31 MARCH 2022

15:00 CEST

IWA Specialist Group on Modelling and Integrated Assessment Webinar Series

State-of-the-art modelling on anaerobic mixed-culture fermentation

Speakers

Ulf Jeppsson
Lund University

Robbert Kleerebezem
Delft University of Technology

Alberte Regueira
Universidade de Santiago de Compostela

Adam Kovalovszki
DTU Environment

Matthew Scarborough
University of Vermont

The webinar is going to be recorded and shared on the MIA SG Youtube channel afterward.

MIA Welcome Note

IWA Modelling and Integrated Assessment Specialist Group

Dr. Ulf Jeppsson (Chair of MIA SG)

Dr. Elena Torfs (Vice-chair of MIA SG)

inspiring change

LUND UNIVERSITY

GHENT UNIVERSITY

MODELLING AND INTEGRATED ASSESSMENT SPECIALIST GROUP (MIA SG)

“This group targets people from research, consulting companies, institutions and operators to think along ***the use of models and computing tools to support the understanding, management and optimization of water systems****.”*

PRIORITIES

- Interact with other IWA SGs and other professional organizations
- Organize specialized conferences, sessions and workshops
- Engage and activate YWPs in the domain.

CURRENTLY 1900 MEMBERS

How to find us

MODELLING & INTEGRATED ASSESSMENT
A Specialist Group of the International Water Association

MODELLING & INTEGRATED ASSESSMENT
A Specialist Group of the International Water Association

Website: http://iwa-mia.org/

Modelling and Integrated Assessment

Website: http://iwa-mia.org/

https://iwa-connect.org

MIA SG: ACTIVITIES

Task Groups (TGs)

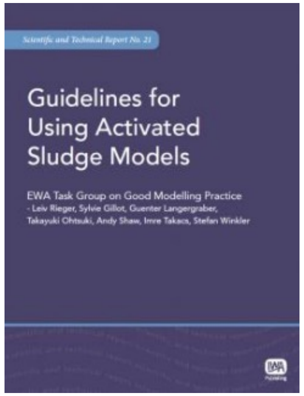
- Benchmarking of Control Strategies for WWTPs (BSM) AND Good Modelling Practice (GMP) AND Design and Operations Uncertainty (DOUT) (All three finished)
- Generalised Physicochemical Modelling (PCM) (almost done)
- Use of Modelling for Minimizing GHG Emissions from Wastewater Systems (GHG) (almost done)
- Membrane Bioreactor Modelling and Control (MBR)
- Good Modelling Practice in Water Resource Recovery Systems

Working Groups (WGs)

- Integrated Urban Water Systems (IUWS)
- Computational Fluid Dynamics (CFD)
- Good Modelling Practice (GMP)

Conferences / Events

- WRRmod
- Watermatex

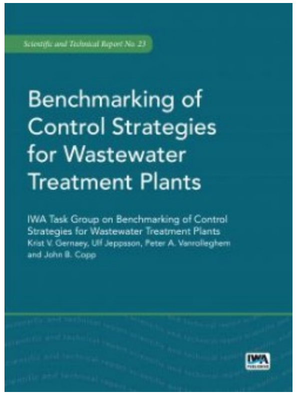


Scientific and Technical Report No. 21

Guidelines for Using Activated Sludge Models

EWA Task Group on Good Modelling Practice
- Leiv Rieger, Sylvie Gillot, Guenter Langergraber,
Takayuki Ohtsuki, Andy Shaw, Imre Takacs, Stefan Winkler

STR
(Sept. 2012)

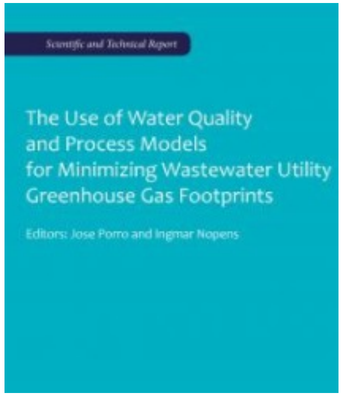


Scientific and Technical Report No. 23

Benchmarking of Control Strategies for Wastewater Treatment Plants

IWA Task Group on Benchmarking of Control Strategies for Wastewater Treatment Plants
Krist V. Gernaey, Ulf Jeppsson, Peter A. Vanrolleghem and John B. Copp

STR
(Sept. 2014)

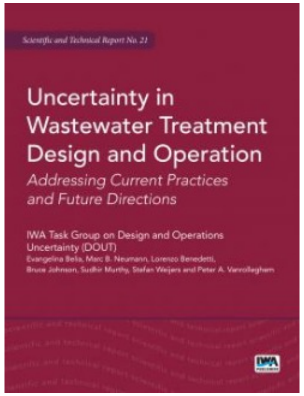


Scientific and Technical Report

The Use of Water Quality and Process Models for Minimizing Wastewater Utility Greenhouse Gas Footprints

Editors: Jose Porro and Ingmar Nopens

STR
(2022)



Scientific and Technical Report No. 21

Uncertainty in Wastewater Treatment Design and Operation

Addressing Current Practices and Future Directions

IWA Task Group on Design and Operations Uncertainty (DOUT)
Evangelina Belia, Marc B. Neumann, Lorenzo Benedetti,
Bruce Johnson, Sudhir Murthy, Stefan Weijers and Peter A. Vanrolleghem

STR
(2022)

MIA SG: UPCOMING CONFERENCES

8th Water Resource Recovery Modelling seminar (WRRmod2022+)

- Location: Stellenbosch, South Africa, 15-18 January 2023
- Chair: Dr. David Ikumi (Univ. Cape Town)

11th Symposium on Modelling and Integrated Assessment (Watermatex2023)

- Location: Québec City, Canada, late summer 2023
- Chair/vice-chair: Prof. Peter Vanrolleghem (Univ. Laval)/Dr. Elena Torfs (Univ. Ghent)

9th Water Resource Recovery Modelling seminar (WRRmod2024), Stowe, Vermont, USA

FIND MIA SG ON SOCIAL MEDIA

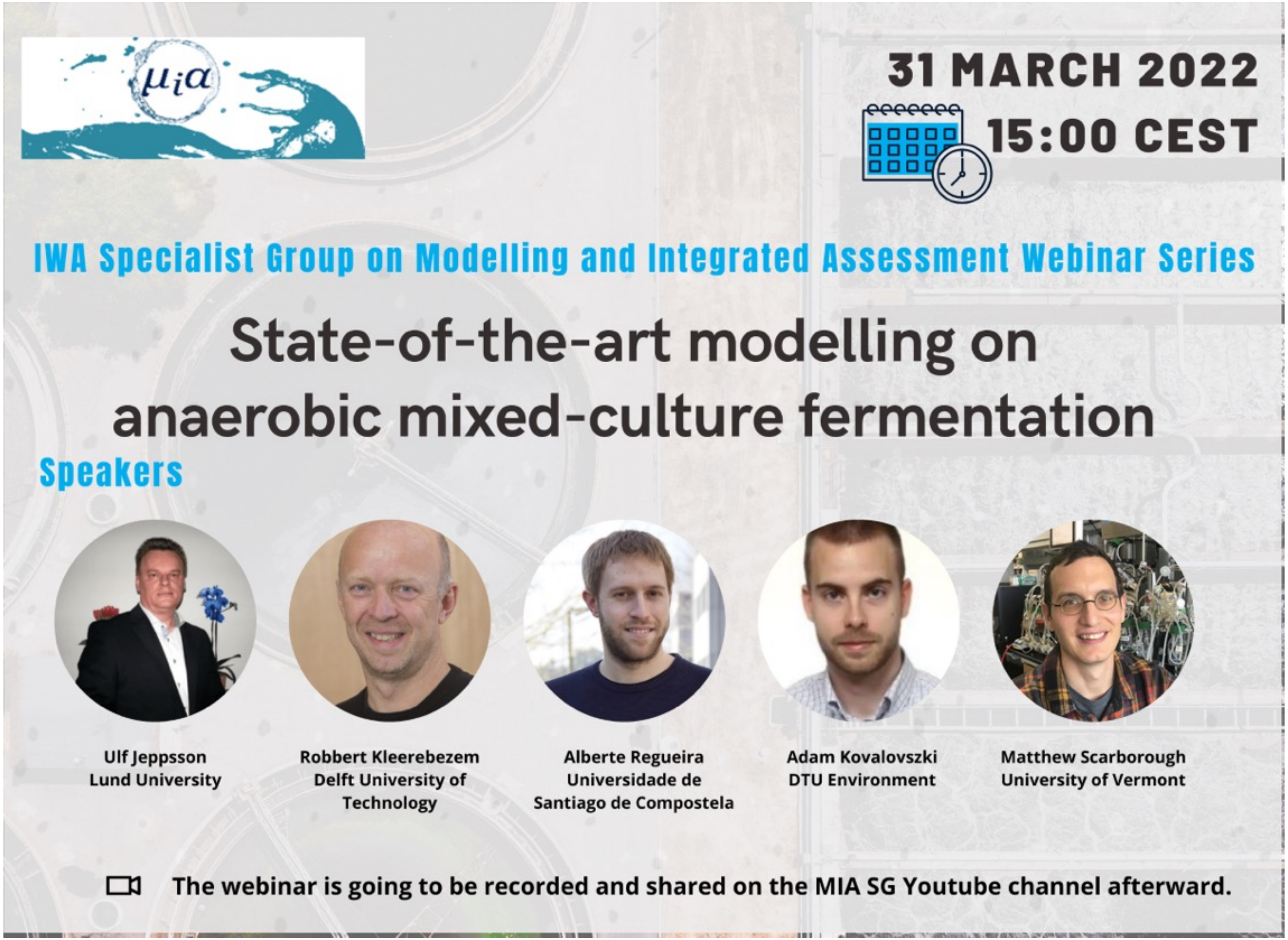
Follow the Modelling and Integrated Assessment Specialist Group on:

- IWA Connect https://iwa-connect.org/group/modelling-and-integrated-assessment-mia/timeline
- https://www.linkedin.com/company/iwa-mia-specialist-group-on-modelling-and-integrated-assessment
- https://twitter.com/iwa_mia_sg
- **MIA SG open web site** http://iwa-mia.org

to get informed about our latest events, publications and news!

MIA Open Group meeting at WWC&E2022, Copenhagen, Denmark

INTRODUCTION TO THE WEBINAR



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Modeling Mixed Culture Fermentations

A short introduction

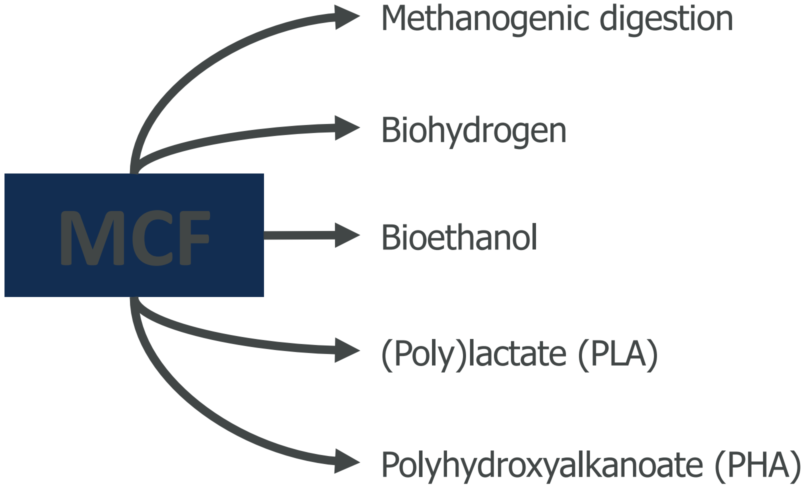
Robbert Kleerebezem (R.Kleerebezem@tudelft.nl)
Delft University of Technology

Modeling ***Wild*** Fermentations

A short introduction

WHY MIXED CULTURE FERMENTATION (MCF)?

Produce building blocks for other processes
Substrate: Carbohydrates, Glycerol…
Products: Acetate, Propionate, Butyrate, Ethanol, Lactate, Hydrogen…



MCF

Methanogenic digestion

Biohydrogen

Bioethanol

(Poly)lactate (PLA)

Polyhydroxyalkanoate (PHA)

WHY MCF IS INTERESTING?

Thermodynamics drive microbial conversions:

The environment selects for maximization of energy harvesting

$$CH_2O \rightarrow 0.5 \cdot CH_4 + 0.5 \cdot CO_2$$

$$NH_4^{+1} + 2 \cdot O_2 \rightarrow NO_3^{-1} + 2 \cdot H^{+1} + H_2O$$

WHY MCF IS INTERESTING?

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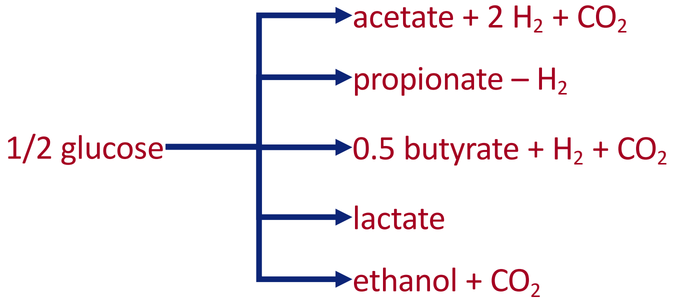
$$NH_4^{+1} + 2 \cdot O_2 \rightarrow NO_3^{-1} + 2 \cdot H^{+1} + H_2O$$

But what determines the end-product of carbohydrate fermentations?



1/2 glucose

acetate + 2 H_2 + CO_2

propionate – H_2

0.5 butyrate + H_2 + CO_2

lactate

ethanol + CO_2

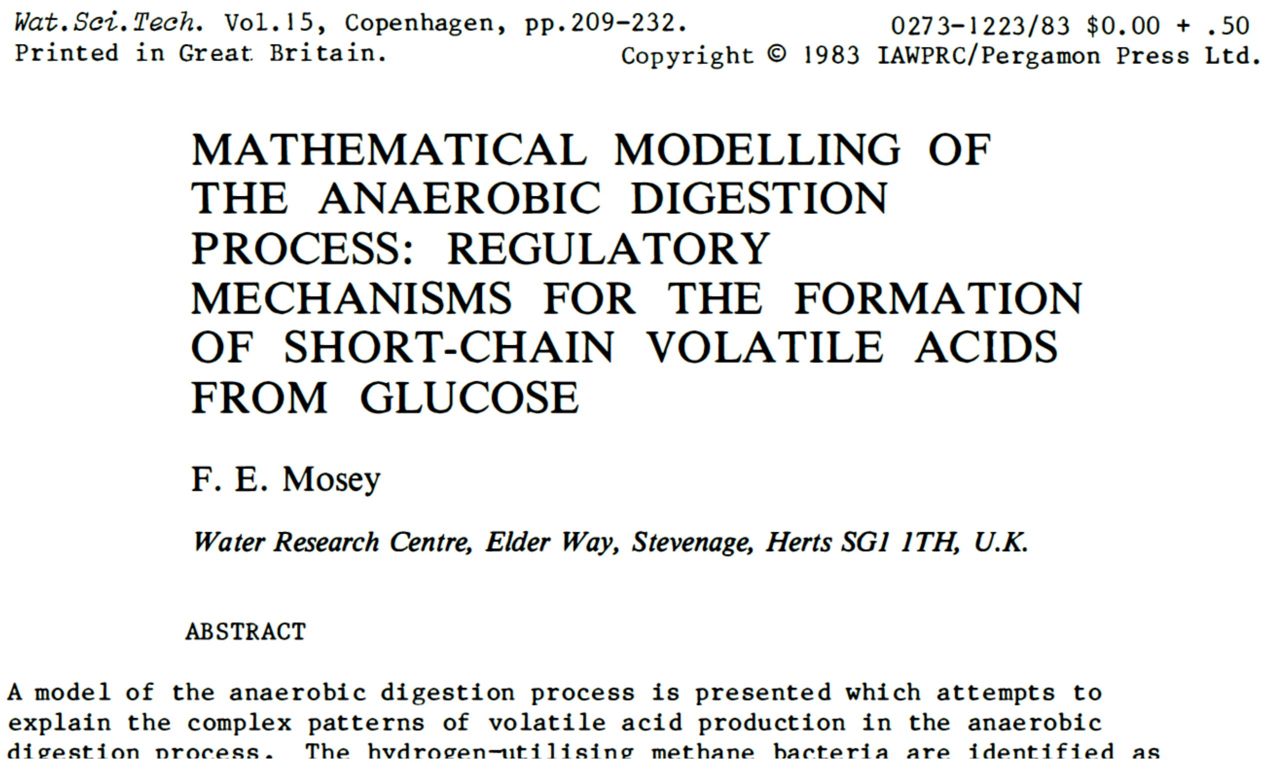
ANAEROBIC CARBOHYDRATE FERMENTATION

Cabbage → Sauerkraut

Grapes → Wine

Potato peel → Butyric acid

HISTORY OF MODELLING MCF



Wat.Sci.Tech. Vol.15, Copenhagen, pp.209-232.
Printed in Great Britain.

0273-1223/83 $0.00 + .50

MATHEMATICAL MODELLING OF THE ANAEROBIC DIGESTION PROCESS: REGULATORY MECHANISMS FOR THE FORMATION OF SHORT-CHAIN VOLATILE ACIDS FROM GLUCOSE

F. E. Mosey

Water Research Centre, Elder Way, Stevenage, Herts SG1 1TH, U.K.

ABSTRACT

A model of the anaerobic digestion process is presented which attempts to explain the complex patterns of volatile acid production in the anaerobic digestion process. The hydrogen-utilising methane bacteria are identified as

HISTORY OF MODELLING MCF

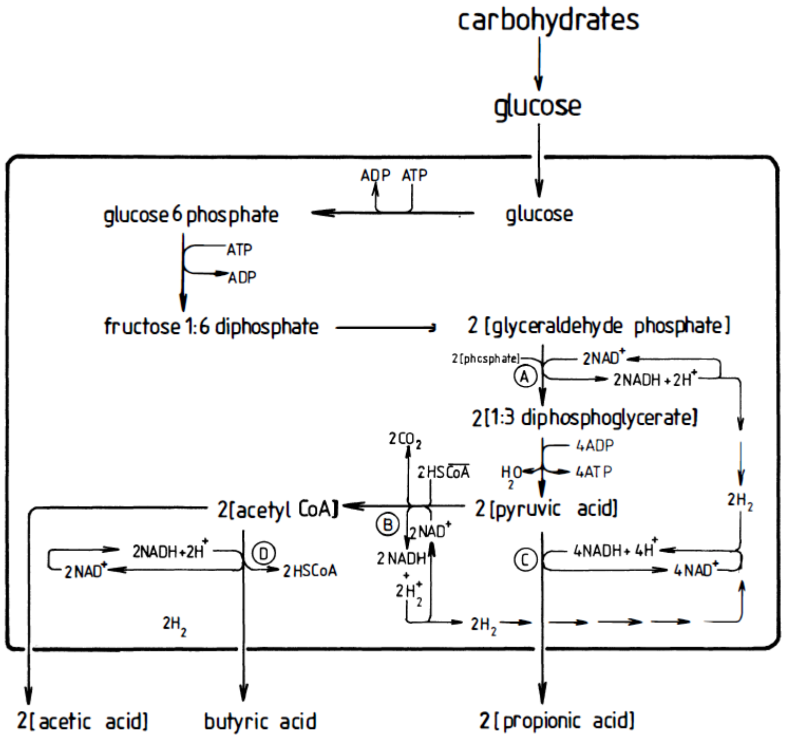


Fig. 2. Metabolic pathways inside acid-forming bacteria

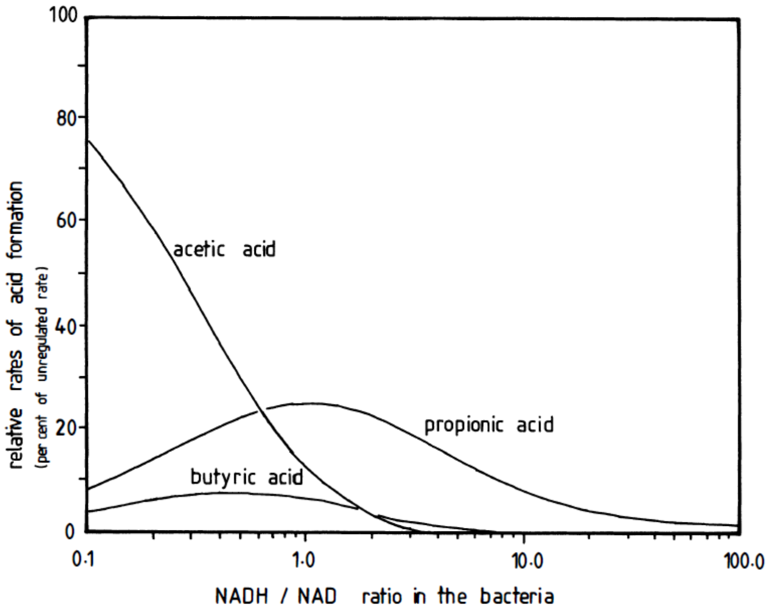


Fig. 3. Relative rates of acid-formation at different NAD$^+$/NADH ratios as predicted by these rate equations

LECTURES OF TODAY!

- Alberte Regueira

  Bioenergetic modelling for predicting the stoichiometry of AF

- Adam Kovalovszki

  Inferring metabolic interactions from genome-scale data: anaerobic digestion 'under the microscope'

- Matthew Scarborough

  Guild-based metabolic modeling for deciphering anaerobic bioprocesses

AGENDA AND HOUSEKEEPING

Speaker 1
Alberte Regueira (Universidade de Santiago de Compostela, Spain)

Speaker 2
Adam Kovalovszki (Technical University of Denmark)

Speaker 3
Matthew Scarborough (University of Vermont, USA)

Q&A Session Moderator: *Robbert Kleerebezem (Delft University of Technology, The Netherlands)*

- This session is being recorded;
- Microphones and cameras have been disabled due to the large number of attendees;
- The normal chat function is disabled;
- Please put any questions and comments you may have in the Q&A (icon to the low right in Zoom) and we will do our best to answer them during the session (in writing or orally).

