A modelling odyssey in the tree of life

Rafael Munoz Tamayo

A modelling odyssey in the tree of life

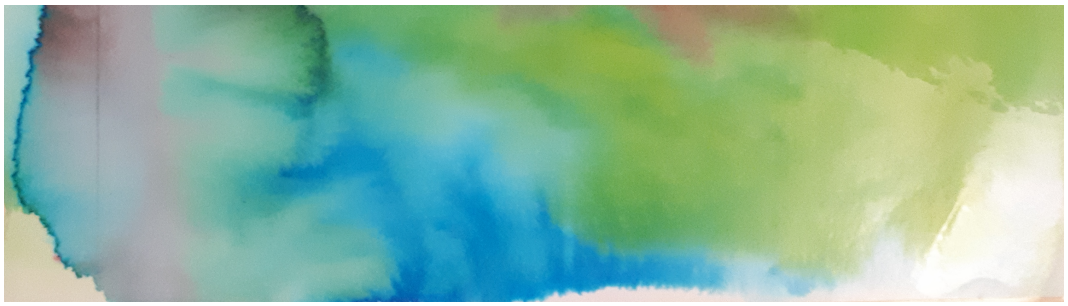
Habilitation à diriger des recherches

Rafael Muñoz-Tamayo

A modelling odyssey in the tree of life

Habilitation à diriger des recherches

Rafael Muñoz-Tamayo



Rapporteurs:

Petra Louis, The Rowett Institute (Aberdeen, United Kingdom)
Veerle Fievez, Ghent University (Ghent, Belgium)
Jérôme Harmand, Inra (Narbonne, France)

Examinateurs:

Sihem Tebbani, CentraleSupélec (Gif Sur Yvette, France)
Sylvie Giger-Reverdin, Inra (Paris, France)
Théodore Bouchez, Irstea (Antony, France)

March 22 2019
Université Paris-Sud

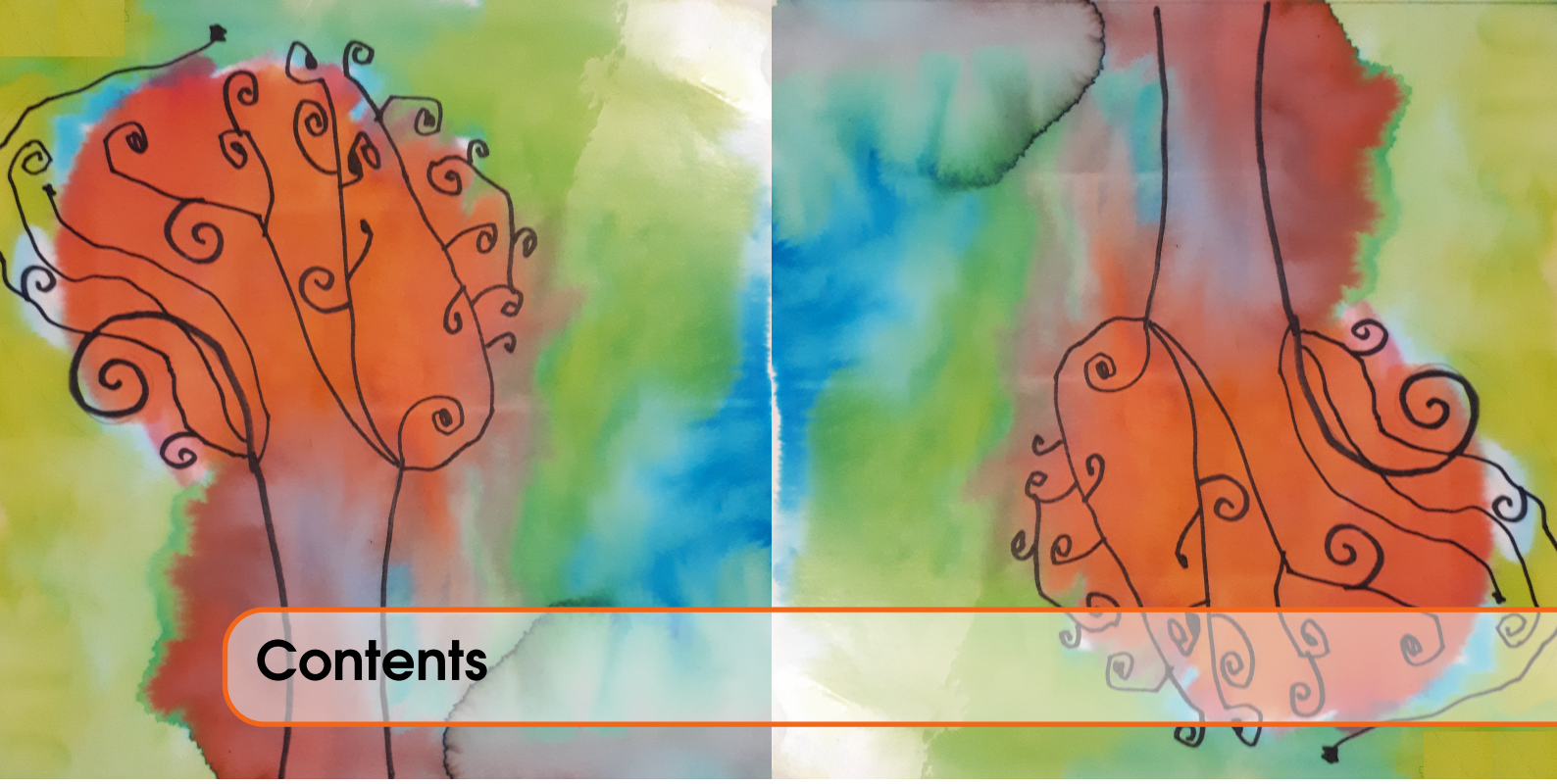
The manuscript was written using The Legrand Orange Book LaTeX Template Version 2.2 (30/3/17)
by Mathias Legrand

A Oriana y David

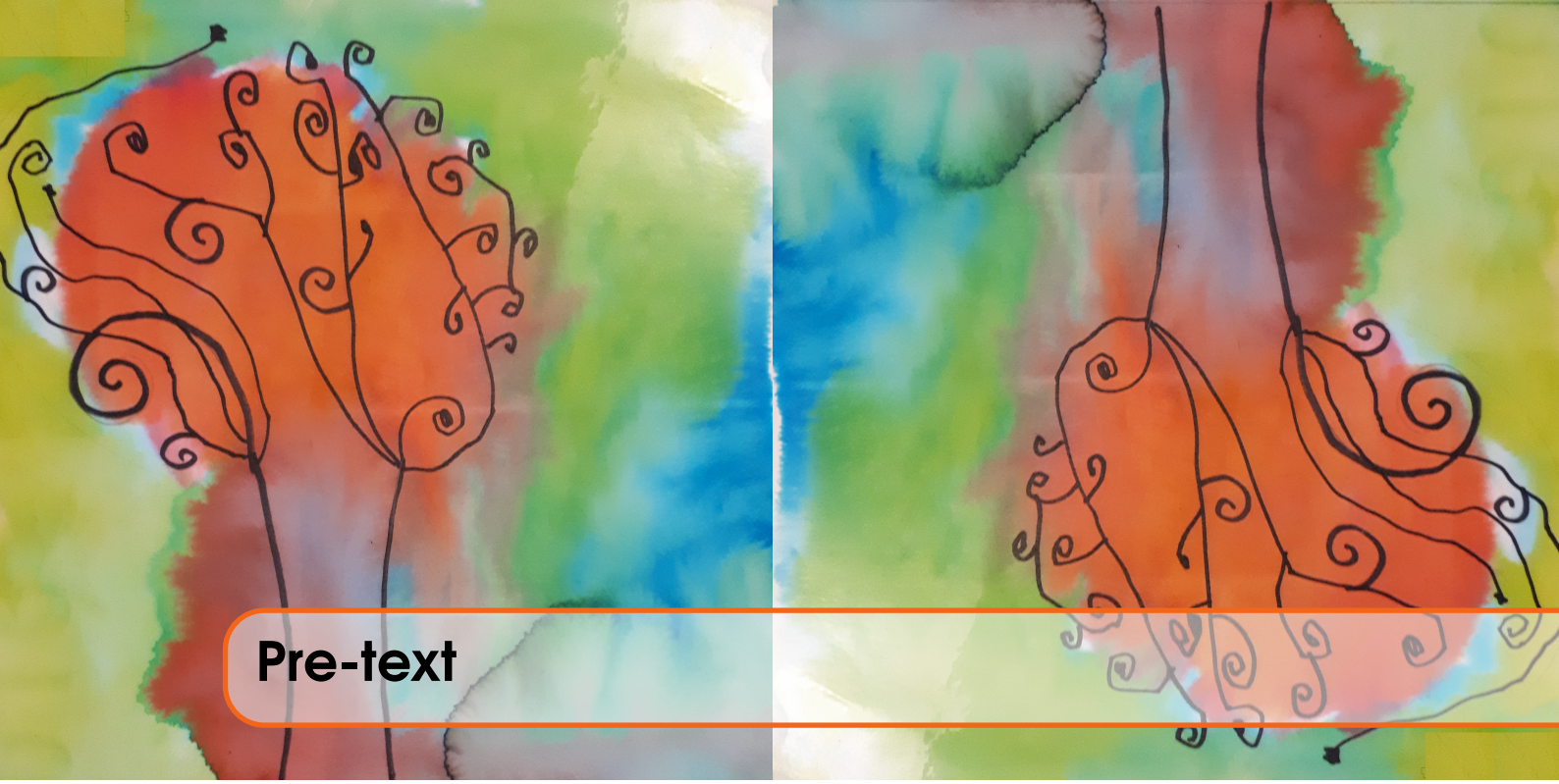
Cuando despertó, el dinosaurio todavía estaba allí

When he woke up, the dinosaur was still there

Augusto Monterroso



Contents



Pre-text

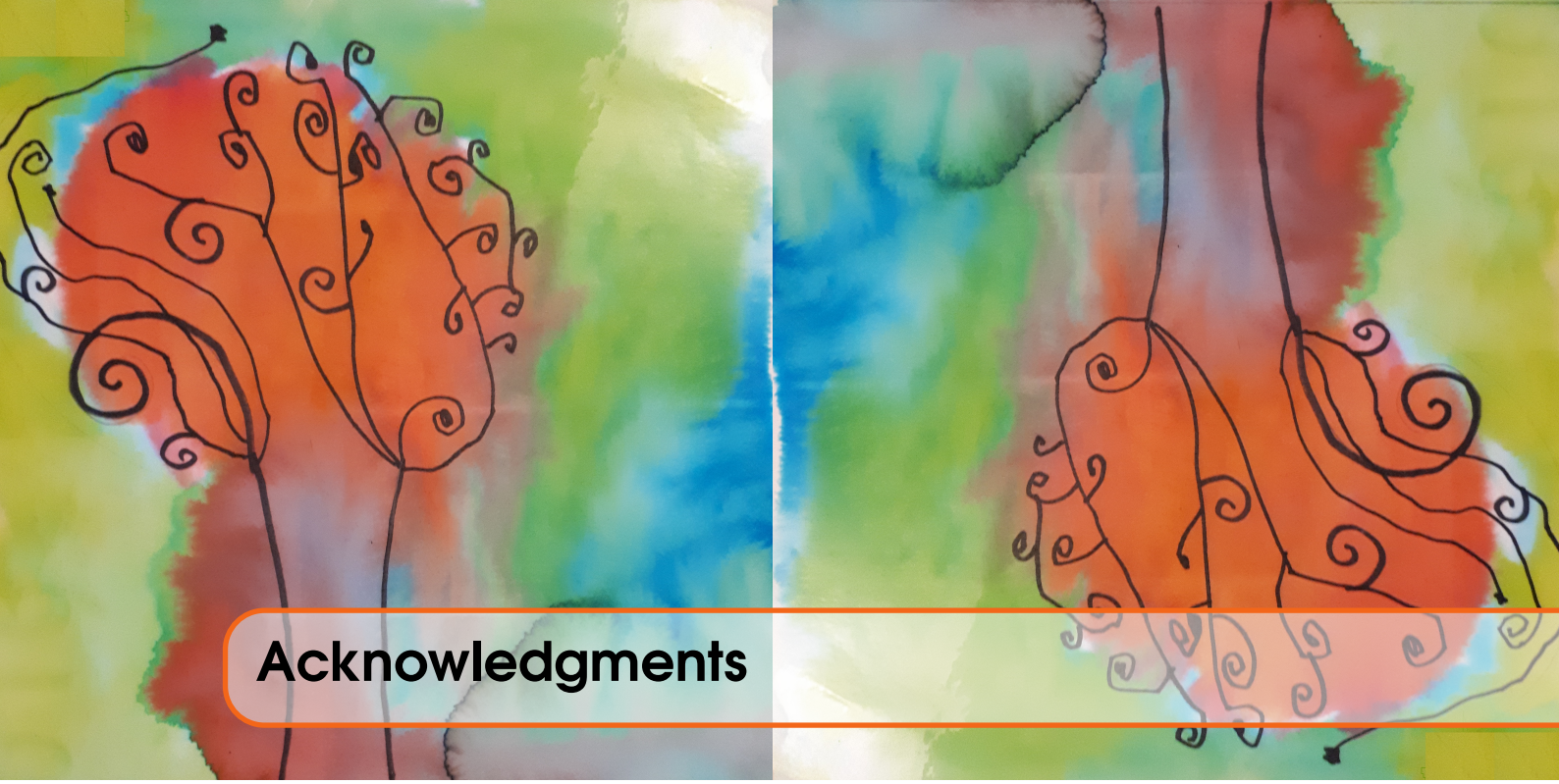
There is really nothing more to say-except why. But since why is difficult to handle, one must take refuge in how.

The bluest eye. Toni Morrison.

The road of science is paved by two kind of tiles. Tiles from the first category are determined by the traditional scientific method once the question (problem) is formulated. Tiles from the second category have, somehow, a rather random origin (the whisper of Serendipity). This metaphor tells that no straight line defines the way A $\Rightarrow$ B, since the road itself is not built in a straight fashion. Our travel therefore may imply that we cross C destinations and that during the route, we might be tempted or even determined in avoiding the original idea of arriving to B and rather modify the compass course towards a new destination D. This nature of science is fascinating.

What is this story about? It is the story of a travel. In the (early) path of this travel, my boat has navigated different seas (domains) and crossed different lands. I have encountered passionate scientists who have made my life of researcher a great adventure. The helm of my boat is called mathematical modelling. I am a mathematical modeller of biological systems with a background in chemical engineering and automatic control. My research motivation is to enhance the understanding of biological processes using modelling to combine and structure information across disciplines. My modelling work has covered all the domains of the Tree of Life, namely Bacteria, Archaea and Eukarya. In particular, I have been interested in modelling microbes in monoculture and in ecosystems. These microbial ecosystems are either natural (animal guts) or designed by humans (engineering bioreactors). Regardless of their specificities, microbial ecosystems are driven by common factors including external forces (e.g., environment), reaction pathways (fermentations), interactions between microbial individuals (e.g., competition and symbiosis). These features make the study of microbial systems a research field in which knowledge from different scientific disciplines is pivotal.

In the infinity sea of knowledge, I am a non-expert. I have been driven by the curiosity of learning from different fields including the fascinating worlds of microbiology and mathematics, but I cannot pretend to be an expert in neither maths nor biology. This situation was source of internal conflicts at the very beginning of my journey. In particular when looking for permanent job opportunities; scarce and targeted to established disciplines. Along my job applications, my profile was categorized as too much applied, to much theoretical. I shared openly my concern by commenting on the point of view of Shapiro (2014) who underscored that most of the tools used to evaluate scientific excellence are biased in favour of established disciplines and against interdisciplinary research. It is in this context that I decided to embrace interdisciplinary science and, with this decision, the fact of not being an expert in any established discipline. I found mathematical modelling as the boat that fits with my curiosity. As it will be almost impossible for a single mind to address the complexity of biological systems, I have undertaken a research work that does not rely exclusively on my own knowledge. I have been consolidating a solid network of collaborations with scientists from different disciplines including microbiology, chemistry, thermodynamics, animal nutrition, process engineering, control and system theory, and computational biology. The curiosity modelling boat has provided me with the background for connecting different disciplines and being the fulcrum in interdisciplinary projects. My research results from these fruitful collaborations. It is why along the manuscript the I must often interpreted as WE.



Acknowledgments

Tengo lo que tenía que tener.

I have what I had to have.

Tengo. Pablo Milanés.

This story has involved many people. I start to express my gratitude to my mentors at the different stages of my scientific career: Adela Londoño Carvajal (Universidad Nacional de Colombia), Fabiola Angulo (Universidad Nacional de Colombia), Carlos Enrique Ruiz (CER, Universidad Naciolal de Colombia), Béatrice Laroche (Inra), Marion Leclerc (Inra), Éric Walter (CNRS), Olivier Bernard (Inria) and Jean-Philippe Steyer (Inra). Thanks to all of you for your unconditional support. Thanks for sharing your love for science. You have been my springboard.

I thank to the Master studens, PhD and postodoc fellows that I have the opportunity to work with: Caroline Baroukh, John Fredy Ramírez Agudelo, Laura Lema Pérez, Emile Dumont and Manuel Revilla. I thank my colleague and friend César A. Aceves-Lara (INSA) who, during my postdoc, promoted a rich space of scientific sharing. It was in this environment that I could participate of passionate discussions within the framework of the PhD thesis of Carlos Eduardo Robles-Rodríguez.

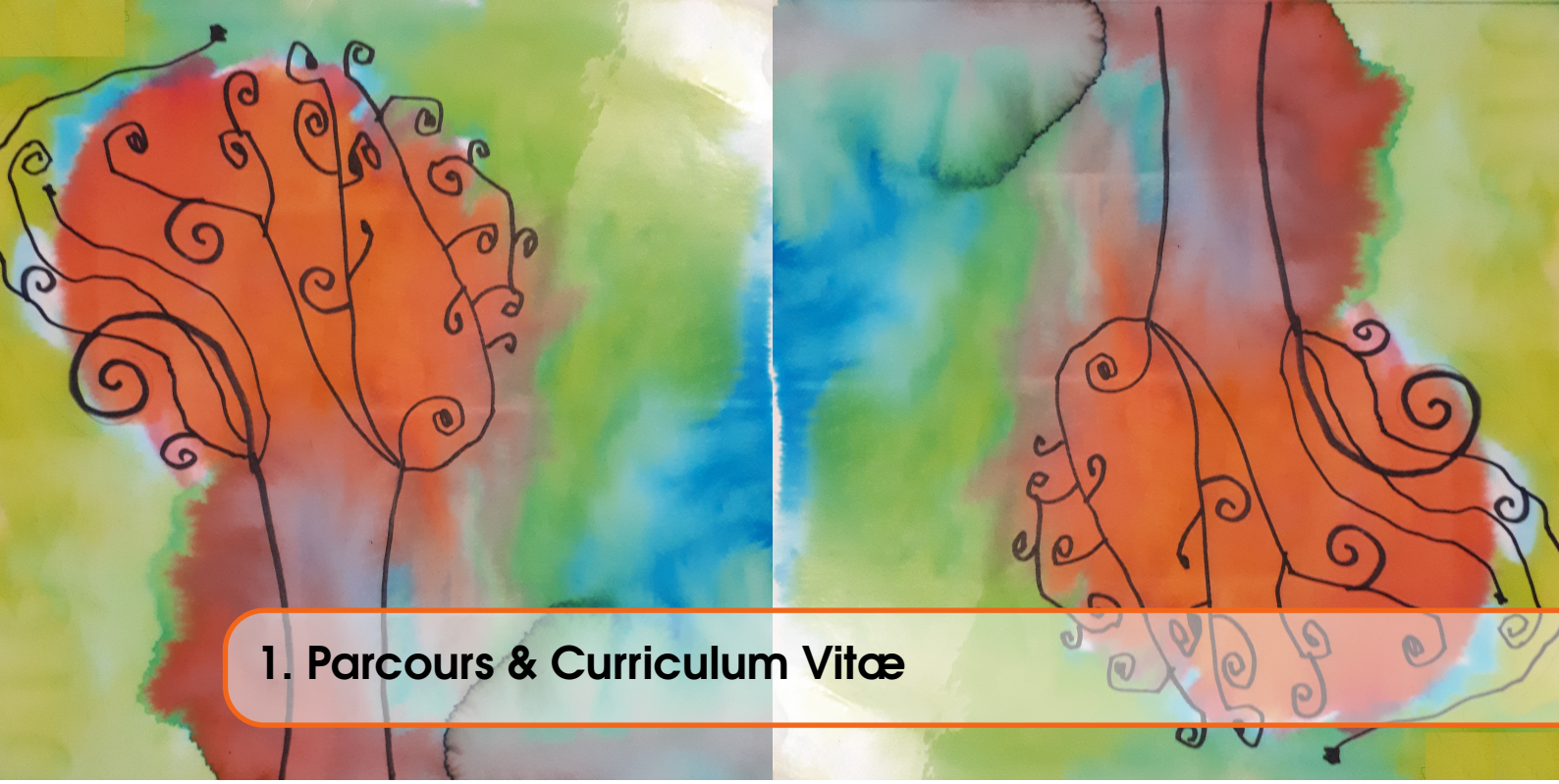
I thank Inra to open me its doors and providing me with resources and support for consolidating my scientific career. I have the privilege of belonging to the MoSAR team. Thanks to all my colleagues for the nice work environment. In particular, I would like to thank Sylvie Giger-Reverdin (Inra) and Daniel Sauvant (AgroParisTech) for introducing me to the fascinating domain of ruminant science. Many thanks to the methane team (Ophélie Dhumez, Alexandra Eymard, Joseph Tessier, Sylvie Giger-Reverdin, Christine Duvaux-Ponter) for their perseverance and commitment to undertake the construction of the phenobox; our experimental facility for phenotyping feeding behaviour and methane production in goats. Our phenobox will be instrumental for my modelling developments. I would like to thank Nicolas Friggens (Inra) for his continuous advice on how to face the challenges of a young scientist. Many thanks Nicolas for your constructive comments on this manuscript.

Thanks to all my colleagues for the fruitful collaborations and their open mindedness along these years. Special thanks to my Inra colleagues: Evelyne Forano, Milka Popova and Diego Morgavi, Nicole Morel (CNRS) and Anne Siegel (CNRS) with who I am undertaking the adventure of decrypting the rumen microbiota by integrating microbiology, thermodynamics, computational biology and modelling.

Many thanks to the members of my HDR committee: Petra Louis (The Rowett Institute, United Kingdom), Veerle Fievez (Professor, Ghent University, Belgium), Jérôme Harmand (Inra, France), Sihem Tebbani (CentraleSupélec, France), Sylvie Giger-Reverdin (Inra, France), and Théodore Bouchez (Irstea, France). I feel honoured of having them in mi jury.

A mi Familia, gracias por la musica, la magia y la palabra.

Parcours



1. Parcours & Curriculum Vitæ

Que sais-je ?

What do I know?

Essais. Montaigne.

1.1 Bio

I am a mathematical modeller of biological systems with a background in chemical engineering and automatic control. I am from Colombia. I did my undergraduate and MSc studies at `Universidad Nacional de Colombia`. I have a PhD degree in applied mathematics from `Université Paris-Sud` (France). I have consolidated my scientific career in Europe participating in research projects in two top institutions in the domain of agriculture and life sciences: `Inra` (France) and `Wageningen University` (The Netherlands), and in the leading institution of computer science `Inria` (France). My research motivation is to develop a quantitative understanding of biological processes using modelling to combine and structure information across disciplines. I have significant experience in being the fulcrum in interdisciplinary projects within a collaboration network of more than 60 international scientists of different domains. In 2014, I was appointed as junior scientist at the `MoSAR` (systemic modelling applied to ruminants) team (`Inra, AgroParisTech, Université Paris-Saclay`). My current research project aims to enhance understanding of the dynamic interplay between the diet, the rumen microbiota and the ruminant animal *via* an interdisciplinary approach covering microbiology, chemistry, thermodynamics, animal nutrition and mathematical modelling.

1.2 Education

2010 Doctor in Science (applied mathematics)
Université Paris-Sud, Supélec, INRA, France

Micalis (Microbiologie de l'Alimentation au service de la Santé), Inra, Jouy-en-Josas
MaIAGe (Mathématiques et Informatique Appliquées du Génome à l'Environnement), Inra
L2S (Laboratoire des signaux et systèmes), Supélec, CNRS, Université Paris-Sud
Thesis: Mathematical modelling of carbohydrate degradation in the human colon
PhD director: Eric Walter. Supervisors: Marion Leclerc, Béatrice Laroche

2006 Master in Automatic Control
Universidad Nacional de Colombia (Manizales)
Dissertation: Automatic control of an anaerobic reactor for leachate treatment
Supervisor: Fabiola Angulo

2004 Chemical Engineer
Universidad Nacional de Colombia (Manizales)

1.3 Research experience

Researcher 2014 - now
Modélisation Systémique Appliquée aux Ruminants (MoSAR), Inra, Paris, France
SUBJECT: Mathematical modelling of the rumen ecosystem

Post-doctoral researcher 2013 - 2014
Laboratoire d'Ingénierie des Systèmes Biologiques et des Procédés (LISBP, Inra), Toulouse, France
SUBJECT: Modelling and control of biofuel production by oleaginous yeast

Post-doctoral researcher 2011 - 2013
Biocore, Institut national de recherche en Informatique et en automatique (Inria), Sophia-Antipolis, France
SUBJECT: Modelling and optimization of microalgae growth

Post-doctoral researcher 2010 - 2011
Laboratory of food chemistry, Wageningen University, Wageningen, The Netherlands
SUBJECT: Modelling milk proteins degradation by *Lactococcus lactis*

PhD fellow 2006 - 2010
Université Paris-Sud, Supélec, Inra, Jouy-en Josas, France
THESIS: Mathematical modelling of carbohydrate degradation in the human colon

1.4 Teaching experience

2017-2018 Lecturer
Course: Parameter Identification of Ordinary Differential Equation models
Project OBIO (Optimization of Bioprocesses). PREFALC Masters in Latin-America
Pontificia Universidad Católica del Perú (Lima, Perú), Centro Universitario de la Ciénaga de la Universidad de Guadalajara, Universidad Autónoma de Guadalajara (México), Universidad Nacional de Colombia (Manizales and Medellin Colombia)

2004-2005 Lecturer
Courses: Automatic control, Heat transfer
Universidad Nacional de Colombia (Manizales), Chemical Engineering Department

1.5 Supervision

Master students

Emile Dumont (2018). Internship M1 3 months. Reconstruction of the metabolic network of cellulolytic rumen bacteria: exploration of the degradation pathways of cellulose and hemicellulose. Supervisors: Anne Siegel (CNRS), Méziane Aite (Inria), Evelyne Forano (Inra), Rafael Muñoz-Tamayo

Elizabeth Machado Maturana (2005). Universidad de Caldas. Colombia. Food Engineering undergraduate project. Modelling of a plate heat exchanger for milk processing. Supervisor: Rafael Muñoz-Tamayo

PhD fellows

Laura Lema Pérez (2018-2019). PhD Thesis Universidad Nacional (UN) de Colombia Sede Medellin, Colombia. Parameters interpretability in phenomenological based semi-physical models: a human glucose homeostasis model. Thesis directors: Hernan Dario Alvarez Zapata (UN), Jose Fernando Garcia Tirado (Center for Diabetes Technology, University of Virginia, USA). Co-supervisors: Carlos Builes-Montaño (Hospital Pablo Tobón Uribe, Colombia), Rafael Muñoz Tamayo

John Fredy Ramírez Agudelo (2017-2018). PhD Thesis Universidad de Antioquia (UdeA), Colombia. Monitoring and simulation of enteric methane emissions in dairy cows. Thesis director: Ricardo Rosero Noguera (UdeA). Co-supervisors: Sandra Lucía Posada Ochoa (UdeA), Rafael Muñoz Tamayo

Caroline Baroukh (2011 – 2014). PhD Thesis Inra-Inria, France. Metabolic modelling under non-balanced growth. Application to microalgae for biofuels production. Thesis directors: Olivier Bernard (Inria), Jean-Philippe Steyer (Inra). Co-supervisor: Rafael Muñoz Tamayo.

Postdoc fellows

Manuel Revilla (2017-2019). Integrative biology analysis and modelling of the influence of host and gut microbiota factors on the piglet sensitivity at weaning. Supervisors: Jordi Estellé (Inra), Nicolas Friggens (Inra), Rafael Muñoz-Tamayo

1.6 Responsibilities for the collectivity

2018- Referee of research proposals of the Research Foundation - Flanders (Fonds Wetenschappelijk Onderzoek - Vlaanderen, FWO), Belgium

2018- Member of the working group Big Data and systemic modelling, Inra-WUR

2017- Member of the working group Holobionte : systèmes microbiens et interactions avec l'hôte. Prospective Scientifique Interdisciplinaire de l'Inra Approches prédictives pour la biologie et l'écologie. France

2017- Reference scientist of MoSAR that ensures an updated bibliography of the team in the open access repositories (HAL, ProdInra).

2017- Scientific coordinator (Animateur) of the projet phare modélisation, prédiction des phénotypes et des réponses adaptatives. Sciences Animales Paris-Saclay (SAPS). France

2017- Scientific coordinator (Animateur) of the group MoMos (Modellers of MoSAR). France

2017 Member of the IFAC's Technical Committee on Bioprocesses and Biosystems `https://tc.ifac-control.org/8/4`

2017 Member of Jury for PhD thesis. Clothilde Villot. Recherche d'indicateurs périphériques de l'acidose ruminale chez la vache laitière. Inra, Clermont-Ferrand, France

2016-2018 Member thesis committee. Sonia Roger. Modelling digestion in pigs. Inra, Rennes, France

2016 Member of Jury for open research positions, Inra, Jouy-en-Josas, France

2013 Reviewer of PhD proposals. Universidad Nacional de Colombia, Manizales, Colombia

2013 Reviewer of research projects. Universidad del Valle; Cali, Colombia

2005-2006 Board member of the Colombian Association of Chemical Engineering

Reviewer of the following journals and conferences

Journals: The Isme Journal, Frontiers in Microbiology, Applied and Environmental Microbiology, International Journal of Food Microbiology, Anaerobe, Biotechnology and Bioengineering, Biotechnology Progress, Computers and Electronics in Agriculture, Food Biophysics, Methods in Ecology and Evolution, Journal of Animal Science, Journal of Dairy Science, Animal Feed Science and Technology

Conferences: World Congress of the International Federation of Automatic Control (IFAC), IFAC Symposium on Dynamics and Control of Process Systems (DYCOPS), European Control Conference, IEEE Mediterranean Conference on Control and Automation, International Symposium on the Nutrition of Herbivores (ISNH)

1.7 Projects

Inra

Genome-scale metabolic modelling of the predominant cellulolytic rumen bacterium *Fibrobacter succinogenes*: towards a novel paradigm for modelling the rumen microbiome (2017)[1]. Call: Crédit Incitatif PHASE. Role: coordinator

[1] Pojects in which I have participated in the proposal construction

Deciphering the Rumen Ecosystem with Advanced Modelling (2018-2019)[1]. Call: Metaprogramme MEM. Role: coordinator

France

microFicient: Relationship between digestive microbiota and feed efficiency in cattle. Leader of the WP3: Assessing and modeling interactions among microbiota communities (2016-2017)[1]. Coordinator: Yuliaxis Ramayo-Caldas (Inra). Call: Efficacité Globale de l'Elevage de Ruminants (EGER) d'APIS-GENE. Role: partner

ANR Project PigletBiota: an integrative biology-based study of the influence of intestinal microbiota composition on piglet robustness at weaning in a perspective of a limited use of antibiotics in livestock production (2017-2018). Coordinator : Jordi Estellé (Inra). Role: partner

ANR Project ProBio3: Biocatalytic production of lipidic bioproducts from renewable resources and industrial by-products: Biojet fuel application for bioenergy (2014). Coordinator: Carole Molina-Jouve (Inra/CNRS) . Role: partner

ANR Project Purple Sun: Sharing photos between photovoltainc panels and microalgae . Project leader (2013). Coordinator: Olivier Bernard (Inria). Role: partner

ANR Project Facteur 4: Non GMO enhancement of microalgae performances (2012-2013). Coordinator: Gael Bougaran (Ifremer). Role: partner

Europe

RumenPredict FACCE ERA-GAS project: Predicting appropriate GHG mitigation strategies based on modelling variables that contribute to ruminant environmental impact (2018-2019) [1]. Coordinator: Sharon Huws (Queen's University Belfast, United Kingdom). Role: partner

H2020 MASTER project: Microbiome Applications for Sustainable food systems through Technologies and Enterprise (2018-2022) [1]. Coordinator: Paul Cotter (Teagasc, Ireland). Role: partner.

