \tilde{k}_{ij}$. As well, $\mathcal{O}(e_{ij}^0 \tilde{k}_{ij}) = 1$ when $\varepsilon \rightarrow 0$.

Then, consider the system of equations:

$$\frac{dX_i}{dt} = F_i(t, X_1, \dots, X_n, \varepsilon, \mu) \quad X_i(0) = x_i^0, \quad (\text{B.8})$$

where

$$F_i := I_i(t) + \sum_{\substack{j=1 \\ j \neq i}}^m s(i)_{ij} \cdot e_{ij}^0 k_{ij} \cdot f_{ij}(X_1, \dots, X_m) + \sum_{j=m+1}^n e_{ij}^0 \frac{\tilde{k}_{ij}}{\varepsilon} \frac{X_j}{X_j + K_{ij}} \\ - \sum_{\substack{j=1 \\ j \neq i}}^n s(i)_{ji} \cdot e_{ji}^0 k_{ji} \cdot f_{ji}(X_1, \dots, X_m) - \mu X_i$$

for $i = 1, \dots, m$, and

$$F_i := I_i(t) + \sum_{j=1}^m s(i)_{ij} \cdot e_{ij}^0 k_{ij} \cdot f_{ij}(X_1, \dots, X_m) + \sum_{\substack{j=m+1 \\ j \neq i}}^n e_{ij}^0 \frac{k_{ij}}{\varepsilon} \frac{X_j}{X_j + K_{ij}} \\ - \sum_{\substack{j=1 \\ j \neq i}}^n e_{ji}^0 \frac{k_{ji}}{\varepsilon} \frac{X_i}{X_i + K_{ji}} - \mu X_i$$

for $i = m + 1, \dots, n$. Without loss of generality, we suppose $I_i(t), f_{ij} \geq 0$. Moreover, in order to have a positively invariant system, we require

$$f_{ji} \Big|_{X_i=0} \geq 0 \quad \forall i = 1, \dots, m, 1 \leq j \leq n, j \neq i.$$

Equation (B.8) is a slow-fast system, where the variables X_1, X_2, \dots, X_m are in the slow part and $X_{m+1}, X_{m+2}, \dots, X_n$ are in the fast part. Indeed, making the change of variable

$$Y_i = \frac{X_i}{\varepsilon}$$

for the fast variables, we obtain

$$\frac{dX_i}{dt} = G_i(t, X_1, \dots, X_m, Y_{m+1}, \dots, Y_n, \varepsilon, \mu) \quad X_i(0) = x_i^0, \quad (\text{B.9})$$

$$\varepsilon \frac{dY_i}{dt} = G_i(t, X_1, \dots, X_m, Y_{m+1}, \dots, Y_n, \varepsilon, \tilde{\mu}) \quad Y_i(0) = y_i^0, \quad (\text{B.10})$$

where $y_i^0 := x_i^0/\varepsilon$ and $\tilde{\mu} := \varepsilon \cdot \mu$ are fixed values (do not change as $\varepsilon \rightarrow 0$),

$$G_i := I_i(t) + \sum_{\substack{j=1 \\ j \neq i}}^m s(i)_{ij} \cdot e_{ij}^0 k_{ij} \cdot f_{ij}(X_1, \dots, X_m) + \sum_{j=m+1}^n e_{ij}^0 \frac{\tilde{k}_{ij}}{\varepsilon} \frac{Y_j}{\varepsilon Y_j + K_{ij}} \\ - \sum_{\substack{j=1 \\ j \neq i}}^n s(i)_{ji} \cdot e_{ji}^0 k_{ji} \cdot f_{ji}(X_1, \dots, X_m) - \mu X_i$$

for $i = 1, \dots, m$, and

$$G_i := I_i(t) + \sum_{\substack{j=1 \\ j \neq i}}^m s(i)_{ij} \cdot e_{ij}^0 k_{ij} \cdot f_{ij}(X_1, \dots, X_m) + \sum_{\substack{j=m+1 \\ j \neq i}}^n e_{ij}^0 k_{ij} \frac{Y_j}{\varepsilon Y_j + K_{ij}} \\ - \sum_{\substack{j=1 \\ j \neq i}}^n e_{ji}^0 k_{ji} \frac{Y_i}{\varepsilon Y_i + K_{ji}} - \tilde{\mu} Y_i$$

for $i = m + 1, \dots, n$.

Evaluating the functions G_i in $\varepsilon = 0$, we have

$$G_i(t, X_1, \dots, X_m, Y_{m+1}, \dots, Y_n, 0, \mu) := \\ I_i(t) + \sum_{\substack{j=1 \\ j \neq i}}^m s(i)_{ij} \cdot e_{ij}^0 k_{ij} \cdot f_{ij}(X_1, \dots, X_m) + \sum_{j=m+1}^n \frac{e_{ij}^0 \tilde{k}_{ij}}{K_{ij}} Y_j \\ - \sum_{\substack{j=1 \\ j \neq i}}^n s(i)_{ji} \cdot e_{ji}^0 k_{ji} \cdot f_{ji}(X_1, \dots, X_m) - \mu X_i$$

for $i = 1, \dots, m$, and

$$G_i(t, X_1, \dots, X_m, Y_{m+1}, \dots, Y_n, 0, \tilde{\mu}) := \\ I_i(t) + \sum_{j=1}^m s(i)_{ij} \cdot e_{ij}^0 k_{ij} \cdot f_{ij}(X_1, \dots, X_m) + \sum_{\substack{j=m+1 \\ j \neq i}}^n \frac{e_{ij}^0 k_{ij}}{K_{ij}} Y_j - \sum_{\substack{j=1 \\ j \neq i}}^n \frac{e_{ji}^0 k_{ji}}{K_{ji}} Y_i - \tilde{\mu} Y_i$$

for $i = m + 1, \dots, n$.

Moreover, Equation (B.10) evaluated in $\varepsilon = 0$ is equivalent to the algebraic equation

$$0 = G_i(t, X_1, \dots, X_m, Y_{m+1}, \dots, Y_n, 0, \tilde{\mu}) \quad \forall i = m + 1, \dots, n,$$

which can be rewritten in the matrix form

$$K \cdot \begin{pmatrix} Y_{m+1} \\ Y_{m+2} \\ \vdots \\ Y_n \end{pmatrix} = - \begin{pmatrix} I_{m+1}(t) + \sum_{j=1}^m s(m+1)_{m+1,j} \cdot e_{m+1,j}^0 k_{m+1,j} \cdot f_{m+1,j}(X_1, \dots, X_m) \\ I_{m+2}(t) + \sum_{j=1}^m s(m+2)_{m+2,j} \cdot e_{m+2,j}^0 k_{m+2,j} \cdot f_{m+2,j}(X_1, \dots, X_m) \\ \vdots \\ I_n(t) + \sum_{j=1}^m s(n)_{nj} \cdot e_{nj}^0 k_{nj} \cdot f_{nj}(X_1, \dots, X_m) \end{pmatrix}.$$

where

$$K := \begin{pmatrix} -\sum_{\substack{j=1 \\ j \neq m+1}}^n \frac{e_{j,m+1}^0 k_{j,m+1}}{K_{j,m+1}} - \tilde{\mu} & \frac{e_{m+1,m+2}^0 k_{m+1,m+2}}{K_{m+1,m+2}} & \cdots & \frac{e_{m+1,n}^0 k_{m+1,n}}{K_{m+1,n}} \\ \frac{e_{m+2,m+1}^0 k_{m+2,m+1}}{K_{m+2,m+1}} & -\sum_{\substack{j=1 \\ j \neq m+2}}^n \frac{e_{j,m+2}^0 k_{j,m+2}}{K_{j,m+2}} - \tilde{\mu} & \cdots & \frac{e_{m+2,n}^0 k_{m+2,n}}{K_{m+2,n}} \\ \vdots & \vdots & \ddots & \vdots \\ \frac{e_{n,m+1}^0 k_{n,m+1}}{K_{n,m+1}} & \frac{e_{n,m+2}^0 k_{n,m+2}}{K_{n,m+2}} & \cdots & -\sum_{\substack{j=1 \\ j \neq n}}^n \frac{e_{j,n}^0 k_{j,n}}{K_{j,n}} - \tilde{\mu} \end{pmatrix}. \quad (\text{B.11})$$

REMARK B.1. Notice that K is stable matrix, since it is strictly column diagonally dominant (see Corollary 2.1). Also remark that we only need the matrix K representing the fast equations to be strictly column diagonally dominant. Thus, a transformation (as described in Section 2.5) of the complete stoichiometric matrix has not to be imposed in this approach and it can be restricted to the fast reactions between the metabolites in QSS.