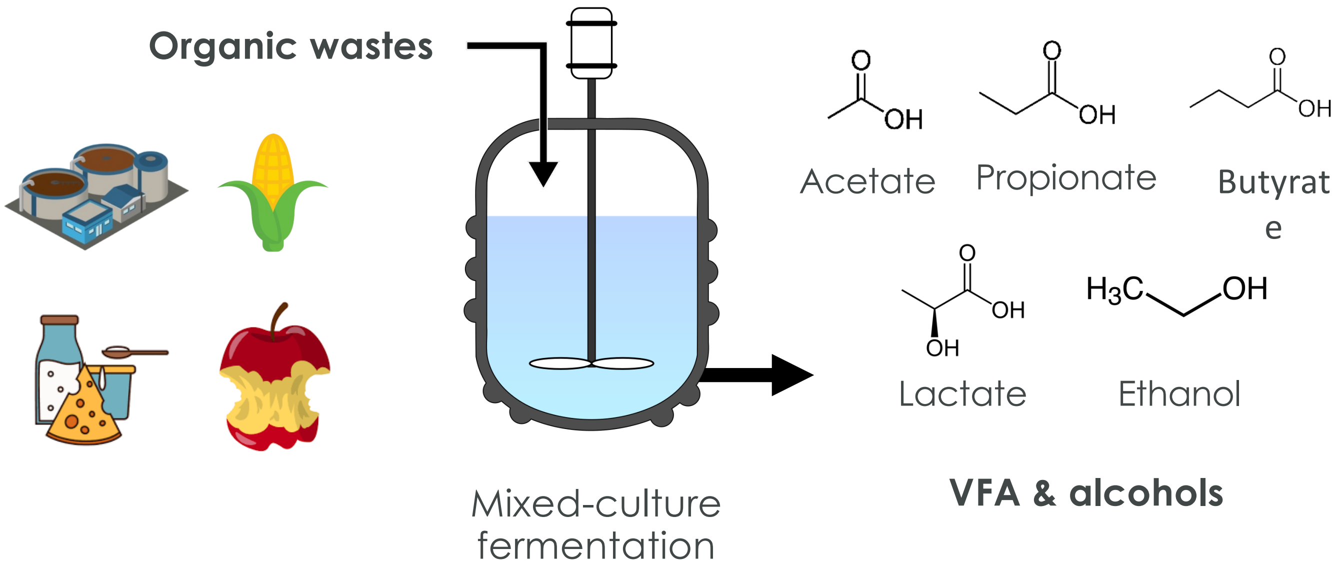
BIOENERGETIC MODELLING FOR PREDICTING THE STOICHIOMETRY OF ANAEROBIC FERMENTATION

Alberte Regueira (Alberte.RegueiraLopez@UGent.be)
Universidade de Santiago de Compostela

Bioenergetic modelling for predicting the stoichiometry of anaerobic fermentation

Alberte Regueira López

Anaerobic fermentation for VFA production



Organic wastes

Mixed-culture fermentation

Acetate

Propionate

Butyrate

Lactate

Ethanol

VFA & alcohols

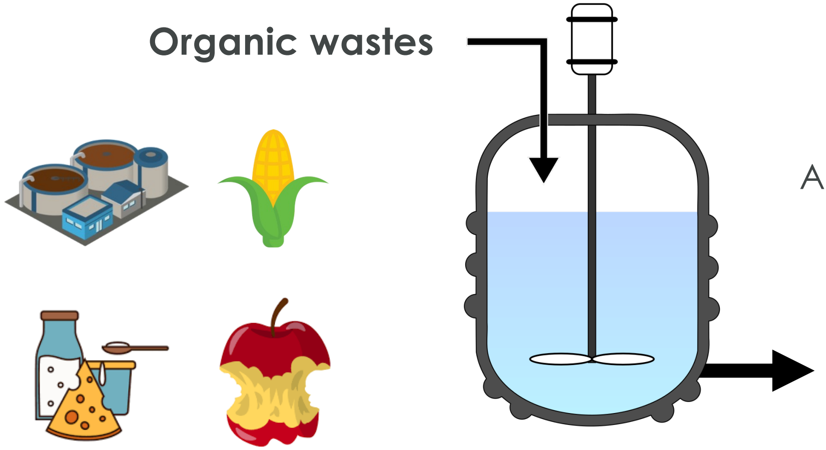
HRT

Substrate

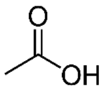
pH

Anaerobic fermentation for VFA production

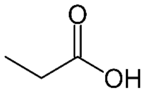
Organic wastes



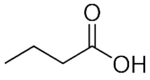
Mixed-culture fermentation



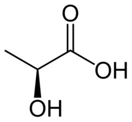
Acetate



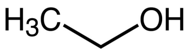
Propionate



Butyrate



Lactate



Ethanol

VFA & alcohols

✖ O_2 $\longrightarrow$ $\Delta G^0 \approx 0$

$$\Delta G = \Delta G^0 + RT\ ln \frac{[P_1] \cdot [P_2]}{[S_1] \cdot [S_2]}$$

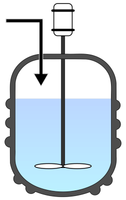
$\longleftrightarrow$ Standard value

$\longleftrightarrow$ Environmental conditions

What are bioenergetic models?

Kinetic unstructured models

- ✓ Biomass is a black box
- ✓ Solve the macroscopic mass balances
- ✓ Variable selectivity is not addressed

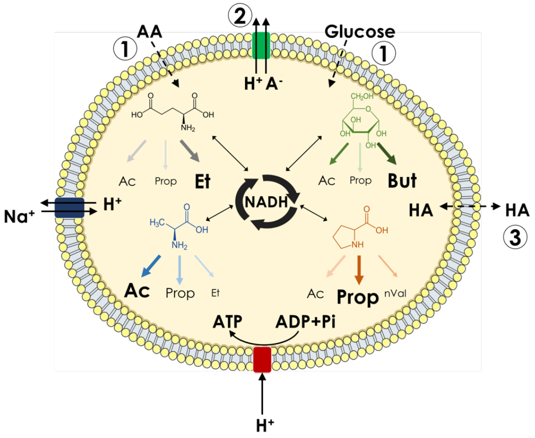


$$\frac{dC_i}{dt} = D \cdot \left(C_i^{IN} - C_i\right) + r_{j,i}$$

$$r_{j,i} = r_{max,j} \cdot \frac{C_i}{C_i + K_{j,i}} \cdot X$$

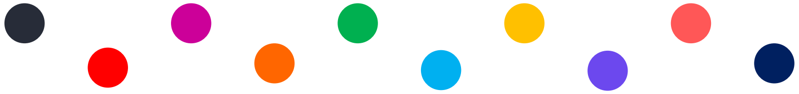
Bioenergetic models

- ✓ Intracellular processes are modelled
- ✓ Cell-environmental interactions
- ✓ Their task is limited to predict the process stoichiometry



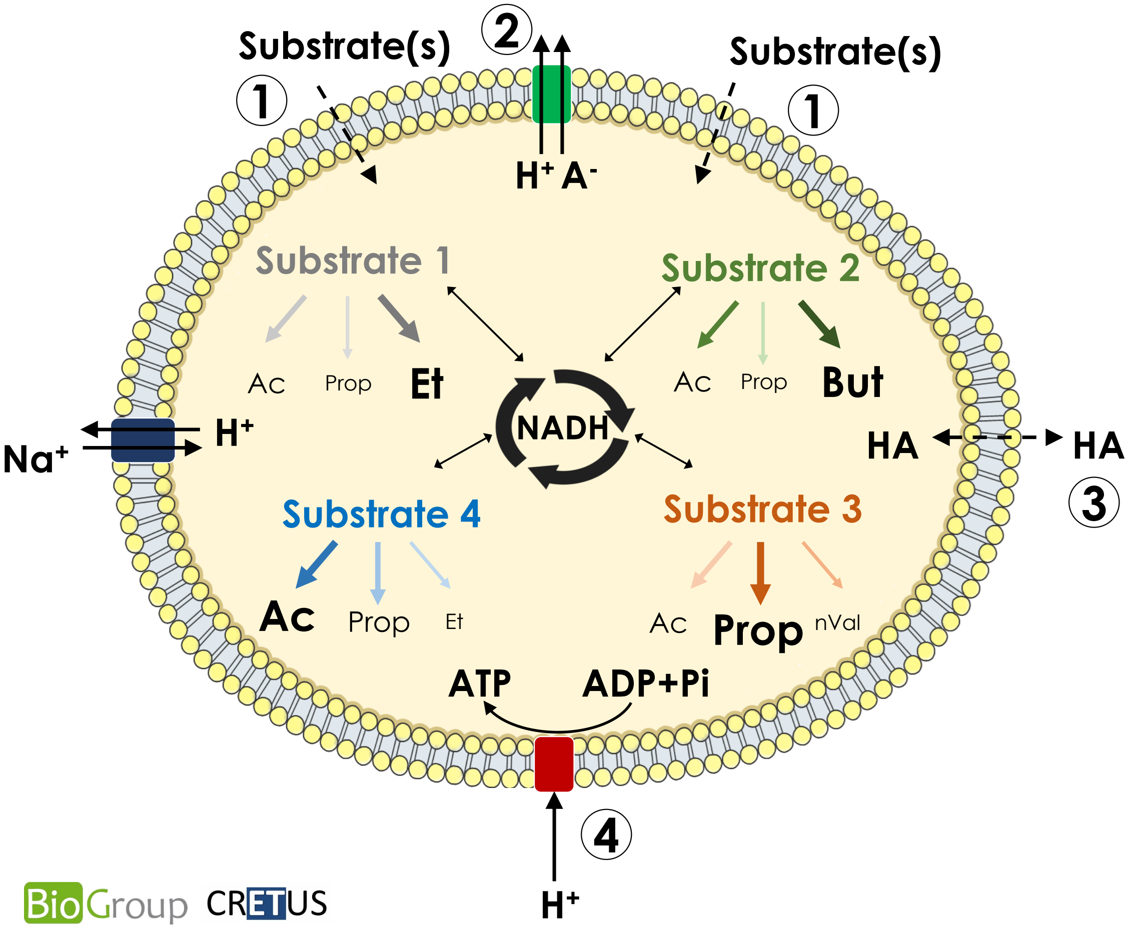
The microbial community is modelled as an enzyme soup

Reality: **Multiple** species performing different or similar metabolic functions



Model: **One** virtual species is able of performing **all** the metabolic functions of the community

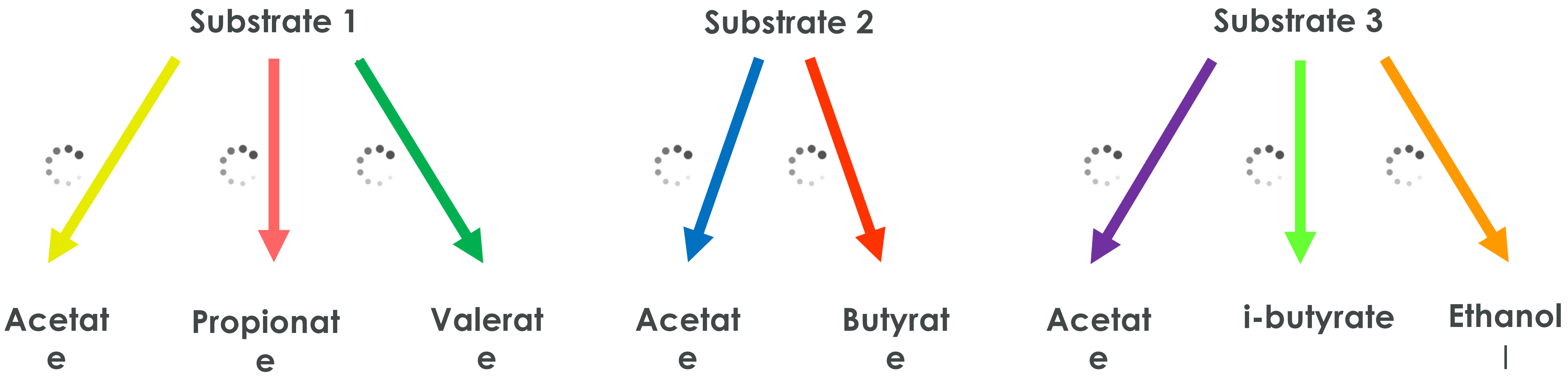
One virtual microorganism does all the possible processes



1. Substrate transport
2. Active transport of products
3. Passive transport of products
4. Energy conservation via proton morive force

Flux balance analysis to determine product selectivity

- FBA determines the metabolite flow throught the pathways of the metabolic network
- The flow distribution maximises a given objective (e.g. maximum growth rate)



Substrate 1

Acetate **Propionate** **Valerate**

Substrate 2

Acetate **Butyrate**

Substrate 3

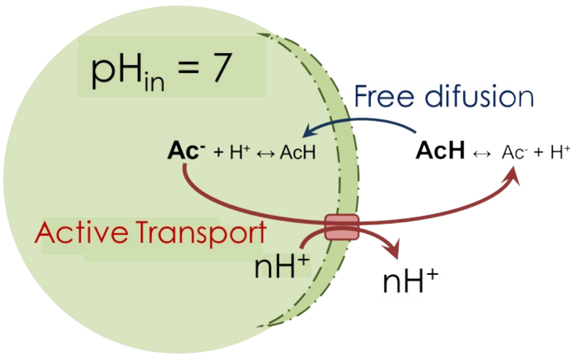
Acetate **i-butyrate** **Ethanol**

Microorganisms behave in an efficient way

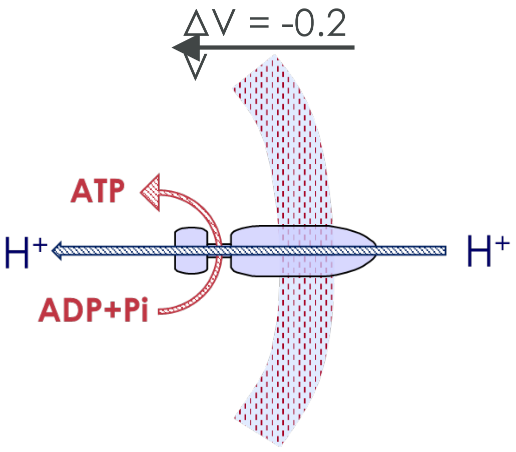
ATP production from the substrate is **maximised**

Pathway selection →

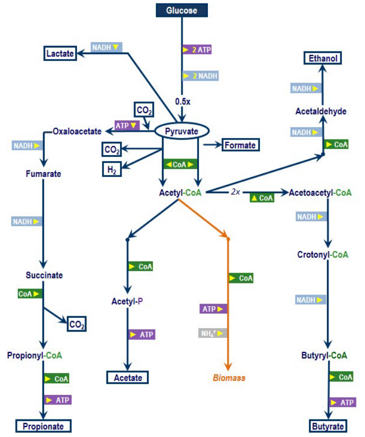
$$r_{ATP} = r_{TRANSPORT} + r_{PMF} + r_{CAT}$$



Transport



Proton translocations



Catabolic ATP

Bioenergetic models are dynamic flux balance analysis models

$$\frac{dC}{dt} = D \cdot \left(C^{in} - C\right) + r(z, C)$$

$$z(t)/\ \max_{z}\ r_{ATP}\big(C(t)\big)$$

Maximise ATP production

$$s.t. \qquad r_{NADH}(z) = 0$$

NADH is conserved

$$g(z) \leq 0$$

$$h(z) = 0$$

Environmental conditions

✓ Intracellular conditions can also vary

Model solution

Environmental conditions

Bioenergetic models in literature

- Glucose

  Rodríguez *et al.* (2006): First bioenergetic model

  Zhang *et al.* (2013): Modifications on Rodríguez's model

  González-Cabaleiro *et al.* (2015): State-of-the-art approach

- Protein

  Regueira *et al.* (2020): First bioenergetic model for protein fermentation

- Protein and glucose cofermentation

  Regueira *et al.* (2020): First bioenergetic model for co-fermentation

Bioenergetic models in literature

- Glucose

  Rodríguez *et al.* (2006): First bioenergetic model

  Zhang *et al.* (2013): Modifications on Rodríguez's model on electron carriers

  González-Cabaleiro *et al.* (2015): State-of-the-art approach

- Protein

  Regueira *et al.* (2020): First bioenergetic model for protein fermentation

- Protein and glucose cofermentation

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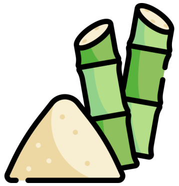
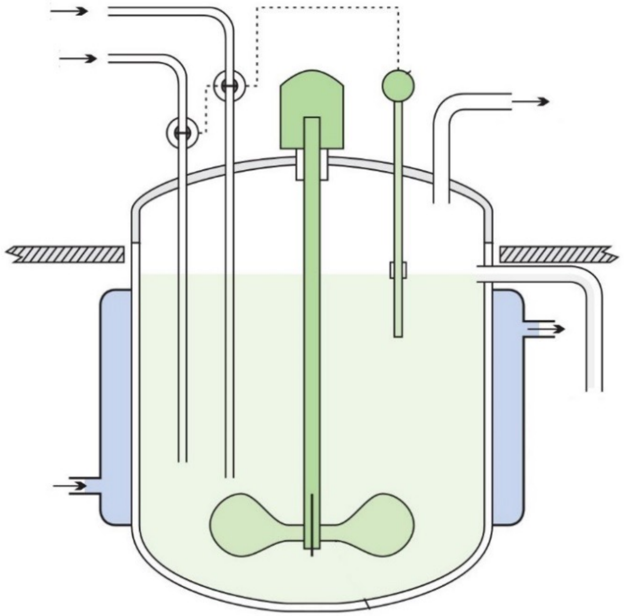
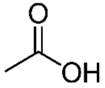
Bioenergetic model for glucose fermentation



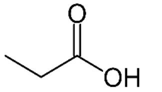
Glucose



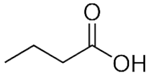
pH: 4-8.5



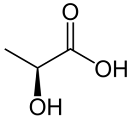
Acetate



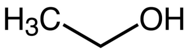
Propionate



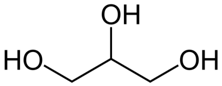
Butyrate



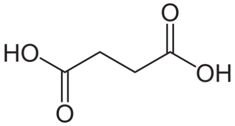
Lactate



Ethanol

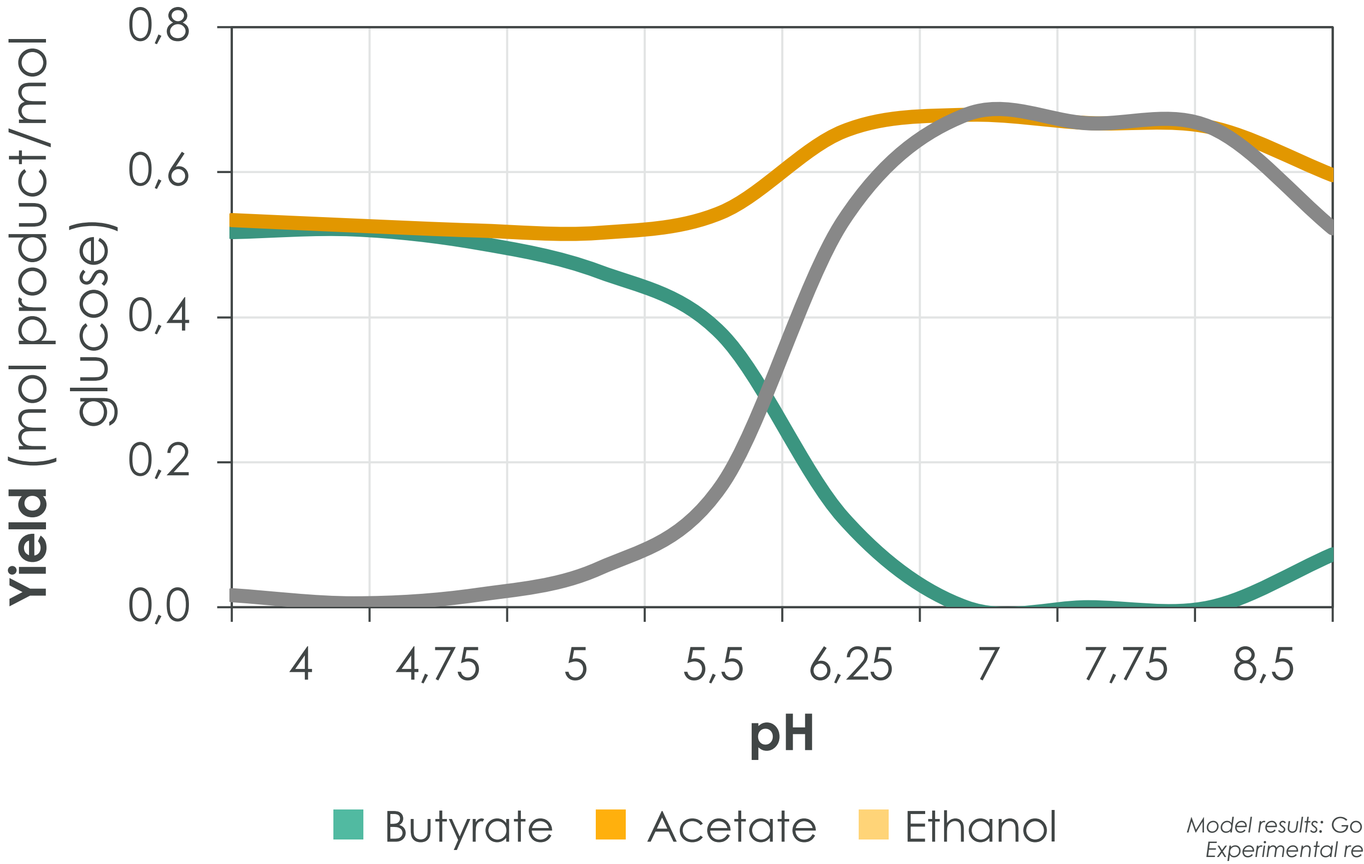


Glycerol



Succinate

The model predicts a shift with pH

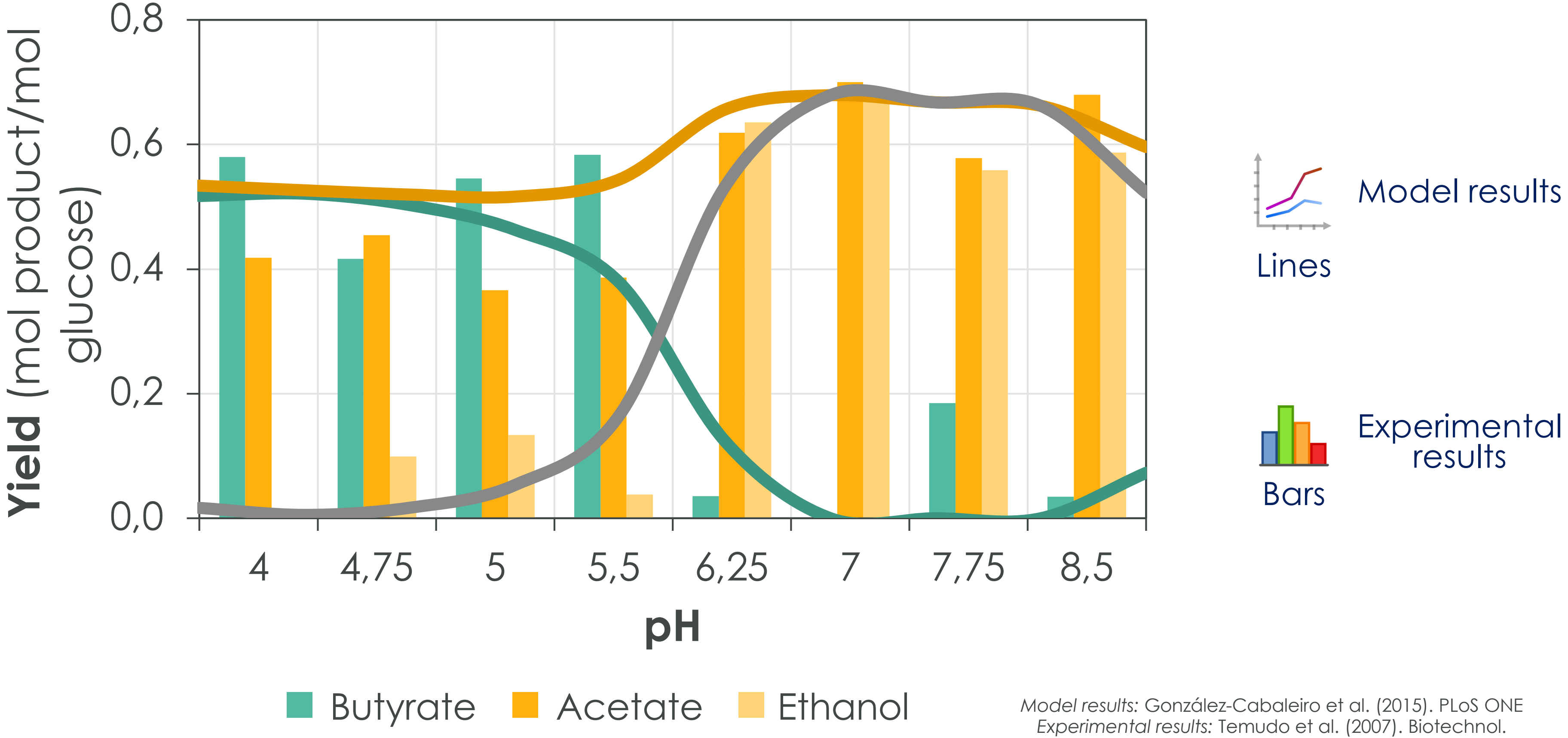


Model results

Lines

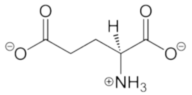
Model results: González-Cabaleiro et al. (2015). PLoS ONE
Experimental results: Temudo et al. (2007). Biotechnol. Bioeng.