1.8 Publications

1.8.1 Research articles

Muñoz-Tamayo, R., Ramírez Agudelo, J. F., Dewhurst, R.J., Miller, G., Vernon, T., and Kettle, H. (2019). A parsimonious software sensor for estimating the individual dynamic pattern of methane emissions from cattle. Animal 13,1180-1187.

Revilla, M., Friggens, N. C., Broudiscou, L. P., Lemonnier, G., Blanc, F., Ravon, L., Mercat M. J., Billon, Y., Rogel-Gaillard, C., Le Floch, N., Estellé, J. and **Muñoz-Tamayo, R.**. (2019). Towards the quantitative characterization of piglets' robustness to weaning: A modelling approach. Animal.

Lema-Perez, L., **Muñoz-Tamayo, R.**, Garcia-Tirado, J., and Alvarez, H. (2019). On parameter interpretability of phenomenological-based semiphysical models in biology. Informatics in Medicine Unlocked 15, 100158.

Robles-Rodríguez, C. E., **Muñoz-Tamayo, R.**, Bideaux, C., Gorret, N., Guillouet, S. E., Molina-Jouve, C., Roux, G. and Aceves-Lara, C. A. (2018). Modeling and optimization of lipid accumulation by *Yarrowia lipolytica* from glucose under nitrogen depletion conditions. Biotechnology and Bioengineering 115, 1137-1151.

Muñoz-Tamayo, R., Giger-Reverdin, S., and Sauvant, D. (2016). Mechanistic modelling of *in vitro* fermentation by rumen microbiota. Animal Feed Science and Technology 220, 1-21.

Baroukh, C., **Muñoz-Tamayo, R.**, Bernard, O., and Steyer, J.-P. (2016). Reply to the comment on Mathematical modeling of unicellular microalgae and cyanobacteria metabolism for biofuel production" by Baroukh et al. [Curr. Opin. Biotechnol. 2015, 33:198–205]. Current Opinion in Biotechnology 38, 200-202.

Mairet, F., **Muñoz-Tamayo, R.**, and Bernard, O. (2015). Adaptive control of light attenuation for optimizing microalgae production. Journal of Process Control 30, 117-124.

Muñoz-Tamayo, R., Martinon, P., Bougaran, G., Mairet, F., and Bernard, O. (2014). Getting the most out of it: Optimal experiments for parameter estimation of microalgae growth models. Journal of Process Control 24(6), 991-1001.

Baroukh, C., **Muñoz-Tamayo, R.**, Steyer, J.P., and Bernard, O. (2014). DRUM: a new framework for metabolic modeling under non-balanced growth. Application to the carbon Metabolism of unicellular microalgae. PLoS One 9(8). doi: ARTN e104499.

Muñoz-Tamayo, R., Mairet, F., and Bernard, O. (2013). Optimizing microalgal production in raceway systems. Biotechnology Progress 29, 543-552.

Muñoz-Tamayo, R., de Groot, J., Wierenga, P.A., Gruppen, H., Zwietering, M.H., and Sijtsma, L. (2012). Modeling peptide formation during the hydrolysis of beta-casein by *Lactococcus lactis*. Process Biochemistry 47(1), 83-93.

Muñoz-Tamayo, R., de Groot, J., Bakx, E., Wierenga, P.A., Gruppen, H., Zwietering, M.H., and Sijtsma, L. (2011). Hydrolysis of beta-casein by the cell-envelope-located P-I-type protease of *Lactococcus lactis*: A modelling approach. International Dairy Journal 21(10), 755-762.

Muñoz-Tamayo, R., Laroche, B., Walter, E., Dore, J., Duncan, S.H., Flint, H.J., and Lecler, M. (2011). Kinetic modelling of lactate utilization and butyrate production by key human colonic bacterial species. FEMS Microbiology Ecology 76, 615-624.

Muñoz-Tamayo, R., Laroche, B., Walter, E., Dore, J., and Leclerc, M. (2010). Mathematical modelling of carbohydrate degradation by human colonic microbiota. Journal of Theoretical Biology. 266, 189-201.

Tap, J., Mondot, S., Levenez, F., Pelletier, E., Caron, C., Furet, J.P., Ugarte, E., **Muñoz-Tamayo, R.**, Paslier, D.L., Nalin, R., Dore, J., and Leclerc, M (2009). Towards the human intestinal microbiota phylogenetic core. Environmental Microbiology 11, 2574-2584.

Muñoz-Tamayo, R. Angulo, F. 2006. Aproximación de estimación de estados en un reactor UASB (An approach of state estimation in a UASB reactor). Revista Colombiana de Tecnologias de Avanzada. Vol 1, No. 7, 27-33.

Muñoz-Tamayo, R., Toro-Garcia, N. 2006. Propuesta de controlador MPC para un reactor UASB (A MPC Controller Proposal for a UASB Reactor). Scientia et Technica, No. 30, 99-104.

1.8.2 Reviews

Muñoz-Tamayo, R., Puillet, L., Daniel, J.B., Sauvant, D., Martin, O., Taghipoor, M., and Blavy, P. (2018). Review: To be or not to be an identifiable model. Is this a relevant question in animal science modelling? Animal 12, 701-712.

Huws Sharon A., Creevey Christopher J., Oyama Linda B., Mizrahi Itzhak, Denman Stuart E., Popova Milka, **Muñoz-Tamayo Rafael,** Forano Evelyne, Waters Sinead M., Hess Matthias, Tapio Ilma, Smidt Hauke, Krizsan Sophie J., Yañez-Ruiz David R., Belanche Alejandro, Guan Leluo, Gruninger Robert J., McAllister Tim A., Newbold C. Jamie, Roehe Rainer, Dewhurst Richard J., Snelling Tim J., Watson Mick, Suen Garret, Hart Elizabeth H., Kingston-Smith Alison H., Scollan Nigel D., do Prado Rodolpho M., Pilau Eduardo J., Mantovani Hilario C., Attwood Graeme T., Edwards Joan E., McEwan Neil R., Morrisson Steven, Mayorga Olga L., Elliott Christopher, Morgavi Diego P. (2018). Addressing global ruminant agricultural challenges through understanding the rumen microbiome: Past, present and future. Front. Microbiol. 9, 2161

Baroukh, C., **Muñoz-Tamayo, R.**, Bernard, O., and Steyer, J.P. (2015). Mathematical modeling of unicellular microalgae and cyanobacteria metabolism for biofuel production. Current Opinion in Biotechnology 33, 198-205.

Baroukh, C., **Muñoz-Tamayo, R.**, Stever, J.P., and Bernard, O. (2015). A state of the art of metabolic networks of unicellular microalgae and cyanobacteria for biofuel production. Metabolic Engineering 30, 49-60.

1.8.3 Preprints

Muñoz-Tamayo, R., Popova, M., Tillier M., Morgavi, D. P., Morel, J. P., Fonty, G. and Morel-Desrosiers, N. (2018). Hydrogenotrophic methanogens of the mammalian gut: functionally similar, thermodynamically different. A modelling approach. bioRxiv. `https://www.biorxiv.org/content/early/2018/10/17/445171`

1.8.4 Conference proceedings

Muñoz-Tamayo, R., Popova, M., Morgavi, D. P., Graviou, D., Morel, J. P., Fonty, G., and

Morel-Desrosiers, N. (2018). Rumen methanogens exhibit specific energetic and growth dynamics: a modelling approach. 10th Int. Symp. Nutr. Herbiv. Adv. Anim. Biosci. 9, 367.

Muñoz-Tamayo, R., Ramírez Agudelo, J. F., Bedoya-Mazo S., Dewhurst, R.J., Miller, G., Rosero-Noguera R., Posada-Ochoa S.L., and Kettle, H. Two parsimonious software sensors for estimating the individual dynamic pattern of methane production by cattle. (2018). 10th Int. Symp. Nutr. Herbiv. Adv. Anim. Biosci. 9, 403.

Muñoz-Tamayo, R., Aceves-Lara, C.A., and Bideaux, C. (2014). Optimization of lipid production by oleaginous yeast in continuous culture. IFAC Papersonline 47, 6210-6215.

Muñoz-Tamayo, R., Popova, M., Morgavi, D. P., Graviou, D., Morel, J. P., Fonty, G., and Morel-Desrosiers, N. (2018). Rumen methanogens: functionally similar, thermodynamically different. A modelling approach. 11th Rowett-INRA joint symposium on gut microbiology. Aberdeen, United Kingdom.

Muñoz-Tamayo, R., Martinon, P., Bougaran, G., Mairet, F. and Bernard, O. 2013. Design of optimal experiments for parameter estimation of microalgae growth models. In Proc. 12th IFAC symposium on Computer Applications in Biotechnology. Mumbai, India.

Baroukh, C., **Muñoz-Tamayo, R.**, Steyer, J.P. and Bernard, O. 2013. A new framework for metabolic modeling under non-balanced growth. Application to carbon metabolism of unicellular microalgae. In Proc. 12th IFAC symposium on Computer Applications in Biotechnology. Mumbai, India.

Mairet, F., **Muñoz-Tamayo, R.**, and Bernard, O. 2013. Adaptive control for optimizing microalgae production. In Proc. 12th IFAC symposium on Computer Applications in Biotechnology. Mumbai, India.

Muñoz-Tamayo, R., Mairet, O. and Bernard, O.. 2013. Driving microalgal production in raceway systems to near optimal productivities. In Proc. 12th European Control Conference. Zurich, Switzerland.

Mairet, F. **Muñoz-Tamayo, R.**, and Bernard, O. 2013. Driving Species Competition in a Light-limited Chemostat. In Proc. 9th IFAC symposium on Nonlinear Control Systems. Toulouse, France. 175-180.

Muñoz-Tamayo, R., Laroche, B., Leclerc, M., and Walter, E. (2009). IDEAS: a Parameter Identification Toolbox with Symbolic Analysis of Uncertainty and its Application to Biological Modelling, in: Proc. 15th IFAC Symposium on System Identification, Saint-Malo, France. 1271-1276.

Muñoz-Tamayo, R., Laroche, B., Leclerc, M., and Walter, E. (2008). Modelling and Identification of in vitro Homoacetogenesis by Human-Colon Bacteria, in: Proc. 16th IEEE Mediterranean Conference on Control and Automation, Ajaccio, France. 1717–1722.

Muñoz-Tamayo, R., Steyer, J.P., Laroche, B., and Leclerc, M. (2007). Human colon: a

complex bioreactor. Conceptual modelling for the anaerobic digestion of the functional trophic chain., in: Proc. 11th World Congress Anaerobic Digestion Bio-energy for our Future, Brisbane, Australia: 6 pages on CD-Rom proceedings).

Angulo, F. **Muñoz, R.** and Olivar, G. 2007. Control of a bioreactor using feedback linearization. In Proc. 15th IEEE Mediterranean Conference on Control and Automation. Athens, Greece. 1368-1373.

Muñoz-Tamayo, R., Angulo, F. and Marín J.E. 2005. Una perspectiva sobre el modelado e identificación de sistemas de degradación anaerobia para el tratamiento de aguas residuales (A perspective about modelling and identification of anaerobic digestion systems for wastewater treatment). In Proc. 23th Colombian Congress of Chemical Engineering: The Productive Chains. Manizales, Colombia. 2005. 10 pages (CD-Rom proceedings).

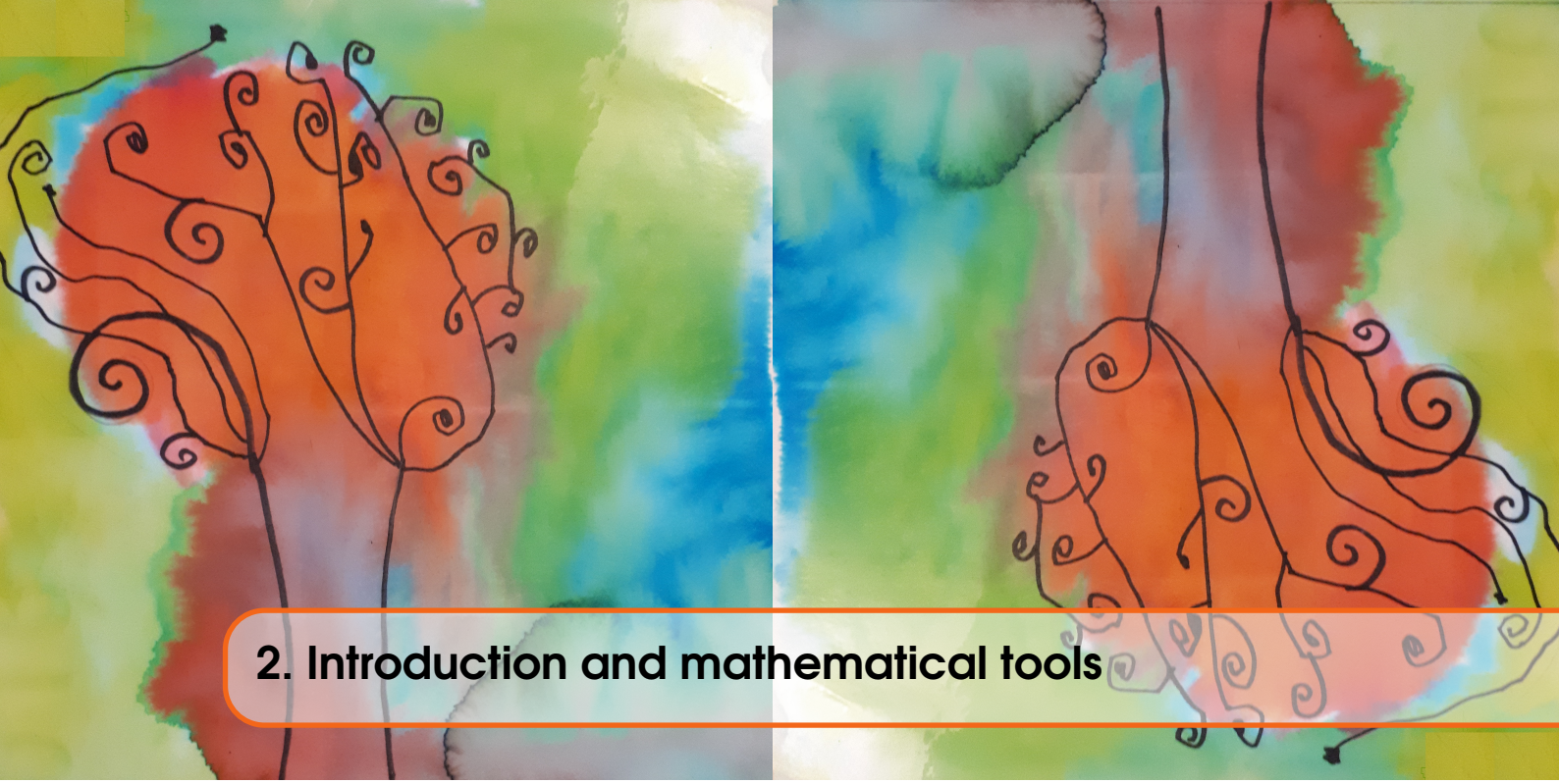
Muñoz-Tamayo, R. Angulo, F., and Marín, J.E. 2005. Modelado y control automático de Temperatura en un reactor anaerobio de manto de lodos de flujo ascendente UASB para el tratamiento de lixiviados en el rango mesofílico. Un estudio teórico (Modeling and automatic control of temperature in a UASB reactor for leachate treatment in the mesophilic range. A theoretical study). In Proc. 2th Symposium on Bio-factories: Advances of Biotechnology in Colombia. Medellín, Colombia. 12 pages (CD-Rom proceedings).

1.8.5 Popular science

Muñoz-Tamayo, R. (2016). Breve elogio a la lentitud en ciencia (A brief tribute to slowness in science), in Ciencia y Humanismo 50 años Revista Aleph (1996-2016), ed. C.E. Ruiz. (Manizales, Colombia Editorial Universidad de Caldas), 473-478. An updated translated version is available at `https://osf.io/preprints/socarxiv/n9wpg/`

Muñoz-Tamayo, R. (2014). Bacterias: pequeños organismos que gustan de las matemáticas (Bacteria: little organisms that like mathematics), in Días fantásticos. La ciencia de pasarla bien. Antología. Colleción Luminautas, ed. L.M.G. Sepúlveda. (Manizales, Colombia: Editorial Universidad de Caldas), 311-317.

II The bedrock: introductory concepts and PhD thesis



2. Introduction and mathematical tools

Algo me han dicho
la tarde y la montaña.
Ya lo he perdido.

The afternoon. The mountain.
What they told me.
Already it's gone.

Diecisiete Haiku. Jorge Luis Borges.

Biology provides countless examples of extremely complex nonlinear dynamic systems. The complexity of biological systems is determined by a large numbers of elements with diverse functionalities that interact nonlinearly to produce coherent behaviors (Kitano, 2002) in an admirable regularity and orderliness (Schrödinger, E, 1944). Mathematical modelling has come to play a central role in biology (May, 2004) by its capability of providing rational and formal representations tools for enhancing the understanding of biological systems (Legay, 1997; Bailey, 1998; Stelling, 2004). My research work has been mainly devoted to the construction of knowledge-based models of biological systems. To get onto our subject, I first define some key concepts of the modelling endavour. The concepts here discussed will be ubiquitous along these pages and in the collection of articles that structure the manuscript. These concepts are extracted mainly from a review article that resulted from a discussion that I led within the group MoMos (Modellers of MoSAR) about parameter identifiability (Muñoz-Tamayo et al., 2018b). In Sections 2.1-2.3, I briefly describe the mathematical tools that I have exploited in my modelling developments.

Definition 2.0.1 — System. A system is a conceptual abstraction and simplification of the object under study (reality). A system consists of a set of inter-related components that interact and react as a whole to external or internal stimuli (Spedding, 1988). The system is delimited by spatial and temporal boundaries. The definition of a system sets the basis for model construction. It is of common usage to refer to the object under study as a system. Hence, we talk about system dynamics, system behaviour, etc.

Definition 2.0.2 — Model. Set of mathematical equations derived from an abstraction and simplification of the real world (Spedding, 1988). A model is therefore a subjective formalization of knowledge on the system under study.

Definition 2.0.3 — Model structure. The model structure refers to the set of mathematical functions that specify the coupling between the state variables, the inputs and model outputs (observables) (Bellman and Astrom, 1970). A structural property is derived from the model equations and is (almost) independent of the values of the parameters (Walter and Pronzato, 1997).

2.1 The construction of mathematical models $\mathscr{M}(\cdot)$

The construction of a model responds to a specific goal. Overall, model construction results from two main motivations: (*i*) understanding the functions of the system under study (curiosity driven approach) and (*ii*) predicting the response of a set of variables for a given set of inputs (solution oriented approach). When the modelling target is that of understanding system functioning, model construction intends to describe at least partly the mechanisms that underlie the behaviour of the system under study by describing some individual elements of the system and their mutual inter-relation. In this case, the resulting model is referred to as a mechanistic (white box) or phenomenological-based model. On the other hand, empirical (black box) models are derived to quantify relationships between variables of interest. The mathematical formulation of black models do not integrate knowledge of the phenomena taking place in the system. A model with mechanistic and empirical components is termed a grey box model. I have developed both white and grey box models. This latter appears useful for providing simplified model structures and for handling lack of knowledge on the representation of the system's phenomena. In the following, I will denote $\mathscr{M}(\cdot)$ as the mathematical model structure.

My research work has been focused on the development of dynamic models described by ordinary differential equations (ODE). This dynamic component implies that when studying a biological variable x, I attempt to describing how x changes in time, that is to formulate the function that represents its time derivative $\frac{\mathrm{d}x}{\mathrm{d}t}$. Dynamic models are often derived from fundamentals laws (e.g., the law of conservation of mass) that govern the system behaviour. The construction of these models results from a stepwise process that include the formulation of verbal hypotheses on the system under study, the definition of the level of detail of the model structure (Schaber and Klipp, 2011; Lema Perez et al., 2019), the identification of the numerical values of the parameters and the model selection (Walter and Pronzato, 1997; Juillet et al., 2006). Whilst the law of conservation of mass is universal, its mathematical representation can lead to multiple $\mathscr{M}(\cdot)$ candidates. When multiple model candidates are available, we might be interested in selecting the $\mathscr{M}(\cdot)$ that balances predictive capability and model complexity (Akaike, 1974). The latter property is associated to the principle of parsimony. In microbial ecosystems, where the progress on

the -omics characterisation of microbes results in a large body of heterogeneous data, the parsimony principle should be taken into consideration in microbial ecosystem modelling research (Wade et al., 2016) to avoid over-parameterization (Baranyi et al., 1996).

The models that I have developed follow

$$\frac{\mathrm{d}\mathbf{x}}{\mathrm{d}t} = \dot{\mathbf{x}} = \mathbf{f}(\mathbf{x},\mathbf{u},\mathbf{p}),\ \mathbf{x}(0) = \mathbf{x}_0(\mathbf{p}) \tag{2.1}$$

where $\mathbf{x}$ is the vector of state variables ($\mathbf{x} \in \mathbb{R}^{n_x}$), $\mathbf{u}$ is the input vector ($\mathbf{u} :\in \mathbb{R}^{n_u}$), $\mathbf{p}$ is the parameter vector ($\mathbf{p} \in \mathbb{R}^{n_p}$), and $\mathbf{f}$ is a function vector ($\mathbf{f} \in \mathbb{R}^{n_x}$). In biological systems, physical and bio-chemical process occur. Equation 2.1 can thus be detailed as

$$\frac{\mathrm{d}\mathbf{x}}{\mathrm{d}t} = \mathbf{S}\nu\left(\mathbf{x},\mathbf{u},\mathbf{p}\right) + \gamma(\mathbf{x},\mathbf{u},\mathbf{p}) \tag{2.2}$$

where $\mathbf{x}$ refers to the vector of concentration of metabolites, $\mathbf{S}$ is the stoichiometry matrix that links metabolites and biochemical reactions, $\mathbf{p}$ is the parameter vector, $\nu(\cdot)$ is the vector of reaction rates, $\mathbf{u}$ is the input vector, and $\gamma(\cdot)$ is the vector of transport functions (*e.g.*, liquid-gas transfer rate). The level of detail of a biological system model is mainly determined by the dimensions of the reaction rate vector $\nu(\cdot)$ which can comprise thousands of intracellular and extracellular reactions (Reed and Palsson, 2003) or a limited number of macroscopic reactions (Bernard et al., 2006; Mairet et al., 2012; Baroukh et al., 2014). This level of detail is determined by the purpose the model is intended to answer, *e.g*, data representation, hypothesis testing, control. The model developments presented here respond to such different applications as it will be discussed later.

2.2 Parameter identification and optimal experiment design

One of the main applications of the model construction process is to describe the dynamics of a system measured by time series experimental data. The link between the reality (the data) and the virtual world $\mathscr{M}(\cdot)$ is made possible by the parameter identification (or estimation); a mathematical and numerical routine consisting in finding the value of unknown parameters of a model that best fit an experimental data set (Fig. 2.1). The inverse problem of finding the model parameters is formulated as the minimization of an adequate measure of the distance between the model observables and the experimental data.

A widely used approach to to tackle the parameter identification problem is the Maximum Likelihood (ML) approach. Let us denote $\mathbf{y}(t_i)$ the vector of data collected at time t_i and assume that it can be modelled as the sum of two components: a deterministic and a stochastic one

$$\mathbf{y}(t_i) = \mathbf{y}_\mathrm{m}(t_i,\mathbf{p}^*) + \boldsymbol{\varepsilon}_i,\ i = 1,...,n_\mathrm{t}, \tag{2.3}$$

where $\mathbf{y}_\mathrm{m}(t_i,\mathbf{p}^*)$ is the predicted output of a deterministic model $\mathscr{M}(\cdot)$ with $\mathbf{p}^*$ the true value of the parameter vector, and $\boldsymbol{\varepsilon}_i$ the vector that represents the measurement errors with n_t the number of observation times. We will assumed that the erros are independent, homoscedastic, zero mean and Gaussian ($\boldsymbol{\varepsilon}_i \sim \mathbf{N}(\mathbf{0},\boldsymbol{\Sigma})$), where $\boldsymbol{\Sigma}$ is the covariance matrix of the errors. The ML estimator maximizes the probability density $\pi_y(\mathbf{y}|\mathbf{p})$ of the observed data $\mathbf{y}$ under the assumption that they are generated by Eq. (2.3) for $\mathbf{p} = \mathbf{p}^*$. The cost function to be optimized depends on the hypothesis made on the covariance matrix $\boldsymbol{\Sigma}$

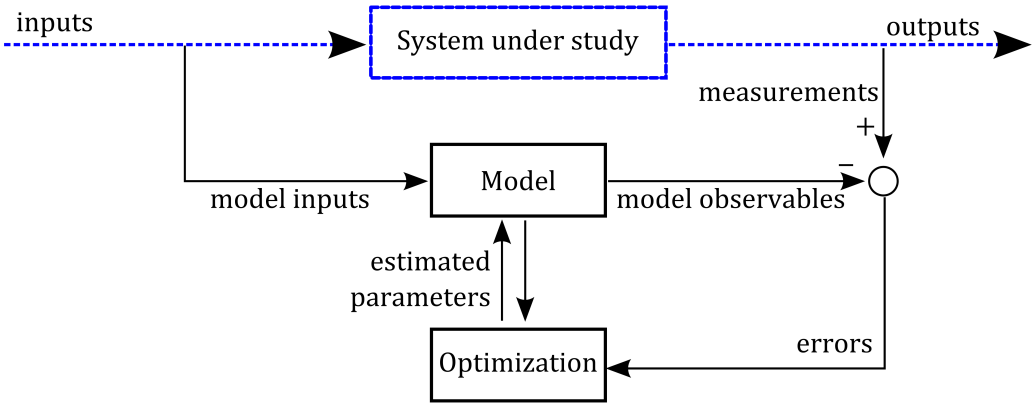


Figure 2.1: Parameter identification process. A model structure has been defined to represent the dynamics of a system under study. Dashed lines represent the system (real world) and solid lines represent the virtual mathematical/numerical world. By an experimental protocol, dynamic measurements of some quantities characterizing the system behaviour have been collected. The model parameters are identified by an optimization algorithm that minimizes the distance between the measured quantities and the model observables (outputs).

(Walter and Pronzato, 1997). For instance, If $\mathbf{\Sigma}$ is known, the ML estimator corresponds to the Gauss-Markov estimator, which minimizes the cost function

$$J_1(\mathbf{p}) = \sum_{i=1}^{n_t} [\mathbf{y}(t_i) - \mathbf{y}_m(t_i, \mathbf{p})]^T \mathbf{\Sigma}^{-1} [\mathbf{y}(t_i) - \mathbf{y}_m(t_i, \mathbf{p})]. \tag{2.4}$$

The minimization Eq. (2.4) is performed by using an optimization algorithm that can be either local (*e.g.* the Quasi-Newton method) (Walter and Pronzato, 1997) or global (*e.g.* scatter search population-based methods) (Egea et al., 2006). There are a wealth of software packages for tackling the parameter identification problem (Maiwald and Timmer, 2008; Muñoz-Tamayo et al., 2009; Balsa-Canto and Banga, 2011).

Before attempting a numerical estimation of the model parameters, we might be interested in determining if the parameter identification problem is well-posed, that is to assess if there is any chance of success in estimating a unique best value of the model parameters from available measurements. This question of uniqueness is referred to as structural identifiability and it is independent of the real experimental data.

Let $\mathscr{M}(\mathbf{p})$ represent the relationship between the inputs and outputs of the model. Let us denote by $\mathscr{M}(\mathbf{p}) = \mathscr{M}(\mathbf{p}^*)$ the equality of the input–output behaviour of the model structure obtained for the two vectors of parameters $\mathbf{p}, \mathbf{p}^*$. The structural identifiability analysis consists in determining whether this identical input-output behavior implies that the parameter vector $\mathbf{p}$ is equal to the parameter vector $\mathbf{p}^*$. We will then state that the parameter p_i is structurally identifiable if

$$\mathscr{M}(\mathbf{p}) = \mathscr{M}(\mathbf{p}^*) \Rightarrow p_i = p_i. \tag{2.5}$$

The structural identifiability analysis resolves the equality $\mathscr{M}(\mathbf{p}) = \mathscr{M}(\mathbf{p}^*))$, expressed into a set of equations in $\mathbf{p}$. Methods for structural indentifiability testing include the Laplace transform, Taylor series, generating series, similarity transformation and differential algebra (Walter and Pronzato, 1997; Chis et al., 2011b; Raue et al., 2014). Structural

identifiability analysis can turn out to be a technical difficult task. This technical hurdle has been overcome by the development of dedicated software tools (Bellu et al., 2007; Chis et al., 2011a; Karlsson et al., 2012).

The mathematical framework of parameter identification is of great usefulness for the design of highly informative experiments devoted to enhance understanding of system response. The information content of an experiment can be formalised mathematically by the capability of providing data allowing an accurate parameter identification. This aspect is addressed by practical identifiability which takes into consideration the available measurements quality. Maximizing the informative content of an experiment is the realm of optimal experiment design (OED) for parameter identification (Walter and Pronzato, 1997; Balsa-Canto et al., 2008). Classical OED approaches are based on the optimization of a scalar function of the Fisher information matrix (FIM), since this matrix is the core for the calculation of the confidence intervals of the parameter estimates. Using a local design approach applied for the nominal vector $\widehat{\mathbf{p}}$, The FIM is computed as

$$\mathbf{F}(\widehat{\mathbf{p}}) = \frac{1}{\sigma^2}\int_0^{t_\mathrm{f}} \left[\frac{\partial y_\mathrm{m}}{\partial \mathbf{p}}\right]^\mathrm{T}_{(t_i,\widehat{\mathbf{p}})} \left[\frac{\partial y_\mathrm{m}}{\partial \mathbf{p}}\right]_{(t_i,\widehat{\mathbf{p}})} dt. \tag{2.6}$$

Once the FIM is calculated, the covariance matrix $\mathbf{P}$ of the estimator can be approximated to

$$\widehat{\mathbf{P}} = \mathbf{F}^{-1}(\widehat{\mathbf{p}}). \tag{2.7}$$

The square root η_j of the jth diagonal element of $\widehat{\mathbf{P}}$ is an estimate of the standard deviation of the paramater $\widehat{p}_j$.