The solution to the algebraic problem is

$$\begin{pmatrix} Y_{m+1} \\ Y_{m+2} \\ \vdots \\ Y_n \end{pmatrix} = K^{-1} \cdot \begin{pmatrix} -\left(I_{m+1}(t) + \sum_{j=1}^m s(m+1)_{m+1,j} \cdot e_{m+1,j}^0 k_{m+1,j} \cdot f_{m+1,j}(X_1, \dots, X_m) \right) \\ -\left(I_{m+2}(t) + \sum_{j=1}^m s(m+2)_{m+2,j} \cdot e_{m+2,j}^0 k_{m+2,j} \cdot f_{m+2,j}(X_1, \dots, X_m) \right) \\ \vdots \\ -\left(I_n(t) + \sum_{j=1}^m s(n)_{nj} \cdot e_{nj}^0 k_{nj} \cdot f_{nj}(X_1, \dots, X_m) \right) \end{pmatrix}.$$

B.4.2.1. *Reduced System.* To deduce the reduced system after Tikhonov's Theorem, define for $i = 1, \dots, m$

$$\begin{aligned} \bar{G}_i(t, \bar{X}_1, \dots, \bar{X}_m, 0, \mu) &:= G_i(t, \bar{X}_1, \dots, \bar{X}_m, \bar{Y}_{m+1}, \dots, \bar{Y}_n, 0, \mu) = \\ &I_i(t) + \sum_{\substack{j=1 \\ j \neq i}}^m s(i)_{ij} \cdot e_{ij}^0 k_{ij} \cdot f_{ij}(\bar{X}_1, \dots, \bar{X}_m) - \sum_{\substack{j=1 \\ j \neq i}}^n s(i)_{ji} \cdot e_{ji}^0 k_{ji} \cdot f_{ji}(\bar{X}_1, \dots, \bar{X}_m) - \mu \bar{X}_i \\ &+ \sum_{j=m+1}^n \frac{e_{ij}^0 \tilde{k}_{ij}}{K_{ij}} \left[\sum_{j'=m+1}^n |\mathbf{b}_{jj'}| \cdot \left(I_{j'}(t) + \sum_{i'=1}^m s(i')_{j'i'} \cdot e_{j'i'}^0 k_{j'i'} \cdot f_{j'i'}(\bar{X}_1, \dots, \bar{X}_m) \right) \right], \end{aligned}$$

where

$$\mathbf{b}_{ij} = \frac{C_{ji}}{\det K}$$

is an entry of K^{-1} and C_{ji} a cofactor of K (see Appendix B.3.3). Thus, the QSSR is

$$\frac{d\bar{X}_i}{dt} = \bar{G}_i(t, \bar{X}_1, \dots, \bar{X}_m, 0, \mu) \quad \bar{X}_i(0) = x_i^0, \quad (\text{B.12})$$

for all $i = 1, \dots, m$. For the variables in QSS,

$$\bar{X}_i = \varepsilon \cdot \left[\sum_{j'=m+1}^n |\mathbf{b}_{ij'}| \cdot \left(I_{j'}(t) + \sum_{j=1}^m s(j')_{j',j} \cdot e_{j',j}^0 k_{j',j} \cdot f_{j',j}(\bar{X}_1, \dots, \bar{X}_m) \right) \right], \quad (\text{B.13})$$

for all $i = m + 1, \dots, n$.

The following proposition is a consequence of Tikhonov's Theorem [39].

PROPOSITION B.3 (Tikhonov's Theorem). *Let (X_i) be the solution of (B.8) and (\bar{X}_i) defined by (B.12)-(B.13). If there is a solution for (B.12), then*

$$X_i(t) = \bar{X}_i(t) + \mathcal{O}(\varepsilon) \quad t \in [0, T_1], \forall i = 1, \dots, m,$$

and there exists $0 \leq T_0$ such that

$$X_i(t) = \bar{X}_i(t) + \varepsilon \cdot [\mathcal{O}(\varepsilon)] \quad t \in [T_0, T_1], \forall i = m + 1, \dots, n.$$

Now we see that $|\mathbf{b}_{ij'}| = 0$ if there is not a flux of fast reactions from $X_{j'}$ to X_i .

PROPOSITION B.4. *Let K be the matrix defined in (B.11) and consider metabolite $X_{j'}$. With out loss of generality suppose $1 \leq j' \leq r$ and rewrite this matrix as*

$$K = \begin{pmatrix} [K']_{r \times r} & [C_1]_{r \times s} \\ 0_{s \times r} & [C_2]_{s \times s} \end{pmatrix}, \quad r + s = n - m, \quad (\text{B.14})$$

where K' is the matrix representing the metabolites with a flux of fast reactions from $X_{j'}$ (including $X_{j'}$) and C_2 is the square block corresponding to metabolites not reached by any flux of fast reactions from $X_{j'}$. Then,

$$\det(K) = \det(K') \cdot \det(C_2).$$

Furthermore, its minors satisfy

$$(K)_{j'i} = (K')_{j'i} \cdot \det(C_2) \quad \forall i = 1, \dots, r,$$

with $(K')_{j'i}$ a minor of K' , and

$$(K)_{j'i} = 0 \quad \forall i = r + 1, \dots, r + s.$$

Proof. Since K defined in (B.14) is a square block triangular matrix, its determinant is the product of the determinants of the diagonal blocks [10]. Then,

$$\det(K) = \det(K') \cdot \det(C_2).$$

For $i = 1, \dots, r$, the submatrix obtained from deleting the j' -th row and the i -th column of K is also a square block triangular matrix. Then, its determinant is

$$(K)_{j'i} = \begin{vmatrix} (K')_{j'i} & [C'_1] \\ 0 & [C_2] \end{vmatrix} = (K')_{j'i} \cdot \det(C_2) \quad \forall i = 1, \dots, r,$$

where $(K')_{j'i}$ is a minor of K' and $[C'_1]$ is the matrix C_1 without its first row. On the other hand, for $i = r + 1, \dots, (r + s)$, the minor $(K)_{j'i}$ is also the determinant of a square block triangular matrix, i.e.

$$(K)_{j'i} = \begin{pmatrix} K' & C_1 \\ 0 & C_2 \end{pmatrix}_{j'i} = 0 \quad \forall i = r + 1, \dots, r + s,$$

as a consequence of the block of zeros below K' . We conclude

$$(K)_{j'i} = 0 \quad \forall i = r + 1, \dots, (r + s).$$

□

Proposition B.4 states that the only elements in the sum

$$\sum_{j'=m+1}^n |\mathbf{b}_{ij'}| \cdot \left(I_{j'}(t) + \sum_{j=1}^m s(j')_{j',j} \cdot e_{j',j}^0 k_{j',j} \cdot f_{j',j}(\bar{X}_1, \dots, \bar{X}_m) \right)$$

which are different from zero are those with index j' such that there is a flux of fast reactions from $X_{j'}$ to X_i (i.e. when $|\mathbf{b}_{ij'}| \neq 0$). Furthermore, notice that in

$$\sum_{j=1}^m s(j')_{j',j} \cdot e_{j',j}^0 k_{j',j} \cdot f_{j',j}(\bar{X}_1, \dots, \bar{X}_m)$$

the parameter $e_{j',j}^0 k_{j',j} \neq 0$ if and only if there is a reaction consuming X_j and producing $X_{j'}$, where X_j is in the slow part and $X_{j'}$ is in the fast part of the system.

B.4.2.2. *Magnitude of Concentration Order.* Assuming

$$\mathcal{O}(e_{ij}^0 k_{ij}) = \mathcal{O}(e_{i'j'}^0 k_{i'j'}) = 1 \quad \text{as } \varepsilon \rightarrow 0$$

for every $i, i' \in \{1, \dots, n\}$ and $j, j' \in \{m + 1, \dots, n\}$, and $\mathcal{O}(K_{ij}) = 1$ for every i, j , we can prove

$$0 \leq \mathbf{b}_{ij} \cdot (-e_{ij}^0 k_{ij}) = \mathcal{O}(1) \quad \forall i = 1, \dots, m, \forall i = m + 1, \dots, n,$$

if $\mathbf{b}_{ij} \neq 0$ and Y_i is not in a *flux trap* for a flux from X_j . Indeed,

$$\mathbf{b}_{ij} = \mathcal{O}\left((e_{ij}^0 k_{ij})^{-1}\right). \quad (\text{B.15})$$

Thus, we write every Y_i as a linear combination of the generic kinetics in the slow-part and the inputs on the fast part (if there exists):

$$\frac{X_i}{\varepsilon} = Y_i = \sum_{j'=m+1}^n |\mathbf{b}_{ij'}| \cdot \left(I_{j'}(t) + \sum_{j=1}^m s(j')_{j',j} \cdot e_{j',j}^0 k_{j',j} \cdot f_{j',j}(X_1, \dots, X_m) \right).$$

Suppose that $f_{j',j}$ is bounded by $0 \leq B_{j',j}$. It follows

$$X_i \leq \varepsilon \cdot \left[\sum_{j'=m+1}^n |\mathbf{b}_{ij'}| \cdot \left(I_{j'}(t) + \sum_{j=1}^m s(j')_{j',j} \cdot e_{j',j}^0 k_{j',j} \cdot B_{j',j}(X_1, \dots, X_m) \right) \right],$$

for $i = m+1, \dots, n$. Moreover, according to (B.15),

$$X_i \leq \varepsilon \cdot \mathcal{O} \left(\sum_{j'=m+1}^n \left[(e_{ij'}^0 k_{ij'})^{-1} \cdot I_{j'}(t) + \sum_{j=1}^m H_{j',j} \cdot B_{j',j}(X_1, \dots, X_m) \right] \right),$$

where

$$H_{j',j} = \begin{cases} 1 & |\mathbf{b}_{ij'}| \cdot \left(s(j')_{j',j} \cdot e_{j',j}^0 k_{j',j} \right) \neq 0 \\ 0 & |\mathbf{b}_{ij'}| \cdot \left(s(j')_{j',j} \cdot e_{j',j}^0 k_{j',j} \right) = 0 \end{cases}.$$

NOTE B.4. According to Proposition B.4, the value

$$|\mathbf{b}_{ij'}| \cdot \left(s(j')_{j',j} \cdot e_{j',j}^0 k_{j',j} \right) \neq 0$$

if and only if there is a flux of fast reactions from the fast metabolite $X_{j'}$ to X_i , or $i = j'$, and a reaction from the slow metabolite X_j to $X_{j'}$. On the other hand,

$$(e_{ij'}^0 k_{ij'})^{-1} \cdot I_{j'} \neq 0$$

if and only if there is an external input to the fast metabolites $X_{j'}$ and a reaction consuming $X_{j'}$ and producing X_i . Moreover, the order of this factor depends on the input $I_{j'}$. For example, if we suppose that the input is slow, we have that

$$I_{j'} = \mathcal{O}(e_{ij'}^0 k_{ij'})$$

and, as a consequence,

$$(e_{ij'}^0 k_{ij'})^{-1} \cdot I_{j'} = \mathcal{O}(1).$$

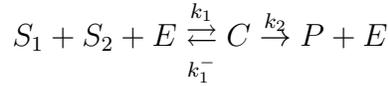
EXAMPLE B.1. In the particular case where slow reactions are of type Michaelis-Menten, we have that the bounds are $B_{j',j}(X_1, \dots, X_m) = X_j$ for every $j = 1, \dots, m$ (see B.4.1.2). If we also suppose that there is no input to any fast metabolite, then

$$X_i \leq \varepsilon \cdot \mathcal{O} \left(\sum_{j' \in \mathbf{F}_i} \left[\sum_{j \in \mathbf{F}_{j'}} X_j \right] \right) = \varepsilon \cdot \mathcal{O} \left(\sum_{\substack{j \in \mathbf{F}_{j'} \\ j' \in \mathbf{F}_i}} X_j \right),$$

where $j' \in F_i$ denotes that there is a flux of fast reactions from $X_{j'}$ to X_i , and $j \in F_{j'}$ that there is a reaction with substrate X_j (slow metabolite) and product $X_{j'}$.