The model was validated with experimental results

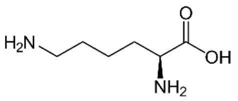


Model results: González-Cabaleiro et al. (2015). PLoS ONE
Experimental results: Temudo et al. (2007). Biotechnol. Bioeng.

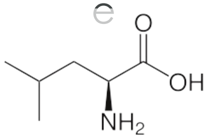
Bioenergetic model for protein fermentation



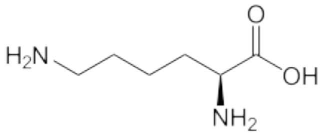
Glutamate



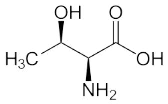
Lysine



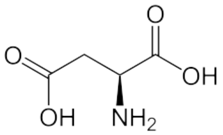
Leucine



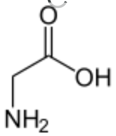
Lysine



Serine

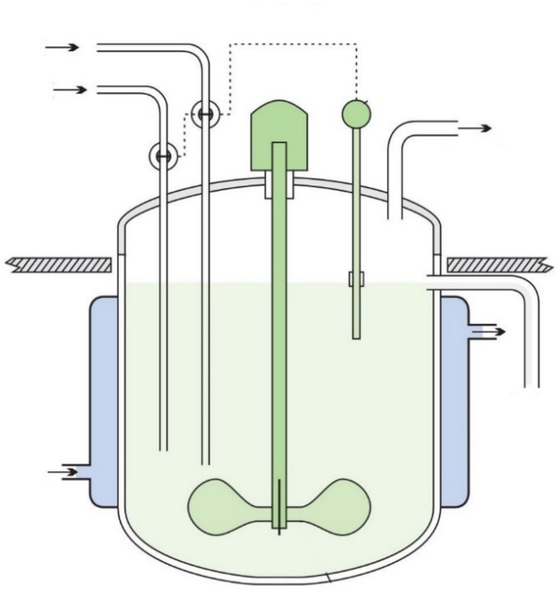


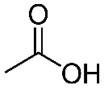
Aspartate



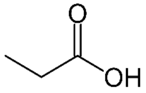
Glycine

...

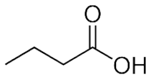




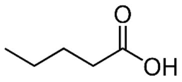
Acetate



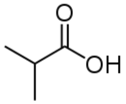
Propionate



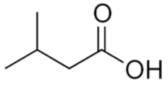
Butyrate



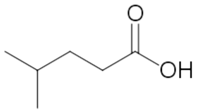
Valerate



Iso-butyrate



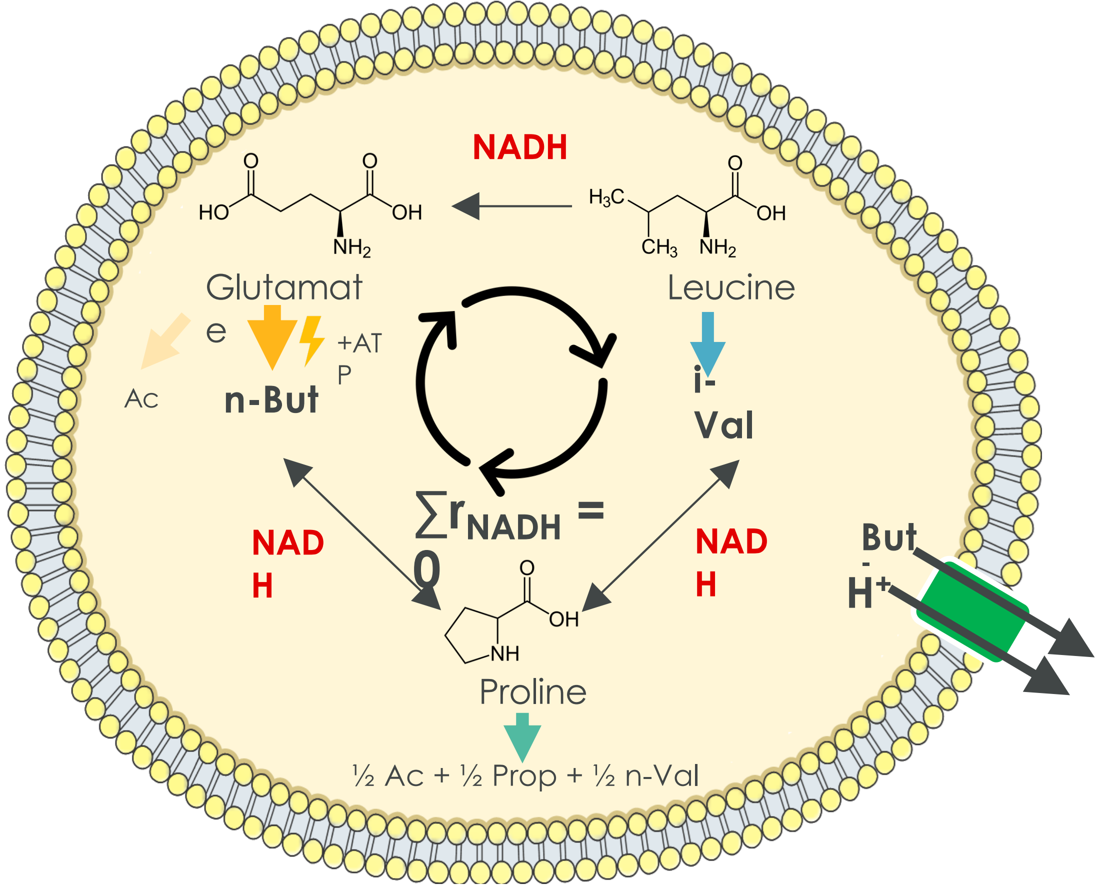
Iso-valerate



Isocaproate

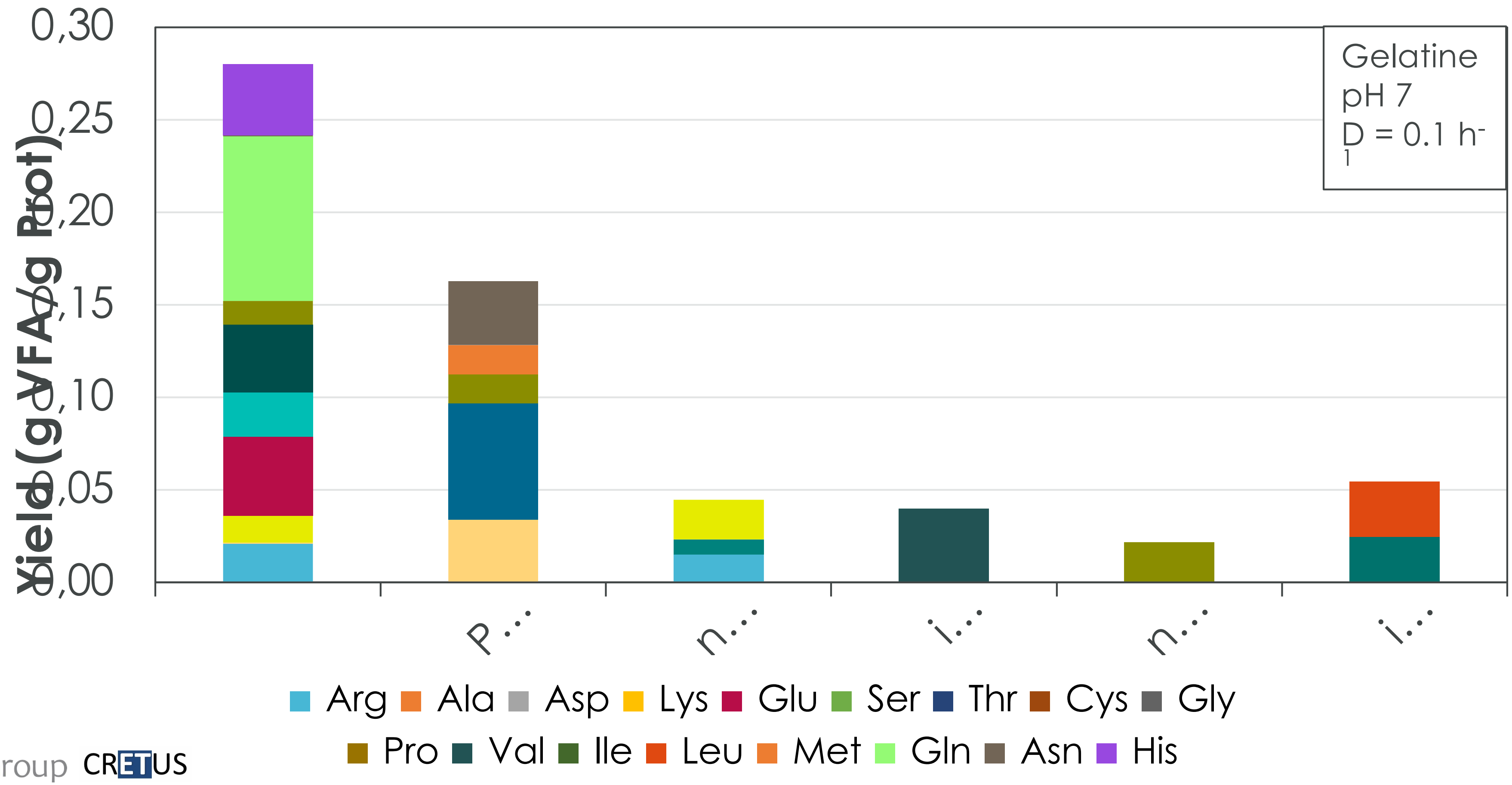
pH: 4-9

Enzyme soup approach: amino acids are converted jointly



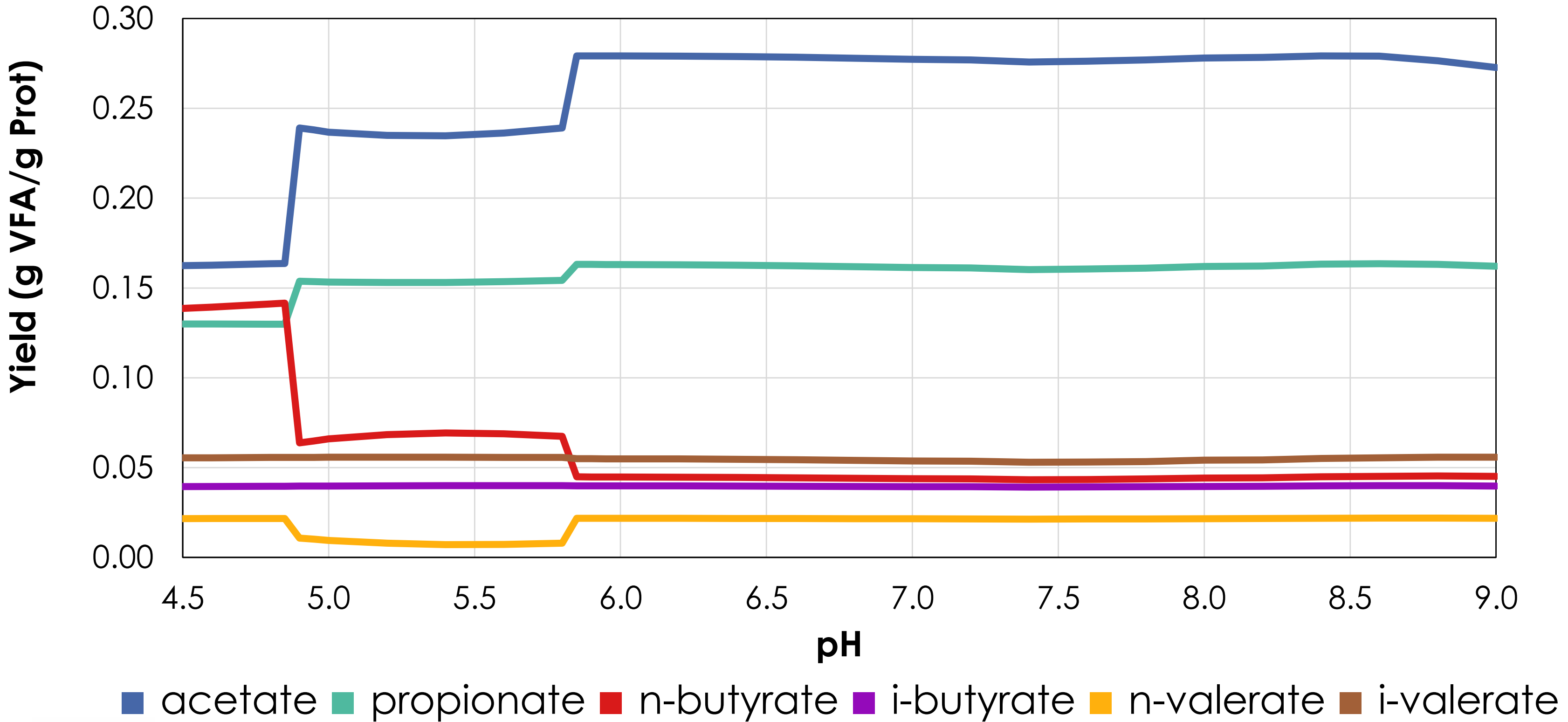
- Amino acids **interact** and **share** common metabolites (e.g. NADH) and affect common processes (e.g. VFA transport)

Predicted VFA yields from gelatine fermentation at pH 7

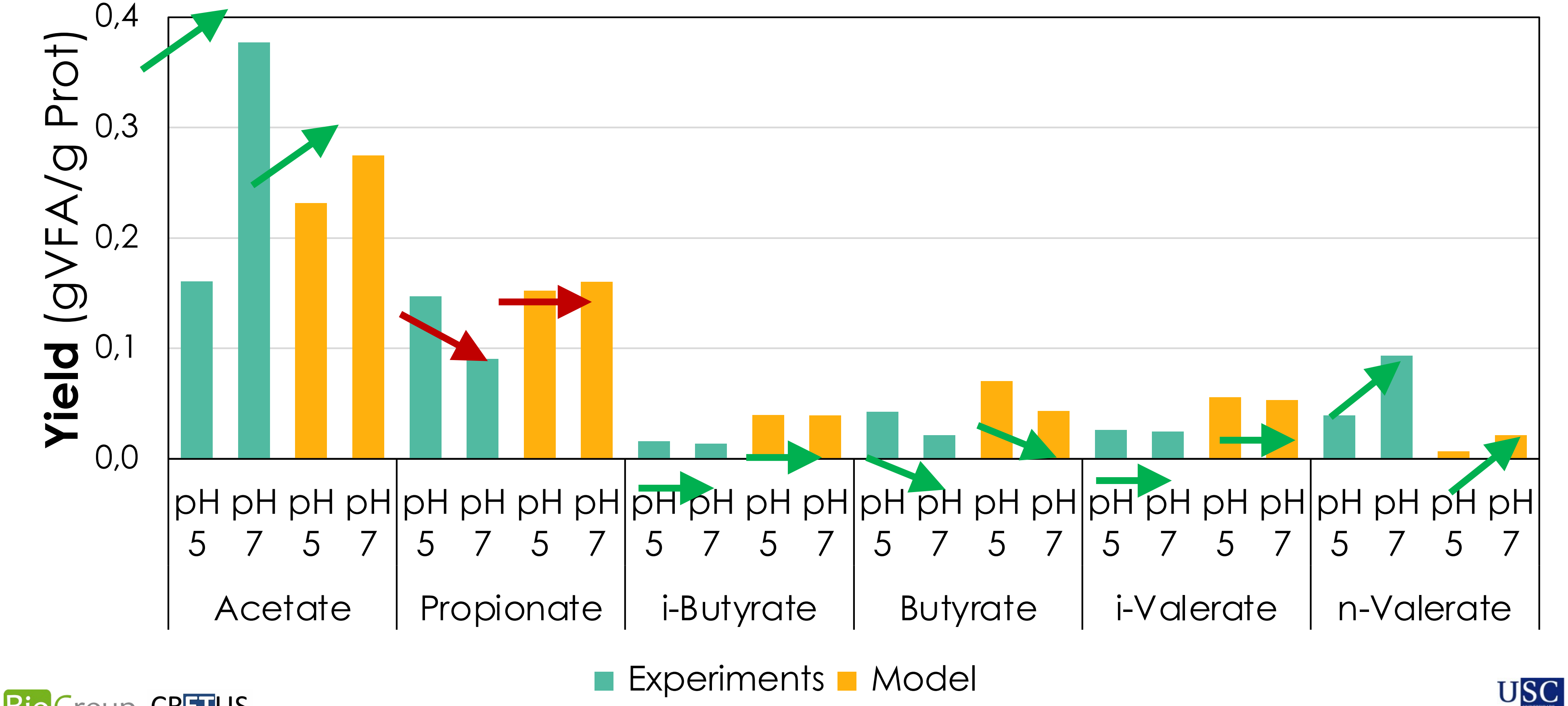


The product spectrum is sensitive to pH

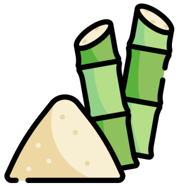
- Higher proton motive force energy available at low pH favours butyrate production



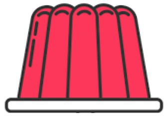
The model captures the tendencies observed with pH



Modelling the cofermentation of gelatine and glucose



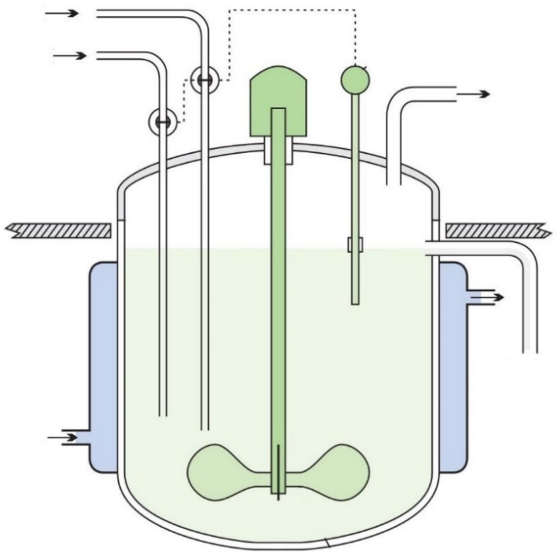
+

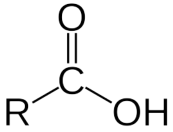


Glucose

Gelatine
(17 aminoacids)

Total concentration = 10 g/L



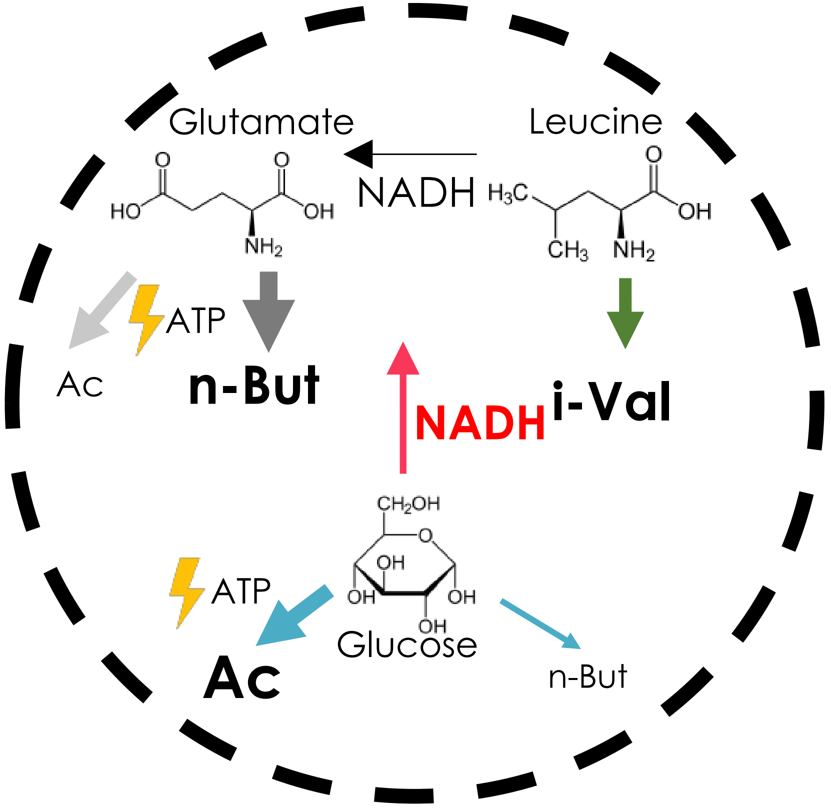


VFA

Substrate proportions

pH

Cofermentation is not just adding two mono-fermentations



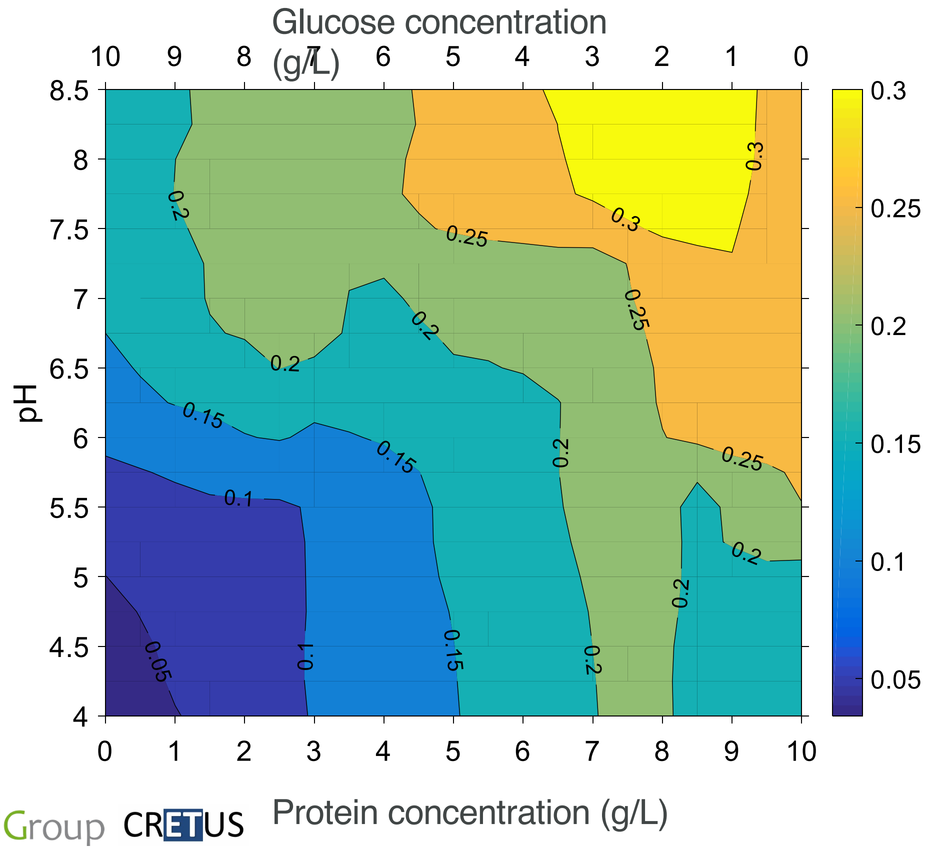
Cofermentation

$$Max(r_{ATP,Prot} + r_{ATP,Glucose})$$

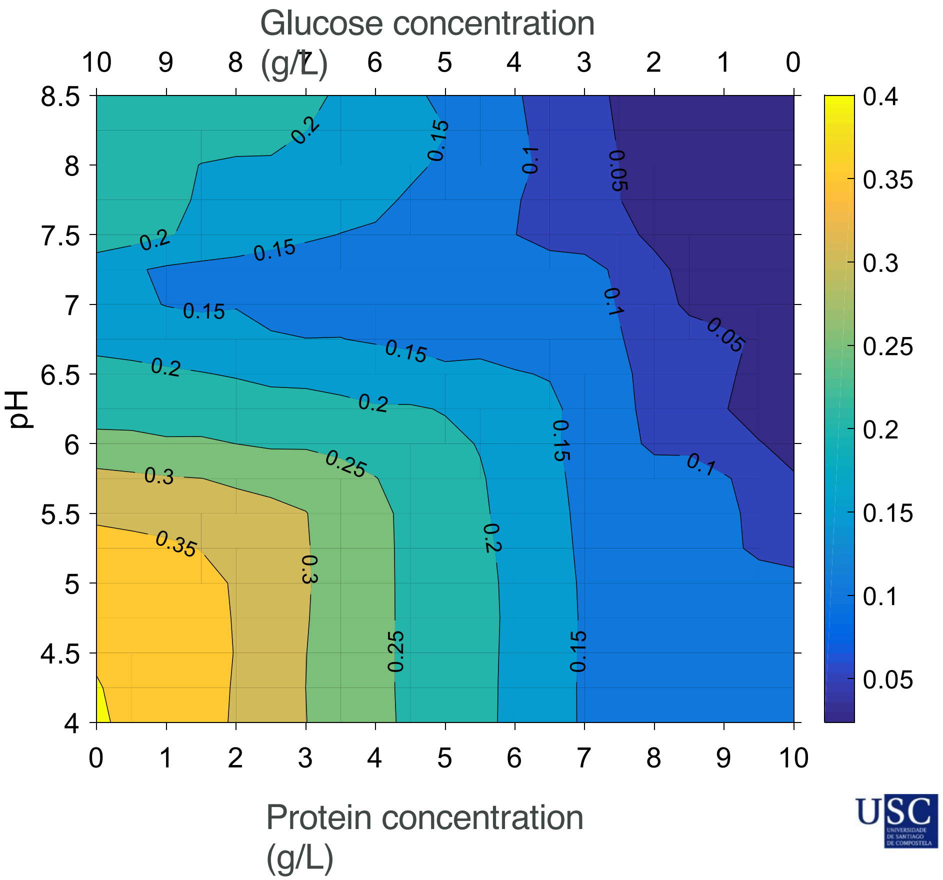
$$\sum r_{NADH,Prot} + \sum r_{NADH,Glu} = 0$$