The OED problem can be then formulated as an optimization problem where an optimal configuration of the experimental protocol must be found. Such an experimental configuration is defined by the decision vector $\boldsymbol{\phi}_\mathrm{d}$ that contains, for instance, the sampling times, the initial conditions, temperature and pH conditions, etc. The ODE problem is then defined as

$$\begin{aligned} &\max_{\boldsymbol{\phi}_\mathrm{d}} \; j(\mathbf{F}(\widehat{\mathbf{p}})) \\ &\text{s.t.} \\ &\quad \boldsymbol{\phi}_{\mathrm{d,min}} \leq \boldsymbol{\phi}_\mathrm{d} \leq \boldsymbol{\phi}_{\mathrm{d,max}} \\ &\quad \mathscr{M}(\widehat{\mathbf{p}}). \end{aligned} \tag{2.8}$$

where $j(\mathbf{F})$ is the cost function to be maximized. Typical cost functions are the determinant of the FIM (D-optimality criterion) and the smallest eigenvalue of the FIM (E-optimality criterion). A D-optimal design minimizes the volume of the confidence ellipsoids for the parameters, while an E-optimal design minimizes the maximum diameter of the confidence ellipsoids for the parameters (Walter and Pronzato, 1997). T

2.3 System optimization

Mathematical models are instrumental for devising control strategies to drive the systems towards optimal operation. Such an optimal operation is formulated by an optimal control

problem that consists in finding the time evolution of the manipulated variables (*e.g.*, feeding rate) maximizing a given criterion (*e.g.*,biomass productivity) on a finite time horizon t_f. Let ψ denote the criterion (performance index) to be optimized and $\mathbf{u}$ the control (manipulated) variables. The optimal control problem of a system represented by $\mathscr{M}(\cdot)$ can be formulated as follows

$$\begin{aligned} &\max_{\mathbf{u}} \int_{t_0}^{t_f} \psi(t,\mathbf{u})\, dt. \\ &\text{s.t.} \\ &\quad \mathbf{u}_{\min} \leq \mathbf{u} \leq \mathbf{u}_{\max} \\ &\quad \mathscr{M}(\cdot). \end{aligned} \tag{2.9}$$

This problem can be solved by indirect methods such as the Pontryagin's maximum principle (Pontryagin et al., 1962) that establishes the set of necessary conditions to be satisfied by the optimal control. This theoretical development turns out to be rather difficult for applications with complex models. Often, the only possibility is then to use methods based on numerical optimization by discretization of the control variables. These methods are the control vector parameterization (CVP) and the total discretization approach. For the CVP approach, the control variables are approximated by a set of basis functions that depend on a finite number of real parameters. In the total discretization approach, all state and control variables are discretized w.r.t. time (Chachuat et al., 2006, 2009). Sotware tools are availabe for both the `CVP` (Hirmajer et al., 2009) and the `total discretization approach` (Bonnans et al., 2017).

2.4 Outline

I was strongly inspired by the HDR of Madalena Chaves (2013) to structure this manuscript. The following chapters briefly summarize my research contribution by referring to a collection of selected articles. To get a whole picture of my research, the interested reader can access to the papers by the links provided in the electronic version of this report or by writing me an e-mail to rafael.munoz-tamayo@inra.fr. My modelling odyssey in the tree of life covers research on bacteria, microalgae, yeast and animals.

In **Chapter 3**, I investigate the dynamics of fermentation by human colonic microbiota.

Chapter 4 studies the hydrolysis of milk protein by a *Lactococcus lactis* bacterium.

Chapter 5 analyzes microalgae processes.

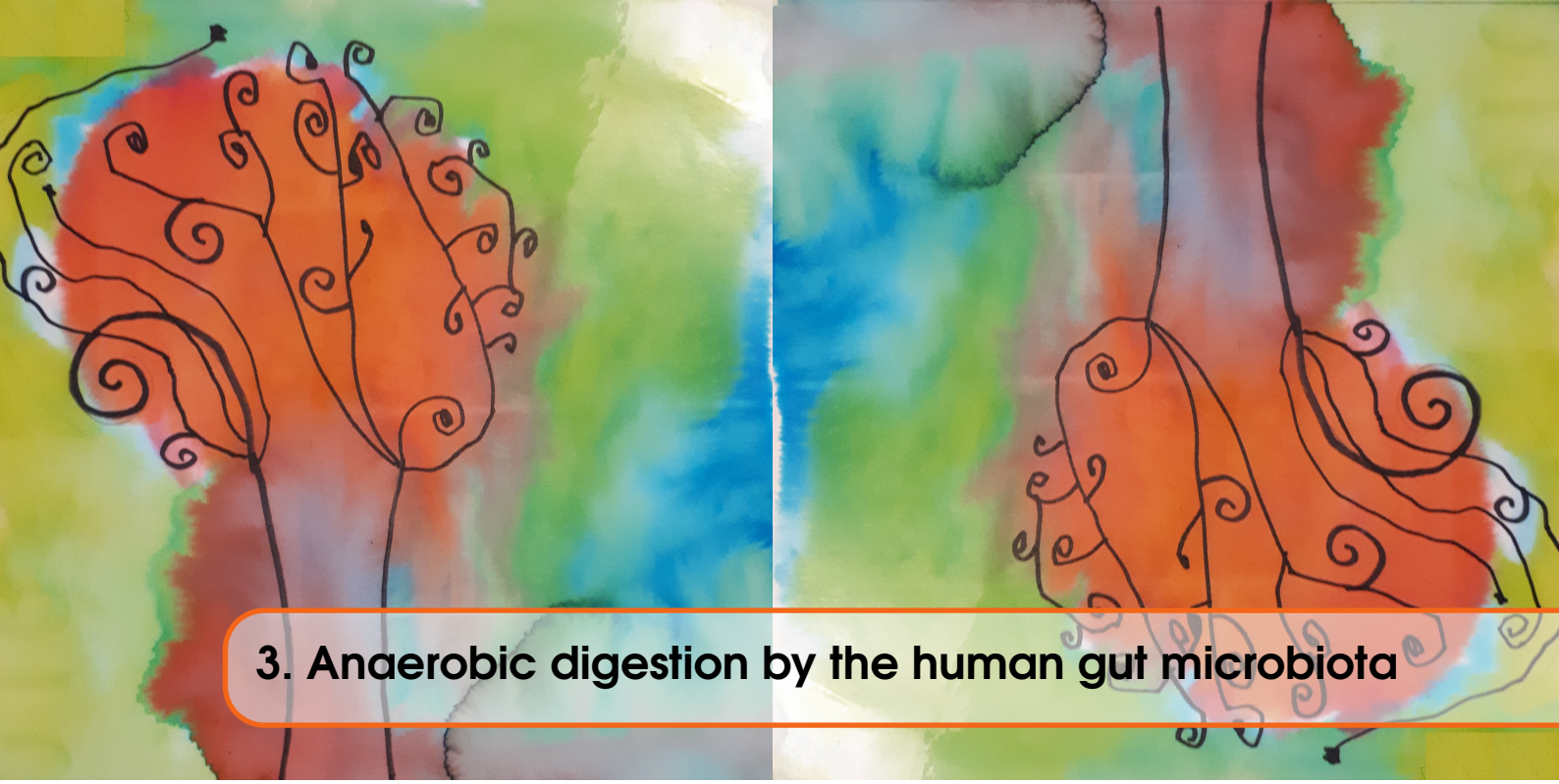
Chapter 6 studies a process of lipid yeast production.

In **Chapter 7**, I address the fermentation and methanogenesis by rumen microbiota and analyze the link between animal feeding behaviour and methane production.

In **Chapter 10**, I establish my prospective research roadmap.

In **Chapter 11**, I present a brief essay about the need of a slow science.

Chapter 12 closes the manuscript with concluding remarks.



3. Anaerobic digestion by the human gut microbiota

- Mais c'est pour approcher du ciel, que votre frère reste là-haut?
- Mio fratello sostiene, risposi, che chi vuole guardare bene la terra deve tenersi alla distanza necessaria.

- But is it to be nearer the sky that your brother stays up there?.
- My brother considers that anyone who wants to see the earth properly must keep himself at a necessary distance from it.

Il Barone rampante. Italo Calvino.

This chapter synthesises the research work of my PhD thesis (Inra, Jouy-en-Josas, France).

I started the research path during my Master studies on the topic of anaerobic digestion (AD) for wastewater treatment (Muñoz-Tamayo et al., 2005; Muñoz-Tamayo and Angulo, 2006; Muñoz-Tamayo and Toro García, 2006; Angulo et al., 2007). This first step was fundamental for my career, since I became passionate about AD and more importantly to interdisciplinary approaches that integrate biochemistry, mathematical modelling and automatic control. My Master project drove me to another fascination anaerobic system: the human gut.

The human colon houses a complex and subject-specific community of microorganisms that forms the human colonic microbiota. Because of the large repertoire of metabolic functions performed by this microbial consortium, it has been proposed to consider the human colonic ecosystem as an organ. The human colonic microbiota has been recognized as an important player in gastrointestinal tract homeostasis, because of its involvement in the development of immune function. Furthermore, this microbial community has been shown to be related to pathologies such as inflammatory bowel disease and obesity. Gut microbes are responsible of the breakdown of polysaccharides that are not digested in

the upper intestine, mediating many of the effects of diet upon gut health (Flint et al., 2007). Fermentation produces essential vitamins, co-factors, and metabolites with health-promoting effects such as short chain fatty acids (SCFA), mainly acetate, propionate and butyrate. Acetate is utilized by the brain, the heart and in peripheral tissues. Propionate is taken by the liver. It is a precursor of glucogenesis and protein synthesis. Butyrate is the preferred energy source for colonic epithelial cells (Zoetendal et al., 2008).

For obvious ethical reasons and technical limitations, the human colon is almost inaccessible for experimentation. This, in conjunction with the complexity of the system, makes the large intestine an ecosystem largely unexplored. Hence, the understanding of the mechanisms underlying the interplay between diet, microbiota and human health is far to be complete. The purpose of the thesis project was to develop a mathematical model of carbohydrate degradation by the human colonic microbiota. We aimed at providing an *in silico* approach to contribute to a better understanding of the fermentation pattern in the human colon.

The model building process was typical of a situation where the experimental data are very limited and where various and heterogeneous sources of information must be used in combination to get a usable model. I compiled and analysed a large body of information about the human colon from literature review (Macfarlane and Cummings, 1991) and discussion with microbiologists. This enabled me to establish the knowledge basis of the model. The above information included physiology of the intestine, metabolic reactions catalysed by the human colonic microbiota and transport phenomena in the system. We looked at the human colon as a bioreactor and defined the conceptual framework of the model structure which includes three main features, namely (*i*) hydraulic behaviour, (*ii*) transport phenomena and (*iii*) reaction pathway (Muñoz-Tamayo et al., 2007). I took advantage of the extensive research on anaerobic digestion modelling to construct the model of the human colonic fermentation. The anaerobic digestion model ADM1 (Batstone et al., 2002) represents the state of the art in the modelling of anaerobic digestion processes. I made use of ADM1 as basis and modified it to account for the specific characteristics of the human colon. The metabolic conversions were represented in an aggregated pathway. Because of the high number of bacterial species ($\approx$1000), the explicit incorporation of individual species into the model would have led to a high dimension structure both in state and parameter vectors. Instead, I represented the microbiota in functional groups according to their role on the metabolic pathway. This assumption is in line with the observation that, despite subject- specificity in the colonic microbiota, humans share a common core of microbial functions as identified in our work (Tap et al., 2009). To make the model development possible, knowledge-based simplification, control theoretic tools, identifiability testing and multivariable identification were satisfactorily applied for dealing with the simplification of the model and assignment of the numerical values of its parameters. The model did not take into account some aspects that are important on the human colon dynamics. To enhance the mechanistic of the model, further extensions should include (*i*) the effect of fibre on the retention time, (*ii*) the discrimination of the diet into its components: xylan, starch, inulin, etc, (iv) the incorporation of microbial genomic information, and (v) consideration of fluid mechanics. This latter aspect has been recently tackled by my colleagues (Labarthe et al., 2018).

Since dynamic data on fermentation in the human colon were not available, we decided to

exploit information from *in vitro* bacterial experiments for providing prior estimates for the whole fermentation model. To perform the parameter estimation of submodels associated to the *in vitro* experiments, we developed the Matlab® toolbox IDEAS (IDEntification and Analysis of Sensitivity), described in the **article 3.1** (Muñoz-Tamayo et al., 2009). Our interest in developing this toolbox was to provide an easy-to-use tool with open source code. IDEAS carries out the parameter estimation of ODE models via the maximum likelihood approach. The optimization step is carried out via the optimization algorithms included in Matlab®, such as the Quasi-Newton method. The main originality of the IDEAS resides on the symbolic computation of the sensitivity functions, which are further utilized to calculate the Fisher Information Matrix (FIM) and the confidence intervals of the parameter estimates. IDEAS is freely available at `http://genome.jouy.inra.fr/logiciels/IDEAS`.

We analyzed *in vitro* experiments covering two mechanisms of the full fermentation pathway, namely homoacetogenesis and butyrate production by lactate utilizing bacteria. The **article 3.2** (Muñoz-Tamayo et al., 2008) tackled the modelling of the homoacetogenesis pathway by *Blautia hydrogenotrophica*, where hydrogen reacts with carbon dioxide to produce acetate.This mechanism is very important in the removal of the hydrogen produced by the oxidation of organic matter contained in food. This work includes a structural identifiability analysis using the approach developed by Denis-vidal and Joly-blanchard (2004). The parameter estimation was performed using experimental data from Bernalier et al. (1996). The **article 3.3** (Muñoz-Tamayo et al., 2011b) tackled the modelling of lactate utilisation pathway using experimental data from Duncan et al. (2004) with two clostridial members: *Eubacterium hallii* L2-7 and *Anaerostipes coli SS2/1*. The biological relevance of this pathway relates to the role of butyrate as the preferred energy source for colonocytes. In contrast, accumulation of high concentrations of lactate is detrimental to gut health.

Finally, the **article 3.4** (Muñoz-Tamayo et al., 2010) presents the whole model of colonic fermentation. The schematics of our model is displayed in Figure 3.1 taking together the hydraulic representation of the system and a macroscopic description of the fermentation pathway. Our model provides a dynamic and spatial picture of the fermentation pattern along the colon. The model was formulated by an ODE model derived from mass balances and implemented in Simulink/Matlab®. The code is available for academic purposes. Model simulations provided an adequate qualitative representation of the human colon. The spatial concentration of short chain fatty acids, total microbial concentration, gas production and excretion frequency as predicted by the model were found to be in agreement with information reported in literature. Furthermore, the model was used to address questions that are difficult to elucidate by means of experimentation. In particular, we used the model to determine the role of the mucus on the physiology of the human colon. The mucus provides conditions for microbial aggregation. It is also an additional carbon source for the microbiota and finally, the microorganisms in the mucus can enrich the luminal microbial community. From the simulations, it was suggested that the microbial aggregation mechanism is the most relevant factor by which the mucus contributes to the maintaining of physiological conditions.

Our model is the first knowledge-based model describing carbohydrate degradation in the human colon. This work has set an important basis for further developments on gut modelling (Widder et al., 2016) and has inspired different works (Moorthy and Eberl,

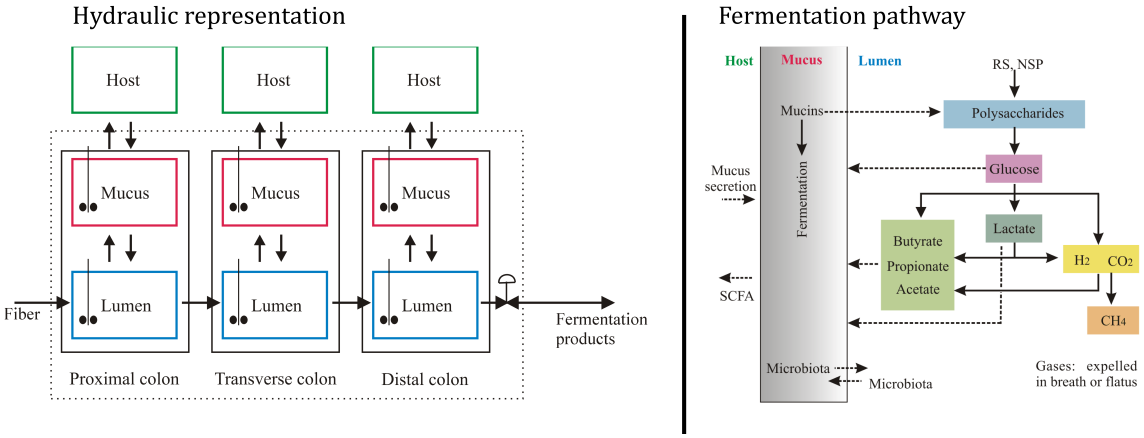


Figure 3.1: Schematics of our human colon model.

2014; Moorthy et al., 2015; Labarthe et al., 2018), including my own developments on rumen fermentation modelling (Muñoz-Tamayo et al., 2016a) that will be discussed in **Chapter 7**.

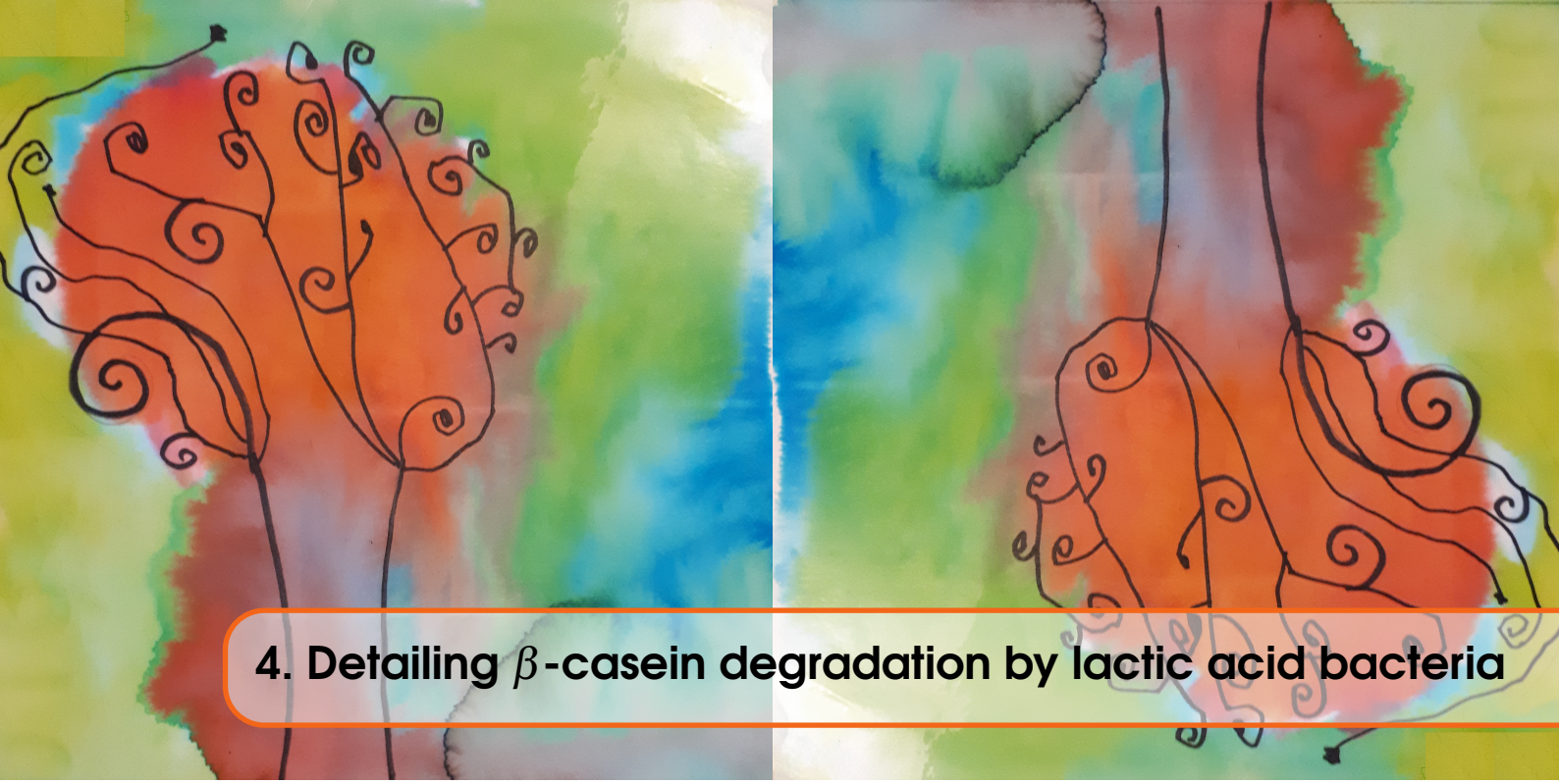
3.1 IDEAS: a parameter identification toolbox with symbolic analysis of uncertainty and its application to biological modelling. Muñoz-Tamayo, R., Laroche, B., Leclerc, M., and Walter, E. (2009) In: Proc. 15th IFAC Symposium on System Identification, Saint-Malo, France. 1271-1276.

3.2 Modelling and identification of *in vitro* homoacetogenesis by human-colon bacteria. Muñoz-Tamayo, R., Laroche, B., Leclerc, M., and Walter, E. (2008). In: Proc. 16th IEEE Mediterranean Conference on Control and Automation, Ajaccio, France. 1717–1722.

3.3 Kinetic modelling of lactate utilization and butyrate production by key human colonic bacterial species. Muñoz-Tamayo, R., Laroche, B., Walter, E., Dore, J., Duncan, S.H., Flint, H.J., and Leclerc, M. (2011). FEMS Microbiology Ecology 76, 615-624.

3.4 Mathematical modelling of carbohydrate degradation by human colonic microbiota. Muñoz-Tamayo, R., Laroche, B., Walter, E., Dore, J., and Leclerc, M. (2010). Journal of Theoretical Biology. 266, 189-201.

III Synthesis of research activities



4. Detailing β-casein degradation by lactic acid bacteria

If you surrendered to the air, you could ride it.

Song of Solomon. Toni Morrison.

This chapter synthesises the research work of my first postdoc (Wageningen University, Wageningen, The Netherlands).

Lactic acid bacteria (LAB) are widely used as starter cultures in dairy fermentation processes, most notably cheeses. Thanks to their proteolytic machinery, LAB are able to use milk proteins (mainly caseins) as nitrogen sources. Casein hydrolysis is the first step in the proteolysis. The hydrolysis of caseins is mediated by cell-envelope located proteases such as PrtP_I. The degradation of β-casein by PrtP_I can lead to the formation of more than 100 peptides with a wide range of lengths. These peptides contribute to the sensory and functional characteristics of the dairy products, *i.e.*, flavour, texture and nutritional supply. In this project, we aimed at providing a mathematical description of the dynamics of peptides formation and hydrolysis of β-casein by the enzyme PrtP_I of LAB. Experiments were carried out with *Lactococcus lactis* IM17, a strain that is deficient for autolysin and for the oligopeptide transport system. The plasmid pLP712, encoding PrtPI was incorporated into *L. lactis* IM17. Experiments were performed to characterize the hydrolysis of β-casein. I built a kinetic model for describing the hydrolysis of intact β-casein. The resulting model was effective in describing the hydrolysis in a broad range of initial protein conditions. These results are detailed in the **article 4.1** (Muñoz-Tamayo et al., 2011a).

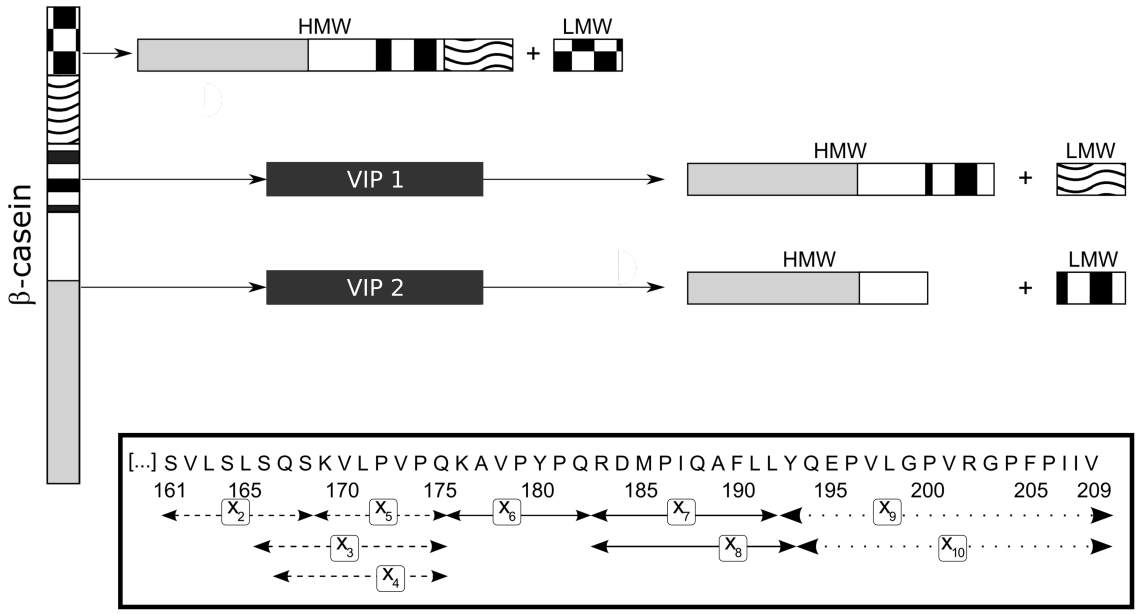
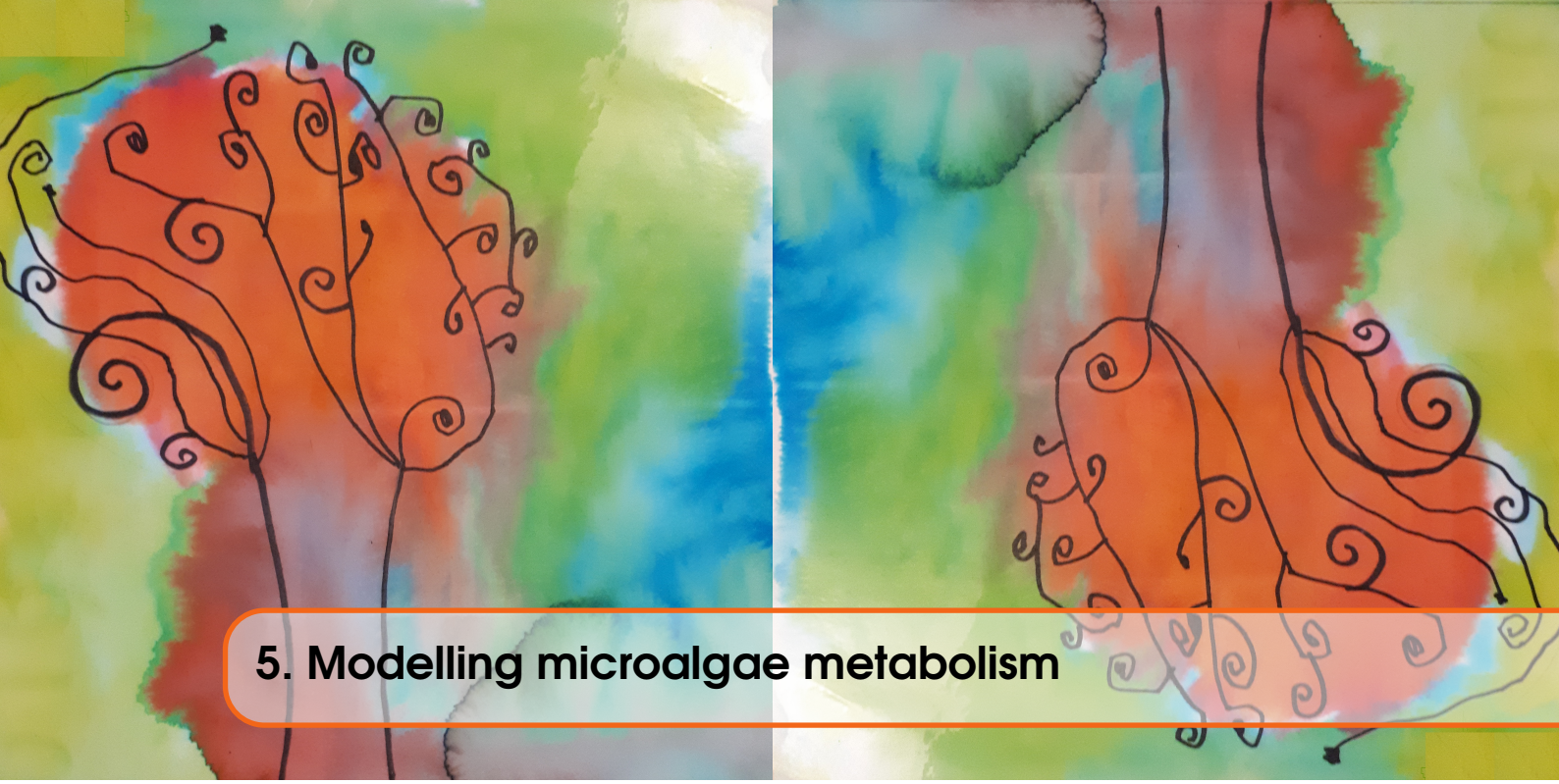


Figure 4.1: The top plot is the graphical representation of our VIP model to describe the dynamics of LMW and HMW peptides during β-casein hydrolysis. The boxes represent a set of either LMW or HMW peptides in a specific region of the β-casein sequence. The bottom box shows the location of nine quantified LMW peptides in the β-casein molecule and their clustering with respect to their dynamics. Only the fragment 161–209 of the primary sequence of β-casein is shown. Three dynamic pools were identified. Dotted arrows: peptides released directly from the breakdown of intact β-casein. The other peptides require one intermediate step to be described. Dashed arrows: peptides released from the breakdown of the VIP 1, solid arrows: peptides released from the breakdown of the VIP 2.

In a second study, I developed a model describing the dynamics of low molecular weight (LMW) and high molecular weight (HMW) peptides. To render our approach useful for practical applications, I strove in the construction of a simple model that could also provide in some extent a mechanistic understanding of process dynamics. Differently to the strategy applied for constructing our human colon model (Muñoz-Tamayo et al., 2010), here I performed an input-output behaviour analysis of the dynamic data of peptides and β-casein to define the model structure. By using the Laplace transform, I determined the structure (number of poles) of the transfer functions for each peptide using the System Identification Toolbox of Matlab® (Ljung, 2007). I integrated further this information into a mass-balance model. I called this hybrid approach as the VIP (virtual intermediate peptides) model. This study is described in the **article 4.2** (Muñoz-Tamayo et al., 2012). Our modelling approach enabled us to describe the dynamics of some peptides and quantify the dependency of the hydrolysis rate of intact β-casein on the initial protein concentration. We suggested that this effect is due to the formation of aggregates (micelles) and the competition between released peptides and intact protein for the active site of the enzyme. In addition, the model was used to cluster the peptides with respect to their dynamic characteristics. This clustering enabled us to hypothesize on the mechanism of action of the enzyme $PrtP_I$. Figure 4.1 displays the representation of the VIP model to describe the formation of LMW and HMW peptides. The Figure also shows the clustering of the peptides with respect to their dynamics.

4.1 Hydrolysis of beta-casein by the cell-envelope-located P-I-type protease of *Lactococcus lactis*: A modelling approach. Muñoz-Tamayo, R., de Groot, J., Bakx, E., Wierenga, P.A., Gruppen, H., Zwietering, M.H., and Sijtsma, L. (2011). International Dairy Journal 21, 755-762.

4.2 Modeling peptide formation during the hydrolysis of beta-casein by *Lactococcus lactis*. Muñoz-Tamayo, R., de Groot, J., Wierenga, P.A., Gruppen, H., Zwietering, M.H., and Sijtsma, L. (2012). Process Biochemistry 47, 83-93.



5. Modelling microalgae metabolism

Los Cronopios vinieron furtivamente, esos objetos verdes y húmedos.
Rodearon al fama y lo compadecían diciéndole así:
- Cronopio cronopio cronopio.
Y el Fama comprendía, y su soledad era menos amarga.

The Cronopios, those wet green objects, came forward furtively and
commiserated with him, speaking like this:
- Cronopio, cronopio, cronopio.
And the Fama understood, and his solitude was less embittered.

Historias de cronopios y de famas. Julio Cortazar.

This chapter synthesises the research work of my second postdoc (Inria, Sophia-Antipolis, France). During the postodoc, I participate in the supervision of the PhD thesis of Caroline Baroukh.