Basically, the concentration of the fast metabolite X_i is bounded by the product of ε and a function of order the sum of the concentrations of slow metabolites which are directly preceding a flux of fast reactions to X_i or such that there is a reaction from the slow metabolite to X_i .

B.4.2.3. Enzymatic reaction with two substrates. As we have mentioned in Chapter 4, with our approach it is possible to consider any kind of non-linear kinetics between the slow metabolites. One of this possibility is to consider an enzymatic reaction with two substrates. Here we propose a QSS for this kind of reaction, obtained with a technique similar to that of the classical Michaelis Menten model. Moreover, we consider α a mass conservation constant for the substrates concentrations. Let



be an enzymatic reaction with double substrate. The ODE associated to this reaction is

$$\begin{aligned} \frac{dS_1}{dt} &= \alpha(-k_1 S_1 S_2 E + k_1^- C) & S_1(0) &= s_1^0 \\ \frac{dS_2}{dt} &= (1 - \alpha)(-k_1 S_1 S_2 E + k_1^- C) & S_2(0) &= s_2^0 \\ \frac{dE}{dt} &= -k_1 S_1 S_2 E + (k_1^- + k_2) C & E(0) &= e_0 \\ \frac{dC}{dt} &= k_1 S_1 S_2 E - (k_1^- + k_2) C & C(0) &= 0 \\ \frac{dP}{dt} &= k_2 C & P(0) &= 0. \end{aligned} \quad (\text{B.16})$$

with $\alpha \in [0, 1]$ a mass conservation constant and s_1^0, s_2^0, e_0 positive initial conditions. Notice that the law of conservation of mass is satisfied by Equation (B.16) if, and only if, $k_1 S_1 S_2 E - (k_1^- + k_2) C = 0$. Moreover,

$$\frac{dE}{dt} + \frac{dC}{dt} = 0.$$

Hence,

$$E = e_0 - C \quad \text{and} \\ C = \frac{e_0 S_1 S_2}{S_1 S_2 + K} \quad \text{with} \quad K = \frac{k_1^- + k_2}{k_1}.$$

Substituting in Equation (B.16), it follows

$$\begin{aligned} \frac{dS_1}{dt} &= -(\alpha) \frac{e_0 k_2 S_1 S_2}{S_1 S_2 + K} & S_1(0) &= s_1^0 \\ \frac{dS_2}{dt} &= -(1 - \alpha) \frac{e_0 k_2 S_1 S_2}{S_1 S_2 + K} & S_2(0) &= s_2^0 \\ \frac{dP}{dt} &= \frac{e_0 k_2 S_1 S_2}{S_1 S_2 + K} & P(0) &= 0. \end{aligned} \quad (\text{B.17})$$

Now we prove that system (B.17) is the Tikhonov reduction of (B.16), after writing (B.16) in the canonical form. The proof is similar to that of the classical Michaelis-Menten model with one substrates [55].

PROPOSITION B.5. *Suppose that $(\overline{S}_1, \overline{S}_2, \overline{P})$ is the solution of Equation (B.17) and*

$$\begin{aligned} \overline{C} &= \frac{e_0 \overline{S}_1 \overline{S}_2}{\overline{S}_1 \overline{S}_2 + K} \\ \overline{E} &= e_0 - \overline{C}. \end{aligned}$$

Then, the solution of system (B.16) is approximated by these functions with an error of order $\varepsilon := e_0/(s_1^0 s_2^0)$. To be more precise,

$$\begin{aligned} S_1(t) &= \overline{S}_1(t) + \mathcal{O}(\varepsilon) & \forall t \in [0, T] \\ S_2(t) &= \overline{S}_2(t) + \mathcal{O}(\varepsilon) & \forall t \in [0, T] \\ P(t) &= \overline{P}(t) + \mathcal{O}(\varepsilon) & \forall t \in [0, T], \end{aligned}$$

for $T > 0$, and there exists $T_0 > 0$ such that

$$\begin{aligned} C(t) &= \overline{C}(t) + \mathcal{O}(\varepsilon) & \forall t \in [T_0, T], \\ E(t) &= \overline{E}(t) + \mathcal{O}(\varepsilon) & \forall t \in [T_0, T]. \end{aligned}$$

Proof. Define

$$\begin{aligned} \varepsilon &= \frac{e_0}{s_1^0 s_2^0} & \tau &= k_1 e_0 t \\ u_1(\tau) &= \frac{S_1(t)}{s_1^0} & u_2(\tau) &= \frac{S_2(t)}{s_2^0} & v(\tau) &= \frac{C(t)}{e_0}. \end{aligned}$$

Then,

$$\frac{du_1(\tau)}{d\tau} = \frac{\alpha}{k_1 e_0 s_1^0} \left[-k_1 (s_1^0 u_1(\tau))(s_2^0 u_2(\tau))(e_0 - e_0 v(\tau)) + k_1^- e_0 v(\tau) \right] \quad (\text{B.18})$$

$$\frac{du_2(\tau)}{d\tau} = \frac{1 - \alpha}{k_1 e_0 s_2^0} \left[-k_1 (s_1^0 u_1(\tau))(s_2^0 u_2(\tau))(e_0 - e_0 v(\tau)) + k_1^- e_0 v(\tau) \right] \quad (\text{B.19})$$

$$\begin{aligned} \frac{dv(\tau)}{d\tau} &= \frac{1}{k_1 e_0^2} \left[k_1 (s_1^0 u_1(\tau))(s_2^0 u_2(\tau))(e_0 - e_0 v(\tau)) - (k_1^- + k_2) e_0 v(\tau) \right] \\ &= \frac{s_1^0 s_2^0}{e_0} \left[(u_1(\tau))(u_2(\tau))(1 - v(\tau)) - \frac{(k_1^- + k_2)}{k_1 s_1^0 s_2^0} v(\tau) \right] \\ &= \frac{1}{\varepsilon} \left[u_1(\tau) u_2(\tau) (1 - v(\tau)) - \frac{K}{s_1^0 s_2^0} v(\tau) \right] = \frac{1}{\varepsilon} \cdot g(u_1, u_2, v, \tau) \quad (\text{B.20}) \end{aligned}$$

Thus, v is the variable in the slow part of the system and we have to solve algebraically

$$0 = u_1(\tau) u_2(\tau) (1 - v(\tau)) - \frac{K}{s_1^0 s_2^0} v(\tau),$$

whose solution is

$$\bar{v}(\tau) = \frac{s_1^0 s_2^0 u_1(\tau) u_2(\tau)}{s_1^0 s_2^0 u_1(\tau) u_2(\tau) + K}.$$

Substituting in the slow part, we obtain

$$\begin{aligned} \frac{du_1(\tau)}{d\tau} &= \frac{\alpha}{k_1 s_1^0} \left[-k_1 (s_1^0 s_2^0 u_1(\tau) u_2(\tau)) \left(1 - \frac{s_1^0 s_2^0 u_1(\tau) u_2(\tau)}{s_1^0 s_2^0 u_1(\tau) u_2(\tau) + K} \right) \right. \\ &\quad \left. + k_1^- \frac{s_1^0 s_2^0 u_1(\tau) u_2(\tau)}{s_1^0 s_2^0 u_1(\tau) u_2(\tau) + K} \right] \\ &= \frac{\alpha}{k_1 s_1^0} \left[-k_1 \left(\frac{s_1^0 s_2^0 u_1(\tau) u_2(\tau) K}{s_1^0 s_2^0 u_1(\tau) u_2(\tau) + K} \right) + k_1^- \frac{s_1^0 s_2^0 u_1(\tau) u_2(\tau)}{s_1^0 s_2^0 u_1(\tau) u_2(\tau) + K} \right] \\ &= \frac{\alpha}{k_1 s_1^0} \left[- \left(\frac{s_1^0 s_2^0 u_1(\tau) u_2(\tau) (k_1^- + k_2)}{s_1^0 s_2^0 u_1(\tau) u_2(\tau) + K} \right) + k_1^- \frac{s_1^0 s_2^0 u_1(\tau) u_2(\tau)}{s_1^0 s_2^0 u_1(\tau) u_2(\tau) + K} \right] \\ &= \frac{\alpha}{k_1 s_1^0 e_0} \left[-e_0 k_2 \frac{s_1^0 s_2^0 u_1(\tau) u_2(\tau)}{s_1^0 s_2^0 u_1(\tau) u_2(\tau) + K} \right] \\ \frac{du_2(\tau)}{d\tau} &= \frac{1 - \alpha}{k_1 s_2^0 e_0} \left[-e_0 k_2 \frac{s_1^0 s_2^0 u_1(\tau) u_2(\tau)}{s_1^0 s_2^0 u_1(\tau) u_2(\tau) + K} \right] \end{aligned}$$

Reverting the change of variable, this is equivalent to

$$\begin{aligned}\frac{dS_1(t)}{dt} &= -(\alpha) \frac{e_0 k_2 S_1 S_2}{S_1 S_2 + K} \\ \frac{dS_2(t)}{dt} &= -(1 - \alpha) \frac{e_0 k_2 S_1 S_2}{S_1 S_2 + K}.\end{aligned}$$

Applying Tikhonov's Theorem [35], we obtain the conclusion of this Proposition. Indeed, the Jacobian of the fast equation (B.20) satisfies

$$\frac{dg}{d\nu} = -u_1(\tau)u_2(\tau) - \frac{K}{s_1^0 s_2^0} < 0.$$

Analogously for the boundary layer equation

$$\frac{d\hat{\nu}}{d\sigma} = u_1(0)u_2(0) \left[1 - (\hat{\nu}(\sigma) + \bar{\nu}(0)) \right] - \frac{K}{s_1^0 s_2^0} (\hat{\nu}(\sigma) + \bar{\nu}(0)),$$

where $\sigma := \tau/\varepsilon$ and $\hat{\nu}(0) = \nu(0) - \bar{\nu}(0)$. □

APPENDIX C

Complements for Chapter 5

C.1. List of metabolites

Table C.1. List of metabolites for autotrophic microalgae metabolic network.