Changing operational conditions favour different VFA

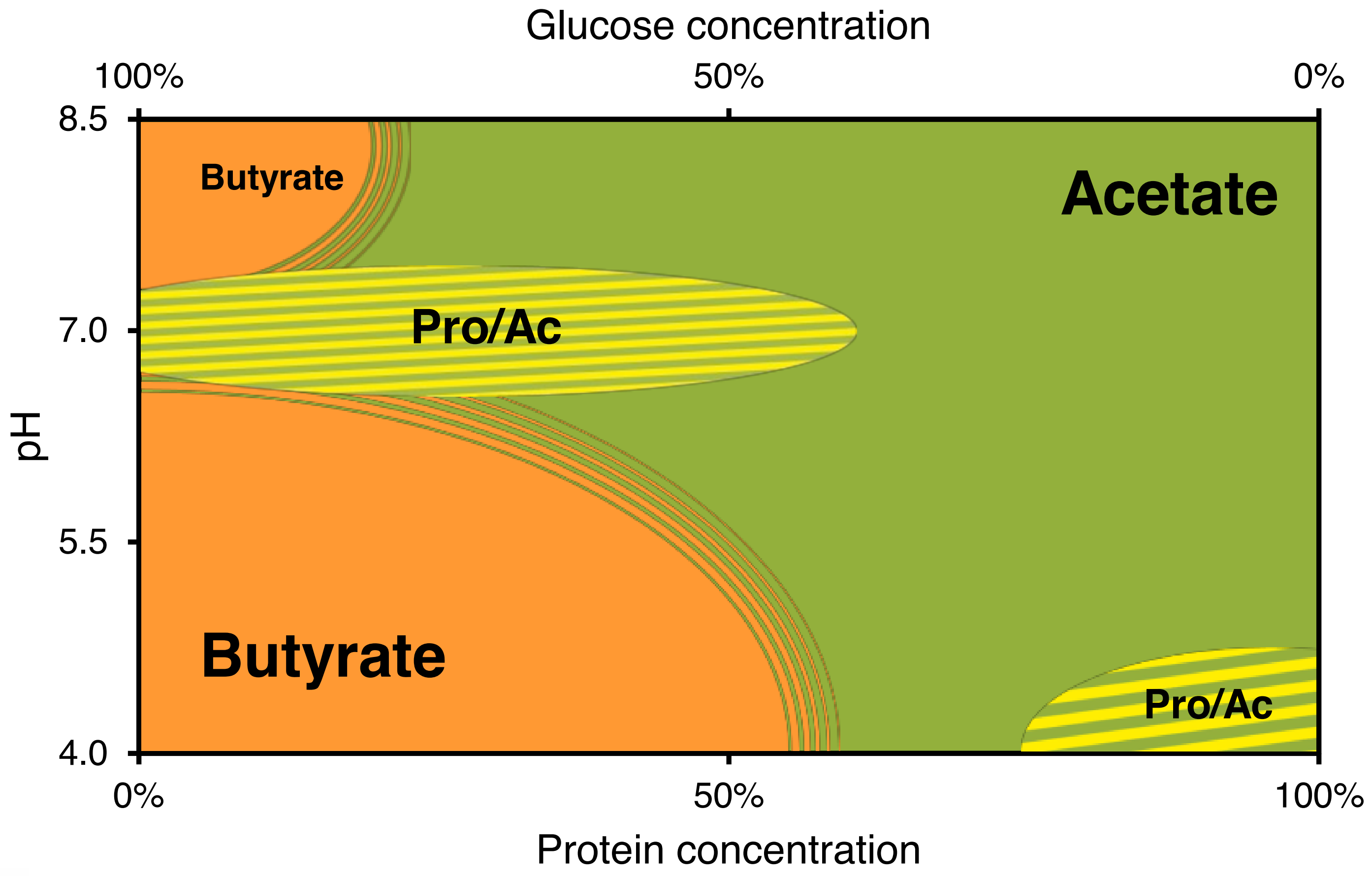
Acetate yield (g/g$_{Feeding}$)



n-Butyrate yield (g/g$_{Feeding}$)



The model can explore the operational space



BioGroup CRETUS

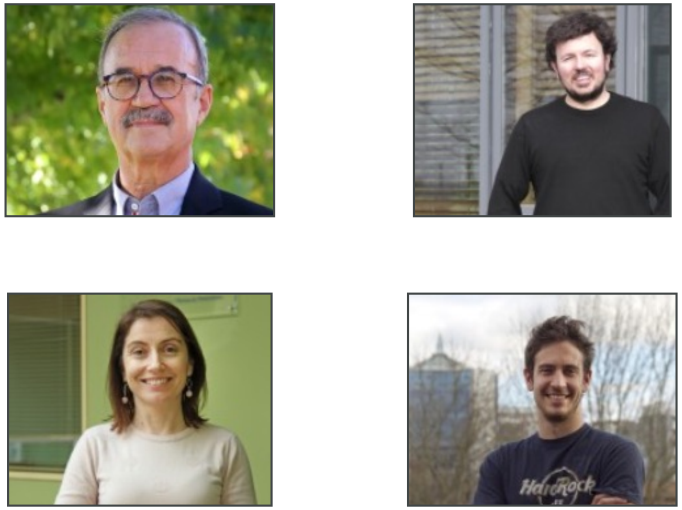
Take home messages

- There are bioenergetic models available for **predicting the fermentation stoichiometry** of glucose, protein and their cofermentation.
- The **effect of main operational conditions** (e.g. pH or substrate proportions) on process selectivity is well captured by the model.
- These models have a direct use as **early-stage design tools** in the context of the carboxylate platform:
    - Select a (mixture of) waste streams to target a particular VFA(s)
    - Select the best pH for targeting a particular VFA(s)

Bioenergetic modelling for predicting the stoichiometry of anaerobic fermentation

Alberte.regueiralopez@ugent.be

@Alberte Regueira



AGENDA AND HOUSEKEEPING

Speaker 1
Alberte Regueira (Universidade de Santiago de Compostela, Spain)

Speaker 2
Adam Kovalovszki (Technical University of Denmark)

Speaker 3
Matthew Scarborough (University of Vermont, USA)

Q&A Session Moderator: *Robbert Kleerebezem (Delft University of Technology, The Netherlands)*

- This session is being recorded;
- Microphones and cameras have been disabled due to the large number of attendees;
- The normal chat function is disabled;
- Please put any questions and comments you may have in the Q&A (icon to the low right in Zoom) and we will do our best to answer them during the session (in writing or orally).

INFERRING METABOLIC INTERACTIONS FROM GENOME-SCALE DATA: ANAEROBIC DIGESTION ‘UNDER THE MICROSCOPE’

Adam Kovalovszki (adko@env.dtu.dk)
DTU Environment –
Technical University of Denmark

AGENDA AND HOUSEKEEPING

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Focus points

☐ What is genome-scale metabolic modeling (GSMM)?

Focus points

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☐ Who’s who in the AD microbiome

Focus points

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- ☐ Who's who in the AD microbiome

↕

AF

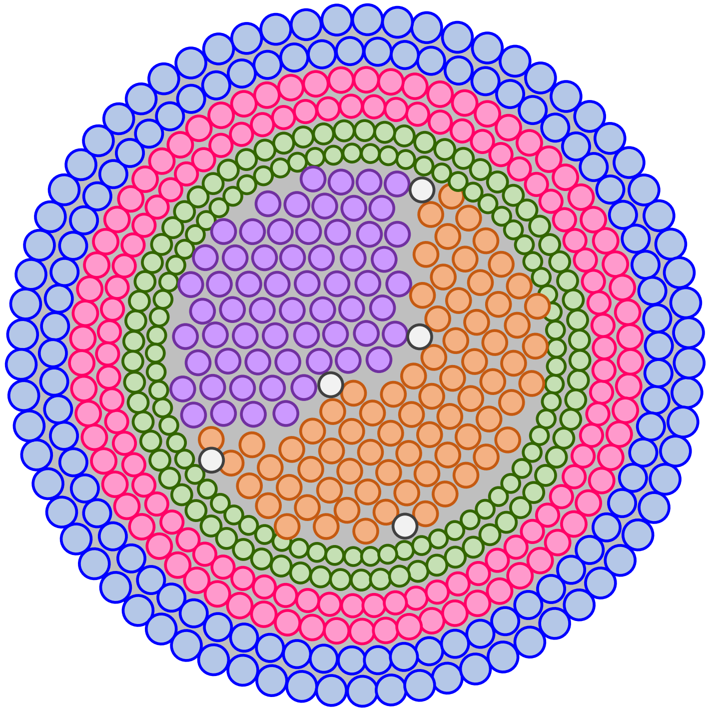
Focus points

☐ What is genome-scale metabolic modeling (GSMM)?

☐ Who’s who in the AD microbiome

☐ Microbial interactions in AD

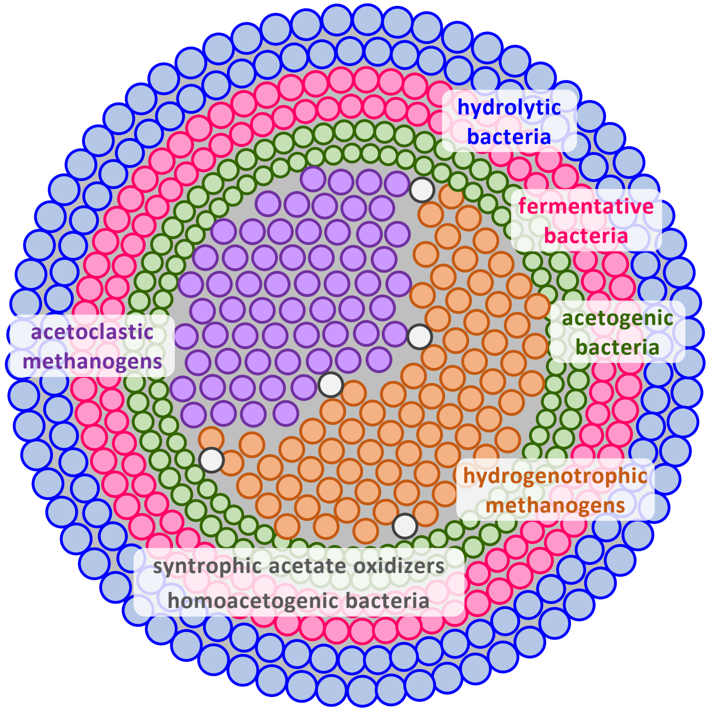
GSMM



METHANOGENIC GRANULE STRUCTURE

(*theoretical*)

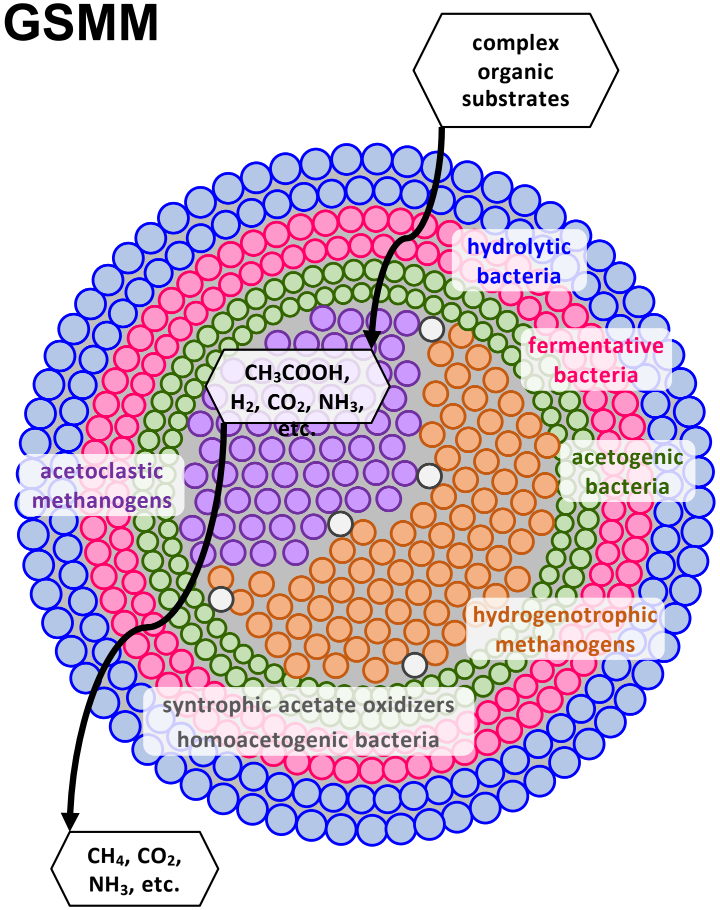
GSMM



METHANOGENIC GRANULE STRUCTURE

(*theoretical*)

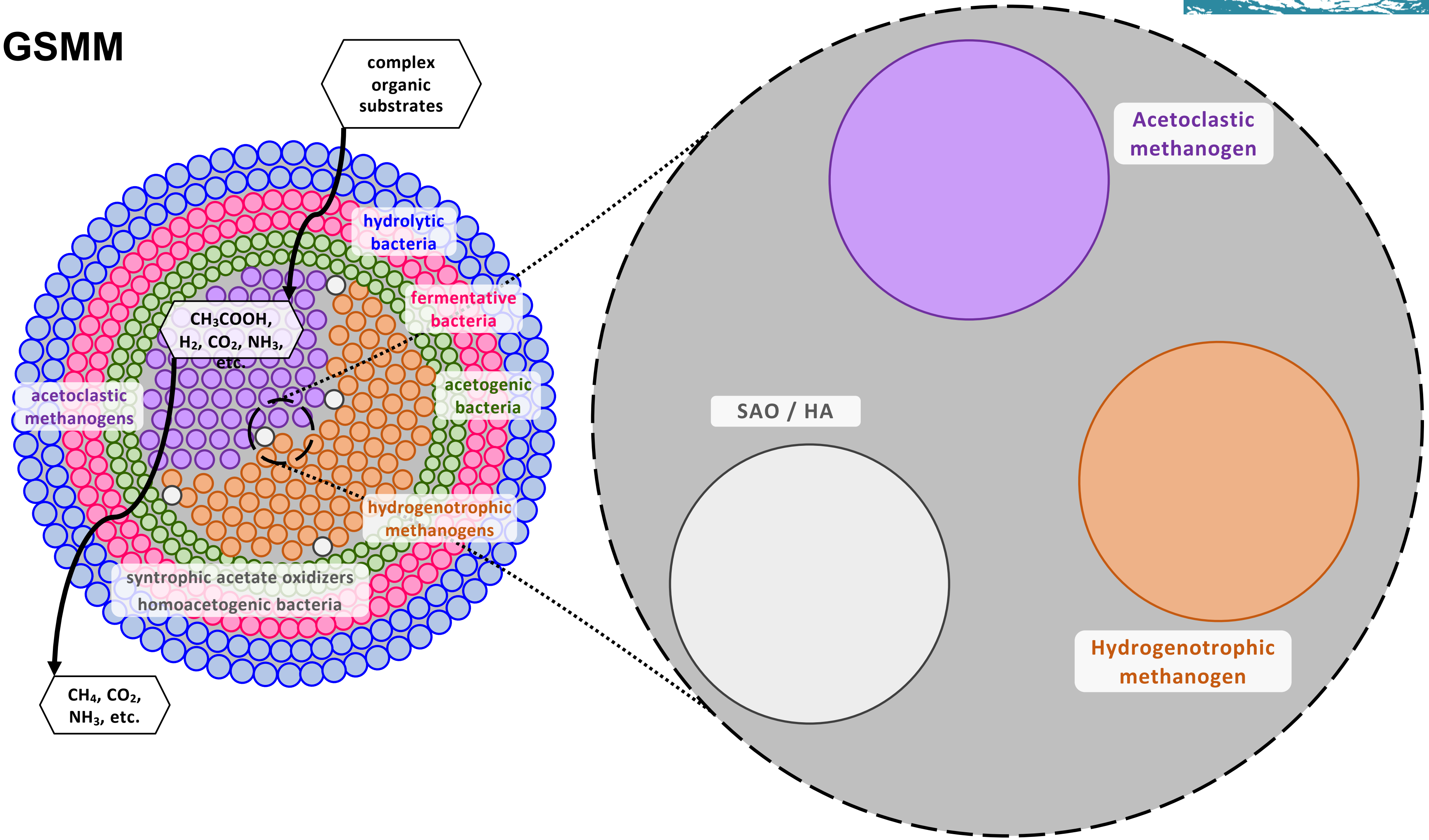
GSMM



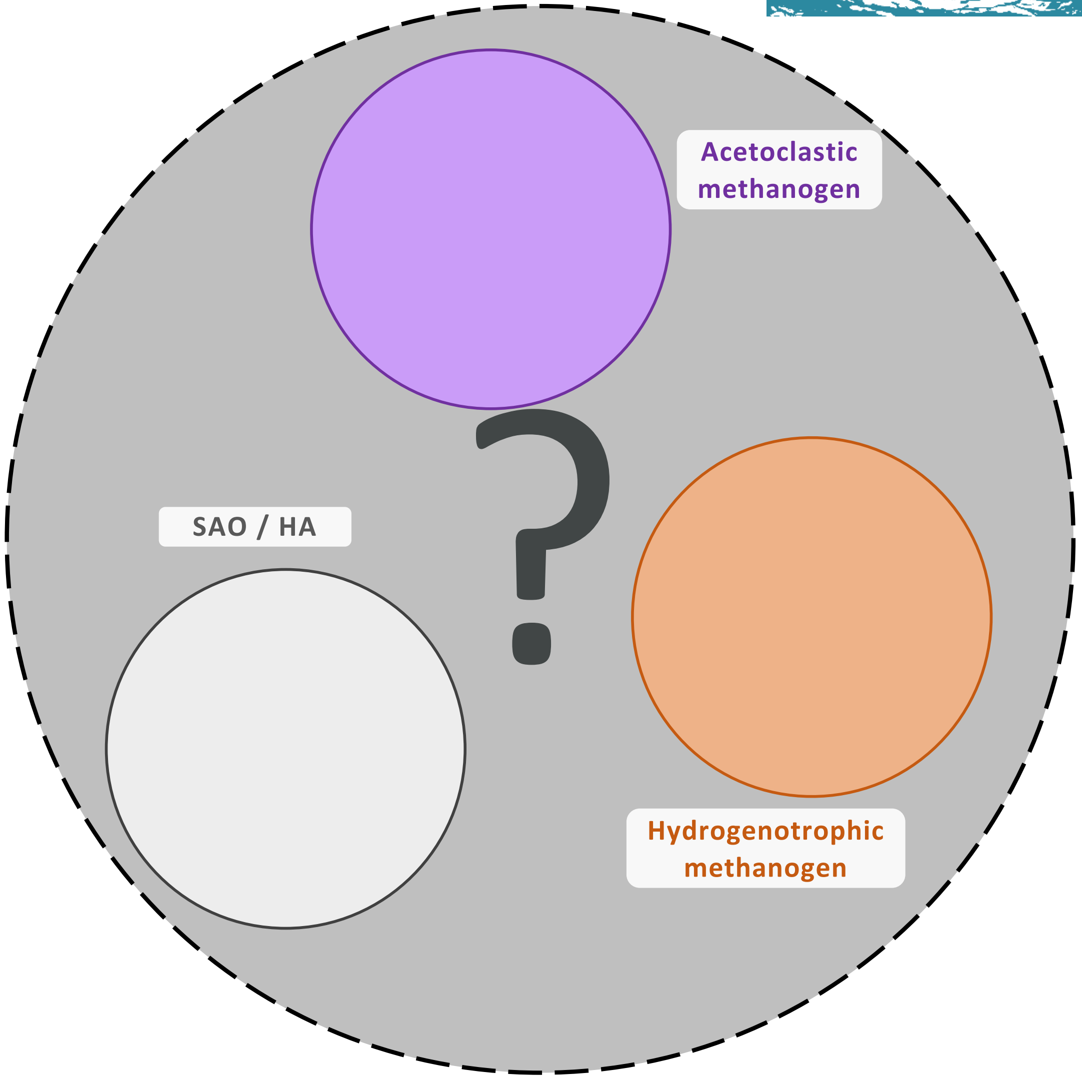
METHANOGENIC GRANULE STRUCTURE

(*theoretical*)