Microalgae have been raised as promising feedstock for the production of high value compounds. The commercial use of microalgae includes applications in food industry and cosmetics. Moreover, some microalgal species have been identified as a potential renewable source for biodiesel production. Microalgae production in large scale takes place in raceway systems (outdoor ponds). Despite the favourable characteristics mentioned above, microalgae production in a sustained and large scale basis is probably carried out far from an optimal working mode. This difficulty results from the nature of a process that is periodically forced by environmental factors such as sunlight and temperature. In this respect, mathematical models offer a powerful tool to be exploited. In this project, we developed mathematical models that account for the main factors affecting microalgae growth in raceway systems. On a model basis, we designed optimal control strategies that render microalgae-based process economically sustainable.

Different mathematical models (Bernard, 2011; Mairet et al., 2011) were integrated to represent the dynamics of microalgae growth and lipid production in raceway systems. Our *in silico* case study was based on the experimental configuration of a pilot-scale open raceway (Algotron) located at Inra (Narbonne, France). The resulting model was used to design strategies for optimal operation in continuous mode. Two strategies were developed. The first one resides in solving numerically an optimal control problem in which the input flow rate of the raceway is calculated such that the productivity in microalgae biomass is maximized on a finite time horizon. This strategy is in open-loop mode, that its the optimal flow rate is calculated without any feedback from the real state of the system. The open-loop controller contrasts with the closed-loop controller in which the manipulated variable is calculated to drive the controllled variable towards a specific target (set point). In the second strategy, we aimed at translating the optimization problem into a regulation problem *i.e.*, by designing a closed-loop controller that regulates a given variable. In this case, the optimal set point can be determined from the solution of the optimization problem (Tebbani et al., 2014). This strategy follows the approach of self-optimizing control (Skogestad, 2000). Since light absorption governs the performance of the system, we proposed to regulate the efficiency of light absorption. The two strategies were compared by means of numerical simulations. Our closed-loop controller performs almost as well as the optimal open loop control and has the advantage of being more robust to perturbations. We further developed an adaptive closed-loop controller to regulate the light attenuation factor for optimizing biomass productivity under realistic day–night cycles (Mairet et al., 2015). Our modelling work has inspired numerical studies on optimal control of raceways (Hurst and Rehbock, 2018). More importantly, our theoretical optimal operational criterion (efficiency of light absorption) has been successfully applied experimentally by other colleagues (Combe et al., 2015). The **article 5.1** (Muñoz-Tamayo et al., 2013) details the modelling developments and optimization strategies for optimal operation using our attenuation factor as operational criterion.

Besides the previous study, we designed an optimal experiment protocol to allow an accurate estimation of the parameters reflecting the influence of light and temperature on microalgae growth. We tackled the problem of optimal experiment design (OED) for parameter estimation under the configuration of a real experimental system. The experimental apparatus, named the TIP is located at Ifremer (Nantes, France). It consists of 18 batch photo-bioreactors with independent regulation of temperature, pH and light intensity (Marchetti et al., 2011). On the basis of a mathematical model of the experimental system, the OED problem was formulated and solved with both static (constant light and temperature) and dynamic (time varying light and temperature) approaches under the D-optimality criterion (maximization of the determinant of of the Fisher information matrix -FIM.). Simulation results indicated that our resulting OED strategy allows for a better accuracy of the parameter estimation than that provided by the existing experimental protocol (Marchetti et al., 2011). Moreover, our study identified that factorial design can lead to practical identifiability problems under inadequate choice of the levels of the factors. Practical identifiability problems might translate into ill-condition (singular) Fisher information matrix, and thus into low reliability on the parameter estimate values. The OED study is presented in the **article 5.2** (Muñoz-Tamayo et al., 2014b). Our results provided guidelines for improving the experiment design of the real system.

During my postdoc project, I had the opportunity of participating in the PhD thesis of Caro-

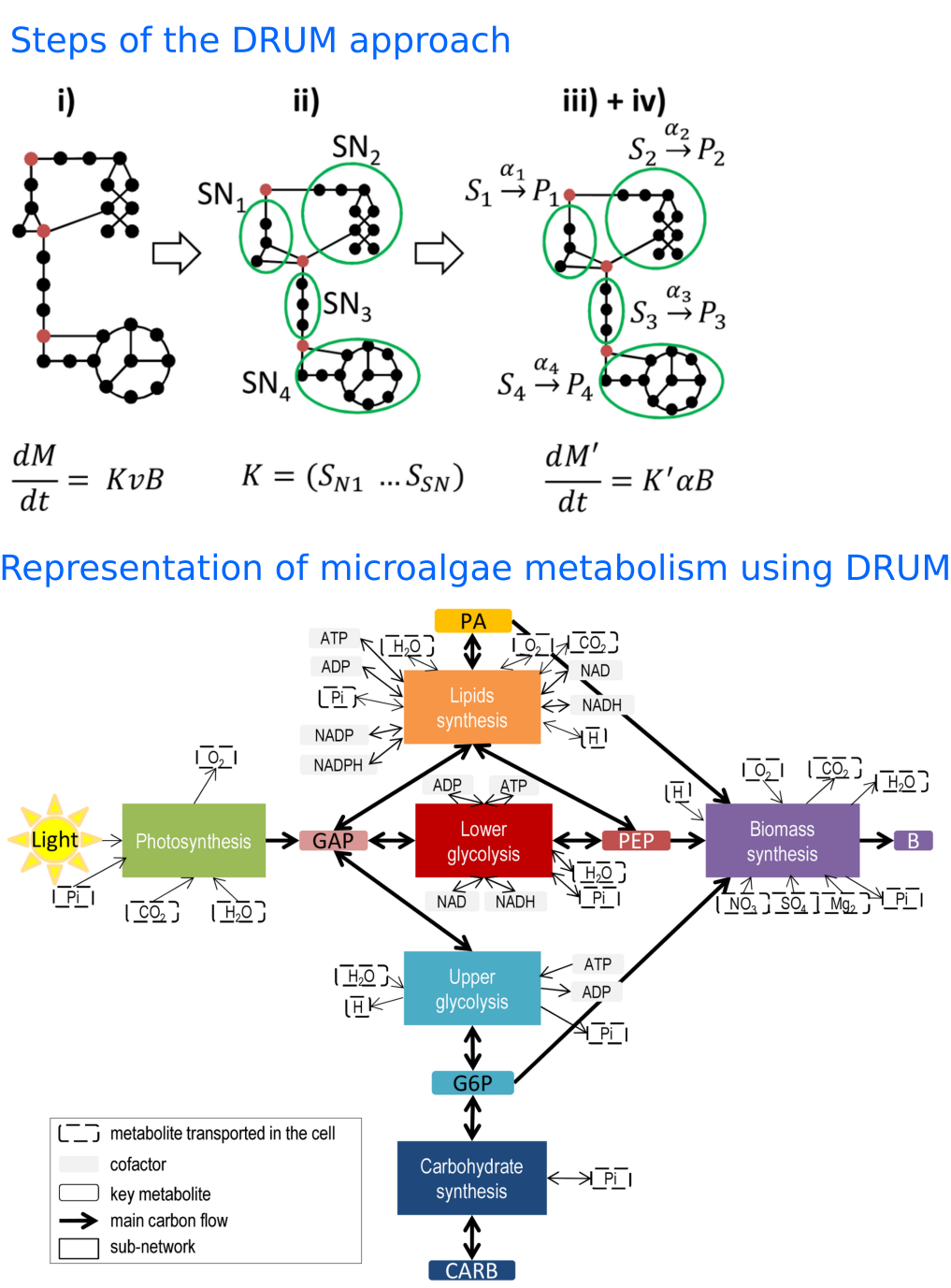


Figure 5.1: Top plot: the DRUM approach. The complete network (step i) is decomposed into sub-networks (SNs) assumed at quasi-steady state (step ii). These SNs are reduced to a set of macroscopic reactions (step iii), whose kinetic rates are defined in step iv. The metabolites that interconnect the SNs (red circles) can accumulate or be further utilized (red circles). In step iv, an ODE model is obtained. Bottom plot. Schematics of our microalgae metabolic model using DRUM. The carbon metabolic network is decomposed into six subnetworks.

line Baroukh (Inra-Inria). This work dealt with the mathematical modelling of microalgae carbon metabolism. The directors of the PhD of Caroline were Olivier Bernard (Inria) and Jean-Philippe Steyer (Inra). I had a role of co-supervisor which can be detailed as follows:

(*i*) Development of theory [20%], (*ii*) Definition of model structure on biological basis [30%], (*iii*) Verification of model implementation [60%], and (*iv*) Writing of articles and thesis manuscript [40%]

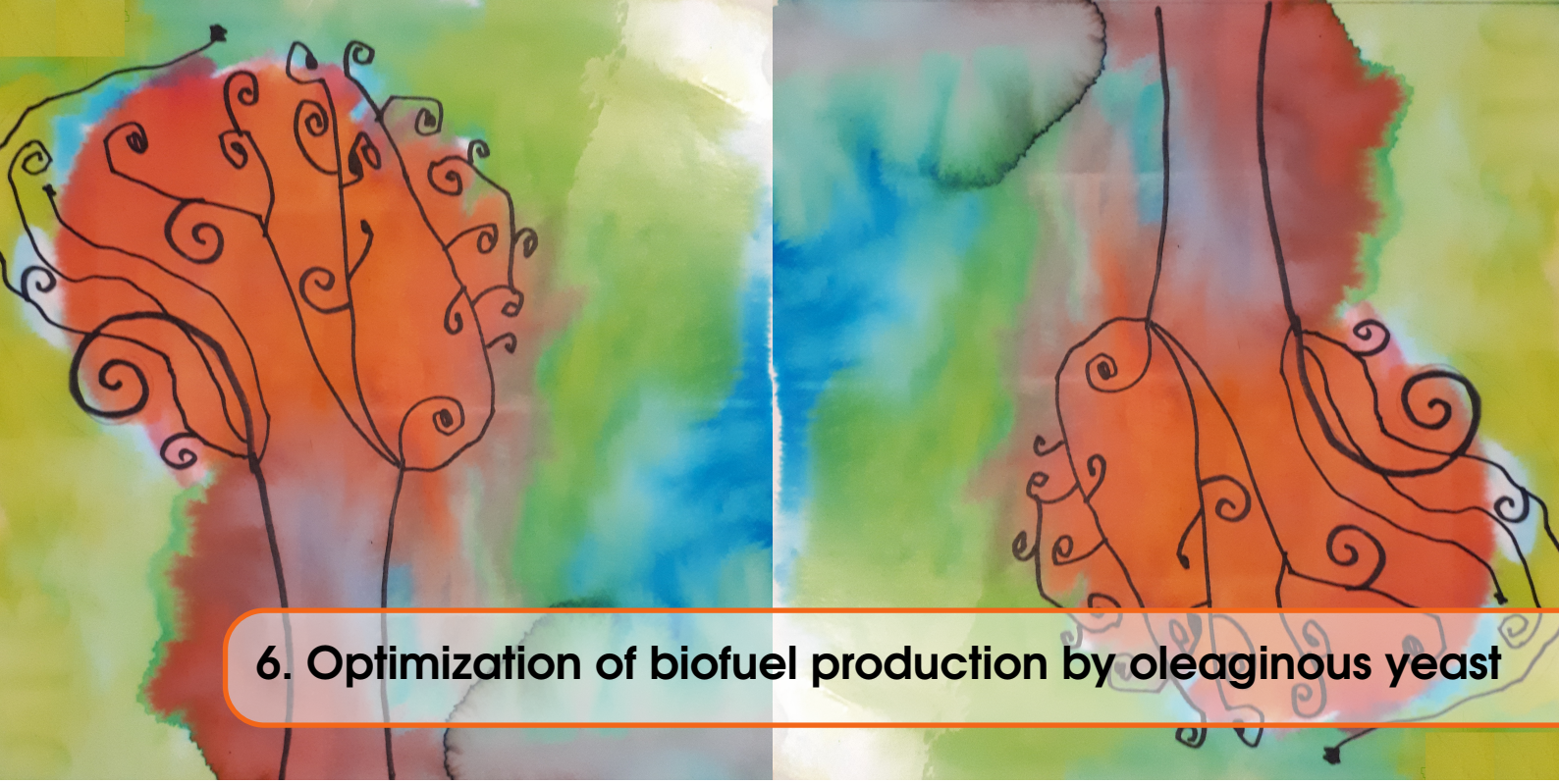
With the support of dedicated literature reviews (Baroukh et al., 2015b,a), we developed a novel mathematical framework that integrates both intracellular and macroscopic scales of microalgae metabolism for better describing how external forces shape microbial dynamics. The methodology is based on the splitting of the full metabolic network into subnetworks that are assumed to follow the balanced growth condition. The model derives from an elementary mode (EFM) analysis (Schuster and Hilgetag, 1994; Provost et al., 2006) applied to metabolic subnetworks that are assumed to follow the balanced growth condition. The resulting framework was named as DRUM (Dynamic Reduction of Unbalanced Metabolism). Our model was applied successfully to describe expxerimental data of the dynamics of microalgae growth and storage of lipids and carbohydrates of *Tisochrysis lutea* in a diurnal cycle (Lacour et al., 2012). The DRUM framework is detailed in the **article 5.3** (Baroukh et al., 2014). Figure 5.1 shows the steps of the DRUM approach and the representation of microalgae metabolism that we used for our model construction. Caroline was recruited at Inra as Junior Scientist (CR) in 2016.

All together, these works contributed in providing model-based tools to predict, optimize and control microalgae growth. These tools can be further deployed to exploit the broad speçtrum of microalage biotechnological applications.

5.1 Optimizing microalgal production in raceway systems. Muñoz-Tamayo, R., Mairet, F., and Bernard, O. (2013). Optimizing microalgal production in raceway systems. Biotechnology Progress 29, 543-552.

5.2 Getting the most out of it: Optimal experiments for parameter estimation of microalgae growth models. Muñoz-Tamayo, R., Martinon, P., Bougaran, G., Mairet, F., and Bernard, O. (2014). Journal of Process Control 24(6), 991-1001.

5.3 DRUM: a new framework for metabolic modeling under non-balanced growth. Application to the carbon Metabolism of unicellular microalgae. Baroukh, C., Muñoz-Tamayo, R., Steyer, J.P., and Bernard, O. (2014). PLoS One 9(8), e104499.



6. Optimization of biofuel production by oleaginous yeast

One good thing about music, when it hits you, you feel no pain.

Trenchtown Rock. Bob Marley.

This chapter synthesises the research work of my third postdoc (Inra, Toulouse, France). During the postodoc, I contributed to the PhD thesis of Carlos Eduardo Robles-Rodríguez.

Oleaginous yeasts are microbial factories capable of converting carbohydrates and fat substrates into neutral lipids (triacylglycerols - TAGs). In this project, we developed mathematical models to represent the dynamics of growth and lipid accumulation by the yeast *Yarrowia lipolytica*, considered as promising microbe for lipid production under nitrogen depletion conditions and excess of the carbon source (Beopoulos et al., 2009). However, under these conditions, *Y. lipolytica* also produces citric acid as result of overflow metabolism (Amribt et al., 2013) decreasing lipid productivity.

We followed a model-based optimization approach to provide guidelines towards optimal lipid productivity. Figure 6.1 displays the macroscopic representation of yeast metabolism used in our model developments. Firstly, we performed a simulation study to investigate optimal lipid productivities at continuous operation mode with two input flow rates for carbon and nitrogen supply. We used the mathematical model developed by Economou et al. (2011) to describe oil production. We determined that driving the inflow carbon/nitrogen (C:N) ratio along the process adequately, allows the system to attain optimal productivity. The C:N modulation was obtained by a simple parametrization of the two input flow rates with piecewise linear functions (Muñoz-Tamayo et al., 2014a). This result was applied in a further modelling study to describe the dynamic metabolism of *Y. lipolytica* from three experimental studies. The results are presented in the **article 6.1** (Robles-Rodríguez et al., 2018). We developed two macroscopic models namely an unstructured model based on Monod and inhibition kinetics, and a quota model based on the

model developed by Droop (1968), where biomass production depends on the fraction of internal nutrient per unit of biomass. This fraction is termed as quota.

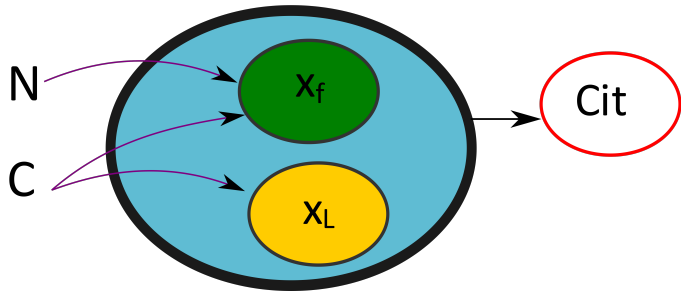
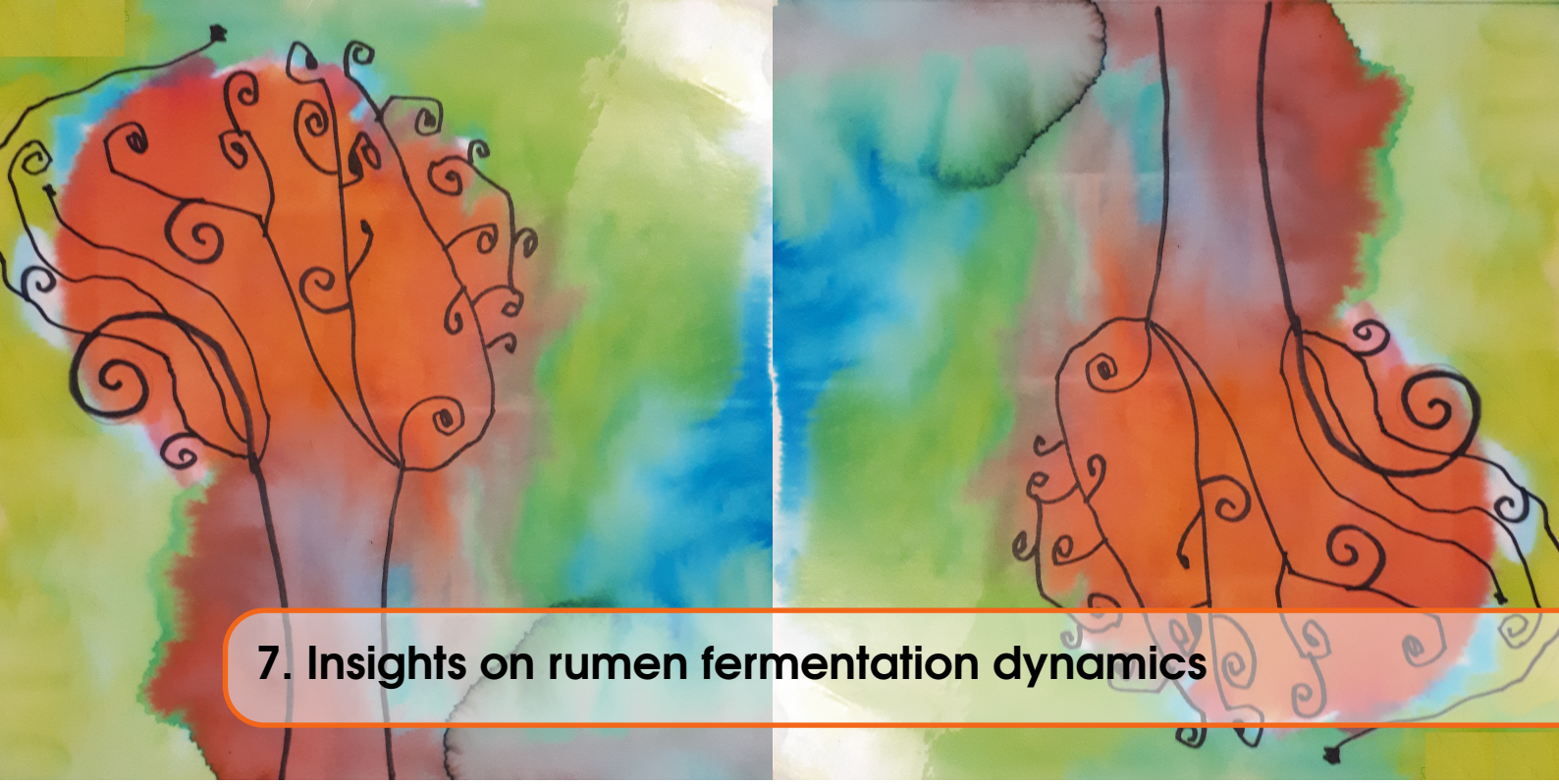


Figure 6.1: Macroscopic representation of yeast metabolism. The distribution of carbon into functional biomass (x_f), neutral lipids (x_L), and citric acid (Cit) is mediated by the nitrogen (N) and carbon (C) uptake fluxes. Nitrogen limitation favours lipid accumulation. Under carbon excess, citric acid is produced and excreted as the result of overflow metabolism

We followed the Droop-based approach developed by Mairet et al. (2011) to model microalgae metabolism. The Droop-based model incorporates the intracellular C:N ratio. It was selected as the best candidate due to its performance to represent experimental data. On the basis of the Droop model, we developed a model-based optimization of lipid accumulation allowing the regulation of the flow rates of glucose and nitrogen and thus the C:N ratio in the reactor. The optimization was performed using the Pattern Search algorithm implemented in the global optimization toolbox of Matlab® (Mathworks, 2018). Optimal feeding strategies were assessed by numerical simulations. Numerical results indicated that it would be possible to attain lipid productivities with higher values than those reported in literature. For example, literature values of lipid content fraction are reported about 0.17 g/g. Our simulations resulted in a lipid content fraction of 0.22 g/g. Further experimental work is needed to validate the simulation results of the presented control optimal strategy.

6.1 Modeling and optimization of lipid accumulation by *Yarrowia lipolytica* from glucose under nitrogen depletion conditions. Robles-Rodríguez, C. E., Muñoz-Tamayo, R., Bideaux, C., Gorret, N., Guillouet, S. E., Molina-Jouve, C., Roux, G. and Aceves-Lara, C. A. (2018). Biotechnology and Bioengineering 115, 1137-1151.

IV Current & future research



7. Insights on rumen fermentation dynamics

São eras sobre eras, e tempos atrás de tempos, e não há mais que andar na circunferência de um círculo que tem a verdade no ponto que está no centro.

They are eras over eras, and times after times, and you just have to walk on the circumference of a circle that houses the truth at the point that is in the center.

A hora do diabo. Fernando Pessoa.

This chapter synthesises my current research work at the MoSAR team (Inra, Jouy-en-Josas, France) on rumen modelling. It includes my involvement in the supervision of the PhD thesis of John Fredy Ramírez Agudelo.

The ultimate goal of my current research is to achieve a system-level understanding of the dynamic interplay between the diet, the animal and the rumen microbiota *via* mathematical modelling. To achieve my long-term goal, I have adopted a Cartesian approach by focusing firstly on enhancing the mathematical representation of the rumen fermentation *in vitro* by analyzing culture systems with mono-cultures and full consortia. I have also started to link animal feeding behaviour with methane production.

Enhancing the mathematical representation of rumen microbial metabolism

The design of optimal nutritional strategies for ruminants with the target of maximizing animal performance and efficiency while reducing enteric methane emissions necessitates a thorough understanding of rumen fermentation. Existing rumen models are mainly based on four model structures (Molly, Karoline, Cornell and Dijkstra models) that have been incrementally improved over the years to determine the nutritional and emission responses for a given diet (Mills et al., 2014; Huhtanen et al., 2015; Van Amburgh et al., 2015; Gregorini et al., 2015). These models have proven to be useful to better understand and

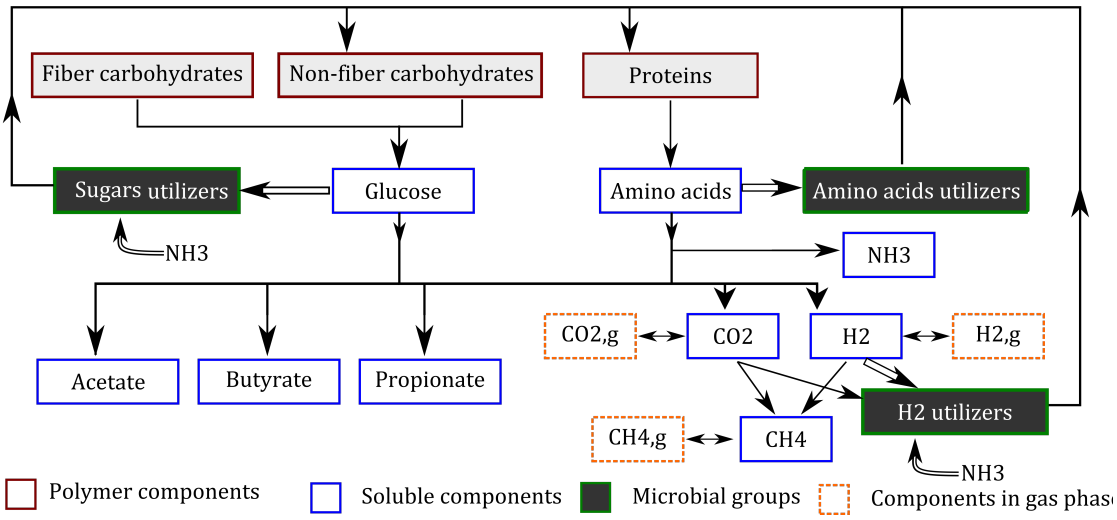


Figure 7.1: Our enhanced model representation of the digestion of feedstuffs by the rumen microbiota (Muñoz-Tamayo et al., 2016a).

represent rumen fermentation. However, they do not provide a detailed description of the rumen microbiota. Expanding the mechanistic description of the rumen microbiota has been identified as a central aspect towards an enhanced understanding of rumen function and development of mathematical models with enhanced prediction capabilities (Ellis et al., 2008; Janssen, 2010). In this context, from a constructive challenger perspective, in the sense defined by Baldwin (2000), we developed an alternative model structure of rumen fermentation under *in vitro* conditions, which is detailed in the **article 7.1** (Muñoz-Tamayo et al., 2016a). Compared to existing rumen fermentation models, our model enlarges the description of the microbiota by including three theoretical microbial functional groups namely sugars utilizers, amino acids utilizers and hydrogen utilizers (methanogens). Figure 7.1 illustrates our model representation of the fermentation performed by the rumen microbiota. To our knowledge, our model is the first one that accounts for the dynamics of methanogens as a key functional group of the fermentation. While previous rumen models describe the pH empirically, our model provides a mechanistic description. The model was calibrated with *in vitro* experimental data (Serment et al., 2016) using the IDEAS toolbox (Muñoz-Tamayo et al., 2009). The model allowed a satisfactory prediction of key variables such as methane production, volatile fatty acids, ammonia and environmental factors such as the pH. Furthermore, the model was instrumental for identifying the factors that explain differences in the fermentation pattern between rumen environments adapted to two types of diets differing in their level of fiber and concentrate. The model was implemented in Matlab®. The code is available for academic purposes. A implementation in the R software was further performed by some of my international collaborators (Kettle et al., 2018). In addition to its scientific contribution, this work represents a cornerstone for my career since it is my first article in the domain of animal science.

With the interest of addressing the interplay between the rumen microbiota and the host, we extended our model of *in vitro* fermentation into a simplified model representing *in vivo* rumen function. Model extensions included transit times, absorption of SCFA and saliva secretion. Our main motivation was to perform a numerical simulation study to analyze why animals subjected to the same diet can exhibit different pH responses (Muñoz-Tamayo

et al., 2016b). Our model was efficiently used to represent the variability of ruminal pH responses extracted from a meta-analysis study (Dragomir et al., 2008). Furthermore, the model was instrumental to identify that saliva secretion along with dry matter intake were the most influential host-related factors of rumen fermentation and pH. This work will be used as scaffold for further construction of a mathematical model representing rumen digestion *in vivo*.

We focused further on the metabolism of methanogens. Methanogenic archaea occupy a unique and functionally important niche in the microbial ecosystem that inhabits the gut of mammals. To enhance understanding of methanogens, we performed a study with the goal of quantitatively characterize the dynamics of methanogenesis by integrating thermodynamics, microbiology and mathematical modelling. For that, *in vitro* growth experiments were performed with key methanogens from the human and ruminant's gut. To facilitate the model calibration step, sampling times were determined by applying an optimal experiment design strategy (framework described in **Section 2.2**). Additional thermodynamic experiments to quantify the methanogenesis heat flux were performed in an isothermal microcalorimeter. On the basis of our rumen model (Muñoz-Tamayo et al., 2016a), we developed a dynamic model of hydrogenotrhophic methanogenesis. Our model uses an energetic-based kinetic function proposed by Desmond-Le Quemener and Bouchez (2014). The developed model captured efficiently the dynamics of H_2, CO_2 and CH_4. Together, data and model enabled us to quantify species-specific metabolism kinetics and energetic patterns within the group of cytochrome-lacking archaea. Using a theoretical exercise, we showed that kinetic information only cannot explain ecological aspects such as microbial coexistence occurring in gut ecosystems. Our results provide new information on the thermodynamics and kinetics of methanogens. This enhanced understanding of methanogens could be useful to (i) construct novel gut models with enhanced prediction capabilities and (ii) devise new feed strategies for promoting health in humans and mitigating methane from ruminants. The **article 7.2** (Muñoz-Tamayo et al., 2018a) details this study.

Linking feeding behaviour and methane production from cattle

The following work is related to a collaboration project that was set up in the context of the PhD thesis of John Fredy Ramírez Agudelo. Fredy started his PhD thesis, entitled Monitoring and simulation of enteric methane emissions in dairy cows, in 2014 at Universidad de Antioquia (UdeA), Colombia. A presentation of the PhD thesis of Fredy is available in the following link: `https://www.youtube.com/watch?v=JxvEPisv6rI`. The director of the PhD project was Ricardo Rosero Noguera, and the supervisor was Sandra Lucía Posada Ochoa (UdeA). My role in the supervision of Fredy's thesis started in January 2017 by electronic exchange and skype meetings ($\approx$ twice per month). In November 2017, Fredy came to MoSAR as a visitor PhD student to work jointly for a period of three months. The thesis project integrates experimental approaches, development of sensors and predictive models of enteric methane emission. The modelling part is 30% of the whole thesis. My supervision covers 90% of the aspects related to model development. During his visit at MoSAR, I trained Fredy in dynamic modelling, parameter estimation and programming in the software Scilab. When Fredy came to MoSAR, I have initiated a collaboration with Helen Kettle (Biomathematics and Statistics Scotland - BioSS, Edinburgh, UK) and Richard Dewhurst (Future Farming Systems, SRUC, Edinburgh, UK) on modelling

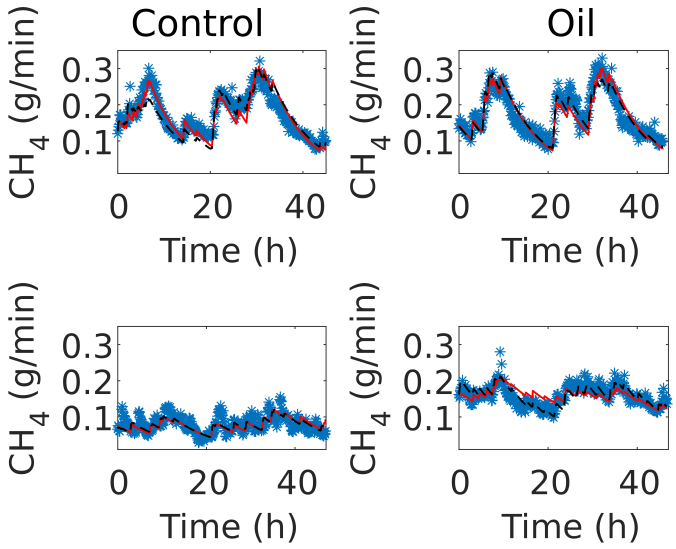


Figure 7.2: Results of our modelling developments for predicting methane production from cattle using feeding behaviour information. Experimental (*) versus predicted methane emissions using DMI (red solid line) and IT (dashed black line) as predictors for control and oil treatments. Top plots are the experiments where model fits were the best. Bottom plots are the experiments where model fits were the poorest. IT is as good predictor as DMI.

methane production in cattle. I invited Fredy to joint the collaboration project, which is briefly detailed below. Fredy will defend his thesis early 2019. He is currently setting up a specialisation training program on precision agriculture at `Institución Universitaria Digital de Antioquia` (Colombia), of which I might also participate as lecturer.