Abbreviation	Metabolite
AcCoA	Acetyl-coenzyme A
AcylACP-chl	Acyl-acyl carrier protein
AcylCoa	Acyl-coenzyme A
AKG	α -Ketoglutarate
ALA	Alanine
APF	Absorbed light photon flux
ARG	Arginine
ASN	Asparagine
ASP	Aspartate
ATP	Adenosine-5'-triphosphate
CAR	Carbohydrates
CYS	Cysteine
CHLO	Chlorophyll
DAG-chl	Diacylglycerol, chloroplast
DAG-ER	Diacylglycerol, endoplasmic reticulum
DNA	Deoxyribonucleic acid
E4P	Erythrose-4-phosphate
E4P-chl	Erythrose-4-phosphate, chloroplast
F6P	Fructose-6-phosphate
F6P-chl	Fructose-6-phosphate, chloroplast
FA	Fatty acid
FA-chl	Fatty acid, chloroplast
FADH2	Flavin adenine dinucleotide, reduced
FUM	Fumarate
G3P	3-Phosphoglycerate
G3P-chl	3-Phosphoglycerate, chloroplast
G6P	Glucose-6-phosphate
G6P-chl	Glucose-6-phosphate, chloroplast
GAP	Glyceraldehyde-3-phosphate
GAP-chl	Glyceraldehyde-3-phosphate, chloroplast
GLN	Glutamine

GLY	Glycine
HIS	Histidine
ILE	Isoleucine
ISOCIT	Isocitrate
LEU	Leucine
LPthOH-chl	Lyso-phosphatidic acid, chloroplast
LPthOH-ER	Lyso-phosphatidic acid, endoplasmic reticulum
LYS	Lysine
MalCoA-chl	Malonyl-coenzyme A
MalACP-chl	Malonyl-acyl carrier protein
MET	Methionine
NADH	Nicotinamide adenine dinucleotide, reduced
N	Nitrate supply
NH ₃	Ammonium
NO ₃	Nitrate
OAA	Oxaloacetate
PEP	Phosphoenolpyruvate
PHE	Phenylalanine
PRO	Proline
PROT	Protein
PtdOH-chl	Phosphatidic acid, chlorophyll
PtdOH-ER	Phosphatidic acid, endoplasmic reticulum
TAG	Triacylglycerol
TAG-chl	Triacylglycerol, chloroplast
TAG-ER	Triacylglycerol, endoplasmic reticulum
R5P	Ribose-5-phosphate
R5Pchl	Ribose-5-phosphate, chloroplast
RNA	Ribonucleic acid
Ru5P	Ribulose-5-phosphate
Ru5Pchl	Ribulose-5-phosphate, chloroplast
RuDP	Ribulose-1,5-bisphosphate
SER	Serine
S7P	Sedoheptulose-7-phosphate
S7Pchl	Sedoheptulose-7-phosphate, chloroplast
STA	Starch
SUCCoA	Succinyl-coenzyme A
THR	Threonine
TRP	Tryptophan
TYR	Tyrosine
VAL	Valine
X5P	Xylulose-5-phosphate
X5Pchl	Xylulose-5-phosphate, chloroplast

C.2. List of reactions

Table C.2. Chemical reactions for an autotrophic microalgae metabolic network. Reference for reactions (R1) - (R84) and (R102) - (R108): [79]. Reference for reactions (R85) - (R101): [46].

Metabolic function (compartment)	Reactions	
Light Reaction (Chloroplast)	$0.125APF + 2NADP \Rightarrow 2.6ATP$	(R1)
Calvin Cycle (Chloroplast)	$CO_2 \Rightarrow 2G3P_{chl}$	(R2)
	$RuDP \Rightarrow 2G3P_{chl}$	(R3)
	$G3P_{chl} \Rightarrow GAP_{chl}$	(R4)
	$ATP \Rightarrow GAP_{chl}$	(R5)
	$G3P_{chl} \Rightarrow NADP$	(R6)
	$ATP \Rightarrow NADP$	(R7)
	$2GAP_{chl} \Rightarrow F6P_{chl}$	(R8)
	$F6P_{chl} \Leftrightarrow G6P_{chl}$	(R9)
	$F6P_{chl} \Rightarrow X5P_{chl}$	(R10)
	$F6P_{chl} \Rightarrow E4P_{chl}$	(R11)
	$E4P_{chl} \Rightarrow S7P_{chl}$	(R12)
	$S7P_{chl} \Rightarrow R5P_{chl}$	(R13)
	$S7P_{chl} \Rightarrow X5P_{chl}$	(R14)
	$R5P_{chl} \Rightarrow Ru5P_{chl}$	(R15)
	$X5P_{chl} \Rightarrow Ru5P_{chl}$	(R16)
	$Ru5P_{chl} \Rightarrow RuDP$	(R17)
	Transport from Chloroplast to Cytoplasm	$GAP_{chl} \Rightarrow GAP$
Glycolytic Pathway and Tricarboxylic Acid Cycle (Cytoplasm and Mitochondrion)	$2GAP \Rightarrow F6P$	(R19)
	$F6P \Leftrightarrow G6P$	(R20)
	$GAP \Leftrightarrow G3P$	(R21)
	$G3P \Leftrightarrow PEP$	(R22)
	$PEP \Rightarrow PYR$	(R23)
	$PYR \Rightarrow AcCoA$	(R24)
	$PEP \Rightarrow OAA$	(R25)
	$OAA + AcCoA \Leftrightarrow ISOCIT$	(R26)
	$ISOCIT \Leftrightarrow AKG$	(R27)
	$AKG \Rightarrow SUCCoA$	(R28)
	$SUCCoA \Leftrightarrow FUM$	(R29)
	$FAD \Leftrightarrow FADH_2$	(R30)
	$FUM \Leftrightarrow OAA$	(R31)
	Transport from Chloroplast to Cytoplasm	$NADP \Rightarrow NADP_{cyt}$
Pentose Phosphate Pathway (Cytoplasm)	$G6P + 2NADP_{cyt} \Rightarrow Ru5P$	(R33)
	$Ru5P \Leftrightarrow R5P$	(R34)
	$Ru5P \Leftrightarrow X5P$	(R35)
	$R5P \Leftrightarrow S7P$	(R36)

	$S7P \rightleftharpoons EAP$	(R37)
	$EAP \rightleftharpoons GAP$	(R38)
Nitrogen supply	$N \Rightarrow NO_3$	(R39)
Assimilation of nitrate (Chloroplast)	$NO_3 \Rightarrow NH_3$	(R40)
	$NO_3 \Rightarrow 3NADP$	(R41)
	$GLU \Rightarrow GLN$	(R42)
	$NH_3 \Rightarrow GLN$	(R43)
	$AKG \Rightarrow 2GLU$	(R44)
	$GLN \Rightarrow 2GLU$	(R45)
Amino acid synthesis (Cytoplasm)	$GLU \Rightarrow PRO$	(R46)
	$ASP \Rightarrow ARG$	(R47)
	$GLN \Rightarrow FUM$	(R48)
	$ASP \Rightarrow LYS$	(R49)
	$GLU \Rightarrow SER$	(R50)
	$SER \Rightarrow GLY$	(R51)
	$SER \Rightarrow CYS$	(R52)
	$GLU \Rightarrow ASP$	(R53)
	$ASP \Rightarrow ASN$	(R54)
	$ASP \Rightarrow THR$	(R55)
	$ASP \Rightarrow MET$	(R56)
	$THR \Rightarrow ILE$	(R57)
	$GLU \Rightarrow ALA$	(R58)
	$GLU \Rightarrow VAL$	(R59)
	$GLU \Rightarrow LEU$	(R60)
	$GLU \Rightarrow PHE$	(R61)
$GLU \Rightarrow TYR$	(R62)	
$SER \Rightarrow TRP$	(R63)	
$ASP \Rightarrow HIS$	(R64)	
Oxidative Phosphorylation (Mitochondrion)	$NADH \Rightarrow 2.5ATP$	(R65)
	$FADH_2 \Rightarrow 1.5ATP$	(R66)
Biosynthesis of Macromolecules (Chloroplast)	$G6P_{chl} \Rightarrow STA$	(R67)
	$8GLU \Rightarrow CHLO$	(R68)
	$G6P \Rightarrow CAR$	(R69)
	$0.08406GLY \Rightarrow PROT$	(R70)
	$0.09959ALA \Rightarrow PROT$	(R71)
	$0.0342ARG \Rightarrow PROT$	(R72)
	$0.02058ASN \Rightarrow PROT$	(R73)
	$0.01046CYS \Rightarrow PROT$	(R74)
	$0.0274HIS \Rightarrow PROT$	(R75)
	$0.02988ILE \Rightarrow PROT$	(R76)
	$0.07003LEU \Rightarrow PROT$	(R77)
	$0.05309LYS \Rightarrow PROT$	(R78)
	$0.01555MET \Rightarrow PROT$	(R79)
	$0.03176PHE \Rightarrow PROT$	(R80)
	$0.04268PRO \Rightarrow PROT$	(R81)