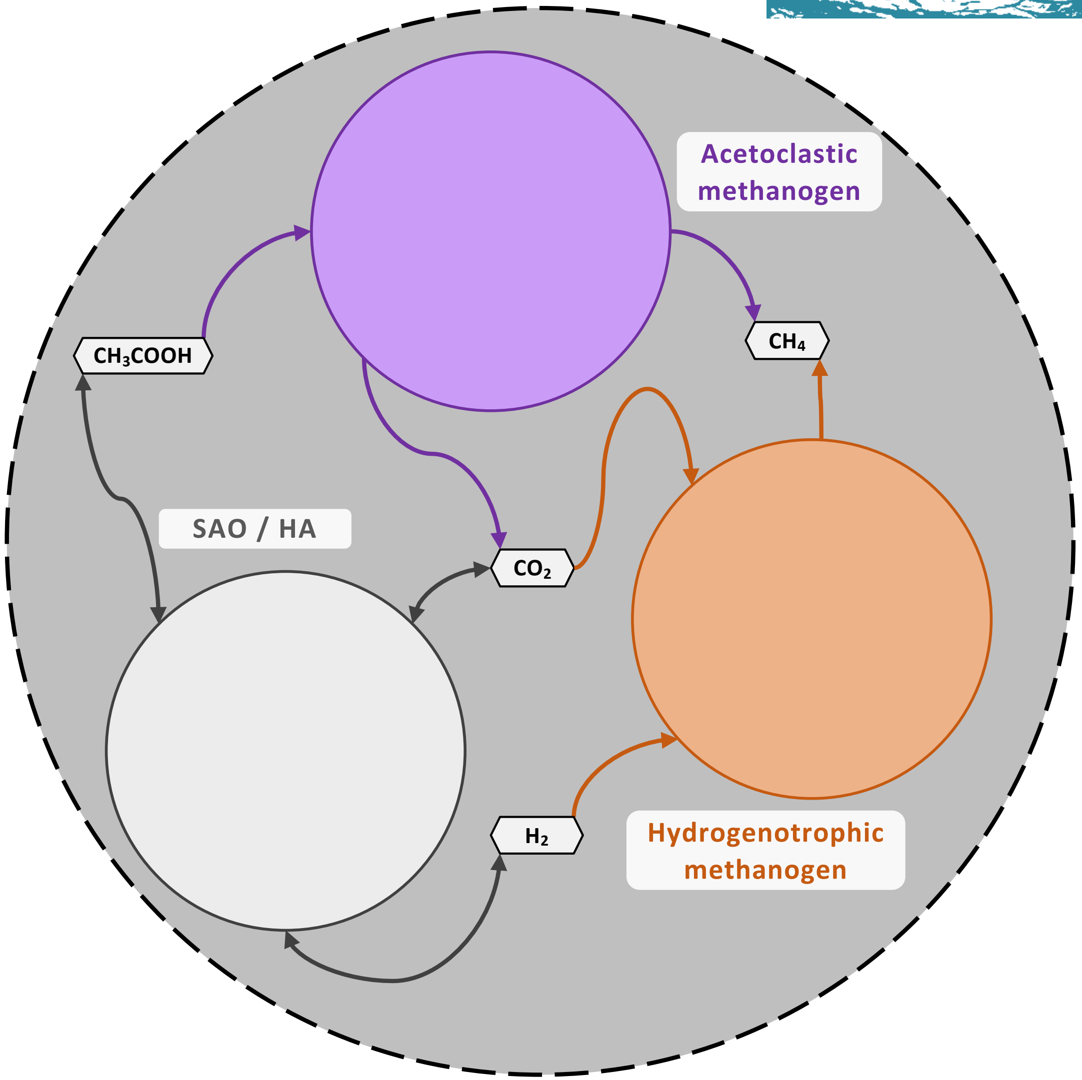
GSMM


complex organic substrates
hydrolytic bacteria
fermentative bacteria
CH_3COOH, H_2, CO_2, NH_3, etc.
acetogenic bacteria
acetoclastic methanogens
hydrogenotrophic methanogens
syntrophic acetate oxidizers
homoacetogenic bacteria
CH_4, CO_2, NH_3, etc.
Acetoclastic methanogen
SAO / HA
Hydrogenotrophic methanogen

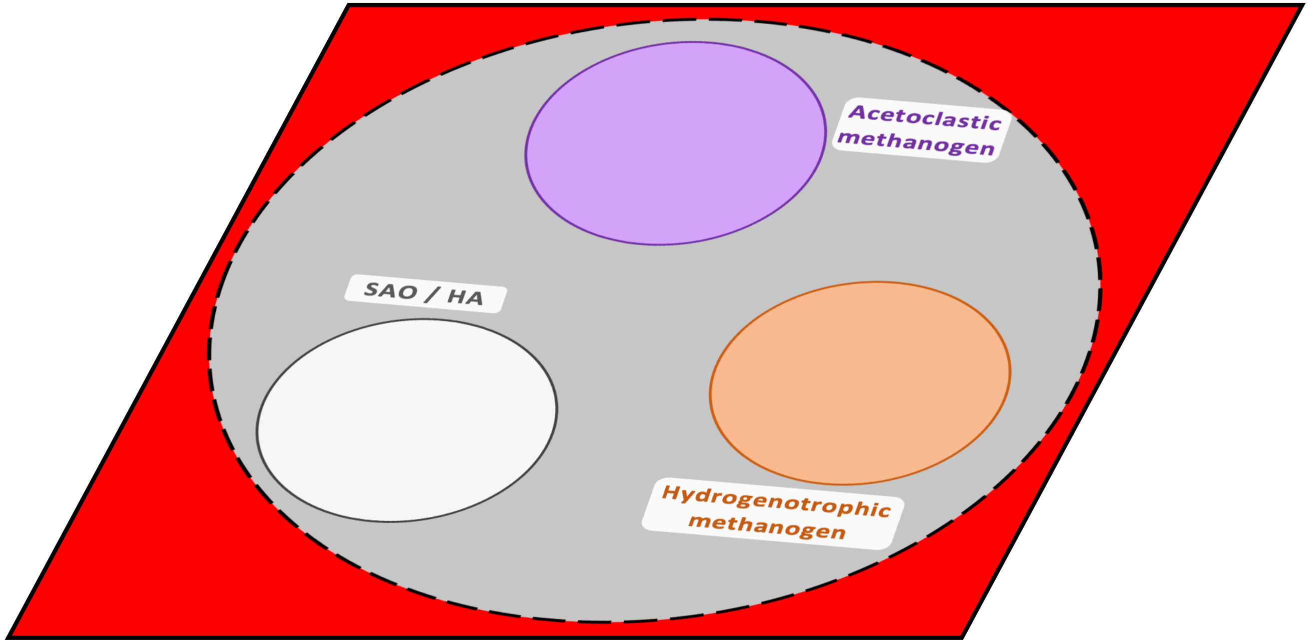
GSMM


Acetoclastic methanogen
SAO / HA
?
Hydrogenotrophic methanogen

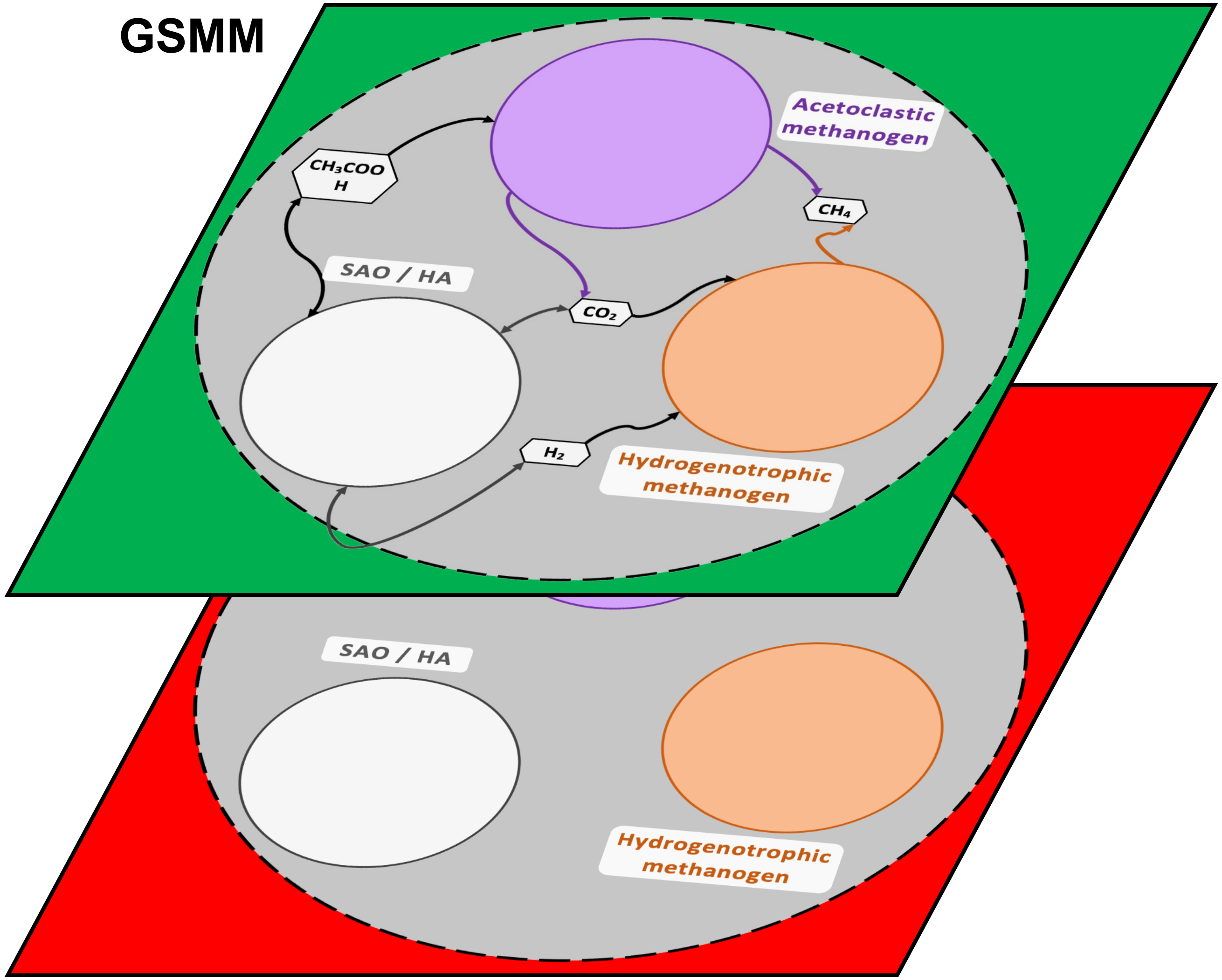
GSMM


Acetoclastic methanogen
CH$_3$COOH
CH$_4$
SAO / HA
CO$_2$
H$_2$
Hydrogenotrophic methanogen

GSMM



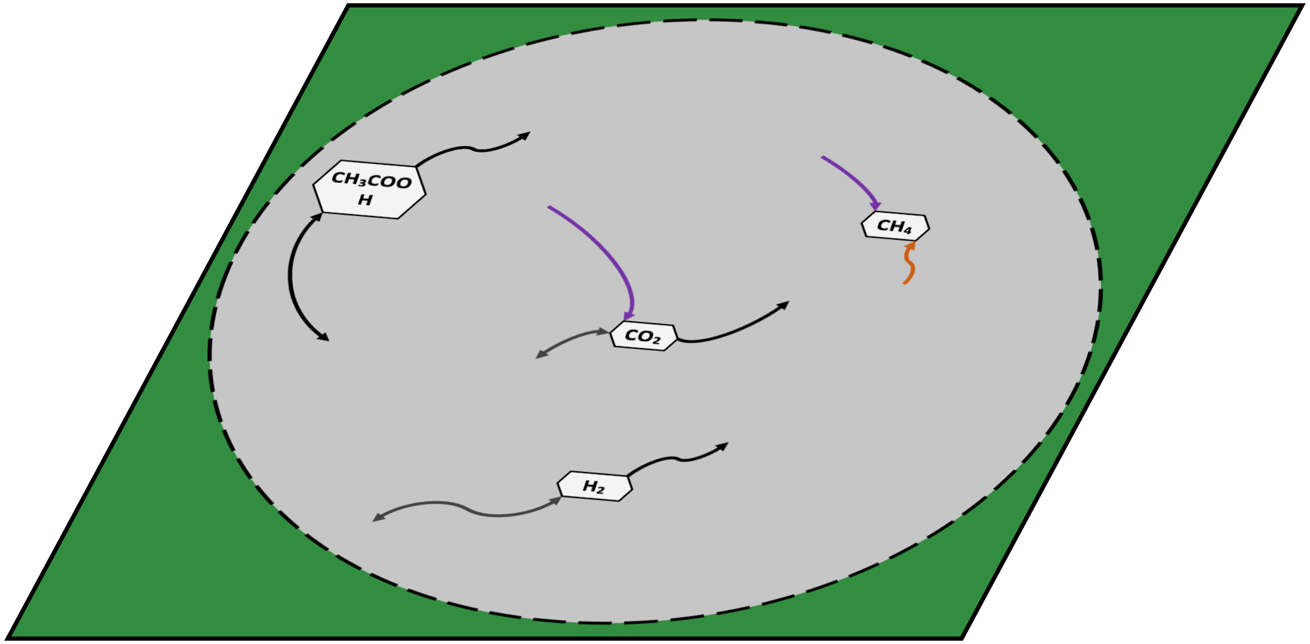
Guilds (functions we assume)



Phenotypes (*what we "see"*)

Guilds (functions we assume)

GSMM

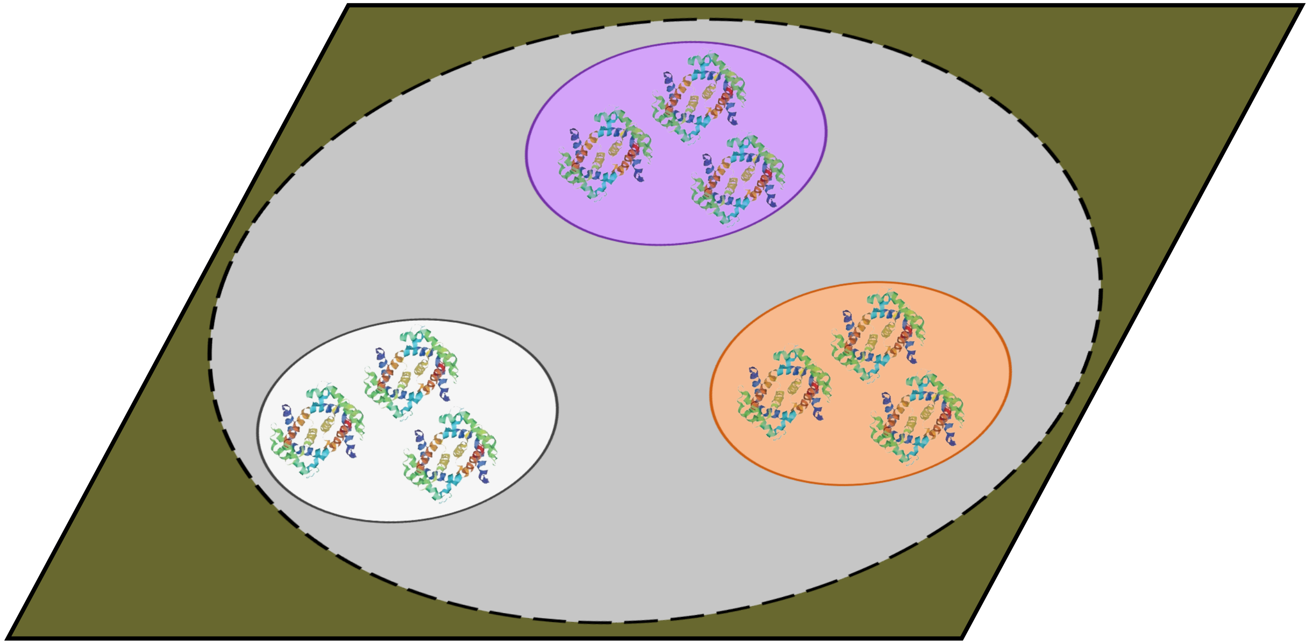


Phenotypes (*what we ”see”*)

Metabolites → metabolomics

Guilds (functions we assume)

GSMM



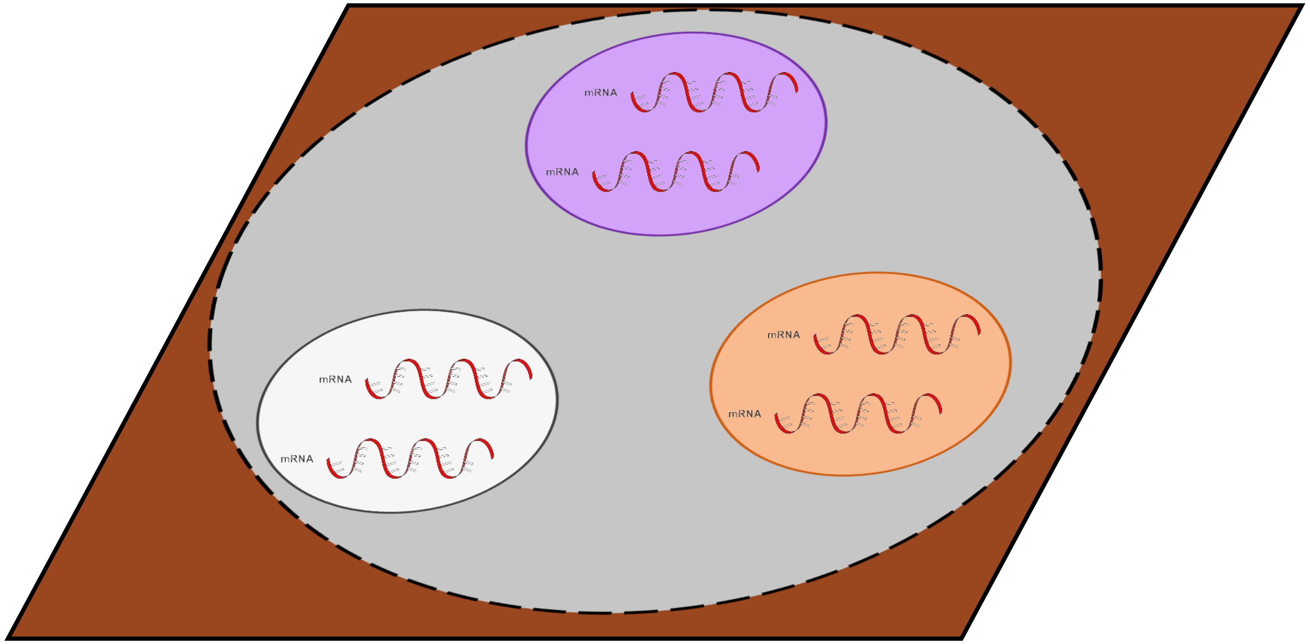
Phenotypes (*what we "see"*)

Metabolites → metabolomics

Proteins → Metaproteomics

Guilds (functions we assume)

GSMM



Phenotypes (*what we "see"*)

Metabolites → metabolomics

Proteins → Metaproteomics

mRNA → Metatranscriptomics

Guilds (functions we assume)

GSMM

Phenotypes (*what we ”see”*)

Metabolites → metabolomics

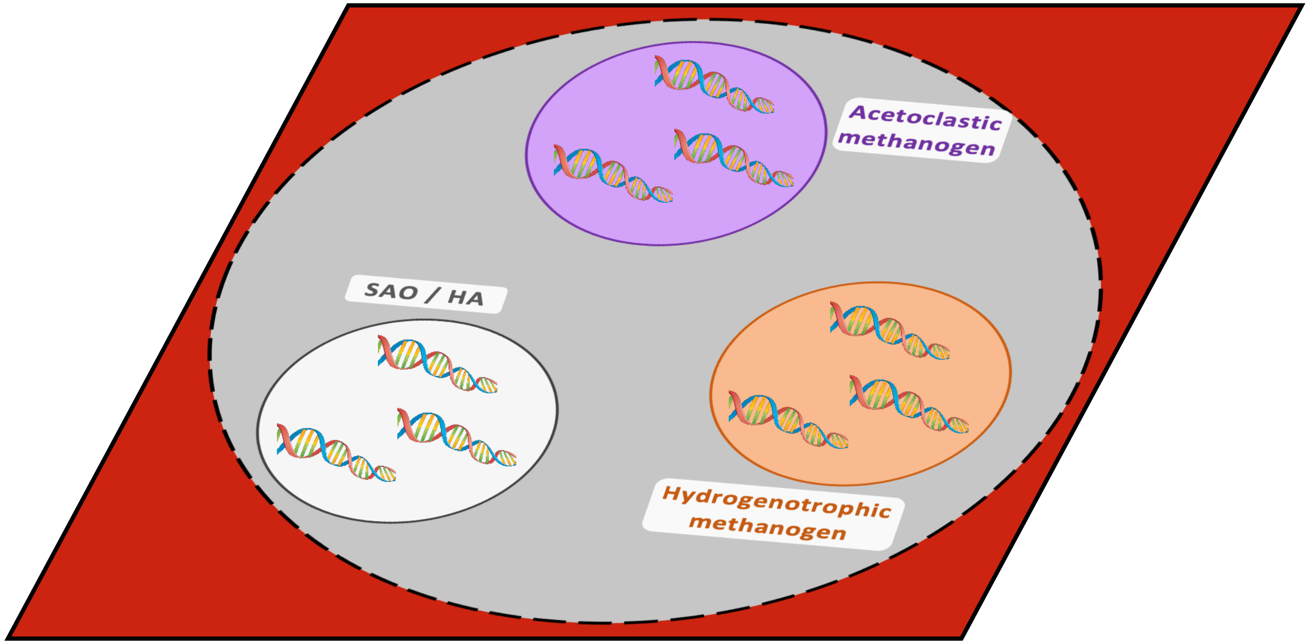
Proteins → Metaproteomics

mRNA → Metatranscriptomics

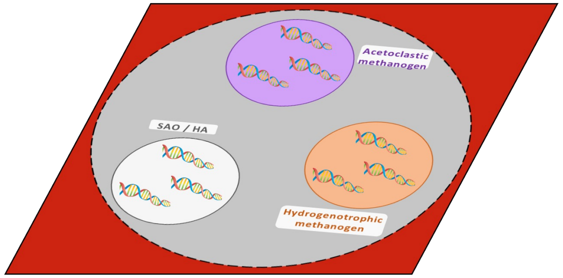
DNA → Metagenomics

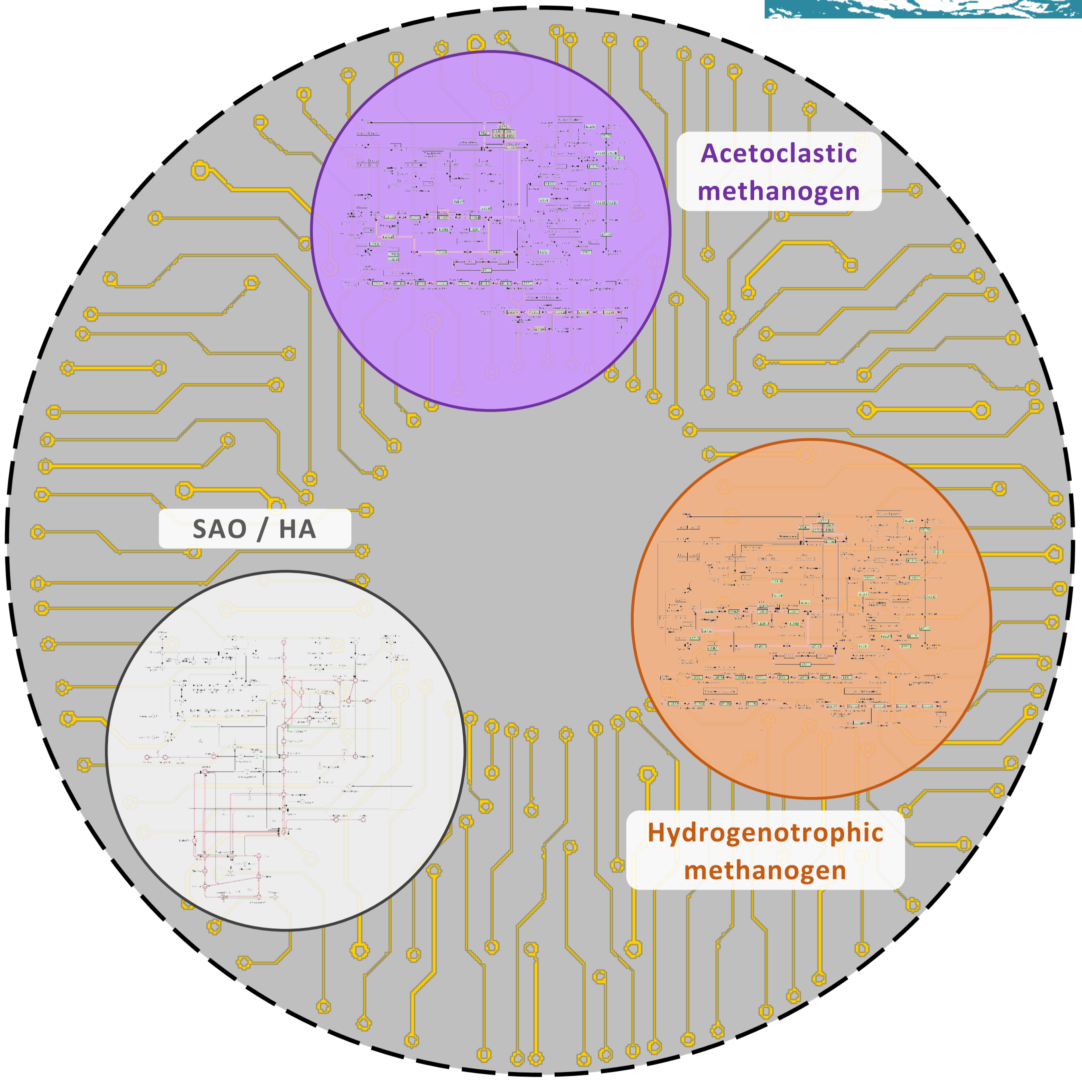
(16S → taxonomy)