Given the primary role of feeding behaviour on methane emissions in cattle (Giger-reverdin et al., 2003; Crompton et al., 2011; Charmley et al., 2016), we investigated the capability of predicting the dynamics of methane production from cattle using only time-series data of feeding behaviour, measured either as Dry Matter Intake (DMI) or Intake Time (IT) as predictors. The objective of this construction was to develop a suitable tool for estimating methane that could be applied at large scale. We developed a dynamic parsimonious grey-box model with the support of experimental data of methane emissions from respiration chambers. The data set comes from a study with finishing beef steers (cross-bred Charolais and purebred Luing finishing) (Troy et al., 2015). Animals received two contrasting basal diets consisting (g/kg DM) of 500:500 and 80:920 forage to concentrate ratios. Within each basal diet, there were two treatments: a control treatment with rapeseed meal as protein source, and an oil treatment with rapeseed cake as protein source to increase dietary oil from 27 (control) to 53 g/kg DM. Figure 7.2 displays the individual dynamic pattern of methane production against model predictions for the best and worst fitting cases. Plots are given for the model using either DMI or IT as predictors applied to both control and oil treatments. As observed, our model provides satisfactory results for predicting the dynamics of methane production with similar levels of performance between DMI and IT as predictors. Since IT measurements are easier to obtain than DMI measurements, our study suggest that a software sensor that integrates our *in silico model* with a real-time sensor providing accurate IT measurements is a viable solution for predicting methane output in a large scale context. Our results are presented in the **article 7.3** (Muñoz-Tamayo

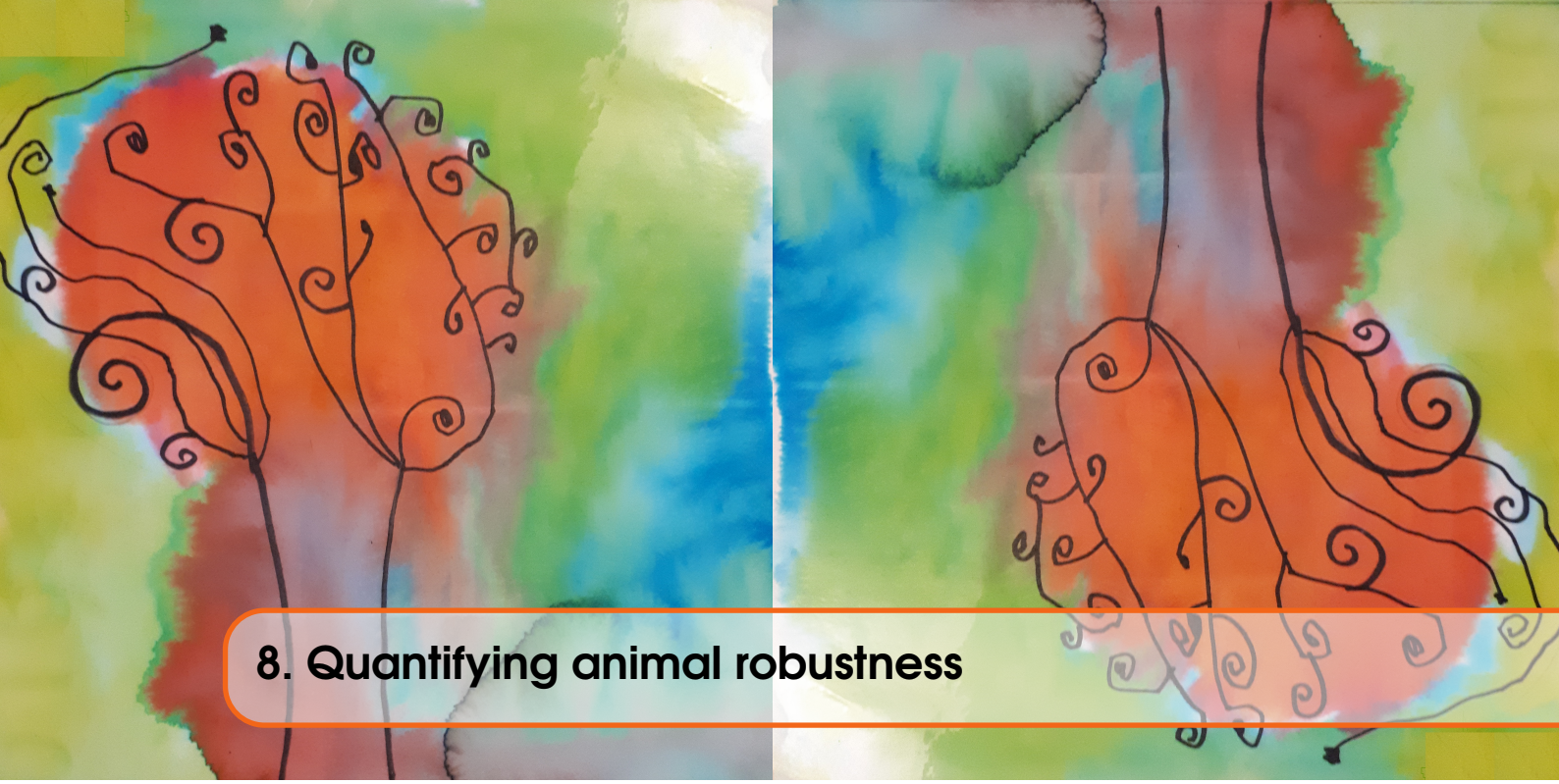
et al., 2019). Fredy intends to apply this model approach to data obtained from steers and dairy cows in the experimental farm of UdeA.

Altogether, our modeling research consolidates a solid basis for enhancing rumen function understanding.

7.1 Mechanistic modelling of *in vitro* fermentation by rumen microbiota. Muñoz-Tamayo, R., Giger-Reverdin, S., and Sauvant, D. (2016). Animal Feed Science and Technology 220, 1-21.

7.2 Hydrogenotrophic methanogens of the mammalian gut: functionally similar, thermodynamically different. A modelling approach. Muñoz-Tamayo, R., Popova, M., Tillier, M., Morgavi, D. P., Graviou, D., Morel, J. P., Fonty, G., Morel-Desrosiers, N. (2018). BioRxiv.

7.3 A parsimonious software sensor for estimating the individual dynamic pattern of methane emissions from cattle. Muñoz-Tamayo, R., Ramírez Agudelo, J. F., Dewhurst, R.J., Miller, G., Vernon, T., and Kettle, H. (2019). Animal 13, 1180-1187.



8. Quantifying animal robustness

Once upon a time, I dreamt I was a butterfly, fluttering hither and thither,
to all intents and purposes a butterfly.
Now I do not know whether I was then a man dreaming I was a butterfly, or
whether I am now a butterfly, dreaming I am a man.

Chuang Tzu.

This chapter synthesises my participation in the Pigletbiota ANR project within the supervision of the postdoc project of Manuel Revilla at the MoSAR team (Inra, Jouy-en-Josas, France).

The quantitative characterization of animal robustness at weaning is a key step for management strategies to improve health and welfare. This characterization is also instrumental for the further design of selection strategies for productivity and robustness. Within the ANR Pigletbiota project- lead by Jordi Estellé (GABI, Inra), we have undertaken an integrative biology approach to elucidate the influence of the host and gut microbiota factors on the piglet sensitivity at weaning. In this context, since 2017, Manuel Revilla conducts his postdoc between MoSAR and GABI on integrating statistical genetics and mathematical tools to help identify drivers for increasing resistance at weaning for piglets fed without antibiotics. The postdoc project is supervised by Jordi Estellé, Nicolas Friggens (MoSAR) and I. My role of supervision covers scientific support in dynamic modelling and parameter estimation (80%) and daily supervision (50%).

The phenotype of robustness is a complex trait composed of multiple components (Friggens et al., 2017) which hampers its quantitative characterisation. We aimed at making a step forward in the quantitative characterization of robustness at weaning. For that, we developed a mathematical modelling approach to describe the body weight of piglets from weaning with the rationale that weight trajectories provide central information to quantify

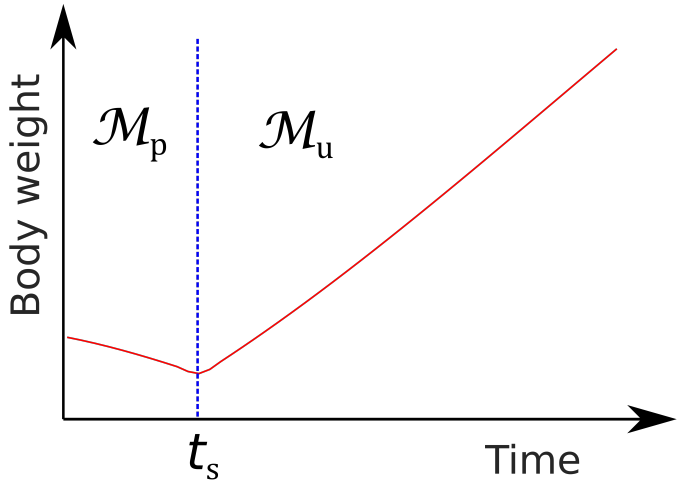


Figure 8.1: The piglet weaning response is partitioned in two time windows defined by the switching time t_s. The dynamics of live weight is represented by a generic perturbed model $\mathscr{M}_p$ that accounts for the detrimental effect of the perturbation. This effect is cancelled when the animal is not longer perturbed. Hence, live weight dynamics follows an unperturbed model $\mathscr{M}_u$.

the capability of the animal to cope with the weaning disturbance (weaning robustness). Our model was based on the Gompertz-Makeham equation (Golubev, 2009) following the rationale that the animal response can be partitioned in two time windows (a perturbation and a recovery window) to represent the moment at which the animal is perturbed and the moment at which it recovers from the perturbation. Figure 8.1 illustrates the modelling concept we applied.

The model reads as follows

$$\begin{aligned}
\frac{dW}{dt} &= (-C+\mu)\cdot W, & W(0) &= W_0,\\
\frac{d\mu}{dt} &= -D\cdot\mu, & \mu(0) &= \mu_0,\\
C &> 0 & \text{if} \quad t &\leq t_s,\\
C &= 0 & \text{if} \quad t &> t_s.
\end{aligned} \tag{8.1}$$

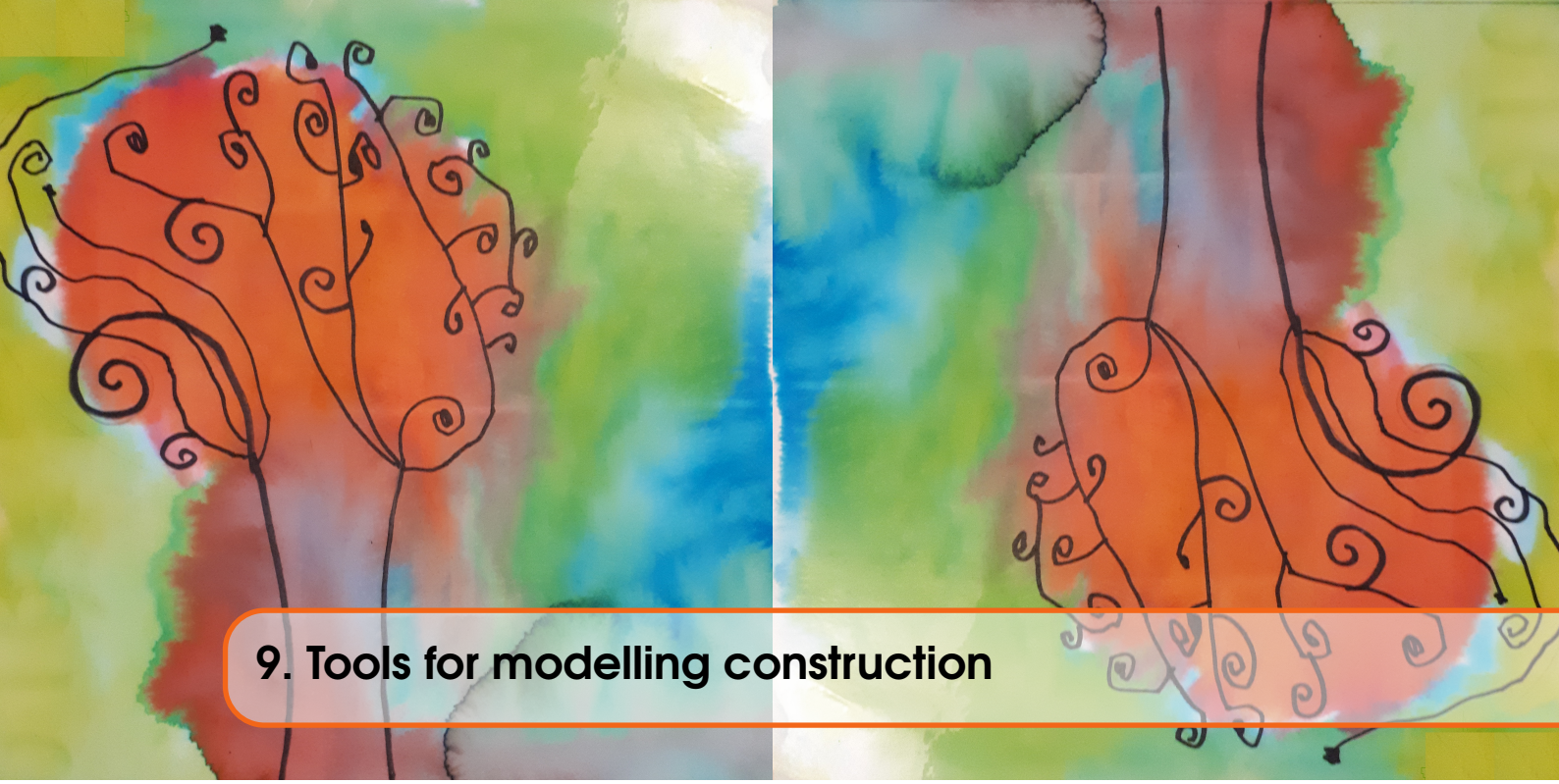
Where W (kg) is the live weight and μ (d^{-1}) is the specific growth rate. The constant D (d^{-1}) is a growth rate coefficient that controls the slope of the growth rate curve, and C (d^{-1}) is a perturbation parameter representing the effect of the environment on the weight change. The transition between the perturbation and recovery phases is determined by the switching time of recovery t_s (d). Together with the initial condition μ_0, the model has four biological meaningful parameters, which are structurally identifiable.

The model was evaluated with data of an experimental study with 325 Large White pigs weaned at 28 days of age and further housed and fed conventionally during the post-weaning period without antibiotic administration. Body weight and diarrhoea scores were recorded before and after weaning, and blood was sampled at weaning and one week later for collecting haematological data. The model captured the weight dynamics of animals at different degrees of perturbation. The utility of the model is that it provides biological

parameters that inform on the amplitude and length of perturbation, and the rate of animal recovery. This study is described in the **article 8.1** (Revilla et al., 2019).

We are currently investigating the construction of a robustness index by integrating our model developments with metaomic data of the gut microbiome. The functional interplay between the gut microbiota and pig physiology has been widely recognised. During weaning transition, gut microbiota disruption is one of the key factors leading to postweaning diarrhea (Gresse et al., 2017). Our challenge is to integrate both statistics and dynamic modelling to analyse data of body weight, pig host genomics and gut microbiome to develop indicators of weaning sensitivity. Host and microbiome omics analysis are being carried out by our Inra collaborators of GABI who participated in the sequencing projects of the pig genome (Groenen et al., 2012) and the pig gut microbiome (Xiao et al., 2016).

8.1 Towards the quantitative characterization of piglets robustness to weaning: A modelling approach. (2019). Revilla, M.,Friggens, N. C., Broudiscou, L. P., Lemonnier, G., Blanc, F., Ravon, L., Mercat, M. J., Billon, Y., Rogel-Gaillard, C., Le Floch, N., Estellé, J., Muñoz-Tamayo, R. Animal.



9. Tools for modelling construction

People never seemed to notice that, by saving time, they were losing something else.

Michel Ende. Momo.

This chapter synthesises my current research work at the MoSAR team (Inra, Jouy-en-Josas, France). It includes my involvement in the supervision of the PhD thesis of Laura Lema Perez.

Modelling construction is a stepwise process that requires dedicated tools. My research belongs to the domain of applied mathematics. I have been focused on the translation of biological knowledge into usable models. When analysing system dynamics and problems associated to model building and parameter identifiability, I have followed a practitioner approach capitalizing on the use of dedicated software tools. For instance, I have exploited the functionality of Matlab® for developing the IDEAS parameter identification toolbox (220 lines of code), which is detailed in **article 3.1** (Muñoz-Tamayo et al., 2009). After its development, IDEAS has been of great usefulness in all my modelling projects. I have used IDEAS as a teaching tool used my lectures on parameter identification. IDEAS has been downloaded more than 100 times and has been used as parameter identification toolbox in at least five publications (without considering my own papers).

In 2014, I embraced the domain of animal science modelling at MoSAR. I realized that problems associated to the structural identifiability of parameters in ODE models were not addressed in the domain. Accordingly, I initiated a discussion about parameter identifiability with the MoSAR colleagues involved in model constructions: Laurence Puillet, Masoomeh Taghipoor, Pierre Blavy, Nicolas Friggens, Daniel Sauvant, Jean-Baptiste Daniel and Olivier Martin. We named this discussion group as the MoMos (MoSAR

Modellers). Since then, the MoMos group has consolidated a rich space of discussion and sharing. The collective MoMos is led by Olivier Martin and I. Coming back to the identifiablity story, we decided to provide a comprehensive explanation of the structural identifiability notion for the community of animal science modelling and introduce existing tools to perform the identifiability analysis without being an expert on the mathematical technicalities associated to this subject (**Section 2.2**). Our aim was to motivate the community to use identifiability analysis in the modelling practice by illustrating examples where such an analysis is instrumental for model construction and experiment design. The result of this work is presented in the **article 9.1** (Muñoz-Tamayo et al., 2018b).

Further, within the PhD project of Laura Lema-Perez, we analysed methodological aspects for building phenomenological-based semiphysical models (PBSM). The PhD thesis of Laura is entitled Parameters interpretability in phenomenological based semi-physical models: a human glucose homeostasis model. Laura started her PhD at Universidad Nacional (UN) de Colombia Sede Medellin in 2015. The thesis directors are Hernan Dario Alvarez Zapata (UN) and Jose Fernando Garcia Tirado (Center for Diabetes Technology, University of Virginia, USA). The co-supervisor is Carlos Builes-Montaño (Hospital Pablo Tobón Uribe, Colombia). In February 2018, Laura came to MoSAR as a visitor PhD student to work jointly during six months. One of the objective of Laura's thesis is to build a methodology for constructing PBSMs that integrates the physical meaning level of the model parameters. We will denote a parameter with physical meaning to be interpretable. The development of such a methodology encounters a first hurdle in the lack of formalism about interpretability as a property of the parameters in a model, there is no consensus about quantifying or measuring such a property. To the best of our knowledge, the concept of interpretability in PBSM has not been deeply discussed, perhaps due to the implicit assumption that interpretability is inherent to the PBSM since they are derived from a phenomenological representation of the system under study. In this work, we propose a conceptual framework that can facilitate the incorporation of interpretability for model construction. My role in the supervision of Laura's thesis has been centred in the integration of structural identifiability property into the modelling building methodology to enhance its level of formalism. My rate of supervision was of 30% during 2018. During her visit at MoSAR, I trained Laura on parameter identifiability concepts, and on the use of software tools dedicated to structural identifiability analysis. We have proposed a conceptual framework to facilitate the incorporation of interpretability for model construction. For that, we used my simple model of $\beta - casein$ hydrolysis by *L. lactis* IM17 (Muñoz-Tamayo et al., 2011a) (**article 4.1**) as an example to elaborate our developments. Figure 9.1 presents a graphical representation of the conceptual framework applied to the model case study.

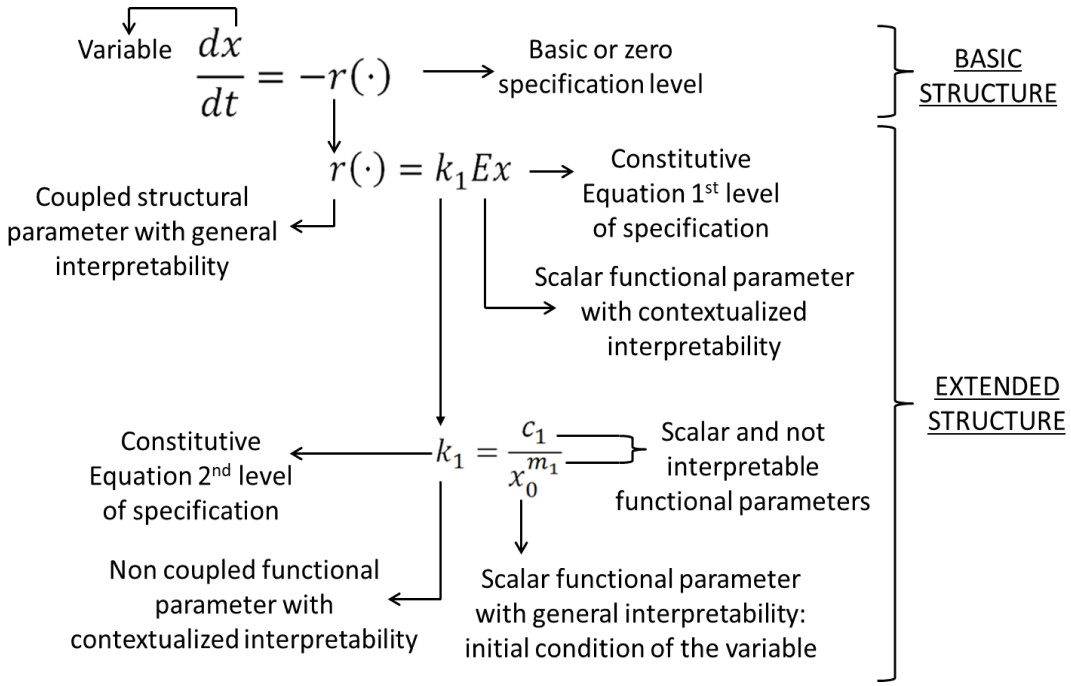
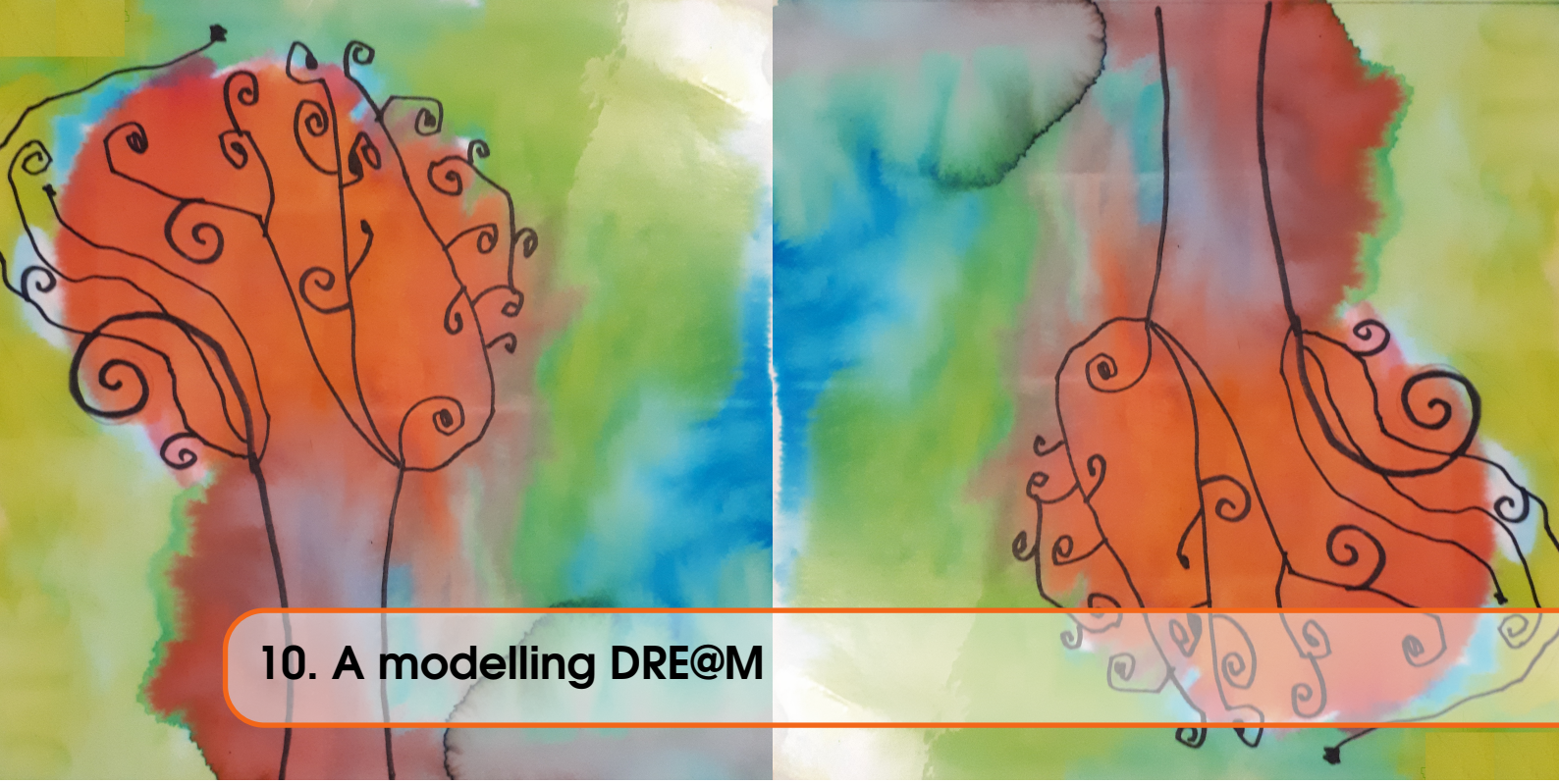


Figure 9.1: Representation of the conceptual framework for parameter interpretability applied in mathematical model of β-casein hydrolysis by a *Lactococcus lactis* bacterium.

9.1 To be or not to be an identifiable model. Is this a relevant question in animal science modelling? Muñoz-Tamayo, R., Puillet, L., Daniel, J. B., Sauvant, D., Martin, O., Taghipoor, M., Blavy, P. (2018). Animal 12, 701-712.

9.2 On parameter interpretability of phenomenological-based semiphysical models in biology. Lema-Perez, L., Muñoz-Tamayo, R., Garcia-Tirado, J., Alvarez, H. (2019). Informatics in Medicine Unlocked 15, 100158.



10. A modelling DRE@M

Yo quiero una princesa convertida en un dragón,
yo quiero el hacha de un brujo para echarla en mi zurrón,
yo quiero un vellocino de oro para un reino,
yo quiero que Virgilio me lleve al infierno,
yo quiero ir hasta el cielo en un fríjol sembrado
y ya.

I want a princess turned into a dragon,
I want a witch's ax to throw it in my shepherd bag,
I want a golden fleece for a kingdom,
I want Virgilio to take me to hell,
I want to go to the sky in a bean sown
and that's it.

La primera mentira. Silvio Rodríguez.

I have defined my prospective research roadmap within a flagship named DRE@M: Deciphering the Rumen Ecosystem with Advanced Modelling. Rumen microbes are essential for the animal by catalysing the degradation of plant polysaccharides, which allows ruminants to harvest nutrients that are otherwise inaccessible. Furthermore, the rumen microbiota harbours a full range of functionalities that represent a tremendous potential for health management. On the other hand, rumen microbes participate in the environmental impact of agriculture by catalysing reactions that govern methane (a potent greenhouse gas) and nitrogen emissions from cattle. To address the trade-offs between the multiple rumen microbial impacts, a thorough understanding of the rumen microbiota is needed to drive rumen metabolism towards optimal ruminant health, productivity and environmental footprint. In this line, I aim at developing research that will contribute to sustainable ruminant production. Considering the ruminant to be a holobiont - entity comprised of the host and its symbiotic microbes (Theis et al., 2016) - I follow the rationale

that the animal phenotype can be partly modulated by its rumen microbiota. By advancing rumen microbiota system-level understanding, my ultimate goal is to use this knowledge to design microbial manipulation strategies for improving ruminant function and promoting a healthy microbial ecosystem for sustainable livestock farming.

Whilst significant technological progress has enhanced our knowledge of the rumen microbiota (Mcsweeney and Mackie, 2012; Pope et al., 2012; Morgavi et al., 2013; McAllister et al., 2015; Li et al., 2018), a deep understanding of the rumen ecosystem function is still limited and consequently, microbial manipulation strategies have been of limited success (Abecia et al., 2013; Yañez-Ruiz et al., 2015; Debruyne et al., 2018).

Microbial ecology, thermodynamics and dynamic systems principles have been proposed to facilitate successful microbial manipulations (Ungerfeld, 2015; Weimer, 2015). However, a holistic approach integrating such principles has not been implemented yet. In addition to identifying the action mechanism of potential drivers of the rumen microbiota, it is important to elucidate the ecosystem dynamics, since central features such as resilience, robustness and microbial adaptation are the resultant of dynamic interactions that shape rumen function and its response to perturbations. Computational omics analyses have allowed to link microbial data to animal phenotypes such as methane production (Kittelmann et al., 2014; Shabat et al., 2016). However, most of these findings are restricted to correlation based approaches. To get the most out of the large body of omics data, mathematical modelling is needed, as it provides a powerful approach for data integration and interpretation towards a system-level understating of the rumen ecosystem that can inform on how to drive microbial metabolism. Mathematical models of rumen function are basically built by representing the action of two central players, namely the animal host and the rumen microbiota. The modelling progresses to be made imply the improvement of the representation of both animal host and microbial elements in the model structure (Offner and Sauvant, 2004; Ellis et al., 2008; Janssen, 2010; Gregorini et al., 2013).

My research roadmap is delineated to developing mathematical models with expanded capabilities of prediction and mechanistic insight of rumen function. I aim at enhancing the understanding of the dynamic interplay between the diet, the animal host and the rumen

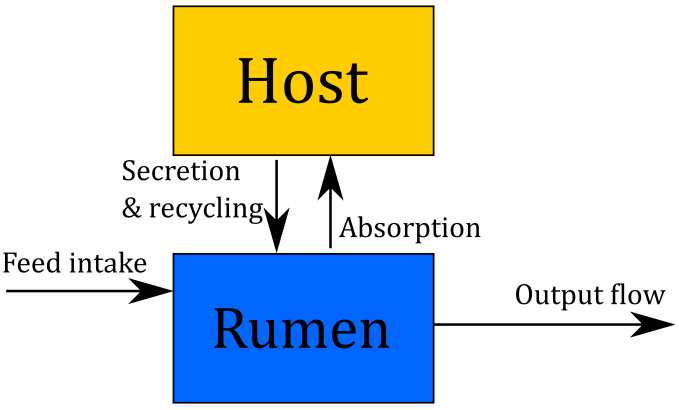


Figure 10.1: Cartoon of my research object (system) representing the interaction of fluxes between the animal host and its rumen ecosystem.