	$0.009932TRP \Rightarrow PROT$	(R82)
	$0.02583TYR \Rightarrow PROT$	(R83)
	$0.05044VAL \Rightarrow PROT$	(R84)
Transport from Mitochondrion to Chloroplast	$AcCoA \Rightarrow AcCoA_{chl}$	(R85)
TAG synthesis (Chloroplast)	$AcCoA_{chl} \Rightarrow MalCoA_{chl}$	(R86)
	$MalCoA_{chl} \Rightarrow MalACP_{chl}$	(R87)
	$MalACP_{chl} \Rightarrow AcylACP_{chl}$	(R88)
	$AcylACP_{chl} \Rightarrow FreeFA_{chl}$	(R89)
	$AcylACP_{chl} \Rightarrow LPtdOH_{chl}$	(R90)
	$LPtdOH_{chl} \Rightarrow PdtOH_{chl}$	(R91)
	$PdtOH_{chl} \Rightarrow DAG_{chl}$	(R92)
	$DAG_{chl} \Rightarrow TAG_{chl}$	(R93)
	$TAG_{chl} \Rightarrow TAG_{LD}$	(R94)
Transport from Chloroplast to Cytoplasm	$FreeFA_{chl} \Rightarrow FreeFA$	(R95)
TAG synthesis (Endoplasmic Reticulum)	$FreeFA \Rightarrow AcylCoa$	(R96)
	$AcylCoa \Rightarrow LPtdOH_{ER}$	(R97)
	$LPtdOH_{ER} \Rightarrow PdtOH_{ER}$	(R98)
	$PdtOH_{ER} \Rightarrow DAG_{ER}$	(R99)
	$DAG_{ER} \Rightarrow TAG_{ER}$	(R100)
	$TAG_{ER} \Rightarrow TAG_{LD}$	(R101)
Biosynthesis of Macromolecules (Nucleus)	$1.2ASP \Rightarrow RNA$	(R102)
	$0.54GLY \Rightarrow RNA$	(R103)
	$R5P \Rightarrow RNA$	(R104)
	$1.2ASP \Rightarrow DNA$	(R105)
	$0.25GLY \Rightarrow DNA$	(R106)
	$R5P \Rightarrow DNA$	(R107)
	$0.25SER \Rightarrow DNA$	(R108)

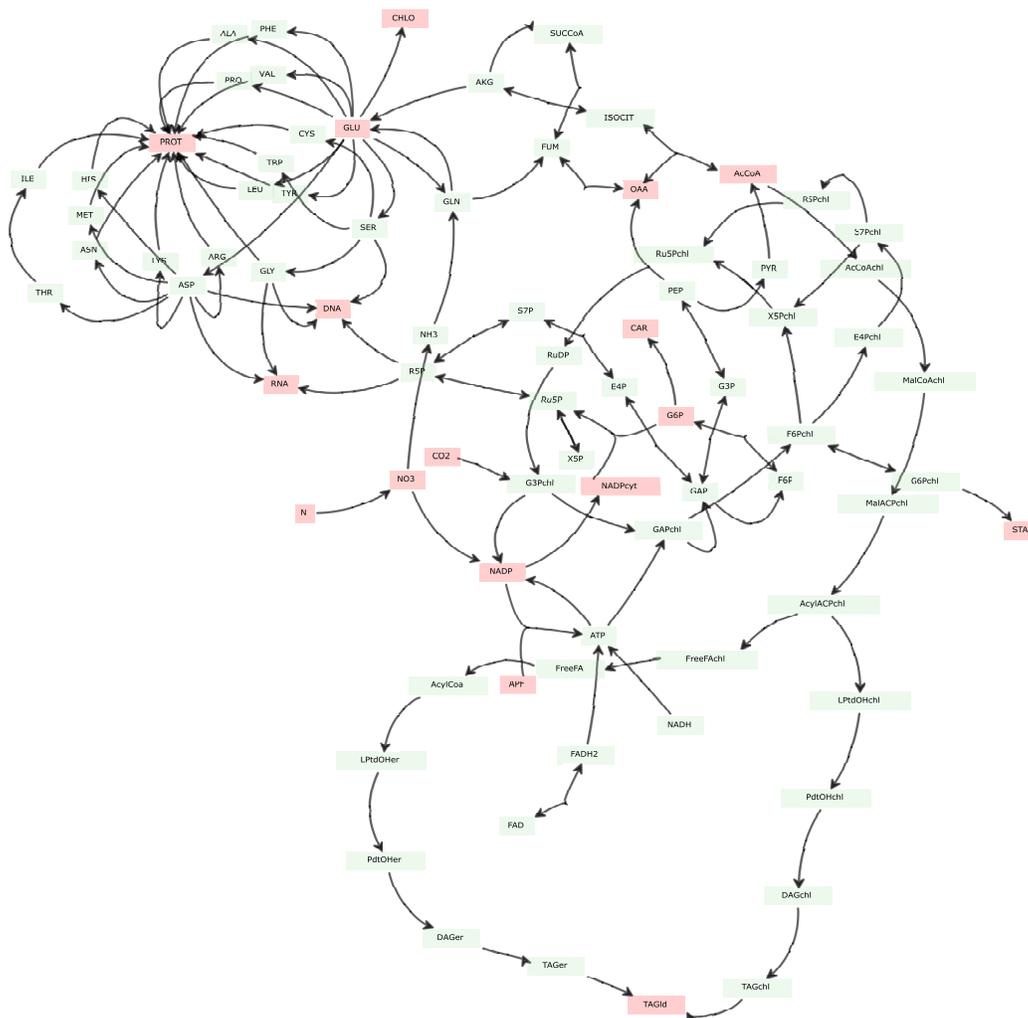


Figure C.1. Autotrophic Microalgae Metabolism Network, based on [79] and [46]. Reactions are listed in Table C.2. Diagram generated automatically with Copasi. The elements in red are inputs, precursors in the macromolecules formation or macromolecules. They are considered to constitute the slow part of the system.

C.3. Left Kernel Positive Vector

In order to obtain a reduced model to describe Carbohydrates, Neutral Lipids and Chlorophyll synthesis in autotrophic microalgae, we consider the network depicted Figure 5.2. To apply the approach described in this manuscript, we suppose that the stoichiometric submatrix of fast reactions between metabolites in QSS has a positive vector in the left kernel (see Section 2.5 and Remark B.1). Indeed, a positive vector in the left kernel was found using the *kernel* tool in Scilab. This vector (β) is described below:

$$\beta = \begin{pmatrix} ATP & 1 \\ GAP & 1 \\ GAPchl & 1 \\ F6Pchl & 2 \\ S7Pchl & 2 \\ Ru5Pchl & 2 \\ AcylACPchl & 3.68 \\ X5Pchl & 2 \\ GLN & 0.19 \\ MalCoAchl & 3.68 \\ PdtOHchl & 3.68 \\ PEP & 1 \\ Ru5P & 1 \\ S7P & 1 \\ FreeFA & 3.68 \\ AKG & 0.19 \\ LPtdOHer & 3.68 \\ DAGer & 3.68 \\ G3Pchl & 1 \\ FADH2 & 1.5 \\ DAGchl & 3.68 \\ SUCCoA & 0.19 \\ G3P & 1 \\ EAP & 1 \\ EAPchl & 2 \\ MalACPchl & 3.68 \\ FreeFAchl & 3.68 \\ RuDP & 2 \\ PdtOHer & 3.68 \\ FUM & 0.19 \\ R5P & 1 \\ LPtdOHchl & 3.68 \\ R5Pchl & 2 \\ AcylCoa & 3.68 \\ FAD & 1.5 \\ PYR & 1 \\ G6Pchl & 2 \\ NH3 & 0.19 \\ X5P & 1 \\ AcCoAchl & 3.68 \\ F6P & 2 \\ TAGer & 3.68 \\ NADH & 2.5 \\ ISOCIT & 0.19 \\ TAGchl & 3.68 \end{pmatrix}$$

The stoichiometry was taken from Yang et al. [79], and Kliphuis et al. [38] for reaction (R3). The fast reaction between metabolites in QSS are: (R3) (R4) (R5) (R8) (R9) (R10) (R11) (R12) (R13) (R14) (R15) (R16) (R17) (R18) (R19) (R21) (R22) (R23) (R27) (R28) (R29) (R30) (R34) (R35) (R36) (R37) (R38) (R43) (R48) (R65) (R66) (R86) (R87) (R88) (R89) (R90) (R91) (R92) (R93) (R95) (R96) (R97) (R98) (R99) (R100)

C.4. List of EFM

#	Net Reaction	Internal Species
1	NADP -> NADPcyt	
2	GLU -> CHLO	
3	G6P -> CAR	
4	NO3 -> NADP	
5	N -> NO3	
6	AcCoA -> TAGld	AcCoAchl, MalCoAchl, AcylACPchl, FreeFAchl, MalACPchl, FreeFA, AcylCoa, LPtdOHer, PdtOHer, DAGer, TAGer
7	AcCoA -> TAGld	AcCoAchl, MalCoAchl, AcylACPchl, LPtdOHchl, PdtOHchl, DAGchl, TAGchl, MalACPchl
8	APF+NADP ->NADP	ATP
9	AcCoA + OAA -> GLU	ISOCIT, AKG
10	AcCoA+OAA ->OAA	ISOCIT, AKG, SUCCoA, FUM
11	GLU->GLU	GLN
12	NO3 -> GLU	NH3, GLN
13	GLU -> OAA	GLN, FUM
14	NO3 -> OAA	NH3, GLN, FUM
15	CO2 -> NADP	G3Pchl
16	APF+NADP ->NADP	ATP, G3Pchl, GAPchl, RuDP, F6Pchl, X5Pchl, Ru5Pchl
17	APF+NADP ->NADP	ATP, G3Pchl, GAPchl, RuDP, F6Pchl, E4Pchl, S7Pchl, R5Pchl, Ru5Pchl
18	APF+NADP ->NADP	ATP, G3Pchl, GAPchl, RuDP, F6Pchl, X5Pchl, E4Pchl, S7Pchl, Ru5Pchl
19	APF + NADP -> G6P	ATP, GAPchl, GAP, F6P
20	NADPcyt +G6P -> G6P	GAP, F6P, Ru5P, R5P, S7P, E4P
21	CO2 -> G6P	G3Pchl, GAPchl, GAP, F6P
22	APF + NADP -> OAA	ATP, GAPchl, GAP, G3P, PEP
23	G6P + NADPcyt -> OAA	GAP, G3P, R5P, PEP, Ru5P, S7P, E4P
24	CO2 -> OAA	G3Pchl, GAPchl, GAP, G3P, PEP
25	APF + NADP -> AcCoA	ATP, GAPchl, GAP, G3P, PEP, PYR
26	G6P + NADPcyt -> AcCoA	GAP, G3P, R5P, PEP, PYR, Ru5P, S7P, E4P
27	CO2 -> AcCoA	G3Pchl, GAPchl, GAP, G3P, PEP, PYR

Table C.3. Elementary flux modes for carbohydrates, neutral lipids and chlorophyll synthesis in autotrophic microalgae. They were computed with Copasi.