Guilds (functions we assume)



GSMM

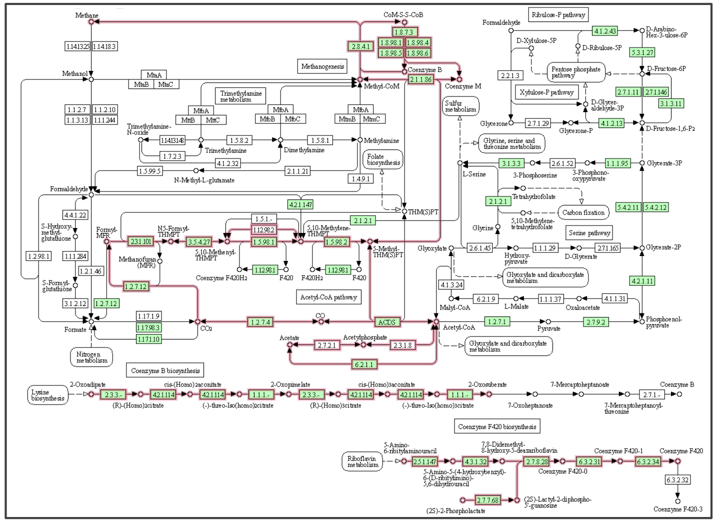




METHANOGENIC COMMUNITY MODEL

(*in silico*)

GSMM

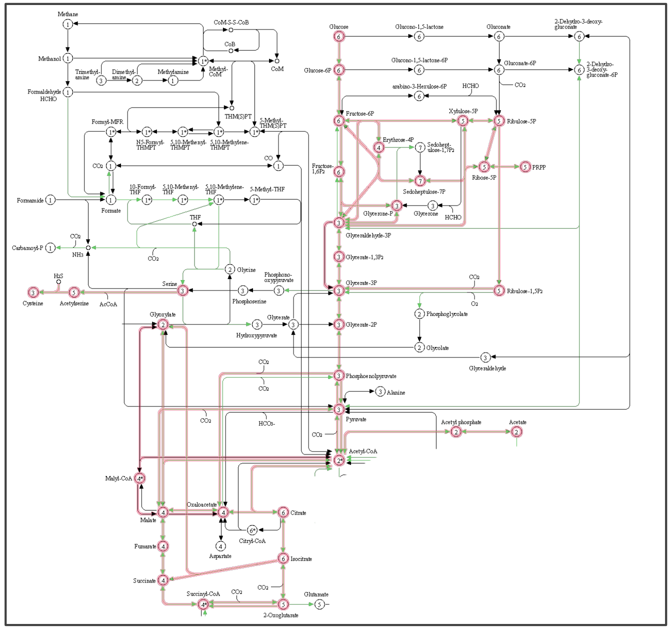


Acetoclastic methanogen

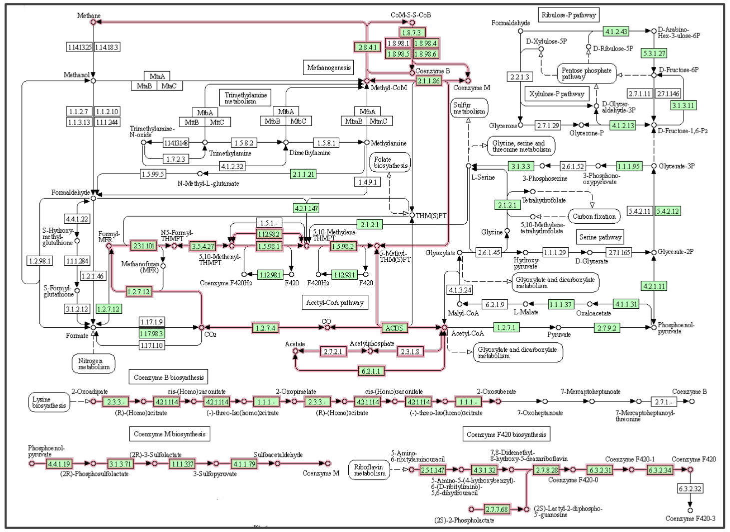
METHANOGENIC COMMUNITY MODEL

(*in silico*)

SAO / HA



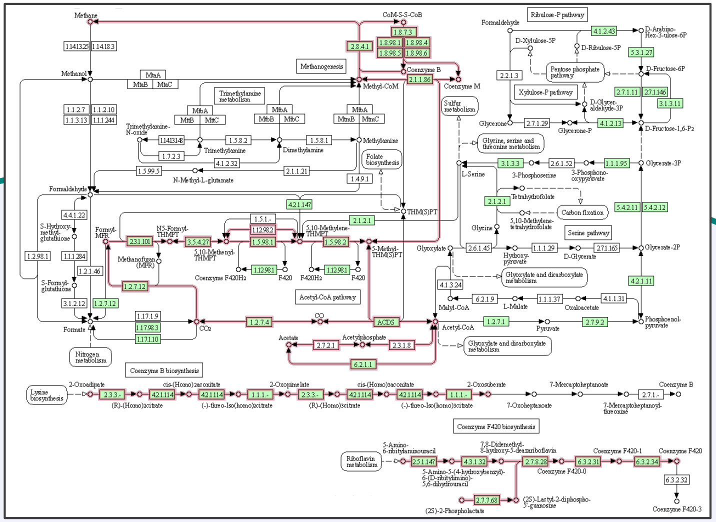
Hydrogenotrophic methanogen



https://www.kegg.jp/kegg/

GSMM

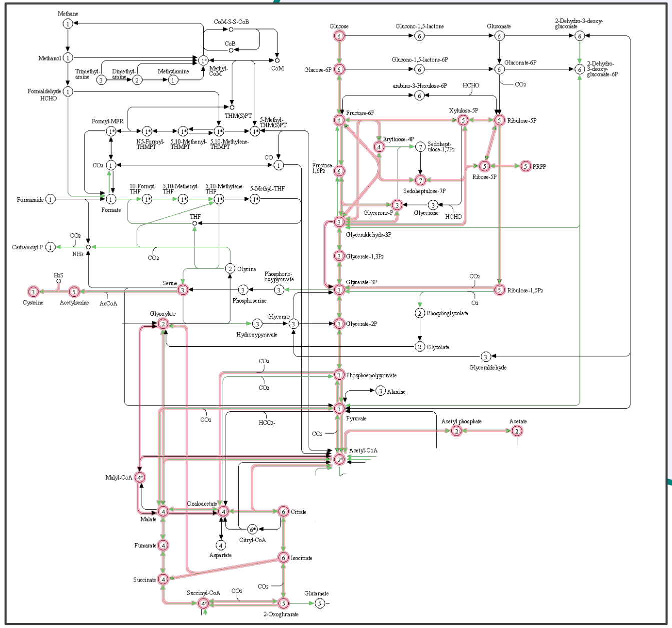
Acetoclastic methanogen



METHANOGENIC COMMUNITY MODEL

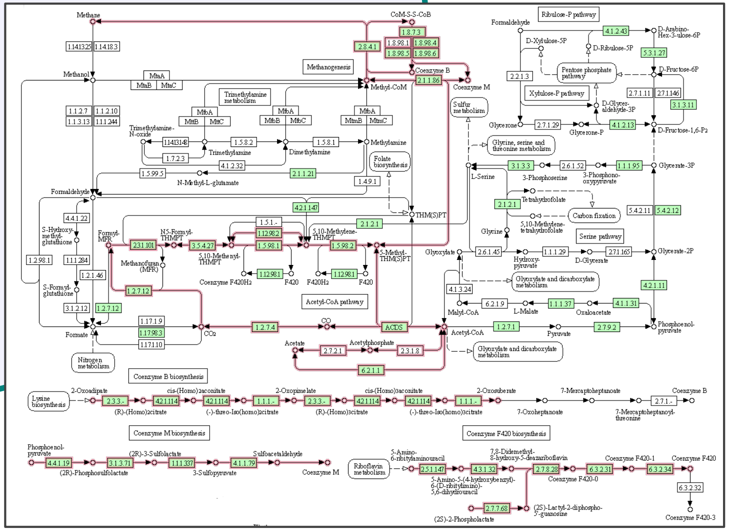
(*in silico*)

SAO / HA

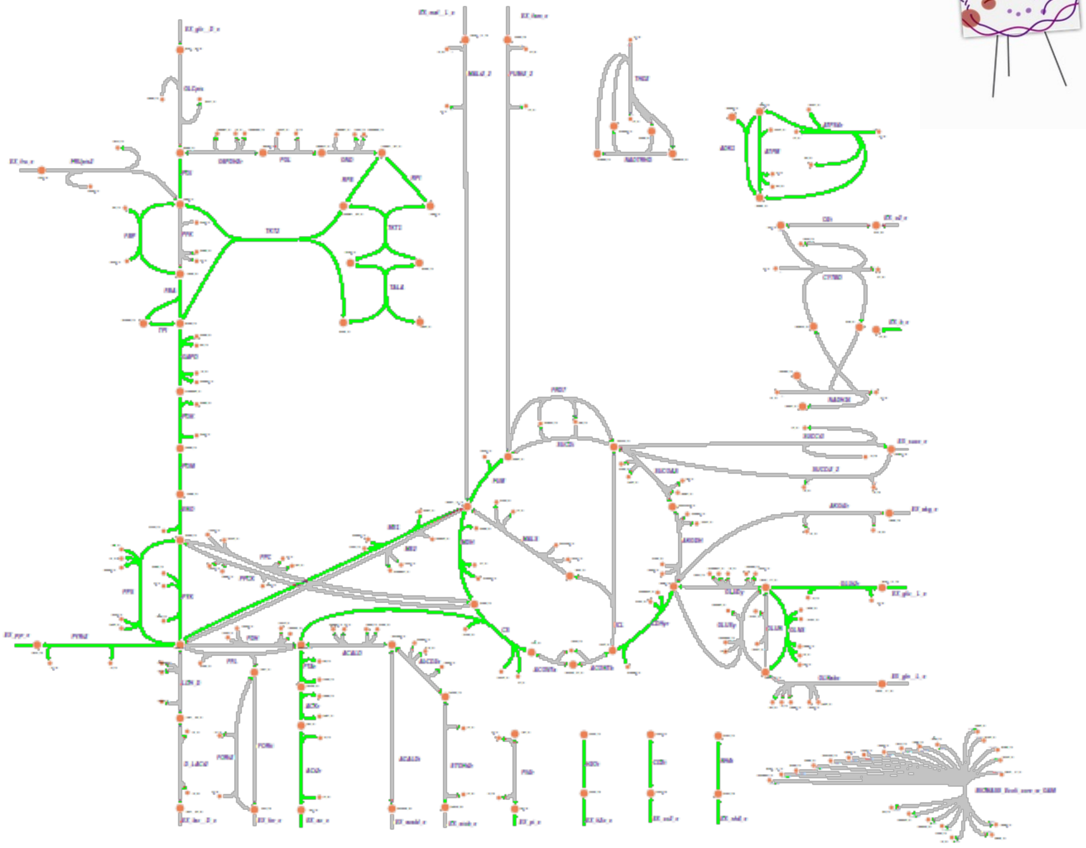


SHARED MEDIUM

Hydrogenotrophic methanogen



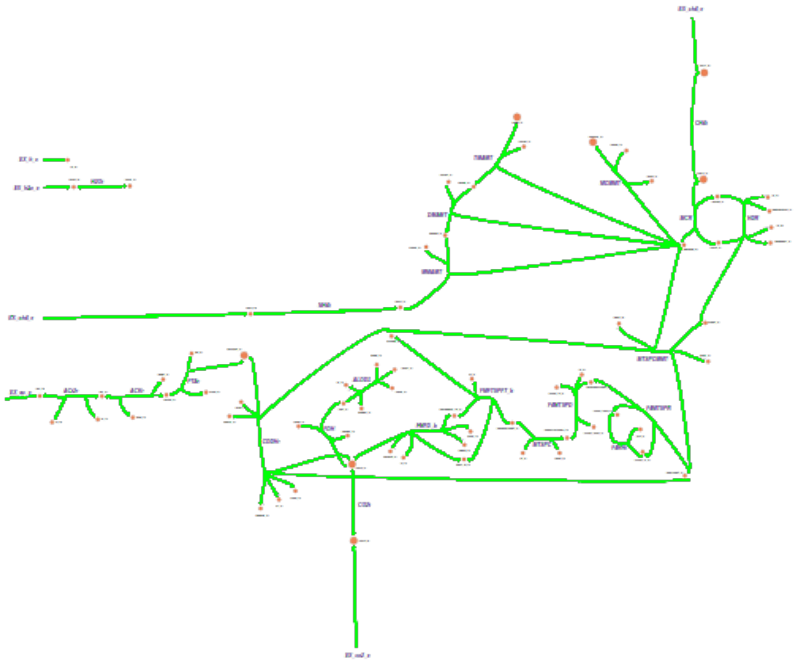
GSMM



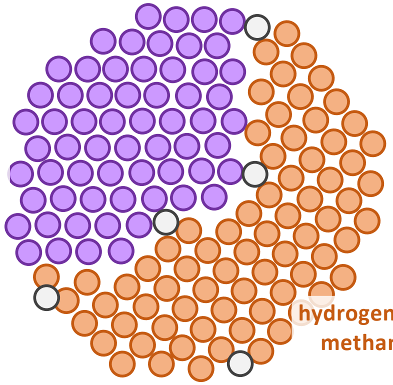
Core carbon metabolism

https://escher.github.io/#/

Methane metabolism



GSMM

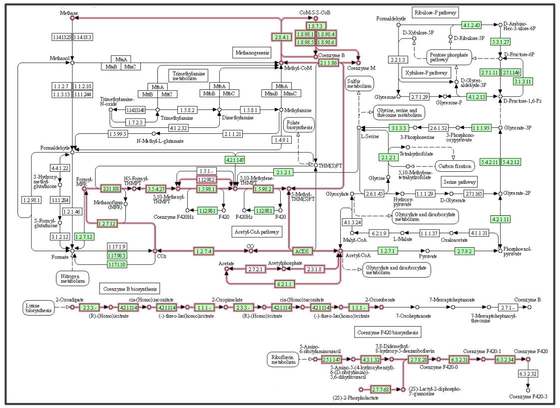


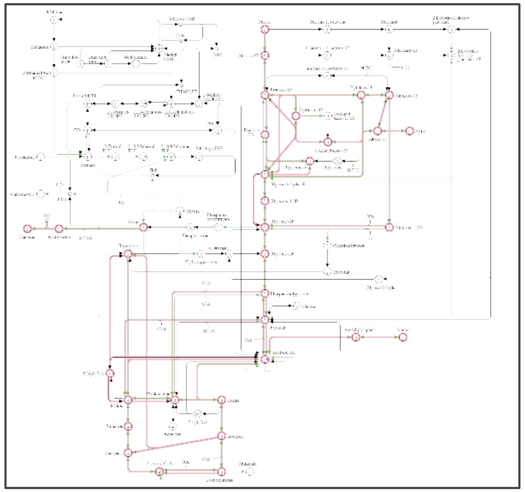
acetoclastic methanogens

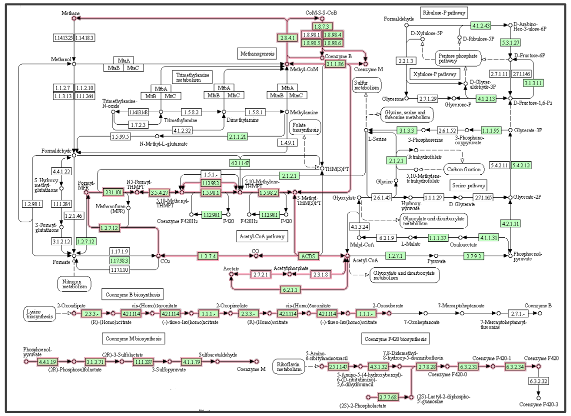
hydrogenotrophic methanogens

syntrophic acetate oxidizers

homoacetogenic bacteria







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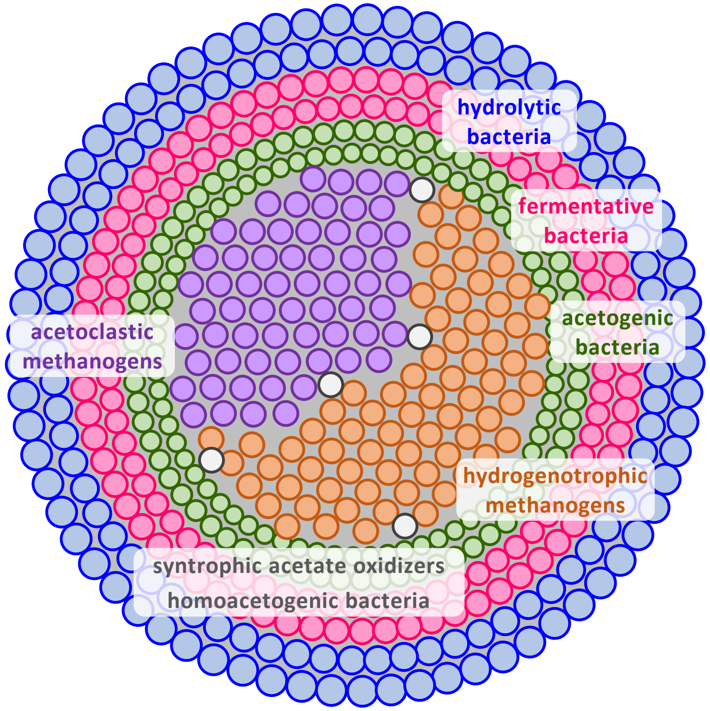
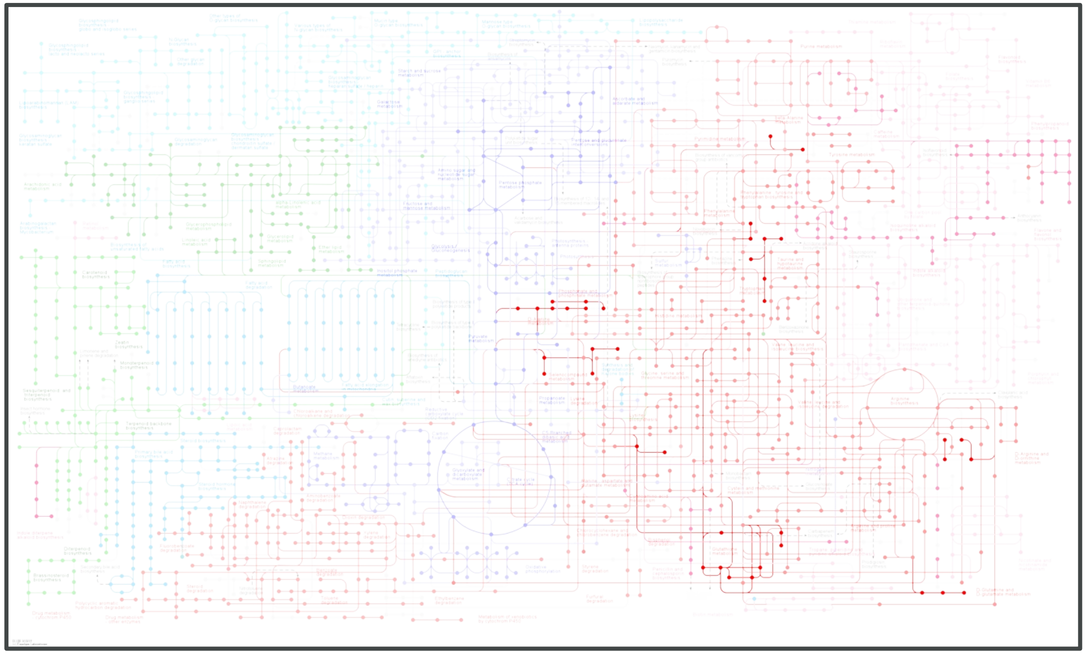
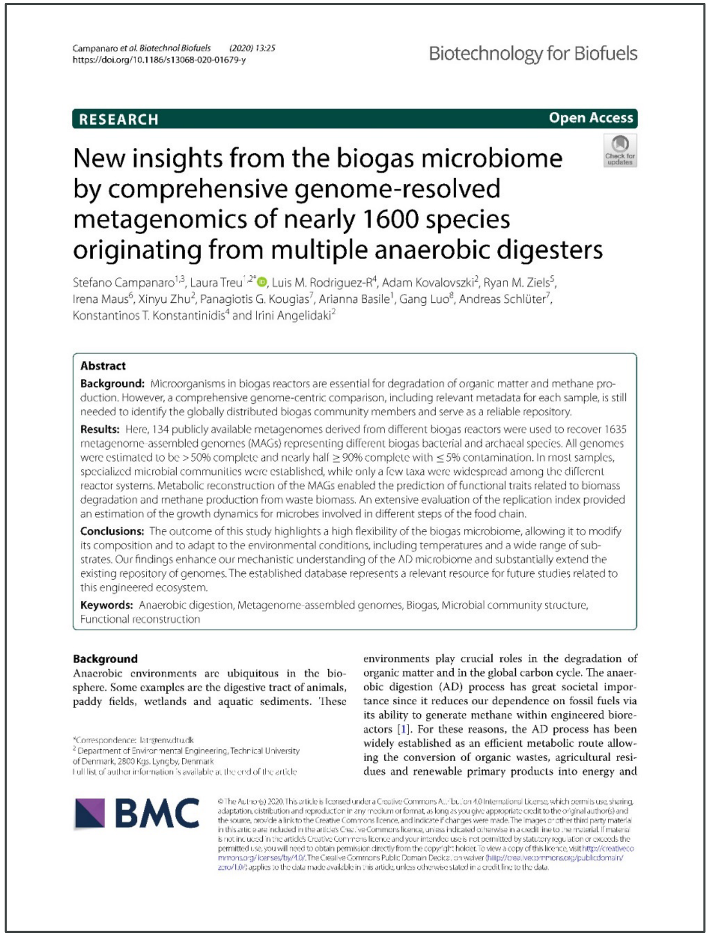




https://www.genome.jp/dbget-bin/www_bget?ko01100

Who’s who in AD



Campanaro *et al. Biotechnol Biofuels* (2020) 13:25
https://doi.org/10.1186/s13068-020-01679-y

Biotechnology for Biofuels

RESEARCH **Open Access**

New insights from the biogas microbiome by comprehensive genome-resolved metagenomics of nearly 1600 species originating from multiple anaerobic digesters

Stefano Campanaro[1,3], Laura Treu[1,2*], Luis M. Rodriguez-R[4], Adam Kovalovszki[2], Ryan M. Ziels[5], Irena Maus[6], Xinyu Zhu[2], Panagiotis G. Kougias[7], Arianna Basile[1], Gang Luo[8], Andreas Schlüter[7], Konstantinos T. Konstantinidis[4] and Irini Angelidaki[2]

Abstract

Background: Microorganisms in biogas reactors are essential for degradation of organic matter and methane production. However, a comprehensive genome-centric comparison, including relevant metadata for each sample, is still needed to identify the globally distributed biogas community members and serve as a reliable repository.

Results: Here, 134 publicly available metagenomes derived from different biogas reactors were used to recover 1635 metagenome-assembled genomes (MAGs) representing different biogas bacterial and archaeal species. All genomes were estimated to be > 50% complete and nearly half ≥ 90% complete with ≤ 5% contamination. In most samples, specialized microbial communities were established, while only a few taxa were widespread among the different reactor systems. Metabolic reconstruction of the MAGs enabled the prediction of functional traits related to biomass degradation and methane production from waste biomass. An extensive evaluation of the replication index provided an estimation of the growth dynamics for microbes involved in different steps of the food chain.