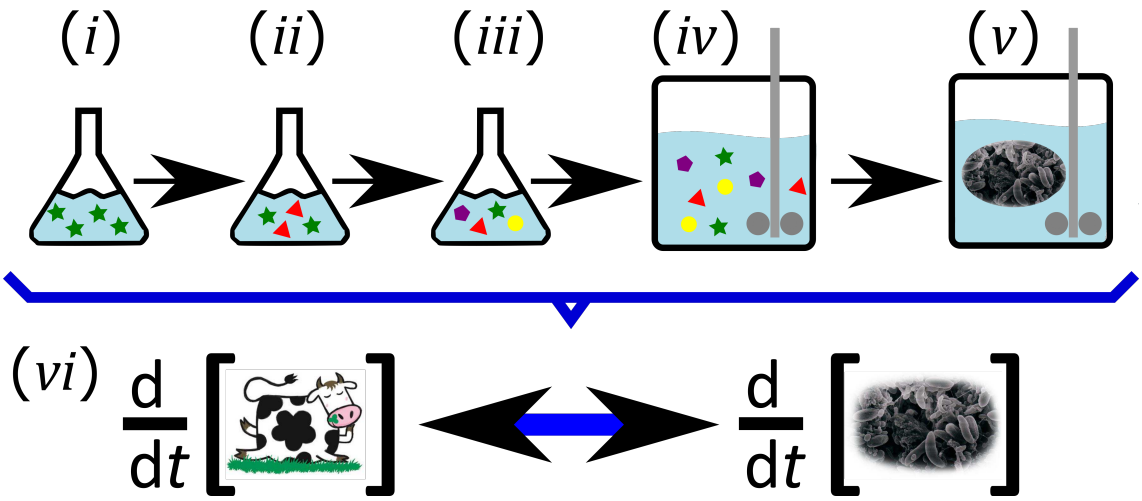


Figure 10.2: Steps to achieve my DRE@M *via* an interdisciplinary approach covering experimental and modelling developments. Experimental *in vitro* batch experiments will be performed at different levels of microbial complexity namely mono-culture (*i*), co-culture (*ii*), and mini-consortia (*iii*). Further, chemostat experiments will be carried out with mini-consortia (*iv*) and the whole rumen microbial consortium (*v*). Mathematical modelling will be pursued in parallel to elaborate a genomic-scale metabolic model of the rumen microbiota. This model will be integrated into a model representing *in vivo* conditions. The resulting model will allow to describe the dynamic interplay between the diet, the host and the rumen microbiota (*vi*).

microbiota. My research object (system) is illustrated in Figure 10.1. To date, I have been mainly focused in the rumen blue box. My strategy consists in constructing firstly a model of rumen microbial metabolism, for a further integration into a whole model of rumen function under *in vivo* conditions. This whole model will incorporate physiological aspects such as transit time, SCFA absorption and saliva secretion.

To pursue my DRE@M, I have been consolidating a strong interdisciplinary collaboration network at the national and international level. My national network comprises (*i*) rumen microbiologists from the Inra teams `MEDIS` (Microbiology, Theix), `UMRH` (Herbivores, Theix), and industrial partners such as `Lallemand SAS` (Animal Nutrition Division, Blagnac), (*ii*) animal scientists and geneticists from `GABI` (Animal genetics and integrative biology, Jouy-en-Josas) and `MoSAR`, (*iii*) process engineers from `LBE` (Laboratory of Environmental Biotechnology, Narbonne), and (*iv*) computational biologists from `Genotoul` (Toulouse) and `Dyliss` (DYnamics, Logics and Inference for biological Systems and Sequences, CNRS/Inria, Rennes). I also collaborate with members of the `Rumen Microbial Genomics network` within the `RumenPredict` and H2020 MASTER projects.

Figure 10.2 displays the successive steps of my DRE@M research roadmap. I will follow a Cartesian approach: *en commençant par les objets les plus simples et les plus aisés à connaître, pour monter peu à peu comme par degrés jusques à la connaissance des plus composés*[1] (Descartes, 1637). My roadmap combines both experimental and modelling developments. The first echelon consists in the study of microbial metabolism of *in vitro* mono-culture experiments under batch conditions. We will follow the study

[1] Discours de la méthode pour bien conduire sa raison, et chercher la vérité dans les sciences. René Descartes.

of co-culture experiments at different levels of complexity towards reconstituted minimal microbial consortia with sequenced rumen microbes. We will further use chemostats to study the dynamics of mini-consortia under conditions closer to real rumen conditions (continuous inflow and outflow), to proceed finally with the whole rumen microbial consortium.

Experimental studies will be followed by modelling developments at all stages to construct in a stepwise fashion a genome-scale metabolic model of the rumen microbiome. Genome-scale modelling wil be pursued with my collaborator Anne Siegel (Dyliss). *In vitro* experiments will be carried out *via* collaboration projects with my colleagues Evelyne Forano (MEDIS), Milka Popova and Diego Morgavi (UMRH), and Elie Le Quéméner, Kim Milferstedt , Éric Trably, Jérôme Hamelin, Jean-Philippe Steyer (LBE). We will characterise quantitatively rumen microbial metabolism using state-of-the-art omics and metaomics technologies. Omics data will be analysed with the support of my collaborators Christophe Klopp (Genotoul) and Jordi Estellé (GABI).

10.1 Improving understanding of rumen microbial dynamics

As we have recently discussed (Huws et al., 2018), existing rumen models do not incorporate genomic microbial knowledge and are thus limited for microbial manipulation applications. The gap between the rumen microbiota available omics data and the existing rumen models needs to be bridged to enhance rumen understanding (Bannink et al., 2016). To improve understanding of rumen ecosystem dynamics, in the coming years, I will lead interdisciplinary projects covering microbiology, thermodynamics, computational biology and mathematical modelling. My hypothesis is that effective strategies of microbial manipulation can be devised using a model-based approach that integrates quantitative knowledge on microbial interactions and ecosystem dynamics.

Basically, two modelling approaches are used to represent microbial metabolism, namely macroscopic and intracellular modelling. While both approaches are mechanistic, they differ in the level of detail used to describe microbial metabolism. Existing models of rumen fermentation fall in the category of macroscopic models. In this approach, microbial metabolism is represented in aggregated form by considering a limited number of major fermentation reactions and by representing the action of the microbiota by few functional groups. The intracellular approach looks for a detailed representation of microbial metabolism at the genome scale. Macroscopic models are essentially dynamic while intracellular models are generally based on a steady state assumption (although extensions are possible to render them dynamic (Mahadevan et al., 2002)). In our projects, we will used both the macroscopic and genome-scale approaches. We will investigate how these two modelling frameworks can be integrated to get the most out of the modelling endeavour. In the following, I briefly describe the directions that I will undertake for enhancing the mathematical representation of rumen function.

10.1.1 Expanding the rumen microbiota representation in macroscopic models

The core of a rumen microbial model is how the microbiota and its metabolism are represented. The level of detail by which the microbiota is represented in a model results from a bet between two motivations namely how simple we want our model, and how close we want the model to mirror the biological complexity. As discussed in **Chapter 7**, we developed a model struture where the rumen microbiota is represented by three

functional groups, namely sugars utilizers, amino acids utilizers and hydrogen utilizers (Muñoz-Tamayo et al., 2016a). The results of the model structure were satisfactory for the objectives traced in the research article. However, model extensions can be pursued to enrich the model capabilities. A potential extension is to incorporate protoza and fungi groups into the model to account for the role of eukaryote members on rumen metabolism. As reviewed in our recent work (Huws et al., 2018), anaerobic fungi are potent fiber-degrading organisms that provide beneficial conditions to other rumen microbes. Fungi penetrate plant structural barriers leading to an increase of plant cell surface area available for microbial colonization. Rumen fungi have been shown to improve feed intake, feed digestibility, feed efficiency, daily weight gain and milk production. With regard to protozoa, their contribution to rumen fermentation dynamics is determined by its metabolic fermenter activity, the endosymbiosis with other microbes (methanogens) and by its engulfment bacteria capacity (Janssen and Kirs, 2008). Different studies have demonstrated the role of protozoa on rumen fermentation and methanogenesis (Ranilla et al., 2007; Mosoni et al., 2011; Belanche et al., 2015; Guyader et al., 2014). The incorporation of eukaryote members into our models developments can use as basis the rumen model developed by Dijkstra (1994) that includes one protozoa group.

It is well recognised the role of lactate accumulation on ruminal of acidosis (Owens et al., 1998; Plaizier et al., 2018). Accordingly, it will be useful to to incorporate lactate utilizers and lactate metabolism into the model to allow the study of scenarios where acidosis takes place. A model extension incorporating homoacetogens will also be useful to better describing hydrogen metabolism and methane production (Joblin, 1999; Klieve, 1999; Ellis et al., 2008; Morgavi et al., 2010). Including homoacetogens into our model developments will be instrumental since this hydrogenotrophic population may be of importance when looking at strategies for methane mitigation. Finally, another challenge to be addressed towards a comprehensive understanding of the rumen microbiome as a whole is the incorporation of bacteriophages. Advances on the function of eukaryote members and rumen viruses might enable to integrate the representation of these groups into a whole rumen microbiota model. The challenges here identified will be tackled on the basis of the modelling frameworks that I have developed which will be used as scaffolds for model extensions. In complement to my own developments, I will enrich my research by using other modelling frameworks and tools such as those developed by my colleague Helen Kettle (Kettle et al., 2015, 2018) that provide a solid basis to undertake a modelling approach with tradeoffs between the biological reality (microbial phylogeny) and model complexity.

Another aspect that opens a key direction of model extensions is the representation of lipid metabolism, which is recognised to impact rumen fermentation, milk and meat quality. In the rumen, lipids undergo hydrolysis, producing unsaturated long-chain fatty acids which can be further biohydrogenated. Lipid metabolism and biohydrogenation reduce the amount of hydrogen available for the methanogenesis (Doreau et al., 2012; Mcsweeney and Mackie, 2012). Despite the importance of lipid metabolism in the rumen, this aspect has been overlooked in existing rumen models (Jenkins et al., 2008; Ellis et al., 2008) and few works have attempted to describe mathematically this process. Moate et al. (2008) developed a kinetic model that represents both *in vitro* lipolysis and biohydrogenation as multistep processes. This model is, to my view, a solid scaffold for my further model developments of rumen lipid metabolism. This model followed a parsimonious and bi-

ological sound approach and, moreover, it has structural identifiability properties (as I demonstrated in Muñoz-Tamayo et al. (2018b)). Experimental data from *in vitro* and *in vivo* studies on lipid metabolism, as described by Fievez et al. (2007), will be central for our model developments.

In addition to functional-based representation of the microbiota, microorganisms can also be distinguished with respect to the size of particle of which they are attached (Baldwin et al., 1987; Lescoat and Sauvant, 1995). The distinction of habitats (small particles, large particles, biofilm, liquid phase) is of biological relevance. For example, the surface area and the microbial competition for adherence sites may be limiting factors of fiber degradation, even more important than the enzymatic activity *per se* (Firkins and Yu, 2015; Huws et al., 2018). With regard to methanogens, these microbes has been found in different niches (epithelium, biofilm, solid and liquid phases) (Pei et al., 2010), indicating the interest in including niche differentiation into mathematical models of the rumen ecosystem. Including niche differentiation will imply to replicate the model structure of metabolism for each microbial habitat as I did in our model of human colonic fermentation (Muñoz-Tamayo et al., 2010).

10.1.2 Incorporating thermodynamics into rumen modelling

Thermodynamics of microbial reactions is one of the hallmarks of my research roadmap. Biochemical reactions are governed by thermodynamics. In the rumen, thermodynamic feasibility of reactions is determined by microbial syntrophism (*e.g.*, hydrogen interspecies transfer) and metabolite absorption (Short Chain Fatty Acids - SCFA -clearance). This latter is, from an engineering point of view, an advanced mechanism that favour the transformation of sugars and amino acids into SCFA, given that SCFA accumulation will tip the position of the thermodynamic equilibrium to the side of the reactants and hence inhibiting sugar and amino acid fermentation. With respect to microbial syntrophism, hydrogen produced by certain microbial species is further utilized by methanogenic archaea and homoacetogens. Lowering the concentration of hydrogen favours the thermodynamic feasibility of fermentation reactions and enhances the overall utilization of substrates (Kohn and Boston, 2000; Janssen, 2010). Another important thermodynamic driver is the pH which exhibits a cause-and-effect role on the fermentation. While the pH impacts the fermentation dynamics, it is also affected by the fermentation pattern. The pH is an important driver of the fermentation, determining the pattern of products (Argyle and Baldwin, 1988; Bannink et al., 2008) and thus the nutrients available for the host (Dijkstra et al., 2012). Very importantly, the animal host exerts a thermodynamic control on rumen fermentation *via* salivary secretion, which acts as a buffer influencing ruminal pH. An imbalance of the actions of the previous drivers can lead to the disruption of homeostasis and the animal can be subject of metabolic disorders (Russell and Rychlik, 2001).

Thermodynamic-based theoretical studies have been carried out to describe the impact of hydrogen concentration on the Gibbs free energy changes of the main reactions of ruminal fermentation and the associated shifts in the fermentation pattern (Janssen, 2010), and also to investigate the impact of alternative hydrogen sinks such as reductive acetogenesis on the SCFA fermentation profile (Ungerfeld, 2013). Some attempts have been done to model ruminal fermentation by using thermodynamic concepts (Offner and Sauvant, 2006; Ghimire et al., 2014; Van Lingen et al., 2016). These works have a great conceptual value. However, further developments are needed for attaining a thermodynamic

and dynamic model with satisfactory prediction capabilities. Theoretical frameworks have been developed to allow stoichiometric and energetic balances of microbial growth from the specification of the anabolic and catabolic reactions of microbial metabolism (Heijnen and Dijken, 1992; Kleerebezem and Van Loosdrecht, 2010), and advances have been done to link thermodynamics to kinetics (Hoh and Cord-Ruwisch, 1996; Rodríguez et al., 2008; Gonzalez-Cabaleiro et al., 2013; Desmond-Le Quemener and Bouchez, 2014; Großkopf and Soyer, 2016). I will exploit these theoretical frameworks for tackling the thermodynamic modelling of rumen microbial metabolism.

10.1.3 A genomic-scale level representation of the rumen microbiota

In my coming projects, we will undertake the development of genome-scale metabolic models (GEMs) of key rumen microbial species and combine them into an interacting community GEM representing the rumen microbiome. This approach will fill the gap that there is no quantitative link between current rumen *in silico* models and microbial metaomics data such as microbial genetic potential (genomics), gene expression profiles (transcriptomics) and metabolism (metabolomics). I expect that this approach will significantly enhance rumen microbial knowledge.

The core of a GEM is the construction of the stoichiometry matrix that links the metabolites to the reactions (Palsson, 2006). The stoichiometry matrix is built on the basis of genome-scale network reconstructions (GENRE). Briefly, for a genome sequenced microorganism, the GENRE is obtained by a protocol that includes functional genome annotation, curation of a draft reconstruction of metabolic reactions and finally translation of the reconstructed network into a computational model (Feist et al., 2009; Oberhardt et al., 2009). Reconstruction of a metabolic network of single organism is a difficult task that can take up to six months. Software tools are currently available to automate some of the steps of the reconstruction (Henry et al., 2010; Prigent et al., 2017; Aite et al., 2018).

A GEM is derived from the application of the steady-state assumption on microbial metabolism that translates in the following equation

$$\mathbf{S}v = \mathbf{0} \tag{10.1}$$

with $\mathbf{S}$ the stoichiometry matrix and v the vector of reaction rates. Since the number of reactions is typically higher than the number of metabolites, Eq. (10.1) is often underdetermined. All admissible solutions of Eq. (10.1) constitute the solution space, that mathematically corresponds to the null space (kernel) of the stoichiometric matrix $\mathbf{S}$.

Given that the capabilities of the microbes are bounded by constraints such as (*i*) mass and charge conservation, (*ii*) substrate and enzyme availability, and (*iii*) thermodynamics, the collection of modelling frameworks that are built on the structure of the stoichiometry matrix $\mathbf{S}$ is known as constraint-based modeling approaches (COBRA). The flux balance analysis (FBA) (Varma and Palsson, 1993; Orth et al., 2010), and elementary flux mode analysis (EFM) (Schuster and Hilgetag, 1994) are the primary frameworks of COBRA approaches. FBA and EFM are the scaffolds of a plethora of approaches that counts with more than 100 methods (Price et al., 2004; Lewis et al., 2012). All together, COBRA methods allow the prediction of the potential phenotypes of a organism on the basis of its genome.

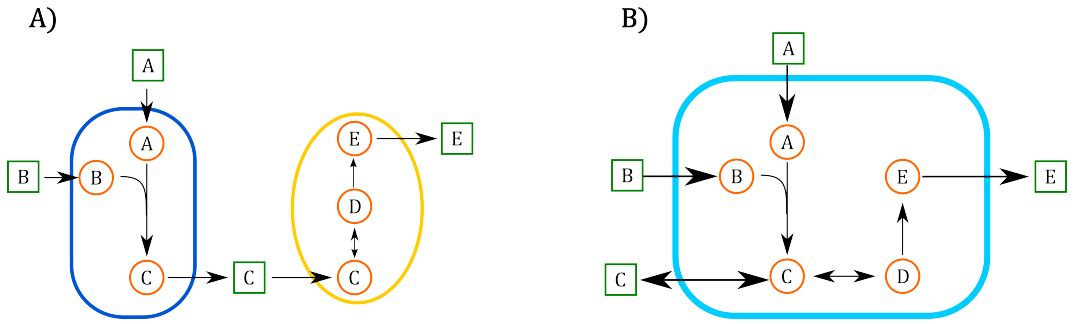


Figure 10.3: Two approaches for modelling microbial communities. The example addresses the modelling of a microbial community composed of two microbes depicted by a rounded blue rectangle and an orange ellipse. In the compartmental approach (A), the microbial species are treated as separate compartments. The two microbes are connected by metabolite cross-feeding. In the supra-organism approach (B), the microbial community is represented by a single microorganism embedded with the metabolic capabilities of the two microbes

GEMs have been extensively applied to single cells, in particular to the well characterized model bacterium *Escherichia coli*, which has been targeted for the development of constrained-based models since 1990 (Reed and Palsson, 2003). However, construction of microbial communities GEMs is still at an early stage. Within the framework of systems biology, the current status of knowledge on the function of microbial communities has been compared to the knowledge of the systems biology of single species ten years ago (Zengler and Palsson, 2012).

The main challenge of building a community model relates to the question of how the species, their metabolic networks, and interspecies interactions should be represented. Tackling this challenge becomes critical when analysing highly diverse ecosystems such as the rumen. Two frameworks, namely the compartmental (Stolyar et al., 2007) and the supra-organism approaches (Rodríguez et al., 2006; Klitgord and Segre, 2011; Borenstein, 2012) have been developed to address the representation of microbial species into a mathematical metabolic model. The two approaches are depicted in Figure 10.3. In the compartmental approach, the metabolic network of each microbial species is treated as a separate compartment, whereas the supra-organism approach assumes that the microbial community behaves as a single microorganism provided with all the metabolic capabilities of the individual species of the consortia. The main weakness of the supra-organism approach is that due to its level of aggregation, it lacks a description of the connectivity principle among species which is a determining factor of the function of the whole community (Walker et al., 2014; Biggs et al., 2015). For highly diverse ecosystems, the compartmental approach in sensu stricto results in a model that is difficultly tractable. To reduce model complexity, an alternative is to represent the microbial community in functional groups rather than in phylogenetic groups. These functional groups can be interpreted as metagenomic species. We will investigate whether the rumen microbiota can be modelled as a mini-consortium of microbial guilds covering a functional core. We will further apply COBRA approaches (FBA, EFM) on the resulting GEMs to link microbial genotype to phenotypes. We will dedicate particular attention in incorporating thermodynamic principles to produce accurate models that integrate primary features such as reaction directionality and feasibility, and allow to predict metabolic shifts driven by

thermodynamic factors. We have already started the GEM construction of the keystone cellulolytic bacterium *Fibrobacter succinogenes*, and to model the metabolism of rumen methanogenic archaea using thermodynamic principles. As mentioned above, model constructions will be supported by the experimental data described in my DRE@M strategy (Figure 10.2).

The resulting research will produce novel modelling frameworks with enhanced predictive capabilities for tackling challenges that cannot be addressed by existing rumen models which do not integrate microbial genomic information and, thus, are not adapted to guide the design of microbial manipulation strategies.

It should be noted that the strategy here described not only applies to the rumen but also to all microbial ecosystems where metabolic reactions take place, including anaerobic reactors for wastewater treatment, and the human gut. It should be said that Hungate (1975) discussed earlier the analogy of the rumen ecosystem with a chemostat. Developments on gut modelling by using the theory of biochemical reactors have been elaborated by Jumars (2000) among others. With this in mind, I expect a mutual benefit of modelling efforts between different domains. While knowledge gained from modelling of engineering reactors can be transferred for modelling digestive ecosystems (Bucci and Xavier, 2014), knowledge on digestive systems could in turn inspire highly efficient bio-processes (Weimer et al., 2009; Godon et al., 2013; Batstone et al., 2015). In fact, enhancing the understanding of microbial systems requires an interdisciplinary approach. I could not find better words than those of Backhed et al. (2005) : *...as microbiotas have coevolved with their animal hosts, this field must coevolve with its academic hosts and their ability to devise innovative ways of assembling interactive interdisciplinary research groups necessary to advance our understanding*. I expect to continue in contributing to the establishment of bridges between multiple disciplines.

10.2 Integrating the rumen microbiota and the animal host

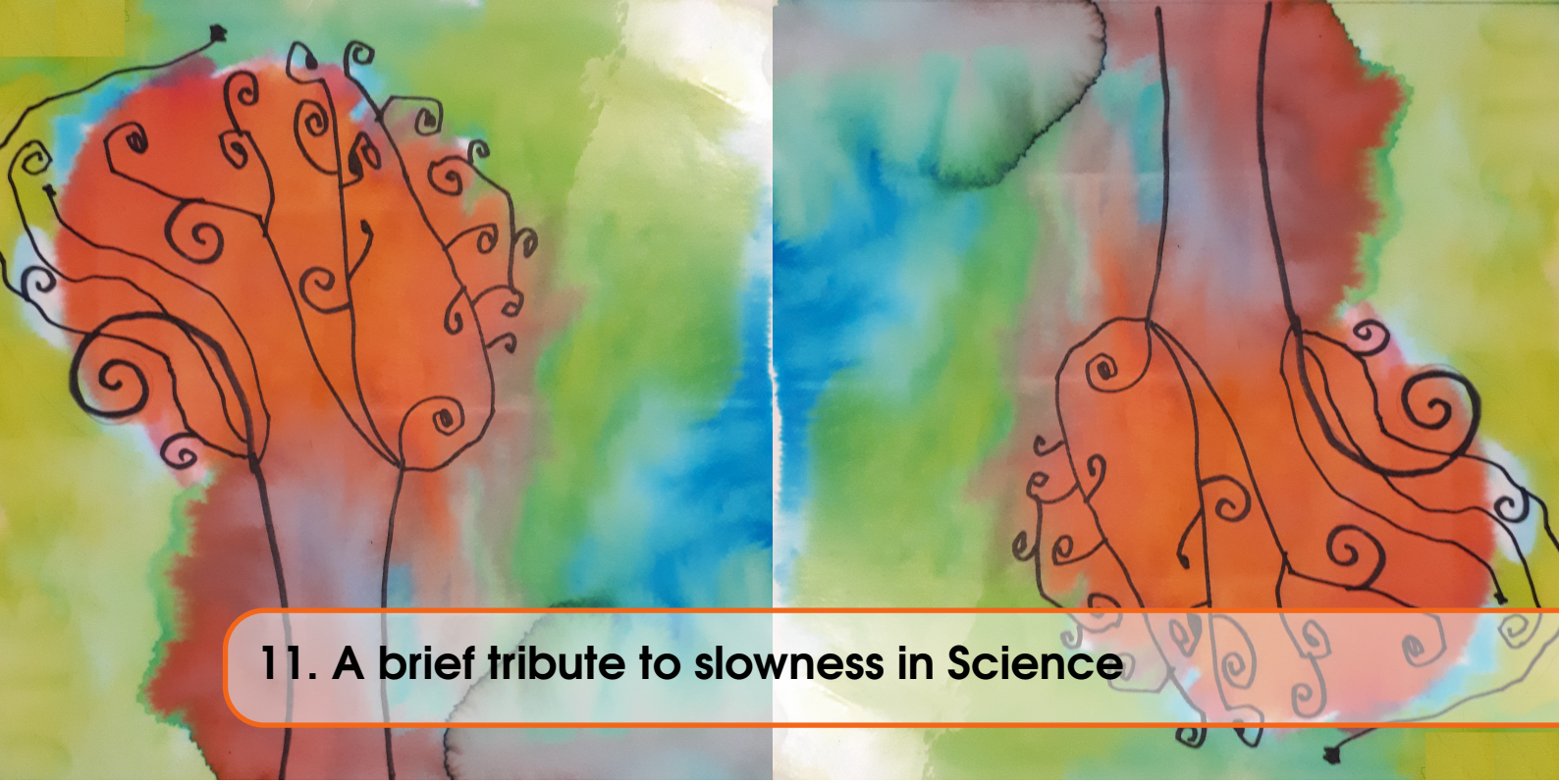
As mentioned before, I have mainly based my research on the rumen microbial component. In the coming years, I will address the animal component. In this direction, to facilitate and support the construction of a mathematical model of rumen function under *in vivo* conditions, I have led, since my arrival at MoSAR, a discussion group for designing and implementing an experimental facility that could provide a dynamic quantitative characterisation of the key phenotypes that influence methane production in ruminants. The discussion group involves technical (Ophélie Dhumez, Alexandra Eymard, Joseph Tessier) and scientific staff (Sylvie Giger-Reverdin, Christine Duvaux-Ponter), and myself. Our experimental device, that we named as the phenobox, allows synchronous measurements of feeding behaviour, water intake and methane production in goats. The phenobox is a battery of four individual boxes whose design is based on the respiration chamber designs of Hart et al. (2014) and Pinares-Patiño et al. (2014). We are currently performing calibration tests. We expect our first experiment to take place in the Fall of 2019. Figure 10.4 displays the prototype of the phenobox device. The animal is located in a transparent box. Inside the box, the ingestion kinetics is recorded using feed bins. Water intake is recorded by a pulse sensor. The air of the box is extracted by the action of two turbines. The air residence time can be set to 6 or 15 min. The humidity of the sampled air is removed by a condensation system to be finally characterised in a MGA3500 gas analyzer

Figure 10.4: Phenobox prototype to measure ingestion kinetics and methane production. This device was designed and implemented by a MoSAR discussion group.

that determines the concentration of methane and carbon dioxide in the box and in the environment background.

Experimental data from the phenobox device and from my national and international projects will be integrated into my model developments. In the long term, I expect to address a genomic-based approach to account for the ruminant holobiont as a whole. The resultant modelling framework will be instrumental for guiding the design of nutritional and microbial manipulation strategies for improving ruminant function within a context of sustainable livestock farming. The design of these optimal nutritional strategies will result from solving a multi-objective mixed-integer dynamic optimization problem addressing central aspects such as feed efficiency, animal health, economic viability and methane emissions. I am already well down this road through the construction of four individual research grants proposals: 1 local (appel Emergences de la ville de Paris), 2 national (ANR) and 1 European (ERC Starting Grant). None of my individual grants got funded but they evolved through European collaborative projects where I lead modelling work packages. While I write this manuscript, I keep fingers crossed for a collaborative research ANR project that I lead as scientific coordinator. Our proposal, that I named as the Syntrophy project has the goal of consolidating the primary pillars of my DRE@M. The Syntrophy project is built upon a collaboration involving 1 industrial partner, 1 CNRS/Inria research team, and 6 Inra research teams from 5 of the 13 scientific Inra divisions.

V A word about Science and conclusions



11. A brief tribute to slowness in Science

Je me souviens des papiers peints où d'énormes formes géométriques oranges et jaunes s'épanouissaient sur fond noir ou marron
Je me souviens «d'un petit pas pour l'homme, mais un grand pas pour l'Humanité »
Je me souviens de la télé en noir et blanc
Je ne me souviens pas du moment de ma naissance
Je me souviens du plumier et des bouteilles d'encre sur la table d'école
Je me souviens des soirées au coin du feu en famille, à raconter nos envies, nos craintes, nos peurs, nos joies, nos colères, nos désaccords, nos émotions, jusqu'à plus de bois.

I remember wallpapers where huge orange and yellow geometric shapes bloomed on a black or brown background.
I remember «a small step for the man, but a big step for Humanity »
I remember black and white TV
I do not remember the moment of my birth
I remember the pencil case and bottles of ink on the school table
I remember the evenings with the family at the corner of the fire, telling our envies, our fears, our joys, our angers, our disagreements, our emotions, until no more wood.

Je me souviens. Georges Pérec.

This text is an updated translated version of the essay: Breve elogio a la lentitud en ciencia. (2016). In: Ciencia y Humanismo 50 años Revista Aleph (1996-2016), pp. 473-478, C.E. Ruiz (Ed.) Ed. Universidad de Caldas, Colombia (Muñoz-Tamayo, 2016). The current manuscript is available at `https://osf.io/preprints/socarxiv/n9wpg/`

Publish or perish: is it the right motto? The answer to this question should be a

strong NO. But we already know that this is not the case. I look at the different research calls in which I have participated to get funding. In the CV section, I find the following phrase: I have a solid track record with W publications, of which X% are published in Q1 journals. I have Y citations and my h-index is Z. Is this the right information that defines my path? Are these the correct measurements of my research? I hope not. Even so, I am the one who has written these phrases and nobody has forced me to write them like that. The why goes with the following essay.

Let's imagine for a moment the following cliché of the scientist: a man with dishevelled hair, wide trousers, the collar of the shirt badly put, the glasses are somewhat fogged. The lenses reflect a lost look towards an apple tree. Time passes slowly. Ideas are carefully woven in a galaxy of neurons. The sun has hidden and our scientist takes the bicycle to go home. The next morning, when passing close to the river, the song of a bird makes him think in certain harmonics.

Well, this image encloses the implicit sense of a spiritual work, guided by the essence of asking, perhaps, by the utopian idea of finding a treasure that might change humankind. For some scientists, the utopia will come true. For others, the utopia will remain as a driving force. However, the reality faced by the scientist, in particular the young (early-career) scientist, is another one.

Metrics and misadventures

As it has occurred in the evaluation process of various public sector institutions (Ogien, 2013), the number has gained an overwhelming importance in the evaluation of research. In principle, the concept of measuring the research work responds to the natural need to have evaluation standards, which is absolutely valid and pertinent. The great difficulty in this procedure is that the measure implies science to be defined as a product and, in this transformation of sense, the legitimacy of science (and of the scientist who performs it) is hereof validated by the measure, in a process that is unscientific, subjective, and secretive (PLoSMedicine-Editors, 2006). But science is not a product.

Currently, the performance of a scientist is mainly measured by their publications: the number of articles published, the number of times their articles are cited by peers, and the prestige of the journals where the articles have been published. The prestige of a journal is often assessed by an indicator known as the impact factor (IF) that measures the average number of citations for each article published in the journal. In addition to the IF, the scientist performance is measured by the H index that attempts to measure the impact of the work published in the scientific community. A H index of value n implies that the scientist has at least n published articles that have been cited at least n times.

The IF, the H index, the number of articles and citations are currently the main criteria for promotion and evaluation both in competitions to obtain research positions and in the calls for research projects and scholarships at national and international level. Although these indicators might correlate with the quality of scientific work, several authors have identified the limitations and dangers of these indicators to assess the value of published research work (Seglen, 1997; PLoSMedicine-Editors, 2006; Lawrence, 2007; Brembs et al., 2013; Paulus et al., 2018). From these criticisms, it is worth to mention the `San Francisco`

Declaration on Research Assessment (DORA) (Cagan, 2013) initiative to discourage and combat the misuse of IF to evaluate the work of individual scientists.