Table C.4. Composition of each EFM in Table C.3.

#	Reactions	Equations
1	1 * R32	NADP -> NADPcyt
2	1 * R68	GLU -> CHLO
3	1 * R69	G6P -> CAR
4	1 * R41	NO3 -> NADP
5	1 * R39	N -> NO3
6	1 * R101	TAGer -> TAGld
1 * R 100	DAGer -> TAGer	
1 * R99	PdtOHer -> DAGer	
1 * R98	LPtdOHer -> PdtOHer	
1 * R97	AcylCoa -> LPtdOHer	
1 * R96	FreeFA -> AcylCoa	
1 * R95	FreeFAchl -> FreeFA	
1 * R 89	AcylACPchl -> FreeFAchl	
1 * R88	MalACPchl -> AcylACPchl	
1 * R87	MalCoAchl -> MalACPchl	

	1 * R86	AcCoAchl -> MalCoAchl
	1 * R85	AcCoA -> AcCoAchl
7	1 * R94	TAGchl -> TAGld
	1 * R93	DAGchl -> TAGchl
	1 * R92	PdtOHchl -> DAGchl
	1 * R91	LPtdOHchl -> PdtOHchl
	1 * R90	AcylACPchl -> LPtdOHchl
	1 * R88	MalACPchl -> AcylACPchl
	1 * R87	MalCoAchl -> MalACPchl
	1 * R86	AcCoAchl -> MalCoAchl
	1 * R85	AcCoA -> AcCoAchl
8	1 * R7	ATP -> NADP
	1 * R1	APF + NADP -> ATP
9	1 * R26	OAA + AcCoA -> ISOCIT
	1 * R27	ISOCIT = AKG
	1 * R44	AKG -> GLU
20	1 * R26	OAA + AcCoA -> ISOCIT
	1 * R27	ISOCIT = AKG
	1 * R29	SUCCoA = FUM
	1 * R31	FUM = OAA
	1 * R28	AKG -> SUCCoA
11	1 * R45	GLN -> GLU
	1 * R42	GLU -> GLN
12	1 * R45	GLN -> GLU
	1 * R43	NH3 -> GLN
	1 * R40	NO3 -> NH3
13	1 * R31	FUM = OAA
	1 * R48	GLN -> FUM
	1 * R42	GLU -> GLN
14	1 * R31	FUM = OAA
	1 * R48	GLN -> FUM
	1 * R43	NH3 -> GLN
	1 * R40	NO3 -> NH3
15	1 * R6	G3Pchl -> NADP
	1 * R2	CO2 -> G3Pchl
16	1 * R6	G3Pchl -> NADP
	1 * R17	Ru5Pchl -> RuDP
	1 * R16	X5Pchl -> Ru5Pchl
	1 * R10	F6Pchl -> X5Pchl
	1 * R8	GAPchl -> F6Pchl
	1 * R5	ATP -> GAPchl
	1 * R3	RuDP -> G3Pchl
	1 * R1	APF + NADP -> ATP
17	1 * R6	G3Pchl -> NADP
	1 * R17	Ru5Pchl -> RuDP
	1 * R15	R5Pchl -> Ru5Pchl
	1 * R13	S7Pchl -> R5Pchl
	1 * R12	E4Pchl -> S7Pchl
	1 * R11	F6Pchl -> E4Pchl
	1 * R8	GAPchl -> F6Pchl
	1 * R5	ATP -> GAPchl
	1 * R3	RuDP -> G3Pchl
	1 * R1	APF + NADP -> ATP
18	1 * R6	G3Pchl -> NADP
	1 * R17	Ru5Pchl -> RuDP
	1 * R16	X5Pchl -> Ru5Pchl
	1 * R14	S7Pchl -> X5Pchl
	1 * R12	E4Pchl -> S7Pchl

	1 * R11	F6Pchl -> E4Pchl
	1 * R8	GAPchl -> F6Pchl
	1 * R5	ATP -> GAPchl
	1 * R3	RuDP -> G3Pchl
	1 * R1	APF + NADP -> ATP
19	1 * R20	F6P = G6P
	1 * R19	GAP -> F6P
	1 * R18	GAPchl -> GAP
	1 * R5	ATP -> GAPchl
	1 * R1	APF + NADP -> ATP
20	1 * R20	F6P = G6P
	1 * R34	Ru5P = R5P
	1 * R36	R5P = S7P
	1 * R37	S7P = E4P
	1 * R38	E4P = GAP
	1 * R33	G6P + NADPcyt -> Ru5P
	1 * R19	GAP -> F6P
21	1 * R20	F6P = G6P
	1 * R19	GAP -> F6P
	1 * R18	GAPchl -> GAP
	1 * R4	G3Pchl -> GAPchl
	1 * R2	CO2 -> G3Pchl
22	1 * R21	GAP = G3P
	1 * R22	G3P = PEP
	1 * R25	PEP -> OAA
	1 * R18	GAPchl -> GAP
	1 * R5	ATP -> GAPchl
	1 * R1	APF + NADP -> ATP
23	1 * R21	GAP = G3P
	1 * R22	G3P = PEP
	1 * R34	Ru5P = R5P
	1 * R36	R5P = S7P
	1 * R37	S7P = E4P
	1 * R38	E4P = GAP
	1 * R33	G6P + NADPcyt -> Ru5P
	1 * R25	PEP -> OAA
24	1 * R21	GAP = G3P
	1 * R22	G3P = PEP
	1 * R25	PEP -> OAA
	1 * R18	GAPchl -> GAP
	1 * R4	G3Pchl -> GAPchl
	1 * R2	CO2 -> G3Pchl
25	1 * R21	GAP = G3P
	1 * R22	G3P = PEP
	1 * R24	PYR -> AcCoA
	1 * R23	PEP -> PYR
	1 * R18	GAPchl -> GAP
	1 * R5	ATP -> GAPchl
	1 * R1	APF + NADP -> ATP
26	1 * R21	GAP = G3P
	1 * R22	G3P = PEP
	1 * R34	Ru5P = R5P
	1 * R36	R5P = S7P
	1 * R37	S7P = E4P
	1 * R38	E4P = GAP
	1 * R33	G6P + NADPcyt -> Ru5P
	1 * R24	PYR -> AcCoA
	1 * R23	PEP -> PYR

27	1 * R21	GAP = G3P
	1 * R22	G3P = PEP
	1 * R24	PYR -> AcCoA
	1 * R23	PEP -> PYR
	1 * R18	GAPchl -> GAP
	1 * R4	G3Pchl -> GAPchl
	1 * R2	CO2 -> G3Pchl

C.5. Lumped parameters

To simplify the notation of system (5.1), we lumped several parameters. Their description is presented here. All parameters a_i are positive, except for $a_{16}, a_{22}, a_{27}, a_{36}, a_{37}, a_{40}$, which can take any real values.

The parameters here denoted by κ represent linear combinations of minors of the stoichiometric matrix (restricted to the fast metabolites). Its description is complex, then we do not give further details on them (see, for example, the results for the toy networks in Chapter 3 and Chapter 4). $e_{ij}^0 k_{ij}$ and K_{ij} is the notation used previously for Michaelis-Menten enzymatic reactions.

The following symbols represent amino acids produced after *GLU*. They are considered for parameter a_{33} :

$$\begin{aligned}
 ac_1 &= GLN; & ac_2 &= PRO; & ac_3 &= SER; & ac_4 &= ASP; & ac_5 &= ALA; \\
 ac_6 &= VAL; & ac_7 &= LEU; & ac_8 &= PHE; & ac_9 &= TYR.
 \end{aligned}$$