Conclusions: The outcome of this study highlights a high flexibility of the biogas microbiome, allowing it to modify its composition and to adapt to the environmental conditions, including temperatures and a wide range of substrates. Our findings enhance our mechanistic understanding of the AD microbiome and substantially extend the existing repository of genomes. The established database represents a relevant resource for future studies related to this engineered ecosystem.

Keywords: Anaerobic digestion, Metagenome-assembled genomes, Biogas, Microbial community structure, Functional reconstruction

Background

Anaerobic environments are ubiquitous in the biosphere. Some examples are the digestive tract of animals, paddy fields, wetlands and aquatic sediments. These environments play crucial roles in the degradation of organic matter and in the global carbon cycle. The anaerobic digestion (AD) process has great societal importance since it reduces our dependence on fossil fuels via its ability to generate methane within engineered bioreactors [1]. For these reasons, the AD process has been widely established as an efficient metabolic route allowing the conversion of organic wastes, agricultural residues and renewable primary products into energy and

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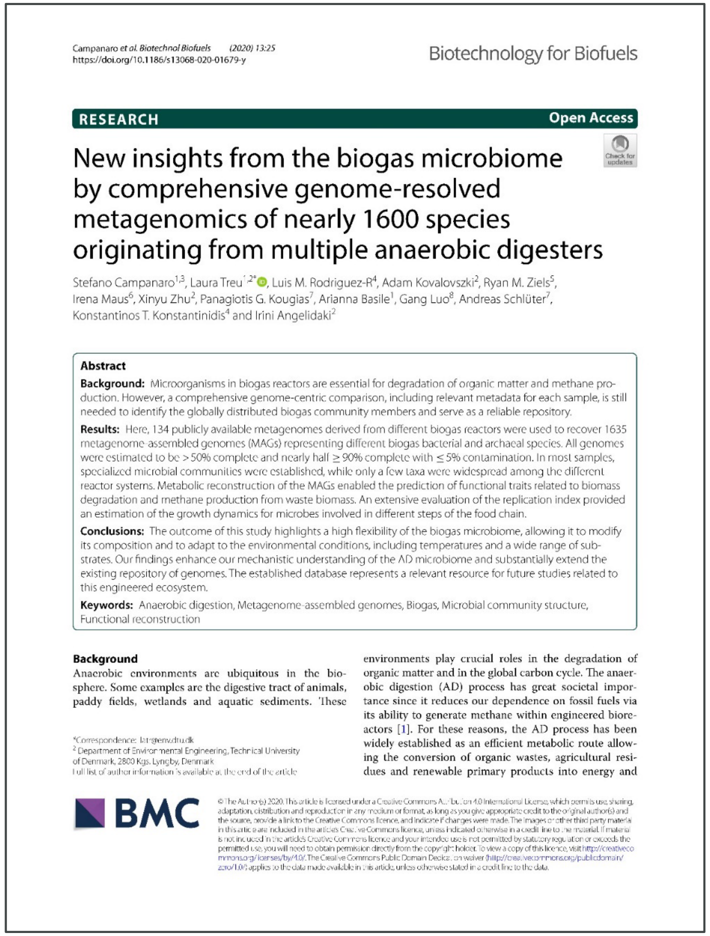
Full list of author information is available at the end of the article

Who’s who in AD

- 18 studies/sources



Campanaro *et al. Biotechnol Biofuels* (2020) 13:25
https://doi.org/10.1186/s13068-020-01679-y

Biotechnology for Biofuels

RESEARCH **Open Access**

New insights from the biogas microbiome by comprehensive genome-resolved metagenomics of nearly 1600 species originating from multiple anaerobic digesters

Stefano Campanaro[1,3], Laura Treu[1,2*], Luis M. Rodriguez-R[4], Adam Kovalovszki[2], Ryan M. Ziels[5], Irena Maus[6], Xinyu Zhu[2], Panagiotis G. Kougias[7], Arianna Basile[1], Gang Luo[8], Andreas Schlüter[7], Konstantinos T. Konstantinidis[4] and Irini Angelidaki[2]

Abstract

Background: Microorganisms in biogas reactors are essential for degradation of organic matter and methane production. However, a comprehensive genome-centric comparison, including relevant metadata for each sample, is still needed to identify the globally distributed biogas community members and serve as a reliable repository.

Results: Here, 134 publicly available metagenomes derived from different biogas reactors were used to recover 1635 metagenome-assembled genomes (MAGs) representing different biogas bacterial and archaeal species. All genomes were estimated to be > 50% complete and nearly half ≥ 90% complete with ≤ 5% contamination. In most samples, specialized microbial communities were established, while only a few taxa were widespread among the different reactor systems. Metabolic reconstruction of the MAGs enabled the prediction of functional traits related to biomass degradation and methane production from waste biomass. An extensive evaluation of the replication index provided an estimation of the growth dynamics for microbes involved in different steps of the food chain.

Conclusions: The outcome of this study highlights a high flexibility of the biogas microbiome, allowing it to modify its composition and to adapt to the environmental conditions, including temperatures and a wide range of substrates. Our findings enhance our mechanistic understanding of the AD microbiome and substantially extend the existing repository of genomes. The established database represents a relevant resource for future studies related to this engineered ecosystem.

Keywords: Anaerobic digestion, Metagenome-assembled genomes, Biogas, Microbial community structure, Functional reconstruction

Background

Anaerobic environments are ubiquitous in the biosphere. Some examples are the digestive tract of animals, paddy fields, wetlands and aquatic sediments. These environments play crucial roles in the degradation of organic matter and in the global carbon cycle. The anaerobic digestion (AD) process has great societal importance since it reduces our dependence on fossil fuels via its ability to generate methane within engineered bioreactors [1]. For these reasons, the AD process has been widely established as an efficient metabolic route allowing the conversion of organic wastes, agricultural residues and renewable primary products into energy and

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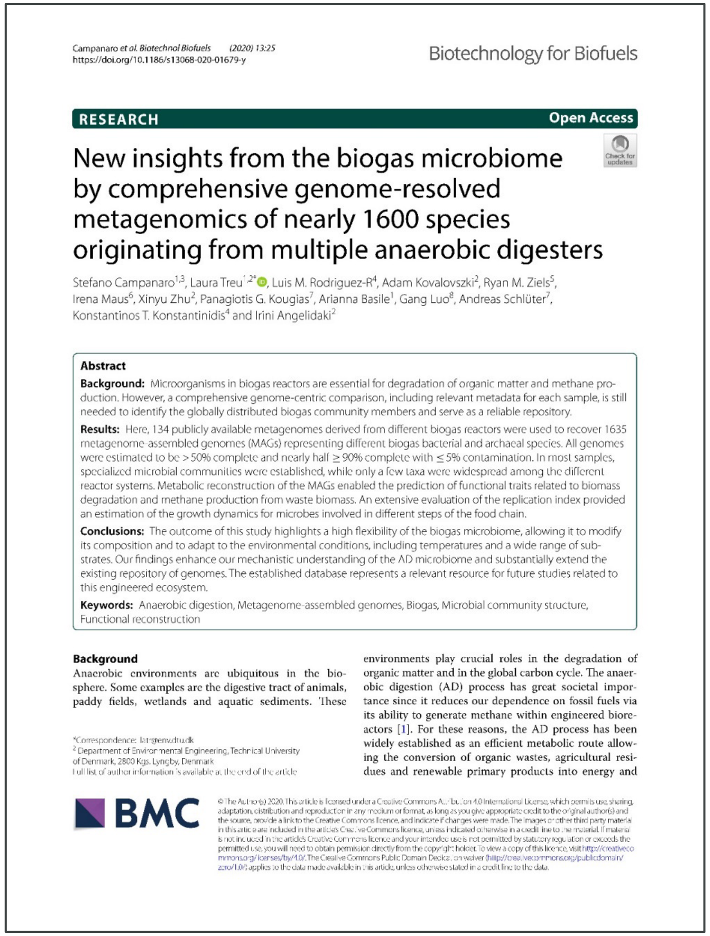

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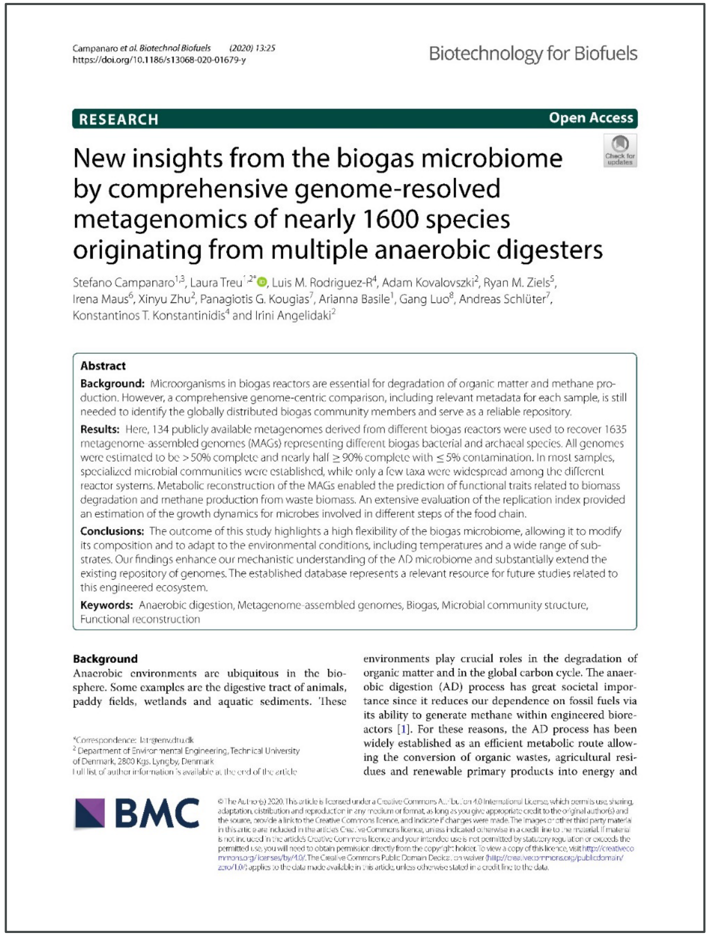

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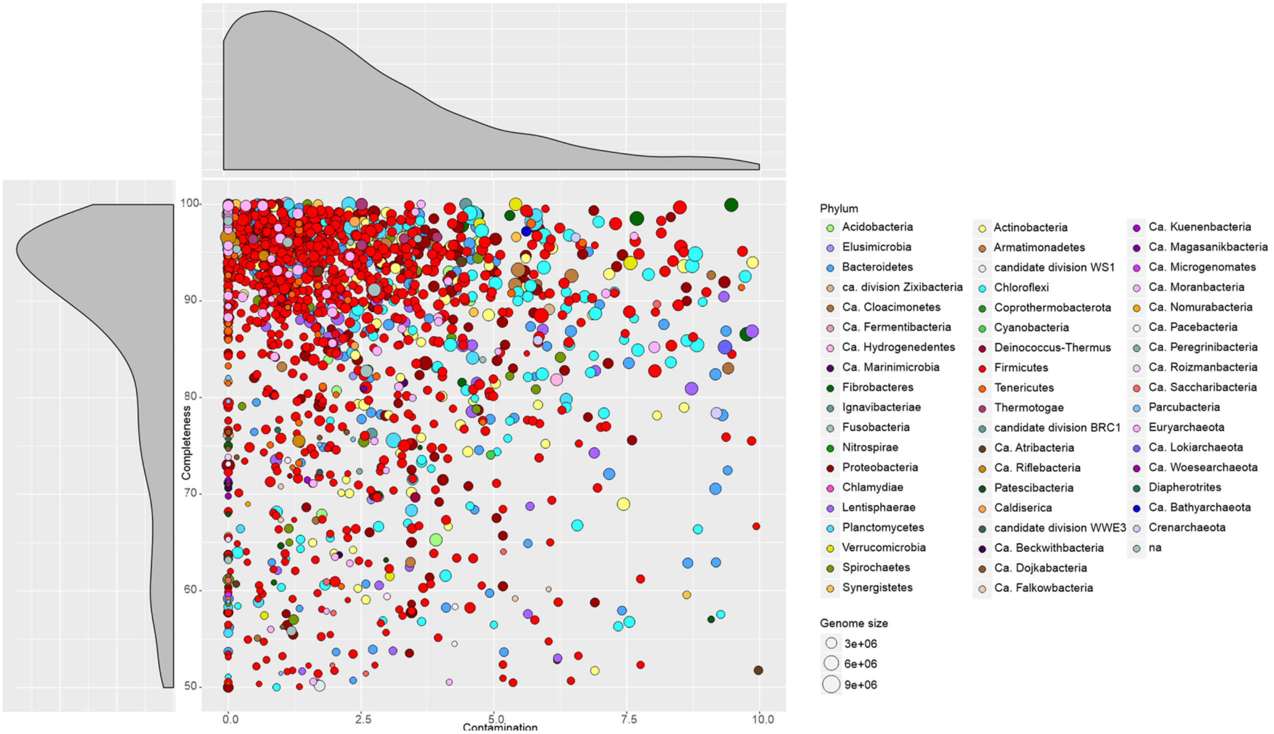

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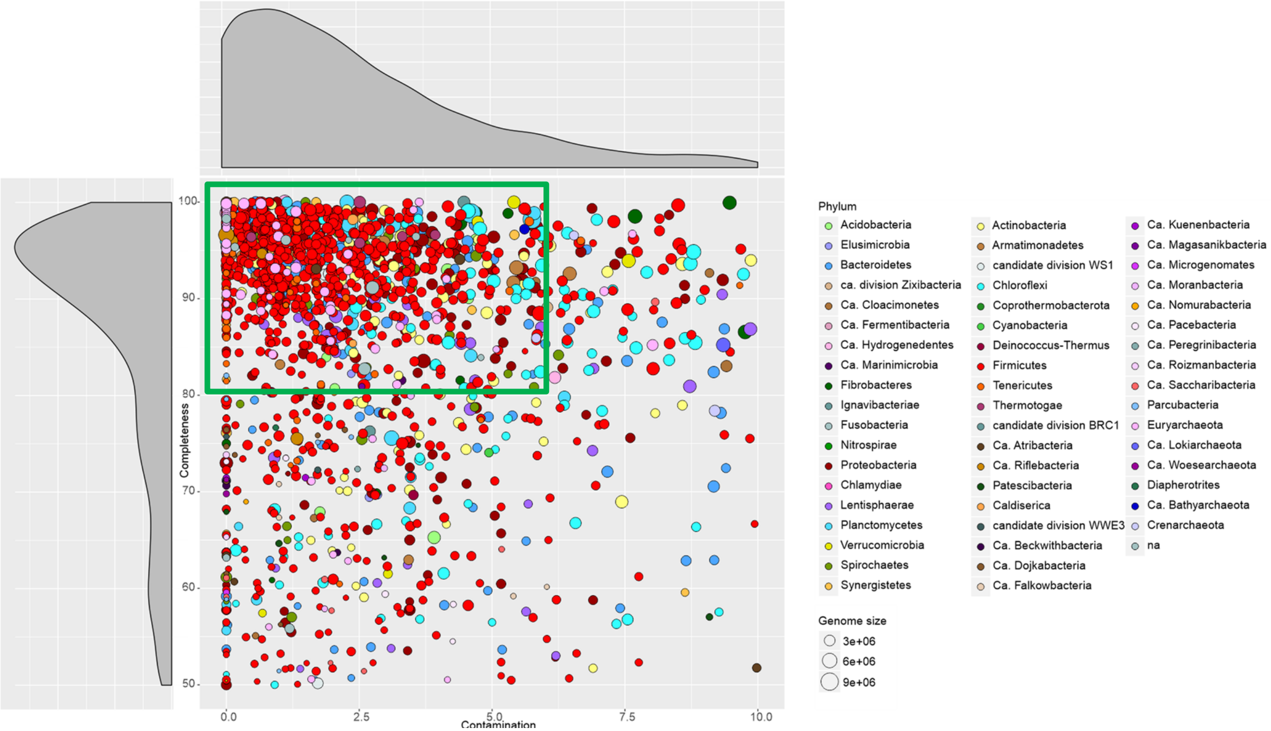

Majority of MAGs with

- High level of completeness
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Microbial interactions in AD

Metabolic Engineering 62 (2020) 138–149

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Original Research Article

Revealing metabolic mechanisms of interaction in the anaerobic digestion microbiome by flux balance analysis

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[d] Department of Computer Science and Information Systems, Teesside University, Middlesbrough, United Kingdom

ARTICLE INFO

Keywords:
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ABSTRACT

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1. Introduction

Microorganisms play an important role in all fields of biological relevance, ranging from human health (Clemente et al., 2012) to biotechnology (Lebuhn et al., 2015). In particular, diverse microbiomes may have various responsibilities, from causing diseases to influencing applied processes (e.g. biogas production) (Zhu et al., 2020) or the synthesis of polymeric substances (Chow et al., 2008). Microbial networks, however, are still poorly understood due to difficulties in isolating most of the microbial species and to the heterogeneous nature of their interactions (Muller et al., 2018). While the inspection of a core microbiome might reveal which species are the key players for a specific process (Faith, 2015), the role of rare members still remains to be clarified (Jousset et al., 2017). Microbial cooperation is extremely important for environmental niche colonization and completing complex activities (Stolyar et al., 2007), which single species could not perform independently (Hay et al., 2004). This is the case in anaerobic digestion (AD), which is a biotechnological process that produces a potent renewable energy carrier called biogas (Yentekakis and Goula, 2017). During biogas production, when acetoclastic methanogenic archaea are inhibited, a pivotal role is played by hydrogenotrophic methanogenic archaea and syntrophic acetate oxidizing bacteria (Mosbæk et al., 2016) (SAOB). An example is the mutualism between the hydrogen-utilizing methanogen *Methanoculleus bourgensis* and the SAOB *Syntrophaceticus schinkii*, *[Clostridium] ultunense*, and *Tepidanaerobacter acetatoxydans* (Westerholm et al., 2019). SAOB oxidise acetate to formate or to H_2 and carbon dioxide (CO_2). The bacteria rely on archaeal activity, because acetate oxidation rapidly becomes endergonic when H_2 accumulates (Stams and Plugge, 2009). Indeed, subsequently H_2-utilizing methanogens convert these substrates to methane (CH_4) (Treu et al., 2018).

Although direct microbial cultivation and phenotyping experiments

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Microbial interactions in AD

- 836 GSMMs

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Microbial interactions in AD

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- Dominant species
    - Mutualistic (+/+)
    - Parasitic (+/-)
    - Commensalistic (+/0)

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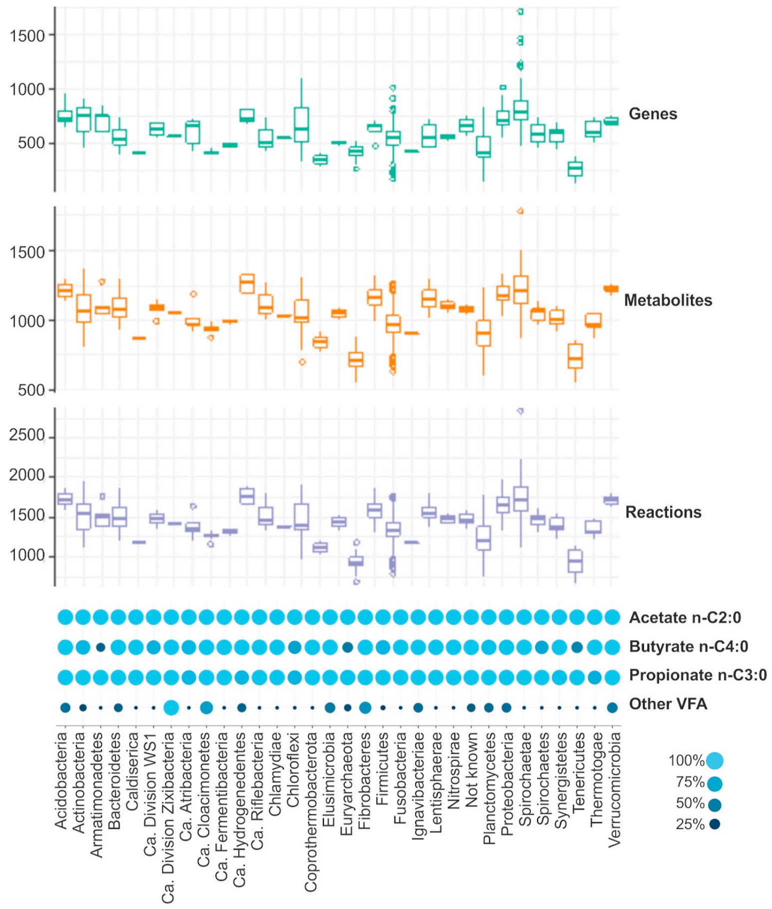

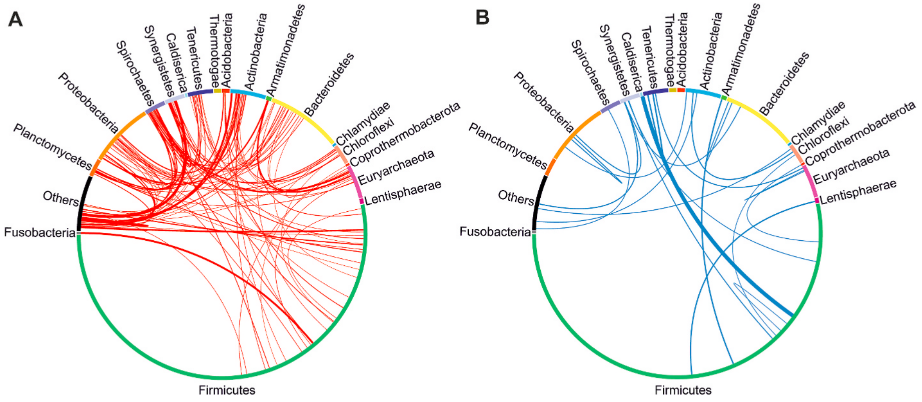

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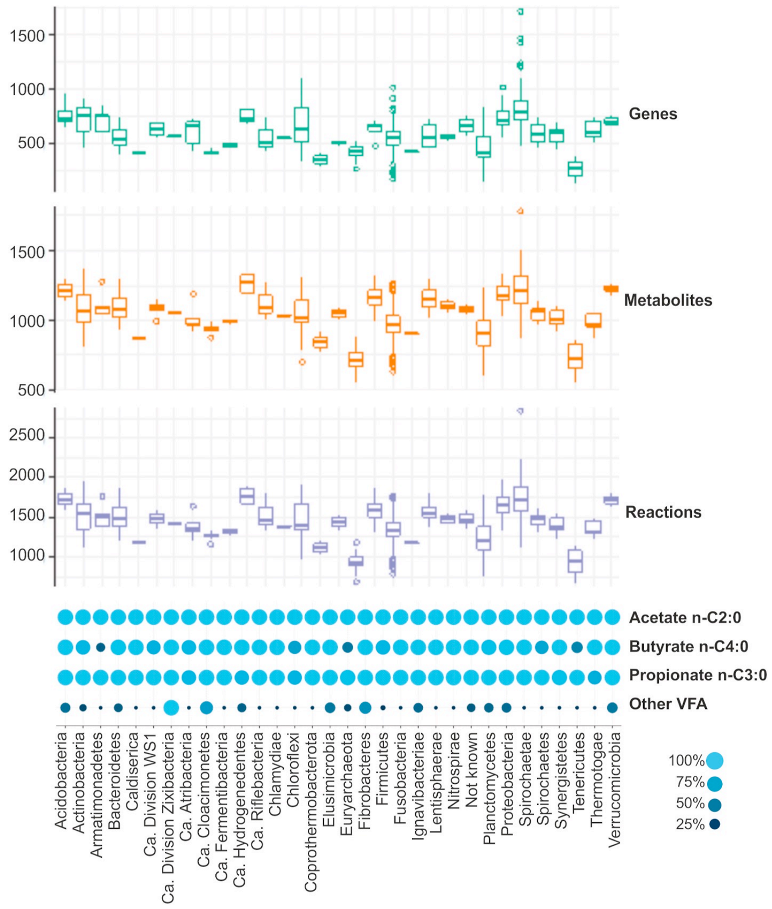