Evaluation is necessary to guarantee the quality of science, and publishing is undoubtedly a central aspect of the scientific work, which also implies a source of satisfaction and recognition. However, publication is not the *raison d'être* of science. The disproportionate importance of publications in the scientific career is a dangerous threat that promotes a science where the discovery of truth is diminished by the desire of publishing (Lawrence, 2007; Park et al., 2014). This phenomenon is recognized in the scientific community under the broadly spread materialistic motto *publish or perish*, and is accompanied by the IF obsession (impactitis) (Casadevall and Fang, 2015). And in this race towards publication, the integrity of science has been jeopardized by ethical misconduct from the scientific community (including Nobel prizes) (Martinson et al., 2005). In fact, a significant number of cases of falsification (Nosek et al., 2012) and data fabrication have been reported in various scientific domains such as the Schön case (2002) in physics, the Stapel case (2011) in psychology and the Voinnet case (2015) in biology (communication by Patricia Volland-Nail http://fr.slideshare.net/pvolland). A gray cloud covers a profession that should enlighten our knowledge of the world.

The pressure exerted by the eagerness to publish affects the entire scientific community, but it shows a marked importance for the young researcher, who must face a hypercompetition to be consolidated as a scientist through funding grants and job competitions in a selection system strongly based on article publication indicators (Schäfer et al., 2011; Farlin and Majewsky, 2013; Schekman, 2013). This system tends to favour short-term applied research projects over long-term basic research projects (Haroche, 2012). The pressure to publish at any price is undoubtedly detrimental to the quality of science and the development of the profile of the researcher, who from the early stage of their career sees how the first utopia becomes overshadowed by mercantile factors. In the silence, the imposter syndrome (Woolston, 2016) comes to disturb our thoughts. In this context, it is very valuable, as statement of principle, the decisions of the ASM journals and eLife of removing the information of the IF on their websites as a declaration of the IF inappropriate use and of the need of alternative research metrics (Schekman and Patterson, 2013; Casadevall et al., 2016). It must be kept in mind, however, that any kind of journal-based assessment will remain flawed in some way and not exempted of the impact factor fallacies discussed by Paulus et al. (2018).

A look at the slowness

The responsibility of changing the engine that drives the scientific activity is not only the resultant of the bureaucratic and political strategies of research centres, and the funding and evaluation agencies of research institutions in each country. The responsibility is also ours who have allowed the implantation of the tremendous reliance on journal citation metrics as indicators of the importance of our research (Casadevall and Fang, 2015). Although this essay illustrates a tendency that is detrimental to the integrity of science, it would be irresponsible to affirm that all scientists follow the doctrine of publishing or perishing and that the conquest of knowledge, as the first motivation, occupies a marginal place. Many researchers continue defending the integrity of science, and resist to the view of science as a mercantile product.

Science will continue its primary role of advancing knowledge and serve society. Modern times call for engaged scientists that, as proposed by the French collective `Sciences en Marche`, defend the scientific method against preconceived opinions and ideologies, and strengthen the dialogue between science and society. Within this context, a switch of direction is needed for promoting beauty and integrity in science. In addition to the necessary actions to undertake in the hiring, promotion and funding decisions adopted in scientific institutions, a change of attitude on the part of the scientific community is needed. We must return to the essential scientific values over the accumulation of publications, and thus be able to eradicate the *impactitis* medical condition (Casadevall and Fang, 2014). We must defend the principles of scientific integrity (Letellier, 2016) and engage in conducting a responsible, reliable and traceable research. And for that, we must beat the rush, not to fall into lethargy, but to strengthen our thinking. Science and scientists need time. Time even to misunderstand each other (`http://slow-science.org/`). Time to read, not only the work of our scientific peers, but also to read and re-read Aristotle, Khant, Khun, Popper, Russell and modern philosophers. Time to read Mafalda, Calvin and Hobbes.

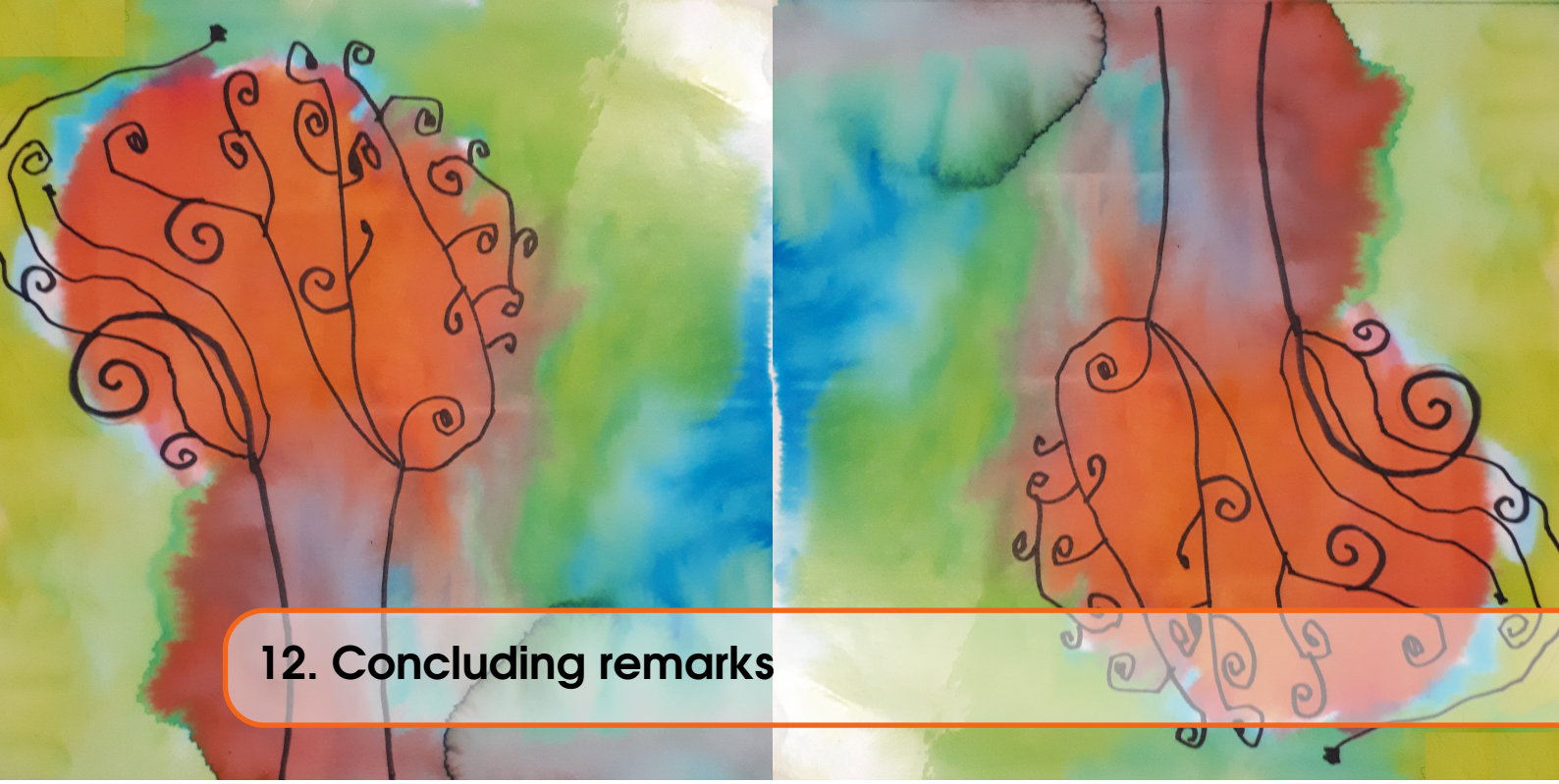
We need to beat the rush, to position utopia as the first place. Promote ethical behaviour through training and discussion spaces for consolidating, at the early stage of the scientist career, an awareness of what the essential scientific values are. An awareness that translates into action of resistance to defend the humanistic role of science.

Beat the rush, to look at science as well as art (Barnes, 1995). Beat the rush, to make the irrational emerge, to give way to creative thinking and give rise to serendipity: that of Alexander Flemming and Isaac Newton, and be able to shout Eureka all over the world.

Beat the rush, to strengthen the genius and keep alive the spirit of a child who asks questions over and over[1]. Beat the rush, to experience the joy of understanding: what is gone, what is on the way.[2].

[1] As the child of the song Escaramujo by Silvio Rodríguez.

[2] From the poem Five Lines by Nazim Hikmet.



12. Concluding remarks

Unus pro omnibus, omnes pro uno.

One for all, all for one.

Motto of the Three Musketeers. Alexandre Dumas père.

This manuscript has traced the path of my travel in the sea of Science. The travel has been a modelling odyssey in the tree of life, in which I have studied different biological systems including bacteria, microalgae, yeast and animals. The collection of articles here discussed demonstrate the power of mathematical modelling to improve understanding of biological systems. These works illustrate that models are not only useful for predicting or describing, but also as powerful tools to formalise knowledge, generate and test hypothesis that are difficult to evaluate experimentally, and to design optimal control strategies to maximize system performance. I want to stress that mathematical modelling do not diminish the importance of experimental work. Models and experiments exhibit a symbiotic relationship for knowledge improvement. Whlist experiments provide the relevant information to model construction, models can be used to substantially enrich the level of information to be extracted from experimentation. Through optimal experimental design, experimental conditions can be identified to provide high quality data to facilitating the quantitative description of system dynamics.

The modelling building process requires a collection of mathematical and informatics tools to provide usable and robust models. Beyond these technicalities, one of the pillars of model construction is the process of knowledge verbalisation and the translation of such a knowledge into a mathematical entity. This process appears often imperceptible in the scientific literature but is the core of the modelling endeavour (a form of art), in particular when dealing with biological systems.

The complexity underlying biology makes it impossible for a single mind to undertake a

research approach covering the multiple components that impact the dynamic behaviour of biological systems. It is here where the interdisciplinary science concept needs to metamorphose from words into actions. I could not be more privileged of having travelled with passionate colleagues from different disciplines that have been keen and willing to share their knowledge, to be patient for taking the time to explain, to listen and constructing a common language. I am deeply indebted to them.

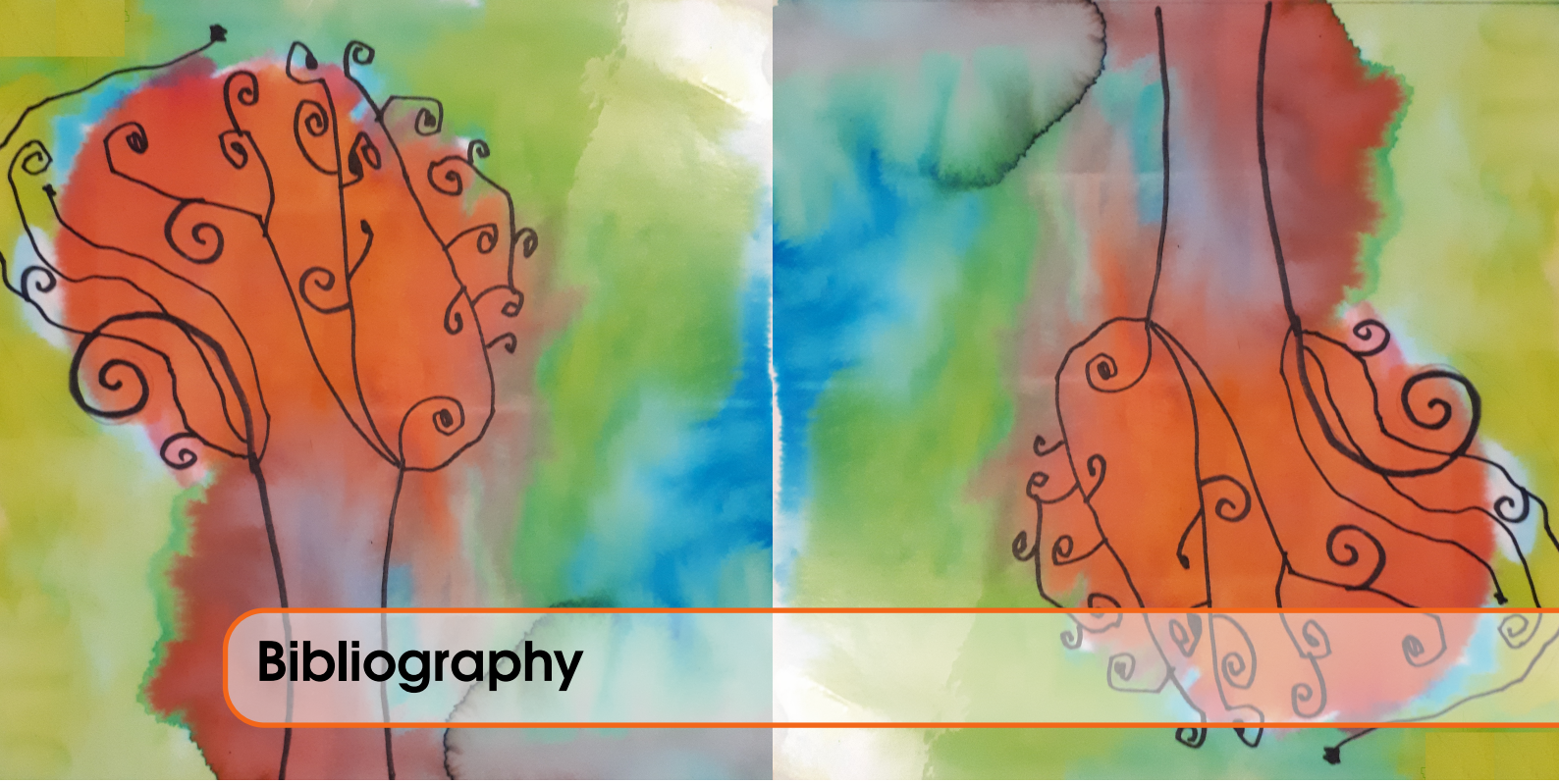
All together, these works converge in setting a solid bedrock for my future research on decrypting the dynamics of the rumen microbiota with the prospect of using such knowledge for the design of microbial manipulation strategies for improving rumen funtion towards sustainable productivity. Incorporating the whole ruminant holobiont entity into mathematical models is my great challenge in the coming years.

Finally, since the questions I address are not exclusive of the rumen and also applies to other microbial ecosystems, I expect my research can also contribute to enhance understanding of artificial engineering reactors and other digestive systems such as the human gut.

I close these pages by saying that a beautiful science needs time to let the orchids bloom.

VI

Bibliography



Bibliography

Abecia, L., Martn-Garcia, A. I., Martinez, G., Newbold, C. J., Yañez-Ruiz, D. R., 2013. Nutritional intervention in early life to manipulate rumen microbial colonization and methane output by kid goats postweaning1. Journal of Animal Science 91, 4832–4840.

Aite, M., Chevallier, M., Frioux, C., Trottier, C., Got, J., Cortés, M. P., Mendoza, S., Carrier, G., Dameron, O., Guillaudeux, N., Latorre, M., Loira, N., Markov, G. V., Maass, A., Siegel, A., 2018. Traceability, reproducibility and wiki-exploration for a-la-carte reconstructions reconstructions of genome-scale metabolic models. Plos Comput Biol. 14, e1006146.

Akaike, H., 1974. A new look at the statistical model identification. IEEE Transactions on Automatic Control 19 (6), 716–723.

Amribt, Z., Niu, H., Bogaerts, P., 2013. Macroscopic modelling of overflow metabolism and model based optimization of hybridoma cell fed-batch cultures. Biochemical Engineering Journal 70, 196–209.

Angulo, F., Munoz, R., Olivar, G., 2007. Control of a bioreactor using feedback linearization. In: Mediterranean Conference on Control & Automation. IEEE, pp. 1–6.

Argyle, J. L., Baldwin, R. L., 1988. Modeling of Rumen Water Kinetics and Effects of Rumen Ph Changes. Journal of Dairy Science 71, 1178–1188.

Backhed, F., Ley, R. E., Sonnenburg, J. L., Peterson, D. A., Gordon, J. I., 2005. Host-bacterial mutualism in the human intestine. Science 307 (5717), 1915–1920.

Bailey, J. E., 1998. Mathematical modeling and analysis in biochemical engineering: past accomplishments and future opportunities. Biotechnology progress 14, 8–20.

Baldwin, R. L., 2000. Introduction: history and future of modelling nutrient utilization in farm animals. In: McNamara France, J., Beever, D.E., J. P. (Ed.), Modelling nutrient utilization in farm animals. CAB International, Wallingford, UK, pp. 1–9.

Baldwin, R. L., Thornley, J. H., Beever, D. E., 1987. Metabolism of the lactating cow. II. Digestive elements of a mechanistic model. J. Dairy Res. 54, 107–131.

Balsa-Canto, E., Alonso, A. A., Banga, J. R., 2008. Computational procedures for optimal experimental design in biological systems. IET Syst Biol 2, 163–172.

Balsa-Canto, E., Banga, J. R., 2011. AMIGO, a toolbox for advanced model identification in systems biology using global optimization. Bioinformatics 27, 2311–2313.

Bannink, A., France, J., Lopez, S., Gerrits, W. J. J., Kebreab, E., Tamminga, S., Dijkstra, J., 2008. Modelling the implications of feeding strategy on rumen fermentation and functioning of the rumen wall. Animal Feed Science and Technology 143, 3–26.

Bannink, A., van Lingen, H. J., Ellis, J. L., France, J., Dijkstra, J., 2016. The Contribution of Mathematical Modeling to Understanding Dynamic Aspects of Rumen Metabolism. Frontiers in Microbiology 7, 1820.

Baranyi, J., Ross, T., McMeekin, T. A., Roberts, T. A., 1996. Effects of parameterization on the performance of empirical models used in 'predictive microbiology'. Food Microbiology 13, 83–91.

Barnes, C. J., 1995. The Art of Catchment Modeling - What Is a Good Model. Environment International 21, 747–751.

Baroukh, C., Muñoz-Tamayo, R., Bernard, O., Steyer, J. P., 2015a. Mathematical modeling of unicellular microalgae and cyanobacteria metabolism for biofuel production. Current Opinion in Biotechnology 33, 198–205.

Baroukh, C., Muñoz-Tamayo, R., Steyer, J. P., Bernard, O., 2014. DRUM: A New Framework for Metabolic Modeling under Non-Balanced Growth. Application to the Carbon Metabolism of Unicellular Microalgae. PLoS One 9 (8).

Baroukh, C., Muñoz-Tamayo, R., Steyer, J.-P., Bernard, O., 2015b. A state of the art of metabolic networks of unicellular microalgae and cyanobacteria for biofuel production. Metabolic Engineering 30, 49–60.

Batstone, D., Puyol, D., Flores-Alsina, X., Rodríguez, J., 2015. Mathematical modelling of anaerobic digestion processes: applications and future needs. Rev Environ Sci Biotechnol, 1–19.

Batstone, D. J., Keller, J., Angelidaki, I., Kalyuzhnyi, S. V., Pavlostathis, S. G., Rozzi, A., Sanders, W. T., Siegrist, H., Vavilin, V. A., 2002. Anaerobic Digestion Model No.1 (ADM1). IWA Publishing, London.

Belanche, A., de la Fuente, G., Newbold, C. J., 2015. Effect of progressive inoculation of fauna-free sheep with holotrich protozoa and total-fauna on rumen fermentation, microbial diversity and methane emissions. FEMS Microbiology Ecology 91 (3).

Bellman, R., Astrom, K. J., 1970. On structural identifiability. Math. Biosci. 7, 329–339.

Bellu, G., Saccomani, M. P., Audoly, S., D'Angio, L., 2007. DAISY: A new software tool to test global identifiability of biological and physiological systems. Computer Methods and Programs in Biomedicine 88, 52–61.

Beopoulos, A., Cescut, J., Haddouche, R., Uribelarrea, J.-L., Molina-Jouve, C., Nicaud, J.-M., 2009. *Yarrowia lipolytica* as a model for bio-oil production. Progress in Lipid Research 48, 375 – 387.

Bernalier, A., Willems, A., Leclerc, M., Rochet, V., Collins, M. D., 1996. *Ruminococcus hydrogenotrophicus* sp. nov., a new H_2/CO_2-utilizing acetogenic bacterium isolated from human feces. Arch Microbiol 166 (3), 176–183.

Bernard, O., 2011. Hurdles and challenges for modelling and control of microalgae for CO2 mitigation and biofuel production. Journal of Process Control 21, 1378–1389.

Bernard, O., Chachuat, B., Hélias, a., Rodriguez, J., feb 2006. Can we assess the model complexity for a bioprocess: theory and example of the anaerobic digestion process. Water Science & Technology 53, 85–92.

Biggs, M. B., Medlock, G. L., Kolling, G. L., Papin, J. A., 2015. Metabolic network modeling of microbial communities. Wiley Interdisciplinary Reviews-Systems Biology and Medicine 7 (5), 317–334.

Bonnans, Frederic, J., Giorgi, D., Grelard, V., Heymann, B., Maindrault, S., Martinon, P., Tissot, O., Liu, J., 2017. Bocop − a collection of examples. Tech. rep., INRIA.

Borenstein, E., 2012. Computational systems biology and in silico modeling of the human microbiome. Briefings in Bioinformatics 13 (6), 769–780.

Brembs, B., Button, K., Munafò, M., 2013. Deep impact: unintended consequences of journal rank. Frontiers in human Neuroscience 7, 291.

Bucci, V., Xavier, J. B., 2014. Towards predictive models of the human gut microbiome. Journal of Molecular Biology 426, 3907–3916.

Cagan, R., 2013. The San Francisco declaration on research assessment. Disease models & mechanisms 6, 869–870.

Casadevall, A., Bertuzzi, S., Buchmeier, M. J., Davis, R. J., Drake, H., Fang, F. C., Gilbert, J., Goldman, B. M., Imperiale, M. J., Matsumura, P., McAdam, A. J., Pasetti, M. F., Sandri-Goldin, R. M., Silhavy, T., Rice, L., Young, J.-A. H., Shenk, T., 2016. ASM journals eliminate impact factor information from journal websites. Applied and Environmental Microbiology 82, 5479–5480.

Casadevall, A., Fang, F. C., 2014. Causes for the persistence of impact factor mania. mBio 5, e00064–e00014.

Casadevall, A., Fang, F. C., 2015. Impacted science: Impact is not importance. mBio 6.

Chachuat, B., Singer, A. B., Barton, P. I., dec 2006. Global Methods for Dynamic Optimization and Mixed-Integer Dynamic Optimization. Industrial & Engineering Chemistry Research 45, 8373–8392.

Chachuat, B., Srinivasan, B., Bonvin, D., oct 2009. Adaptation strategies for real-time optimization. Computers & Chemical Engineering 33, 1557–1567.

Charmley, E., Williams, S. R. O., Moate, P. J., Hegarty, R. S., Herd, R. M., Oddy, V. H., Reyenga, P., Staunton, K. M., Anderson, A., Hannah, M. C., 2016. A universal equation to predict methane production of forage-fed cattle in Australia. Animal Production Science 56 (2-3), 169–180.

Chaves, M., 2013. Predictive analysis of dynamical systems: combining discrete and continuous formalisms. mémoire présenté pour obtenir le diplôme d'habilitation à diriger des recherches. Tech. rep., Université de Nice Sophia Antipolis.

Chis, O., Banga, J. R., Balsa-Canto, E., 2011a. GenSSI: a software toolbox for structural identifiability analysis of biological models. Bioinformatics 27, 2610–2611.

Chis, O. T., Banga, J. R., Balsa-Canto, E., 2011b. Structural Identifiability of Systems Biology Models: A Critical Comparison of Methods. PLoS One 6, e27755.

Combe, C., Hartmann, P., Rabouille, S., Talec, A., Bernard, O., Sciandra, A., 2015. Long-term adaptive response to high-frequency light signals in the unicellular photosynthetic eukaryote *Dunaliella salina*. Biotechnology and Bioengineering 112 (6), 1111–1121.

Crompton, L. A., Mills, J. A. N., Reynolds, C. K., France, J., 2011. Fluctuations in methane emission in response to feeding pattern in lactating dairy cows. Modelling Nutrient Digestion and Utilisation in Farm Animals, 176–180.

Debruyne, S., Ruiz-González, A., Artiles-Ortega, E., Ampe, B., Van Den Broeck, W., De Keyser, E., Vandaele, L., Goossens, K., Fievez, V., 2018. Supplementing goat kids with coconut medium chain fatty acids in early life influences growth and rumen papillae development until 4 months after supplementation but effects on *in vitro* methane emissions and the rumen microbiota are transient. Journal of Animal Science 96, 1978–1995.

Denis-vidal, L., Joly-blanchard, G., 2004. Equivalence and identifiability analysis of uncontrolled nonlinear dynamical systems. Automatica 40, 287 – 292.

Descartes, R., 1637. Discours de la méthode pour bien conduire sa raison, et chercher la vérité dans les sciences.
URL ExtractedfromTheProjectGutenbergcollection(http://www.gutenberg.org)

Desmond-Le Quemener, E., Bouchez, T., 2014. A thermodynamic theory of microbial growth. Isme Journal 8 (8), 1747–1751.

Dijkstra, J., 1994. Simulation of the dynamics of protozoa in the rumen. Br J Nutr 72, 679–699.

Dijkstra, J., Ellis, J. L., Kebreab, E., Strathe, A. B., López, S., France, J., Bannink, A., 2012. Ruminal pH regulation and nutritional consequences of low pH. Animal Feed Science and Technology 172, 22–33.

Doreau, M., Fievez, V., Troegeler-Maynadier, A., Glasser, F., 2012. Ruminal metabolism and digestion of long chain fatty acids in ruminants: recent advances in knowledge. Inra Productions Animales 25 (4), 361–373.

Dragomir, C., Sauvant, D., Peyraud, J. L., Giger-Reverdin, S., Michalet-Doreau, B., 2008. Meta-analysis of 0 to 8 h post-prandial evolution of ruminal pH. Animal 2, 1437–1448.

Droop, M. R., 1968. Vitamin b12 and marine ecology .iv. the kinetics of uptake, growth and inhibition in *Monochrysis lutheri*. J. Mar. Biol. Assoc. 48, 689–773.

Duncan, S. H., Louis, P., Flint, H. J., 2004. Lactate-utilizing bacteria , isolated from human Feces, that Produce butyrate as a major fermentation product. Society 70 (10), 5810–5817.

Economou, C. N., Aggelis, G., Pavlou, S., Vayenas, D. V., may 2011. Modeling of single-cell oil production under nitrogen-limited and substrate inhibition conditions. Biotechnology and bioengineering 108, 1049–55.

Egea, J. a., Rodríguez-Fernández, M., Banga, J. R., Martí, R., 2006. Scatter search for chemical and bio-process optimization. Journal of Global Optimization 37, 481–503.

Ellis, J. L., Dijkstra, J., Kebreab, E., Bannink, A., Odongo, N. E., McBride, B. W., France, J., 2008. Aspects of rumen microbiology central to mechanistic modelling of methane production in cattle. J. Agric. Sci. 146, 213–233.

Farlin, J., Majewsky, M., 2013. Performance indicators: The educational effect of publication pressure on young researchers in environmental sciences. Environmental Science & Technology 47, 2437–2438, pMID: 23461664.

Feist, A. M., Herrgard, M. J., Thiele, I., Reed, J. L., Palsson, B. O., 2009. Reconstruction of biochemical networks in microorganisms. Nature Reviews Microbiology 7 (2), 129–143.

Fievez, V., Vlaeminck, B., Jenkins, T., Enjalbert, F., Doreau, M., 2007. Assessing rumen biohydrogenation and its manipulation *in vivo, in vitro* and *in situ*. European Journal of Lipid Science and Technology 109, 740–756.

Firkins, J. L., Yu, Z., 2015. RUMINANT NUTRITION SYMPOSIUM: How to use data on the rumen microbiome to improve our understanding of ruminant nutrition. Journal of Animal Science 93, 1450–1470.

Flint, H. J., Duncan, S. H., Scott, K. P., 2007. Minireview Interactions and competition within the microbial community of the human colon : links between diet and health. Environmental Microbiology.

Friggens, N. C., Blanc, F., Berry, D. P., Puillet, L., 2017. Review: Deciphering animal robustness. A synthesis to facilitate its use in livestock breeding and management. Animal 11, 2237–2251.

Ghimire, S., Gregorini, P., Hanigan, M., 2014. Evaluation of predictions of volatile fatty acid production rates by the Molly cow model. Journal of Dairy Science 97 (1), 354–362.

Giger-reverdin, S., Morand-Fehr, P., Tran, G., 2003. Literature survey of the influence of dietary fat composition on methane production in dairy cattle. Livestock Production Science 82, 73–79.

Godon, J. J., Arcemisbehere, L., Escudie, R., Harmand, J., Miambi, E., Steyer, J. P., 2013. Overview of the Oldest Existing Set of Substrate-optimized Anaerobic Processes: Digestive Tracts. Bioenergy Research 6 (3), 1063–1081.

Golubev, A., 2009. How could the Gompertz-Makeham law evolve. Journal of Theoretical Biology 258 (1), 1–17.

Gonzalez-Cabaleiro, R., Lema, J. M., Rodriguez, J., Kleerebezem, R., 2013. Linking thermodynamics and kinetics to assess pathway reversibility in anaerobic bioprocesses. Energy & Environmental Science 6, 3780–3789.

Gregorini, P., Beukes, P., Waghorn, G., Pacheco, D., Hanigan, M., 2015. Development of an improved representation of rumen digesta outflow in a mechanistic and dynamic model of a dairy cow, Molly. Ecological Modelling 313, 293–306.

Gregorini, P., Beukes, P. C., Hanigan, M. D., Waghorn, G., Muetzel, S., McNamara, J. R., 2013. Comparison of updates to the Molly cow model to predict methane production from dairy cows fed pasture. Journal of Dairy Science 96 (8), 5046–5052.

Gresse, R., Chaucheyras-Durand, F., Fleury, M. A., Van de Wiele, T., Forano, E., Blanquet-Diot, S., 2017. Gut microbiota dysbiosis in postweaning piglets: understanding the keys to health. Trends Microbiol 25, 851–873.

Groenen, M. A. M., Archibald, A. L., Uenishi, H., Tuggle, C. K., Takeuchi, Y., Rothschild, M. F., Rogel-Gaillard, C., Park, C., Milan, D., Megens, H.-J., Li, S., Larkin, D. M., Kim, H., Frantz, L. A. F., Caccamo, M., Ahn, H., Aken, B. L., Anselmo, A., Anthon, C., Auvil, L., Badaoui, B., Beattie, C. W., Bendixen, C., Berman, D., Blecha, F., Blomberg, J., Bolund, L., Bosse, M., Botti, S., Bujie, Z., Bystrom, M., Capitanu, B., Carvalho-Silva, D., Chardon, P., Chen, C., Cheng, R., Choi, S.-H., Chow, W., Clark, R. C., Clee, C., Crooijmans, R. P. M. A., Dawson, H. D., Dehais, P., De Sapio, F., Dibbits, B., Drou, N., Du, Z.-Q., Eversole, K., Fadista, J., Fairley, S., Faraut, T., Faulkner, G. J., Fowler, K. E., Fredholm, M., Fritz, E., Gilbert, J. G. R., Giuffra, E., Gorodkin, J., Griffin, D. K., Harrow, J. L., Hayward, A., Howe, K., Hu, Z.-L., Humphray, S. J., Hunt, T., Hornshj, H., Jeon, J.-T., Jern, P., Jones, M., Jurka, J., Kanamori, H., Kapetanovic, R., Kim, J., Kim, J.-H., Kim, K.-W., Kim, T.-H., Larson, G., Lee, K., Lee, K.-T., Leggett, R., Lewin, H. A., Li, Y., Liu, W., Loveland, J. E., Lu, Y., Lunney, J. K., Ma, J., Madsen, O., Mann, K., Matthews, L., McLaren, S., Morozumi, T., Murtaugh, M. P., Narayan, J., Nguyen, D. T., Ni, P., Oh, S.-J., Onteru, S., Panitz, F., Park, E.-W., Park, H.-S., Pascal, G., Paudel, Y., Perez-Enciso, M., Ramirez-Gonzalez, R., Reecy, J. M., Rodriguez-Zas, S., Rohrer, G. A., Rund, L., Sang, Y., Schachtschneider, K., Schraiber, J. G., Schwartz, J., Scobie, L., Scott, C., Searle, S., Servin, B., Southey, B. R., Sperber, G., Stadler, P., Sweedler, J. V., Tafer, H., Thomsen, B., Wali, R., Wang, J., Wang, J., White, S., Xu, X., Yerle, M., Zhang, G., Zhang, J., Zhang, J., Zhao, S., Rogers, J., Churcher, C., Schook, L. B., 2012. Analyses of pig genomes provide insight into porcine demography and evolution. Nature 491, 393–398.