$$\begin{aligned}
a_1 &= k_N \text{ (nitrate input rate)} & a_2 &= e_{31}^0 k_{31} & a_3 &= K_{31} \\
a_4 &= e_{NH3,1}^0 k_{NH3,1} & a_5 &= K_{NH3,1} & a_6 &= k_C \text{ (carbon input rate)} \\
a_7 &= c_0 \text{ (absorbed carbon rate)} & a_8 &= e_{G3P,2}^0 k_{G3P,2} & a_9 &= K_{G3P,2} \\
a_{10} &= (\alpha) e_{Ru5P}^0 k_{Ru5P} & a_{11} &= K_{Ru5P} & a_{12} &= \kappa_4 \\
a_{13} &= \kappa_{42} & a_{14} &= e_{84}^0 k_{84} & a_{15} &= K_{84} \\
a_{16} &= (e_{F6P,4}^0 k_{F6P,4} - \kappa_{4,F6P}) \in \mathbb{R} & a_{17} &= K_{F6P,4} & a_{18} &= \kappa_5 \\
a_{19} &= \kappa_{52} & a_{20} &= e_{chl,5}^0 k_{chl,5} & a_{21} &= K_{chl,5} \\
a_{22} &= (\beta) e_{ISO,5}^0 k_{ISO,5} - \kappa_{5,ISO} \in \mathbb{R} & a_{23} &= K_{ISO} & a_{24} &= \kappa_6 \\
a_{25} &= \kappa_{61} & a_{26} &= \kappa_{62} & & \\
a_{27} &= (1 - \beta) e_{ISO,6}^0 k_{ISO,6} - \kappa_{6,ISO} \in \mathbb{R} & a_{28} &= K_{FUM,6} & a_{29} &= \kappa_{71} \\
a_{30} &= \kappa_{75} & a_{31} &= e_{10,7}^0 k_{10,7} & a_{32} &= K_{10,7} \\
a_{33} &= \sum_{\gamma=1}^9 e_{ac\gamma,7}^0 k_{ac\gamma,7} & a_{34} &= K_{ac\gamma,7} & a_{35} &= \kappa_{95} \\
a_{36} &= (e_{FUM,6}^0 k_{FUM,6} - \kappa_{6,FUM}) \in \mathbb{R} & & & & \\
a_{37} &= (1 - \alpha) e_{Ru5P}^0 k_{Ru5P} - \kappa_{4,F6P} \in \mathbb{R} & & & & \\
a_{38} &= \kappa_{53} & a_{39} &= \kappa_{63} & & \\
a_{40} &= (e_{ATP,3}^0 k_{ATP,3} - e_{3,ATP}^0 k_{3,ATP}) \in \mathbb{R} & a_{41} &= e_{11,3}^0 k_{11,3} & & \\
a_{42} &= K_{11,3} & a_{43} &= \kappa_{67} & a_{44} &= K_{GLN,7} \\
a_{45} &= k_{GLN,7} & a_{46} &= \kappa_{32}. & &
\end{aligned}$$

APPENDIX D

Optimal values for homographic functions

DEFINITION D.1. A function $f: \mathbf{I} \subset \mathbb{R} \rightarrow \mathbb{R}$ is homographic with respect to α if it has the form

$$f(\alpha) = \frac{\alpha \cdot a_{11} + a_{12}}{\alpha \cdot a_{21} + a_{22}},$$

where \mathbf{I} is an interval such that $\alpha \cdot a_{21} + a_{22} \neq 0$ for all $\alpha \in \mathbf{I}$, and a_{ij} is a real number for any $i, j \in \{1, 2\}$.

PROPOSITION D.1. Let $f: [t_0, t_1] \rightarrow \mathbb{R}$ be the homographic function

$$f(\alpha) = \frac{\alpha \cdot a_{11} + a_{12}}{\alpha \cdot a_{21} + a_{22}},$$

where $\alpha \cdot a_{21} + a_{22} \neq 0$ for all $\alpha \in [t_0, t_1]$. Define the matrix

$$\mathbf{H} := \begin{pmatrix} a_{11} & a_{12} \\ a_{21} & a_{22} \end{pmatrix}.$$

Then, f is a monotone function and it is increasing if $\det \mathbf{H} \geq 0$ and decreasing if $\det(\mathbf{H}) \leq 0$. Moreover, f has its maximum and minimum at the extremes of the interval.

Proof. The derivative with respect to α is

$$f'(\alpha) = \frac{a_{11}a_{22} - a_{12}a_{21}}{(\alpha \cdot a_{21} + a_{22})^2},$$

and $\det(\mathbf{H}) = a_{11}a_{22} - a_{12}a_{21}$. Thus, $f'(\alpha) \geq 0$ for every $\alpha \in D$ if and only if $\det(\mathbf{H}) \geq 0$ and, in this case, f is increasing, its minimum is $f(t_0)$ and its maximum $f(t_1)$.

Analogously, $f'(\alpha) \leq 0$ for every $\alpha \in D$ if and only if $\det(\mathbf{H}) \leq 0$. In this case f is decreasing and has its maximum in $f(t_0)$ and its minimum in $f(t_1)$. \square

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Bibliography

- [1] B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, and P. Walter. *Molecular Biology of The Cell*. Garland Science, Taylor & Francis Group, fifth edition, 2008.
- [2] J. E. Bailey and D. F. Ollis. *Biochemical Engineering Fundamentals*. Chemical Engineering Series. McGraw-Hill, United States of America, second edition, 1986.
- [3] R. Balakrishnan and K. Ranganathan. *A textbook of graph theory*. Springer Science & Business Media, 2012.
- [4] C. Baroukh, R. Muñoz Tamayo, J. P. Steyer, and O. Bernard. DRUM: a new framework for metabolic modeling under non-balanced growth. application to the carbon metabolism of unicellular microalgae. *PloS one*, 9(8):e104499, 2014.
- [5] C. Baroukh, R. Muñoz Tamayo, J. P. Steyer, and O. Bernard. A state of the art of metabolic networks of unicellular microalgae and cyanobacteria for biofuel production. *Metabolic Engineering*, 30:49–50, 2015.
- [6] M. Baudin, V. Couvert, and S. Steer. *Optimization in scilab*. Scilab Consortium, INRIA Paris-Rocquencourt, 2010.
- [7] P. Benner, R. Findeisen, D. Flockerzi, U. Reichl, K. Sundmacher, and P. Benner. *Large-scale networks in engineering and life sciences*. Springer, 2014.
- [8] A. Berman and R. Plemmons. *Nonnegative matrix in the mathematical science*. Classics in Applied Mathematics. Society for Industrial and Applied Mathematics, Philadelphia, 1994.
- [9] O. Bernard and G. Bastin. Identification of reaction networks for bioprocesses: determination of a partially unknown pseudo-stoichiometric matrix. *Bioprocess and Biosystems Engineering*, 27(5):293–301, 2005.
- [10] D. S. Bernstein. *Matrix mathematics: Theory, facts, and formulas with application to linear systems theory*. Princeton University Press, New Jersey, 2005.
- [11] S. Boie, V. Kirk, J. Sneyd, and M. Wechselberger. Effects of quasi-steady-state reduction on biophysical models with oscillations. *Journal of theoretical biology*, 393:16–31, 2016.
- [12] N. R. Boyle and J. A. Morgan. Flux balance analysis of primary metabolism in *chlamydomonas reinhardtii*. *BMC systems biology*, 3(1):4, 2009.
- [13] L. Brennan and P. Owende. Biofuels from microalgae – a review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable and sustainable energy reviews*, 14(2):557–577, 2010.
- [14] C. Conradi, D. Flockerzi, J. Raisch, and J. Stelling. Subnetwork analysis reveals dynamic features of complex (bio) chemical networks. *Proceedings of the National Academy of Sciences*, 104(49):19175–19180, 2007.
- [15] P.-J. D’Huys, I. Lule, D. Vercaemmen, J. Anné, J. F. Van Impe, and K. Bernaerts. Genome-scale metabolic flux analysis of *streptomyces lividans* growing on a complex medium. *Journal of Biotechnology*, 161(1):1–13, 2012.

- [16] M. L. Eltgroth, R. L. Watwood, and G. V. Wolfe. Production and cellular localization of neutral long-chain lipids in the haptophyte algae *isochrysis galbana* and *emiliania huxleyi*. *Journal of Phycology*, 41(5):1000–1009, 2005.
- [17] J. Fan, C. Andre, and C. Xu. A chloroplast pathway for the de novo biosynthesis of triacylglycerol in *chlamydomonas reinhardtii*. *FEBS letters*, 585(12):1985–1991, 2011.
- [18] L. Farina and S. Rinaldi. *Positive Linear Systems: Theory and Applications*. Pure and Applied Mathematics. John Wiley & Sons, New York, 2000.
- [19] N. Fenichel. Geometric singular perturbation theory for ordinary differential equations. *Journal of Differential Equations*, 31(1):53–98, 1979.
- [20] D. Flockerzi, A. Bohmann, and A. Kienle. On the existence and computation of reaction invariants. *Chemical engineering science*, 62(17):4811–4816, 2007.
- [21] Z. Gerdtzen, P. Daoutidis, and W. Hu. Non-linear reduction for kinetic models of metabolic reaction networks. *Metabolic Engineering*, 6(2):140–154, 2004.
- [22] A. Goeke, C. Schilli, S. Walcher, and E. Zerz. Computing quasi-steady state reductions. *Journal of Mathematical Chemistry*, 50(6):1495–1513, 2012.
- [23] G. H. Golub and C. F. Van Loan. *Matrix computations*. The Johns Hopkins University Press, fourth edition, 2013.
- [24] R. Heinrich, S. Rapoport, and T. Rapoport. Metabolic regulation and mathematical models. *Progress in biophysics and molecular biology*, 32:1–82, 1978.
- [25] R. Heinrich and S. Schuster. *The Regulation of Cellular Systems*. International Thomson Publishing, New York, 1996.
- [26] R. Heinrich and S. Schuster. The modelling of metabolic systems. structure, control and optimality. *BioSystems*, 47:61–77, 1998.
- [27] H. Holzhütter, G. Jacobasch, and A. Bisdorff. Mathematical modelling of metabolic pathways affected by an enzyme deficiency. *The FEBS Journal*, 149(1):101–111, 1985.
- [28] S. Hoops, S. Sahle, R. Gauges, C. Lee, J. Pahle, N. Simus, M. Singhal, L. Xu, P. Mendes, and U. Kummer. Copasi—a complex pathway simulator. *Bioinformatics*, 22(24):3067–3074, 2006.
- [29] F. Hoppensteadt. Stability in systems with parameter. *Journal of Mathematical Analysis and Applications*, 18(1):129–134, 1967.
- [30] F. Hoppensteadt. On systems of ordinary differential equations with several parameters multiplying the derivatives. *Journal of Differential Equations*, 5(1), 5(1):106–116, 1969.
- [31] F. Hoppensteadt. Properties of solutions of ordinary differential equations with small parameters. *Communications on Pure and Applied Mathematics*, 24(6):807–840, 1971.
- [32] R. Horn and C. R. Johnson. *Matrix Analysis*. Cambridge University Press, New York, 1985.
- [33] J. A. Jacquez and C. P. Simon. Qualitative theory of compartmental systems. *Siam Review*, 35(1):43–79, 1993.
- [34] K. J. Kauffman, P. Prakash, and J. S. Edwards. Advances in flux balance analysis. *Current opinion in biotechnology*, 14(5):491–496, 2003.
- [35] H. K. Khalil. *Nonlinear systems*. Prentice Hall, New Jersey, third edition, 2002.
- [36] S. Klamt, O. Hädicke, and A. von Kamp. Stoichiometric and constraint-based analysis of biochemical reaction networks. In *Large-scale networks in engineering and life sciences*, pages 263–316. Springer, 2014.
- [37] S. Klamt, G. Regensburger, M. P. Gerstl, C. Jungreuthmayer, S. Schuster, R. Mahadevan, J. Zanghellini, and S. Müller. From elementary flux modes to elementary