Großkopf, T., Soyer, O. S., 2016. Microbial diversity arising from thermodynamic constraints. ISME Journal 10, 2725–2733.

Guyader, J., Eugene, M., Noziere, P., Morgavi, D. P., Doreau, M., Martin, C., 2014. Influence of rumen protozoa on methane emission in ruminants: a meta-analysis approach. Animal 8 (11), 1816–1825.

Haroche, S., 2012. The secrets of my prizewinning research. Nature News 490, 311.

Hart, K., Yañez-Ruiz, D., Martin-Garcia, A., Newbold, C., 2014. Technical Manual on Respiration Chamber Designs. Wellington, New Zealand: Ministry of Agriculture and Forestry, Ch. Chapter 5: Sheep Methane Chambers at Aberystwyth University (UK) and CSIC (Spain), pp. 77–88.

Heijnen, J. J., Dijken, J. P., 1992. In search of a thermodynamic description of biomass yields for the chemotrophic growth of microorgansims. Biotechnology and Bioengeneering 39, 833–852.

Henry, C. S., DeJongh, M., Best, A. a., Frybarger, P. M., Linsay, B., Stevens, R. L., 2010. High-throughput generation, optimization and analysis of genome-scale metabolic models. Nature biotechnology 28 (9), 977–82.

Hirmajer, T., Balsa-Canto, E., Banga, J. R., 2009. DOTcvpSB, a software toolbox for dynamic optimization in systems biology. BMC Bioinformatics 10, 1–14.

Hoh, C. Y., Cord-Ruwisch, R., 1996. A practical kinetic model that considers endproduct inhibition in anaerobic digestion processes by including the equilibrium constant. Biotechnology and Bioengineering 51 (5), 597–604.

Huhtanen, P., Ramin, M., Uden, P., 2015. Nordic dairy cow model Karoline in predicting methane emissions: 1. model description and sensitivity analysis. Livestock Science.

Hungate, R. E., 1975. The Rumen Microbial Ecosystem. Annual Review of Ecology and Systematics 6, 39–66.

Hurst, T., Rehbock, V., 2018. Optimal control for micro-algae on a raceway model. Biotechnology progress 34, 107–119.

Huws, S. A., Creevey, C. J., Oyama, L. B., Mizrahi, I., Denman, S. E., Popova, M., Muñoz-Tamayo, R., Forano, E., Waters, S. M., Hess, M., Tapio, I., Smidt, H., Krizsan, S. J., Yañez-Ruiz, D. R., Belanche, A., Guan, L., Gruninger, R. J., McAllister, T. A., Newbold, C. J., Roehe, R., Dewhurst, R. J., Snelling, T. J., Watson, M., Suen, G., Hart, E. H., Kingston-Smith, A. H., Scollan, N. D., do Prado, R. M., Pilau, E. J., Mantovani, H. C., Attwood, G. T., Edwards, J. E., McEwan, N. R., Morrisson, S., Mayorga, O. L., Elliott, C., Morgavi, D. P., 2018. Addressing global ruminant agricultural challenges through understanding the rumen microbiome: Past, present, and future. Frontiers in Microbiology 9, 2161.

Janssen, P. H., 2010. Influence of hydrogen on rumen methane formation and fermentation balances through microbial growth kinetics and fermentation thermodynamics. Anim. Feed Sci. Technol. 160, 1–22.

Janssen, P. H., Kirs, M., 2008. Structure of the archaeal community of the rumen. Applied and environmental microbiology 74, 3619–3625.

Jenkins, T. C., Wallace, R. J., Moate, P. J., Mosley, E. E., 2008. Board-invited review: Recent advances in biohydrogenation of unsaturated fatty acids within the rumen microbial ecosystem1. Journal of Animal Science 86, 397–412.

Joblin, K. N., 1999. Ruminal acetogens and their potential to lower ruminant methane emissions. Australian Journal of Agricultural Research 50, 1307–1313.

Juillet, B., Saccomani, M. P., Bos, C., Gaudichon, C., Tome, D., Fouillet, H., 2006. Conceptual, methodological and computational issues concerning the compartmental modeling of a complex biological system: Postprandial inter-organ metabolism of dietary nitrogen in humans. Mathematical Biosciences 204, 282–309.

Jumars, P. A., 2000. Animal guts as nonideal chemical reactors: Partial mixing and axial variation in absorption kinetics. American Naturalist 155 (4), 544–555.

Karlsson, J., Anguelova, M., M, J., 2012. An Efficient Method for Structural Identifiability Analysis of Large Dynamic Systems. pp. 941–946.

Kettle, H., Holtrop, G., Louis, P., Flint, H. J., 2018. microPop: Modelling microbial populations and communities in R. Methods in Ecology and Evolution 9 (2), 399–409.

Kettle, H., Louis, P., Holtrop, G., Duncan, S. H., Flint, H. J., 2015. Modelling the emergent dynamics and major metabolites of the human colonic microbiota. Environmental Microbiology 17, 1615–1630.

Kitano, H., 2002. Systems biology: a brief overview. Science 295 (5560), 1662–1664.

Kittelmann, S., Pinares-Patino, C. S., Seedorf, H., Kirk, M. R., Ganesh, S., McEwan, J. C., Janssen, P. H., 2014. Two Different Bacterial Community Types Are Linked with the Low-Methane Emission Trait in Sheep. PLoS One 9 (7).

Kleerebezem, R., Van Loosdrecht, M. C. M., 2010. A Generalized Method for Thermodynamic State Analysis of Environmental Systems. Critical Reviews in Environmental Science and Technology 40, 1–54.

Klieve, A. V., 1999. Opportunities for biological control of ruminal methanogenesis. Australian Journal of Agricultural Research 50, 1315–1319.

Klitgord, N., Segre, D., 2011. Ecosystems biology of microbial metabolism. Current Opinion in Biotechnology 22 (4), 541–546.

Kohn, R. a., Boston, R. C., 2000. The Role of Thermodynamics in Controlling Rumen Metabolism. Modelling Nutrient Utilization in Farm Animals, 11–24.

Labarthe, S., Polizzi, B., Phan, T., Goudon, T., Ribot, M., B., L., 2018. A mathematical model to investigate the key drivers of the biogeography of the colon microbiota. Preprint HAL.

Lacour, T., Sciandra, A., Talec, A., Mayzaud, P., Bernard, O., 2012. Diel variations of carbohydrates and neutral lipids in nitrogen-sufficient and nitrogen-starved cyclostat cultures of *Isochrysis* sp. Journal of Phycology 48, 966–975.

Lawrence, P. A., 2007. The mismeasurement of science. Current biology 17, R583–R585.

Legay, J. M., 1997. L'expérience et le modèle. Un discours sur la méthode. Collection Sciences en questions. INRA editions.

Lema Perez, L., Muñoz-Tamayo, R., Garcia Tirado, J. F., Alvarez, H., 2019. On parameter interpretability of phenomenological-based semiphysical models. Informatics in Medicine Unlocked 15, 100158.
URL `https://www.biorxiv.org/content/early/2018/10/18/446583`

Lescoat, P., Sauvant, D., 1995. Development of a mechanistic model for rumen digestion validated using the duodenal flux of amino acids. Reprod. Nutr. Dev. 35, 45–70.

Letellier, L., 2016. Integrity and responsibility in research practices. a guide. Tech. rep., CNRS Ethics Committee (COMETS).

Lewis, N. E., Nagarajan, H., Palsson, B. O., 2012. Constraining the metabolic genotype-phenotype relationship using a phylogeny of in silico methods. Nature Reviews Microbiology 10 (4), 291–305.

Li, J., Zhong, H., Ramayo-Caldas, Y., Terrapon, N., Lombard, V., Potocki-Veronese, G., Estelle-Fabrellas, J., Popova, M., Yang, Z., Zhang, H., Li, F., Tang, S., Chen, W., Chen, B., Li, J., Guo, J., Martin, C., Maguin, E., Xu, X., Yang, H., Wang, J., Madsen, L., Kristiansen, K., Henrissat, B., Ehrlich, S. D., Morgavi, D. P., jan 2018. A catalog of microbial genes from the bovine rumen reveals the determinants of herbivory. bioRxiv. URL `http://biorxiv.org/content/early/2018/02/27/272690.abstract`

Ljung, L., 2007. System Identification Toolbox for use with Matlab. Version 7. The Mathworks, Inc.

Macfarlane, G. T., Cummings, J. H., 1991. The Large Intestine: Physiology, Pathophysiology and Disease. Raven Press Ltd, London, Ch. The colonic flora, fermentation, and large bowel digestive function, pp. 51–92.

Mahadevan, R., Edwards, J. S., Doyle, F. J., 2002. Dynamic flux balance analysis of diauxic growth in *Escherichia coli*. Biophysical Journal 83, 1331–1340.

Mairet, F., Bernard, O., Cameron, E., Ras, M., Lardon, L., Steyer, J. P., Chachuat, B., 2012. Three-reaction model for the anaerobic digestion of microalgae. Biotechnol Bioeng 109, 415–425.

Mairet, F., Bernard, O., Masci, P., Lacour, T., Sciandra, A., 2011. Modelling neutral lipid production by the microalga *Isochrysis* aff. *galbana* under nitrogen limitation. Bioresource technology 102 (1), 142–149.

Mairet, F., Muñoz-Tamayo, R., Bernard, O., 2015. Adaptive control of light attenuation for optimizing microalgae production. Journal of Process Control 30, 117–124.

Maiwald, T., Timmer, J., 2008. Dynamical modeling and multi-experiment fitting with PottersWheel. Bioinformatics 24, 2037–2043.

Marchetti, J., Bougaran, G., Le Dean, L., Mégrier, C., Lukomska, E., Kaas, R., Olivo, E., Baron, R., Robert, R., Cadoret, J., 2011. Optimizing conditions for the continuous culture of *Isochrysis* affinis *galbana* relevant to commercial hatcheries. Aquaculture 326-329, 106–115.

Martinson, B. C., Anderson, M. S., de Vries, R., 2005. Scientists behaving badly. Nature 435, 737–738.

Mathworks, 2018. Global Optimization Toolbox. User's Guide. The Mathworks, Inc. Natick, MA, USA.

May, R. M., Feb 2004. Uses and abuses of mathematics in biology. Science 303 (5659), 790–793.

McAllister, T. A., Meale, S. J., Valle, E., Guan, L. L., Zhou, M., Kelly, W. J., Henderson, G., Attwood, G. T., Janssen, P. H., 2015. RUMINANT NUTRITION SYMPOSIUM: Use of genomics and transcriptomics to identify strategies to lower ruminal methanogenesis. J. Anim. Sci. 93, 1431–1449.

Mcsweeney, C., Mackie, R., 2012. Micro-organisms and ruminant digestion: state of knowledge, trends and future prospects. FAO.

Mills, J. A. N., Crompton, L. A., Ellis, J. L., Dijkstra, J., Bannink, A., Hook, S., Benchaar, C., France, J., 2014. A dynamic mechanistic model of lactic acid metabolism in the rumen. J. Dairy Sci. 97, 2398–2414.

Moate, P. J., Boston, R. C., Jenkins, T. C., Lean, I. J., 2008. Kinetics of ruminal lipolysis of triacylglycerol and biohydrogenation of long-chain fatty acids: New insights from old data. Journal of Dairy Science 91 (2), 731–742.

Moorthy, A. S., Brooks, S. P., Kalmokoff, M., Eberl, H. J., 2015. A Spatially Continuous Model of Carbohydrate Digestion and Transport Processes in the Colon. PLoS One 10 (12), e0145309.

Moorthy, A. S., Eberl, H. J., 2014. Assessing the influence of reactor system design criteria on the performance of model colon fermentation units. Journal of Bioscience and Bioengineering 117 (4), 478–484.

Morgavi, D. P., Forano, E., Martin, C., Newbold, C. J., 2010. Microbial ecosystem and methanogenesis in ruminants. Animal 4, 1024–1036.

Morgavi, D. P., Kelly, W. J., Janssen, P. H., Attwood, G. T., 2013. Rumen microbial (meta)genomics and its application to ruminant production. Animal 7 Suppl 1, 184–201.

Mosoni, P., Martin, C., Forano, E., Morgavi, D. P., 2011. Long-term defaunation increases the abundance of cellulolytic ruminococci and methanogens but does not affect the bacterial and methanogen diversity in the rumen of sheep. Journal of Animal Science 89 (3), 783–791.

Muñoz-Tamayo, R., 2016. Breve elogio a la lentitud en ciencia (A brief tribute for slowness in science). In: Ruiz, C. E. (Ed.), Ciencia y Humanismo 50 años Revista Aleph (1996-2016). Editorial Universidad de Caldas, Manizales, Colombia, pp. 473–478.

Muñoz-Tamayo, R., Aceves-Lara, C. A., Bideaux, C., 2014a. Optimization of lipid production by oleaginous yeast in continuous culture. IFAC Proceedings Volumes (IFAC-PapersOnline) 47, 6210–6215.

Muñoz-Tamayo, R., Angulo, F., 2006. Aproximación de estimación de estados en un reactor uasb. Revista Colombiana de Tecnologıas de Avanzada 1, 27–33.

Muñoz-Tamayo, R., Angulo, F., Marín, J. E., 2005. Una Perspectiva sobre el Modelado e Identificación de Sistemas de Degradación Anaerobia para el Tratamiento de Aguas Residuales. p. 10p.

Muñoz-Tamayo, R., de Groot, J., Bakx, E., Wierenga, P. A., Gruppen, H., Zwietering, M. H., Sijtsma, L., 2011a. Hydrolysis of beta-casein by the cell-envelope-located p-i-type protease of *Lactococcus lactis*: A modelling approach. International Dairy Journal 21 (10), 755–762.

Muñoz-Tamayo, R., De Groot, J., Wierenga, P. A., Gruppen, H., Zwietering, M. H., Sijtsma, L., 2012. Modeling peptide formation during the hydrolysis of β-casein by *Lactococcus lactis*. Process Biochemistry 47 (1), 83–93.

Muñoz-Tamayo, R., Giger-Reverdin, S., Sauvant, D., 2016a. Mechanistic modelling of *in vitro* fermentation by rumen microbiota. Anim. Feed Sci. Technol. 220, 1–21.

Muñoz-Tamayo, R., Giger-Reverdin, S., Sauvant, D., 2016b. Predicting ruminal pH dynamics by linking microbial metabolism and the host: a theoretical study. In: 10th INRA-Rowett symposium. Gut Microbiology 20 years and counting. Clerment-Ferrand, France, p. 105.

Muñoz-Tamayo, R., Laroche, B., Leclerc, M., Walter, E., 2008. Modelling and Identification of in vitro Homoacetogenesis by Human-Colon Bacteria. In: Proc. 16th IEEE Mediterranean Conference on Control and Automation, Ajaccio, France. pp. 1717–1722.

Muñoz-Tamayo, R., Laroche, B., Leclerc, M., Walter, E., 2009. IDEAS: A parameter identification toolbox with symbolic analysis of uncertainty and its application to biological modelling. In: Proceedings of the 15th IFAC Symposium on System Identification Saint-Malo, France. Vol. 15. pp. 1271–1276.

Muñoz-Tamayo, R., Laroche, B., Walter, E., Doré, J., Duncan, S. H., Flint, H. J., Leclerc, M., 2011b. Kinetic modelling of lactate utilization and butyrate production by key human colonic bacterial species. FEMS microbiology ecology 76, 615–24.

Muñoz-Tamayo, R., Laroche, B., Walter, E., Doré, J., Leclerc, M., 2010. Mathematical modelling of carbohydrate degradation by human colonic microbiota. Journal of theoretical biology 266, 189–201.

Muñoz-Tamayo, R., Mairet, F., Bernard, O., 2013. Optimizing microalgal production in raceway systems. Biotechnology Progress 29 (2), 543–552.

Muñoz-Tamayo, R., Martinon, P., Bougaran, G., Mairet, F., Bernard, O., 2014b. Getting the most out of it: Optimal experiments for parameter estimation of microalgae growth models. Journal of Process Control 24, 991–1001.

Muñoz-Tamayo, R., Popova, M., Tillier, M., Morgavi, D. P., Morel, J.-P., Fonty, G., Morel-Desrosiers, N., 2018a. Hydrogenotrophic methanogens of the mammalian gut: functionally similar, thermodynamically different. a modelling approach. bioRxiv. URL `https://www.biorxiv.org/content/early/2018/10/17/445171`

Muñoz-Tamayo, R., Puillet, L., Daniel, J. B., Sauvant, D., Martin, O., Taghipoor, M., Blavy, P., 2018b. Review: To be or not to be an identifiable model. Is this a relevant question in animal science modelling? Animal 12, 701–712.

Muñoz-Tamayo, R., Ramirez Agudelo, J. F., Dewhurst, R. J., Miller, G., Vernon, T., Kettle, H., 2019. A parsimonious software sensor for estimating the individual dynamic pattern of methane emissions from cattle. Animal 13, 1180–1187.

Muñoz-Tamayo, R., Steyer, J. P., Laroche, B., Leclerc, M., 2007. Human colon: a complex bioreactor. Conceptual modelling for the anaerobic digestion of the functional trophic chain. In: Proc. 11th World Congress Anaerobic Digestion Bio-energy for our Future, Brisbane, Australia. 6 pages on CD-Rom proceedings.

Muñoz-Tamayo, R., Toro García, N., 2006. Propuesta de controlador mpc para un reactor uasb. Scientia Et Technica 12 (30), 99–104.

Nosek, B. A., Spies, J. R., Motyl, M., 2012. Scientific utopia: II. restructuring incentives and practices to promote truth over publishability. Perspectives on Psychological Science 7, 615–631.

Oberhardt, M. A., Palsson, B., Papin, J. A., 2009. Applications of genome-scale metabolic reconstructions. Molecular Systems Biology 5 (320), 1–15.

Offner, A., Sauvant, D., 2004. Comparative evaluation of the Molly, CNCPS, and LES rumen models. Anim. Feed Sci. Technol. 112 (1), 107–130.

Offner, A., Sauvant, D., 2006. Thermodynamic modeling of ruminal fermentations. Animal Research 55 (5), 343–365.

Ogien, A., 2013. Désacraliser le chiffre dans l'évaluation du service public. Sciences en questions. Quae Editions.

Orth, J. D., Thiele, I., Palsson, B. O., 2010. What is flux balance analysis? Nature Biotechnology 28 (3), 245–248.

Owens, F. N., Secrist, D. S., Hill, W. J., Gill, D. R., 1998. Acidosis in cattle: A review. Journal of Animal Science 76 (1), 275–286.

Palsson, B. O., 2006. Systems Biology. Properties of Reconstructed Networks. Cambridge University Press, New York, USA.

Park, I. U., Peacey, M. W., Munafo, M. R., 2014. Modelling the effects of subjective and objective decision making in scientific peer review. Nature 506, 93–96.

Paulus, F. M., Cruz, N., Krach, S., 2018. The impact factor fallacy. Frontiers in psychology 9, 1487.

Pei, C. X., Mao, S. Y., Cheng, Y. F., Zhu, W. Y., 2010. Diversity, abundance and novel 16S rRNA gene sequences of methanogens in rumen liquid, solid and epithelium fractions of Jinnan cattle. Animal 4, 20–29.

Pinares-Patiño, C., Hunt, C., Martin, R., West, J., Lovejoy, P., Waghorn, G., 2014. Technical Manual on Respiration Chamber Designs. Wellington, New Zealand: Ministry of Agriculture and Forestry, Ch. Chapter 1: New Zealand Ruminant Methane Measurement Centre, AgResearch, Palmerston North, pp. 10–28.

Plaizier, J., Mesgaran, M. D., Derakhshani, H., Golder, H., Khafipour, E., Kleen, J., Lean, I., Loor, J., Penner, G., Zebeli, Q., 2018. Enhancing gastrointestinal health in dairy cows. animal, 1–20.

PLoSMedicine-Editors, Jun. 2006. The impact factor game. it is time to find a better way to assess the scientific literature. PLoS medicine 3, e291.

Pontryagin, L., Boltyansky, V., Gamkrelidze, R., and, E. M., 1962. Mathematical Theory of Optimal Processes. Wiley-Interscience, New York.

Pope, P. B., Mackenzie, A. K., Gregor, I., Smith, W., Sundset, M. A., McHardy, A. C., Morrison, M., Eijsink, V. G. H., 2012. Metagenomics of the Svalbard Reindeer Rumen Microbiome Reveals Abundance of Polysaccharide Utilization Loci. PLoS One 7, e104612.

Price, N. D., Reed, J. L., Palsson, B. O., 2004. Genome-scale models of microbial cells: evaluating the consequences of constraints. Nat Rev Microbiol 2, 886–897.

Prigent, S., Frioux, C., Dittami, S. M., Thiele, S., Larhlimi, A., Collet, G., Gutknecht, F., Got, J., Eveillard, D., Bourdon, J., Plewniak, F., Tonon, T., Siegel, A., 2017. Meneco, a Topology-Based Gap-Filling Tool Applicable to Degraded Genome-Wide Metabolic Networks. Plos Computational Biology 13, e1005276.

Provost, A., Bastin, G., Agathos, S. N., Schneider, Y. J., 2006. Metabolic design of macroscopic bioreaction models: application to Chinese hamster ovary cells. Bioprocess and Biosystems Engineering 29 (5-6), 349–366.

Ranilla, M. J., Jouany, J.-P., Morgavi, D. P., 2007. Methane production and substrate degradation by rumen microbial communities containing single protozoal species in vitro. Lett Appl Microbiol 45, 675–680.

Raue, A., Karlsson, J., Saccomani, M. P., Jirstrand, M., Timmer, J., 2014. Comparison of approaches for parameter identifiability analysis of biological systems. Bioinformatics 30, 1440–1448.

Reed, J. L., Palsson, B. O., 2003. Thirteen years of building constraint-based in silico models of *Escherichia coli*. Journal of Bacteriology 185 (9), 2692–2699.

Revilla, M., Friggens, N. C., Broudiscou, L. P., Lemonnier, G., Blanc, F., Ravon, L., Mercat, M.-J., Billon, Y., Rogel-Gaillard, C., Le Floch, N., Estellé, J., Muñoz-Tamayo, R., 2019. Towards the quantitative characterization of piglets' robustness to weaning: A modelling approach. Animal.

Robles-Rodríguez, C., Muñoz-Tamayo, R., Bideaux, C., Gorret, N., Guillouet, S., Molina-Jouve, C., Roux, G., Aceves-Lara, C., 2018. Modeling and optimization of lipid accumulation by *Yarrowia lipolytica* from glucose under nitrogen depletion conditions. Biotechnology and Bioengineering 115, 1137–1151.

Rodríguez, J., Kleerebezem, R., Lema, J. M., van Loosdrecht, M. C. M., 2006. Modeling product formation in anaerobic mixed culture fermentations. Biotechnol. Bioeng. 93 (3), 592–606.

Rodríguez, J., Lema, J. M., Kleerebezem, R., 2008. Energy-based models for environmental biotechnology. Trends Biotechnol 26 (7), 366–374.

Russell, J. B., Rychlik, J. L., may 2001. Factors That Alter Rumen Microbial Ecology. Science 292 (5519), 1119–1122.

Schaber, J., Klipp, E., 2011. Model-based inference of biochemical parameters and dynamic properties of microbial signal transduction networks. Current Opinion in Biotechnology 22 (1), 109–116.

Schäfer, R. B., Cooke, S. J., Arlinghaus, R., Bonada, N., Brischoux, F., Casper, A. F., Catford, J. a., Rolland, V., nov 2011. Perspectives from early career researchers on the publication process in ecology - a response to Statzner & Resh (2010). Freshwater Biology 56, 2405–2412.

Schekman, R., 2013. How journals like Nature, Cell and Science are damaging science. The Guardian.

Schekman, R., Patterson, M., 2013. Reforming research assessment. eLife 2, e00855, original DateCompleted: 20130524, Original DateCompleted: 20140206.

Schrödinger, E, 1944. What is Life? The Physical Aspect of the Living Cell,. Cambridge University Press,.

Schuster, S., Hilgetag, C., 1994. On elementary flux modes in biochemical reaction systems at steady state. Journal of Biological Systems 2 (2), 165–182.

Seglen, P. O., 1997. Why the impact factor of journals should not be used for evaluating research. BMJ 314, 498502.

Serment, A., Giger-Reverdin, S., Schmidely, P., Dhumez, O., Broudiscou, L. P., Sauvant, D., 2016. *In vitro* fermentation of total mixed diets differing in concentrate proportion: relative effects of inocula and substrates. Journal of the Science of Food and Agriculture 96, 160–168.

Shabat, S. K., Sasson, G., Doron-Faigenboim, A., Durman, T., Yaacoby, S., Berg Miller, M. E., White, B. A., Shterzer, N., Mizrahi, I., 2016. Specific microbiome-dependent mechanisms underlie the energy harvest efficiency of ruminants. Isme Journal 10, 2958–2972.

Shapiro, E., 2014. Correcting the bias against interdisciplinary research. eLife 3, e02576, original DateCompleted: 20140402.

Skogestad, S., 2000. Plantwide control: The search for the self-optimizing control structure. Journal of process control 10 (5), 487–507.

Spedding, C. R. W., 1988. General aspects of modelling and its application in livestock production. In: Korver, S., Van Arendonk, J. A. M. (Eds.), Modelling of livestock production systems. Kluwer Academica Publishers, Dordrecht, The Netherlands, pp. 3–13.

Stelling, J., 2004. Mathematical models in microbial systems biology. Curr Opin Microbiol 7 (5), 513–518.

Stolyar, S., Van Dien, S., Hillesland, K. L., Pinel, N., Lie, T. J., Leigh, J. A., Stahl, D. A., 2007. Metabolic modeling of a mutualistic microbial community. Molecular Systems Biology 3, 92.

Tap, J., Mondot, S., Levenez, F., Pelletier, E., Caron, C., Furet, J. P., Ugarte, E., Muñoz-Tamayo, R., Paslier, D. L., Nalin, R., Dore, J., Leclerc, M., 2009. Towards the human intestinal microbiota phylogenetic core. Environ Microbiol 11 (10), 2574–2584.

Tebbani, S., Lopes, F., Filali, R., Dumur, D., Pareau, D., 2014. Nonlinear predictive control for maximization of co2 bio-fixation by microalgae in a photobioreactor. Bioprocess and Biosystems Engineering 37, 83–97.

Theis, K. R., Dheilly, N. M., Klassen, J. L., Brucker, R. M., Baines, J. F., Bosch, T. C. G., Cryan, J. F., Gilbert, S. F., Goodnight, C. J., Lloyd, E. A., Sapp, J., Vandenkoornhuyse, P., Zilber-Rosenberg, I., Rosenberg, E., Bordenstein, S. R., 2016. Getting the Hologenome Concept Right: an Eco-Evolutionary Framework for Hosts and Their Microbiomes. mSystems 1, e00028–16.

Troy, S. M., Duthie, C. A., Hyslop, J. J., Roehe, R., Ross, D. W., Wallace, R. J., Waterhouse, A., Rooke, J. A., 2015. Effectiveness of nitrate addition and increased oil content as methane mitigation strategies for beef cattle fed two contrasting basal diets. Journal of Animal Science 93, 1815–1823.

Ungerfeld, E. M., 2013. A theoretical comparison between two ruminal electron sinks. Frontiers in Microbiology 4, 319.

Ungerfeld, E. M., 2015. Shifts in metabolic hydrogen sinks in the methanogenesis-inhibited ruminal fermentation: A meta-analysis. Frontiers in Microbiology 6, 37.

Van Amburgh, M. E., Collao-Saenz, E. A., Higgs, R. J., Ross, D. A., Recktenwald, E. B., Raffrenato, E., Chase, L. E., Overton, T. R., Mills, J. K., Foskolos, A., 2015. The Cornell Net Carbohydrate and Protein System: Updates to the model and evaluation of version 6.5. J Dairy Sci 98 (9), 6361–6380.

Van Lingen, H. J., Plugge, C. M., Fadel, J. G., Kebreab, E., Bannink, A., Dijkstra, J., 2016. Thermodynamic driving force of hydrogen on rumen microbial metabolism: A theoretical investigation. PLoS ONE 11 (10), 1–18.

Varma, A., Palsson, B. O., 1993. Metabolic capabilities of *Escherichia-Coli* .2. optimal-growth patterns. Journal of Theoretical Biology 165, 503–522.

Wade, M. J., Harmand, J., Benyahia, B., Bouchez, T., Chaillou, S., Cloez, B., Godon, J. J., Boudjemaa, B. M., Rapaport, A., Sari, T., Arditi, R., Lobry, C., 2016. Perspectives in mathematical modelling for microbial ecology. Ecological Modelling 321, 64–74.

Walker, A. W., Duncan, S. H., Louis, P., Flint, H. J., 2014. Phylogeny, culturing, and metagenomics of the human gut microbiota. Trends Microbiol 22, 267–274.

Walter, E., Pronzato, L., 1997. Identification of Parametric Models from Experimental Data. Springer, London.

Weimer, P. J., 2015. Redundancy, resilience, and host specificity of the ruminal microbiota: implications for engineering improved ruminal fermentations. Frontiers in Microbiology 6.

Weimer, P. J., Russell, J. B., Muck, R. E., 2009. Lessons from the cow: What the ruminant animal can teach us about consolidated bioprocessing of cellulosic biomass. Bioresource technology 100 (21), 5323–5331.

Widder, S., Allen, R. J., Pfeiffer, T., Curtis, T. P., Wiuf, C., Sloan, W. T., Cordero, O. X., Brown, S. P., Momeni, B., Shou, W., Kettle, H., Flint, H. J., Haas, A. F., Laroche, B., Kreft, J. U., Rainey, P. B., Freilich, S., Schuster, S., Milferstedt, K., Van Der Meer, J. R., Grobkopf, T., Huisman, J., Free, A., Picioreanu, C., Quince, C., Klapper, I., Labarthe, S., Smets, B. F., Wang, H., Soyer, O. S., 2016. Challenges in microbial ecology: Building predictive understanding of community function and dynamics. ISME Journal 10 (11), 2557–2568.

Woolston, C., 2016. Psychology: Faking it. Nature 529, 555–557.

Xiao, L., Estellé, J., Kiilerich, P., Ramayo-Caldas, Y., Xia, Z., Feng, Q., Liang, S., Pedersen, A. y., Kjeldsen, N. J., Liu, C., Maguin, E., Dor., Pons, N., Le Chatelier, E., Prifti, E., Li, J., Jia, H., Liu, X., Xu, X., Ehrlich, S. D., Madsen, L., Kristiansen, K., Rogel-Gaillard, C., Wang, J., 2016. A reference gene catalogue of the pig gut microbiome. Nature microbiology 19, 16161.

Yañez-Ruiz, D. R., Abecia, L., Newbold, C. J., 2015. Manipulating rumen microbiome and fermentation through interventions during early life: a review. Frontiers in Microbiology 6, 1133.

Zengler, K., Palsson, B. O., 2012. A road map for the development of community systems (CoSy) biology. Nature Reviews Microbiology 10, 366–372.

Zoetendal, E. G., Rajilic-Stojanovic, M., de Vos, W. M., Nov. 2008. High-throughput diversity and functionality analysis of the gastrointestinal tract microbiota. Gut 57, 1605–1615.