- flux vectors: Metabolic pathway analysis with arbitrary linear flux constraints. *PLoS computational biology*, 13(4):e1005409, 2017.
- [38] A. M. J. Kliphuis, A. J. Klok, D. E. Martens, P. P. Lamers, M. Janssen, and R. H. Wijffels. Metabolic modeling of *Chlamydomonas reinhardtii*: energy requirements for photoautotrophic growth and maintenance. *Journal of applied phycology*, 4(2):253–266, 2012.
- [39] P. Kokotović, H. K. Khalil, and J. O’Reilly. *Singular perturbation methods in control: analysis and design*. Society for Industrial and Applied Mathematics, Philadelphia, 1999.
- [40] T. Lacour, A. Sciandra, A. Talec, P. Mayzaud, and O. Bernard. Diel variations of carbohydrates and neutral lipids in nitrogen-sufficient and nitrogen-starved cyclostat cultures of *isochrysis* sp. *Journal of Phycology*, 48(4):966–975, 2012.
- [41] P. Lancaster and M. Tismenetsky. *The theory of matrices: with applications*. Academic Press, San Diego, California, second edition, 1985.
- [42] L. Lardon, A. Helias, B. Sialve, J. Steyer, and O. Bernard. Life-cycle assessment of biodiesel production from microalgae. *Environmental sciences & technology*, 43(17):6475–6481, 2009.
- [43] J. J. Levin. The asymptotic behaviour of the stable initial manifolds of a system of nonlinear differential equations. *Transactions of the American Mathematical Society*, 85(2):357–368, 1957.
- [44] N. Levinson. Perturbations of discontinuous solutions of non-linear systems of differential equations. *Acta Mathematica*, 82(1):71–106, 1950.
- [45] Y.-H. Lin, F.-L. Chang, C.-Y. Tsao, and J.-Y. Leu. Influence of growth phase and nutrient source on fatty acid composition of *isochrysis galbana* ccmp 1324 in a batch photoreactor. *Biochemical Engineering Journal*, 37(2):166–176, 2007.
- [46] B. Liu and C. Benning. Lipid metabolism in microalgae distinguishes itself. *Current opinion in biotechnology*, 24(2):300–309, 2013.
- [47] C.-P. Liu and L.-P. Lin. Ultrastructural study and lipid formation of *isochrysis* sp. ccmp1324. *Botanical Bulletin of Academia Sinica*, 42, 2001.
- [48] N. Loira, S. Mendoza, M. P. Cortés, N. Rojas, D. Travisany, A. Di Genova, N. Gajardo, N. Ehrenfeld, and A. Maass. Reconstruction of the microalga *nannochloropsis salina* genome-scale metabolic model with applications to lipid production. *BMC systems biology*, 11(1):66, 2017.
- [49] C. López Zazueta, O. Bernard, and J.-L. Gouzé. Analytical reduction of nonlinear metabolic networks accounting for dynamics in enzymatic reactions. *Complexity*, 2018. <https://doi.org/10.1155/2018/2342650>
- [50] C. López Zazueta, O. Bernard, and J.-L. Gouzé. Dynamical reduction of linearized metabolic networks through quasi steady state approximation. *AIChE Journal*, 65(1):18–31, 2019. <https://doi.org/10.1002/aic.16406>
- [51] R. Mahadevan, J. S. Edwards, and F. J. Doyle. Dynamic flux balance analysis of diauxic growth in *Escherichia coli*. *Biophysical Journal*, 83(3):1331–1340, 2002.
- [52] F. Menegus, L. Cattaruzza, A. Chersi, and G. Fronza. Differences in the anaerobic lactate-succinate production and in the changes of cell sap pH for plants with high and low resistance to anoxia. *Plant Physiology*, 90(1):29–32, 1989.
- [53] S. S. Merchant, S. E. Prochnik, O. Vallon, E. H. Harris, S. J. Karpowicz, G. B. Witman, A. Terry, A. Salamov, L. K. Fritz-Laylin, L. Maréchal-Drouard, et al. The *chlamydomonas* genome reveals the evolution of key animal and plant functions. *Science*, 318(5848):245–250, 2007.

- [54] C. D. Meyer. *Matrix analysis and applied linear algebra*. Society for Industrial and Applied Mathematics, Philadelphia, 2000.
- [55] J. Murray. *Mathematical Biology*, volume I. An Introduction. Springer, 2001.
- [56] A. Narang-Siddarth and J. Valasek. *Nonlinear Time Scale Systems in Standard and Nonstandard Forms: Analysis and Control*. Society for Industrial and Applied Mathematics, Philadelphia, 2014.
- [57] J. D. Orth, I. Thiele, and B. O. Palsson. What is flux balance analysis? *Nature biotechnology*, 28(3):245–248, 2010.
- [58] O. Perez-Garcia, F. M. Escalante, L. E. de Bashan, and Y. Bashan. Heterotrophic cultures of microalgae: metabolism and potential products. *Water research*, 45(1):11–36, 2011.
- [59] L. Perko. *Differential equations and dynamical systems*. Texts in Applied Mathematics 7. Springer-Verlag, 1991.
- [60] A. Provost and G. Bastin. Dynamic metabolic modelling under the balanced growth condition. *Journal of Process Control*, 14(7):717–728, 2004.
- [61] O. Radulescu, A. Gorban, A. Zinovyev, and A. Lilienbaum. Robust simplifications of multiscale biochemical networks. *BMC systems biology*, 2(1):86, 2008.
- [62] T. Rapoport, R. Heinrich, and S. Rapoport. The regulatory principles of glycolysis in erythrocytes in vivo and in vitro. a minimal comprehensive model describing steady states, quasi-steady states and time-dependent processes. *Biochemical Journal*, 154(2):449–469, 1976.
- [63] J. N. Rosenberg, G. A. Oyler, L. Wilkinson, and M. J. Betenbaugh. A green light for engineered algae: redirecting metabolism to fuel a biotechnology revolution. *Current opinion in biotechnology*, 19(5):430–436, 2008.
- [64] D. Rotin, B. Robinson, and I. F. Tannock. Influence of hypoxia and an acidic environment on the metabolism and viability of cultured cells: potential implications for cell death in tumors. *Cancer research*, 46(6):2821–2826, 1986.
- [65] M. Sáez, C. Wiuf, and E. Feliu. Graphical reduction of reaction networks by linear elimination of species. *Journal of mathematical biology*, 74(1-2):195–237, 2017.
- [66] M. Schauer and R. Heinrich. Quasi-steady-state approximation in the mathematical modeling of biochemical reaction networks. *Mathematical biosciences*, 65(2):155–170, 1983.
- [67] L. A. Segel. *Modeling dynamic phenomena in molecular and cellular biology*. Cambridge University Press, 1984.
- [68] A. A. Shastri and J. A. Morgan. Flux balance analysis of photoautotrophic metabolism. *Biotechnology progress*, 21(6):1617–1626, 2005.
- [69] B. Sportisse and R. Djouad. Reduction of chemical kinetics in air pollution modeling. *Journal of Computational Physics*, 164(2):354–376, 2000.
- [70] G. Stephanopoulos, A. A. Aristidou, and J. Nielsen. *Metabolic engineering: principles and methodologies*. Academic press, San Diego, California, 1998.
- [71] S. Tebbani, F. Lopes, R. Filali, D. Dumur, and D. Pareau. *CO₂ Biofixation by Microalgae. Modeling, Estimation and Control*. FOCUS. Bioengineering and Health Science Series. ISTE Ltd and Jonh Wiley & Sons, Inc., 2014.
- [72] A. Tikhonov, A. B. Vasil’eva, and A. G. Sveshnikov. *Differential Equations*. Springer-Verlag, Berlin, 1985.
- [73] F. Verhulst. Singular perturbation methods for slow–fast dynamics. *Nonlinear Dynamics*, 50(4):747–753, 2007.

- [74] M. Vitova, K. Bisova, S. Kawano, and V. Zachleder. Accumulation of energy reserves in algae: from cell cycles to biotechnological applications. *Biotechnology advances*, 33(6):1204–1218, 2015.
- [75] E. O. Voit, H. A. Martens, and S. W. Omholt. 150 years of the mass action law. *PLoS computational biology*, 11(1):e1004012, 2015.
- [76] S. Waldherr, D. A. Oyarzún, and A. Bockmayr. Dynamic optimization of metabolic networks coupled with gene expression. *Journal of theoretical biology*, 365:469–485, 2015.
- [77] E. Walter and L. Pronzato. *Identification of parametric models from experimental data*. Springer Verlag, 1997.
- [78] R. H. Wijffels, M. J. Barbosa, and M. H. Eppink. Microalgae for the production of bulk chemicals and biofuels. *Biofuels, Bioproducts and Biorefining: Innovation for a sustainable economy*, 4(3):287–295, 2010.
- [79] C. Yang, Q. Hua, and K. Shimizu. Energetics and carbon metabolism during growth of microalgal cells under photoautotrophic, mixotrophic and cyclic light-autotrophic/dark-heterotrophic conditions. *Biochemical Engineering Journal*, 6(2):87–102, 2000.