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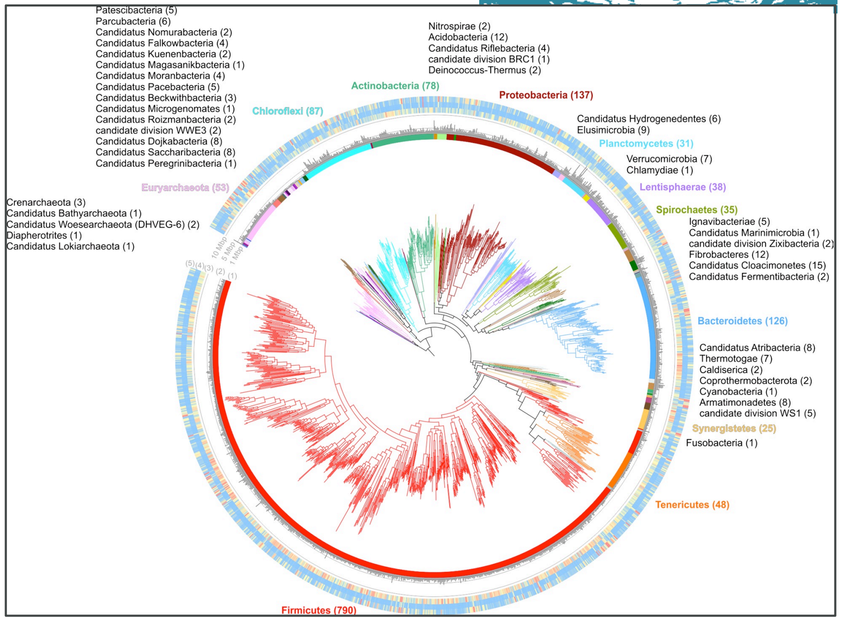

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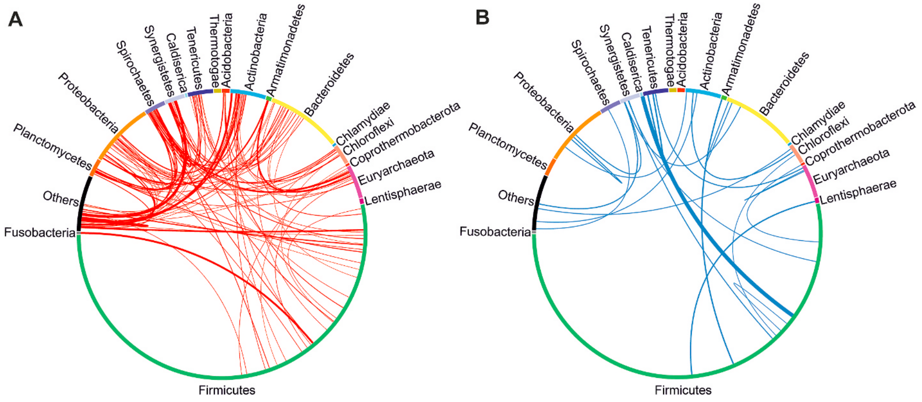

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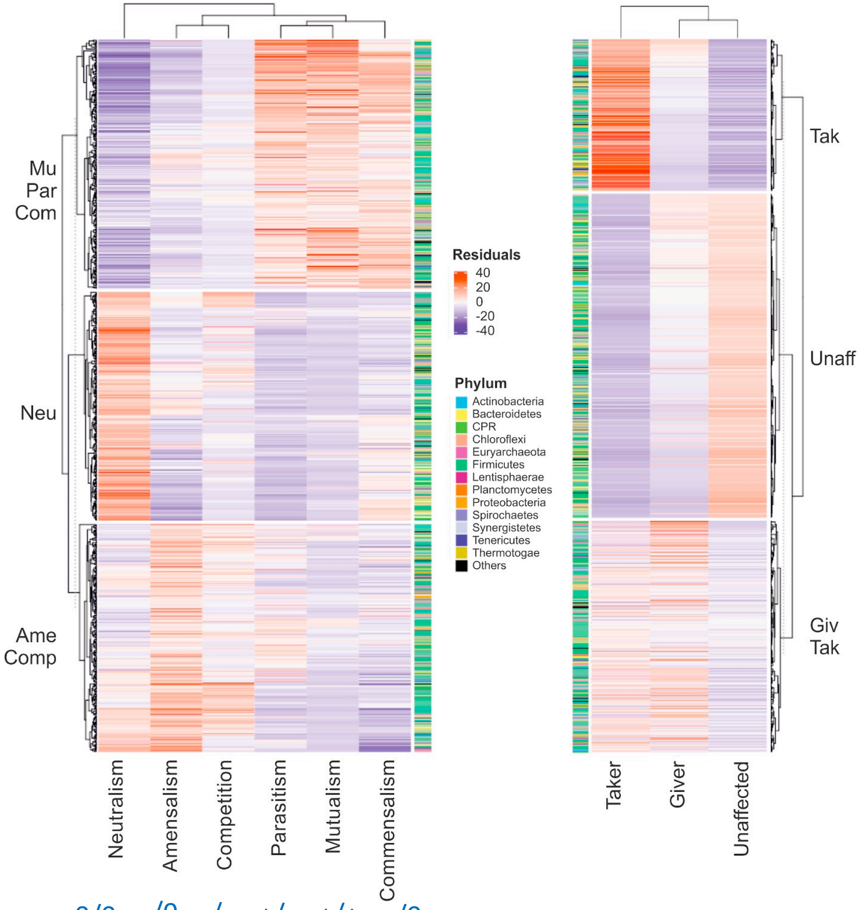

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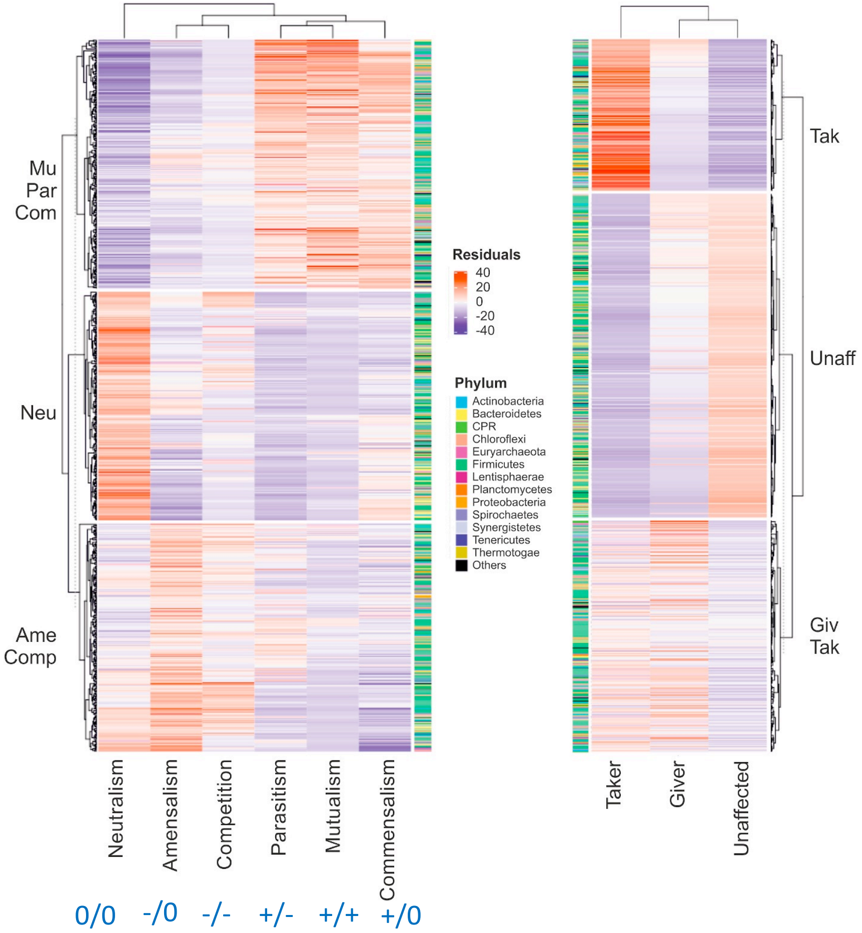

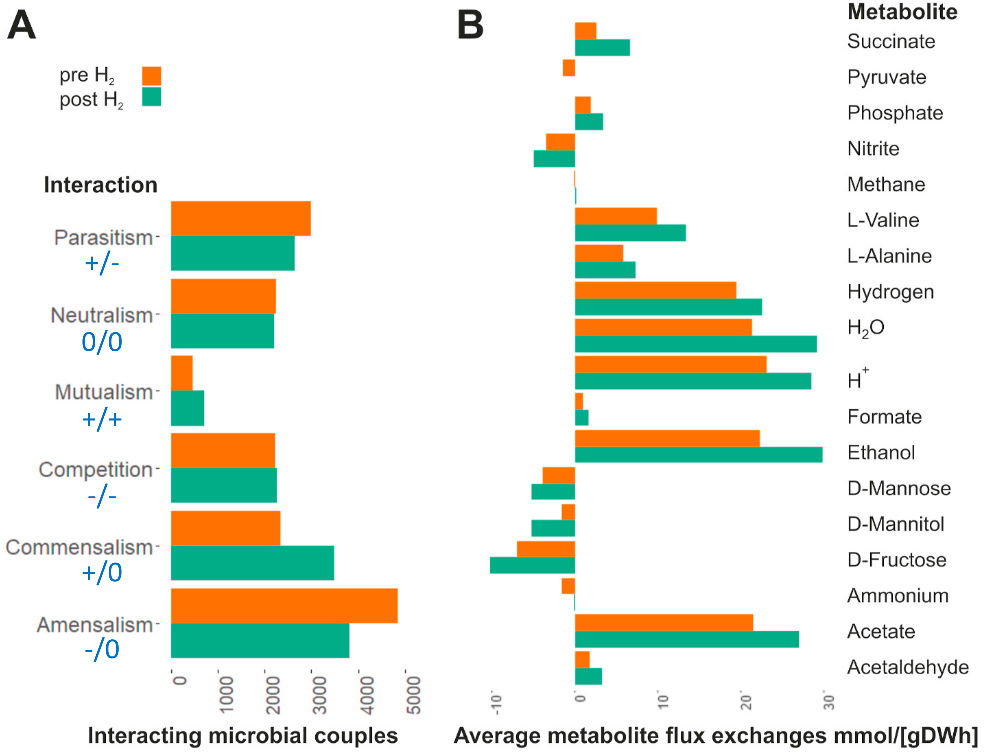

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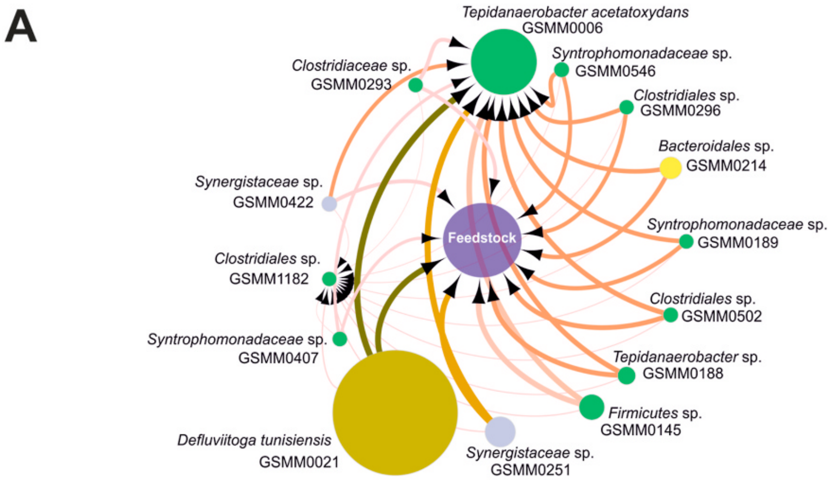
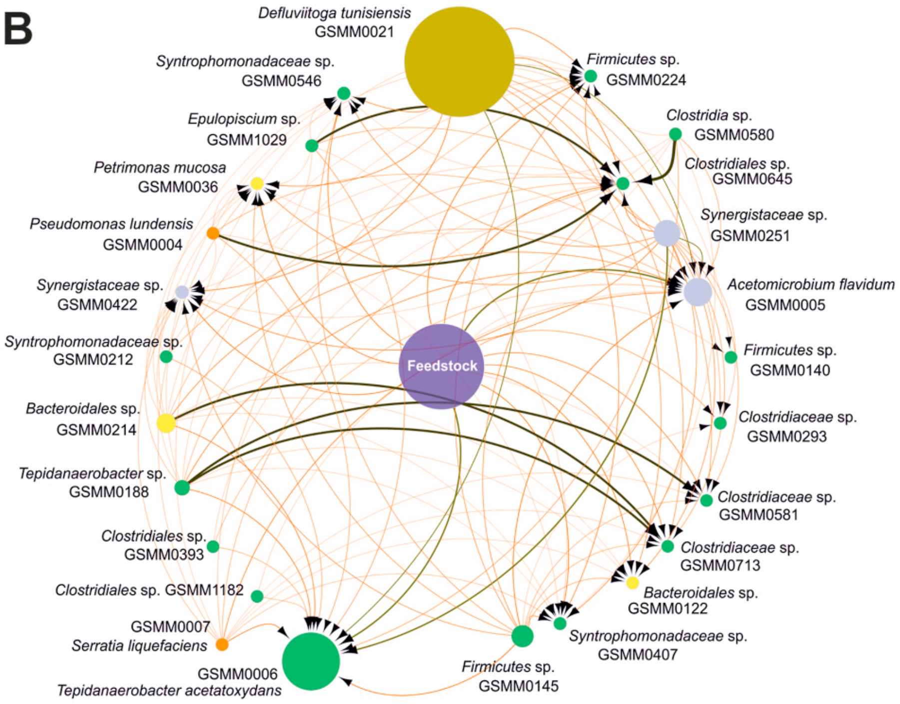
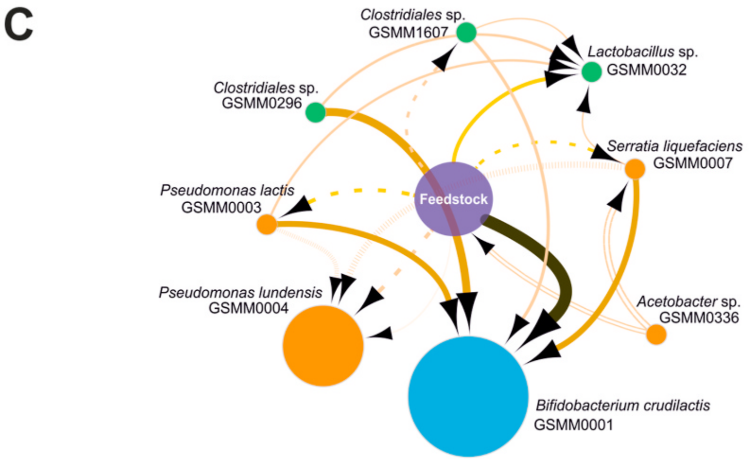
Microbial interactions in AD







Basile *et al.*, 2020, Revealing metabolic …

Final remarks

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GSMM a holy grail?

Final remarks

GSMM a holy grail? → not quite

Final remarks

- Vast search space (*genes missing, or with unknown functions*)

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Final remarks

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- Experimenting is computationally and time-wise intensive (*constraints, futile cycles, sinks*)
- GSMM: what could be and NOT what is → other omics to corroborate
- Productivity vs survival → finding the balance
- (Currently) unpredictable synergistic effects

Thank you for your attention!

References

Campanaro, S., Treu, L., Rodriguez-R, L. M., Kovalovszki, A., Ziels, R. M., Maus, I., Zhu, X., Kougias, P. G., Basile, A., Luo, G., Schlüter, A., Konstantinidis, K. T., Angelidaki, I. (2020). New insights from the biogas microbiome by comprehensive genome-resolved metagenomics of nearly 1600 species originating from multiple anaerobic digesters. *Biotechnology for biofuels*, 13(1), 1-18.

Basile, A., Campanaro, S., Kovalovszki, A., Zampieri, G., Rossi, A., Angelidaki, I., Valle, G., Treu, L. (2020). Revealing metabolic mechanisms of interaction in the anaerobic digestion microbiome by flux balance analysis. *Metabolic Engineering*, 62, 138-149.

GUILD-BASED METABOLIC MODELING FOR DECIPHERING ANAEROBIC BIOPROCESSES

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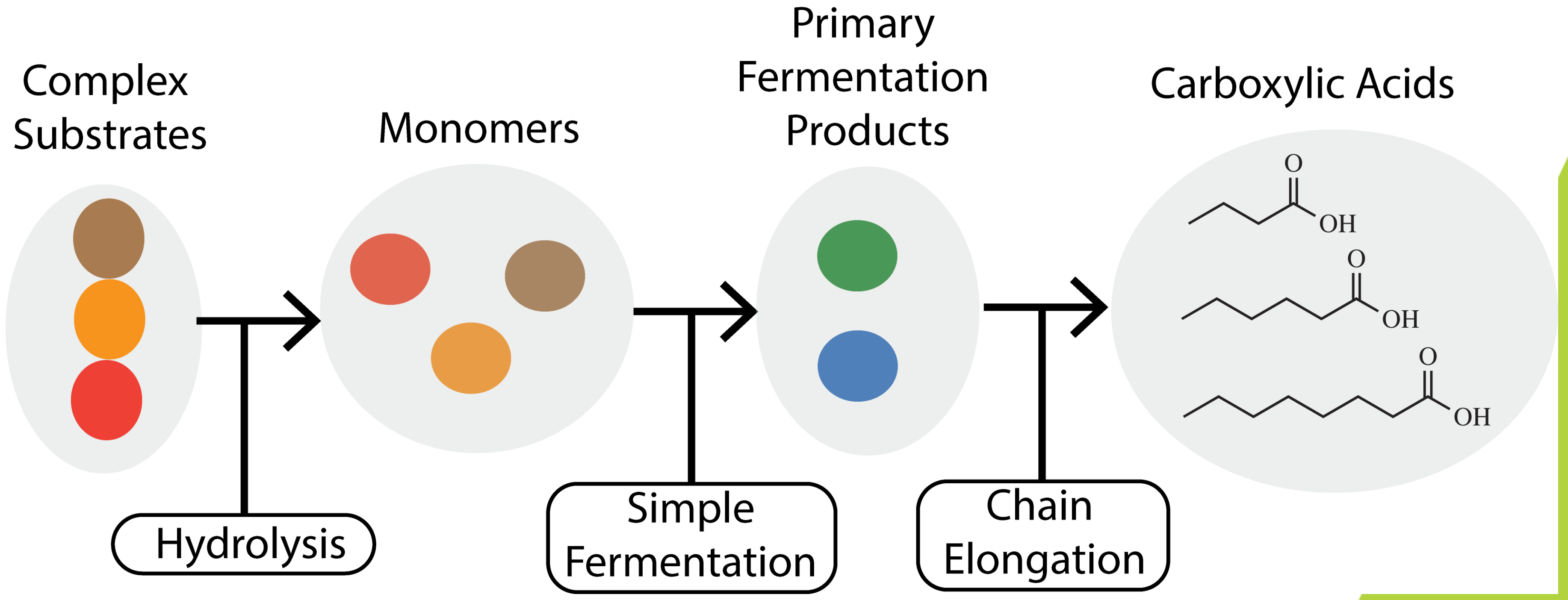
(Matthew.Scarborough@uvm.edu)
University of Vermont

Guild-based metabolic modeling for deciphering anaerobic bioprocesses

Matthew Scarborough, P.E., Ph.D.
Department of Civil and Environmental Engineering
College of Engineering and Mathematical Sciences
The University of Vermont

Chain elongation from complex organic wastes



Complex Substrates

Monomers

Primary Fermentation Products

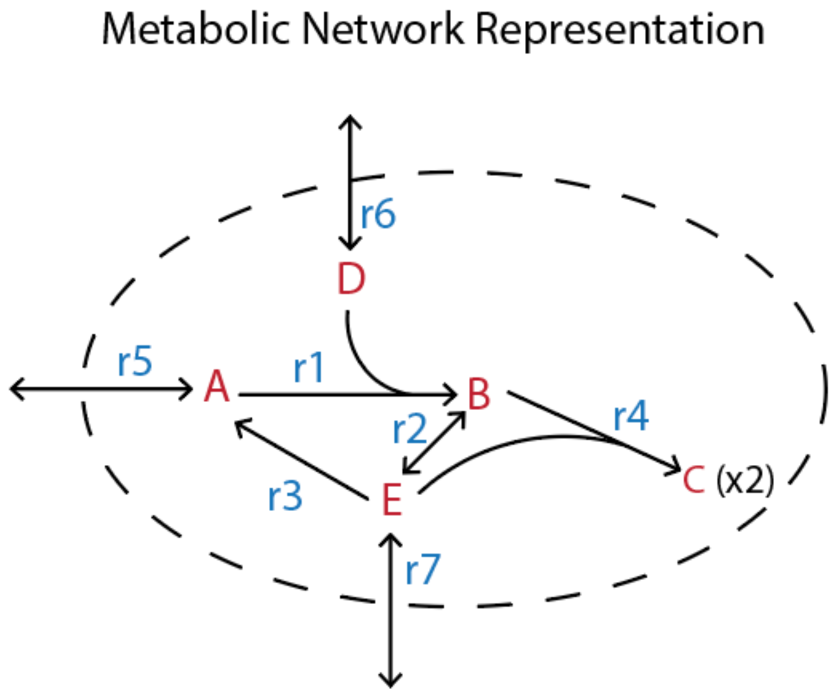
Carboxylic Acids

Hydrolysis

Simple Fermentation

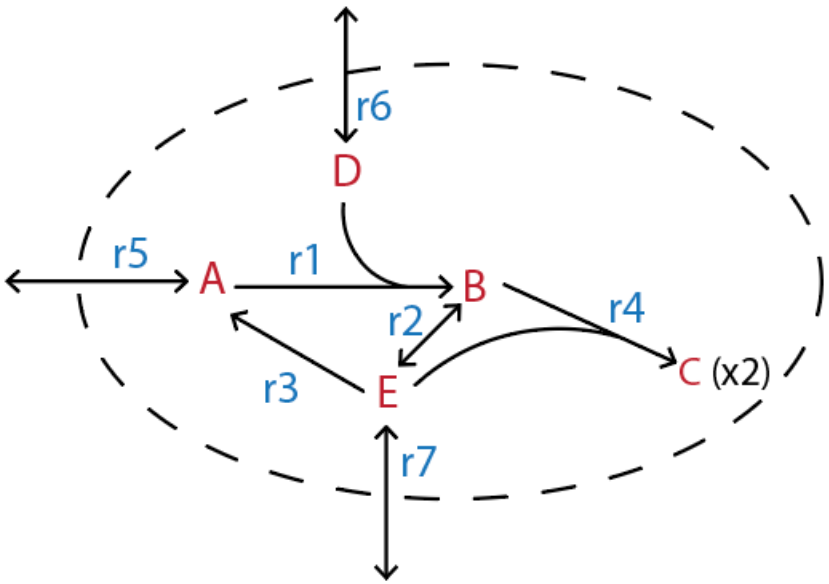
Chain Elongation

A metabolic modeling primer



A metabolic modeling primer

Metabolic Network Representation



	r1	r2	r3	r4	r5	r6	r7
A	-1	0	1	0	1	0	0
B	1	-2	0	-1	0	0	0
C	0	0	0	2	0	0	0
D	-1	0	0	0	0	1	0
E	0	2	-1	1	0	0	1

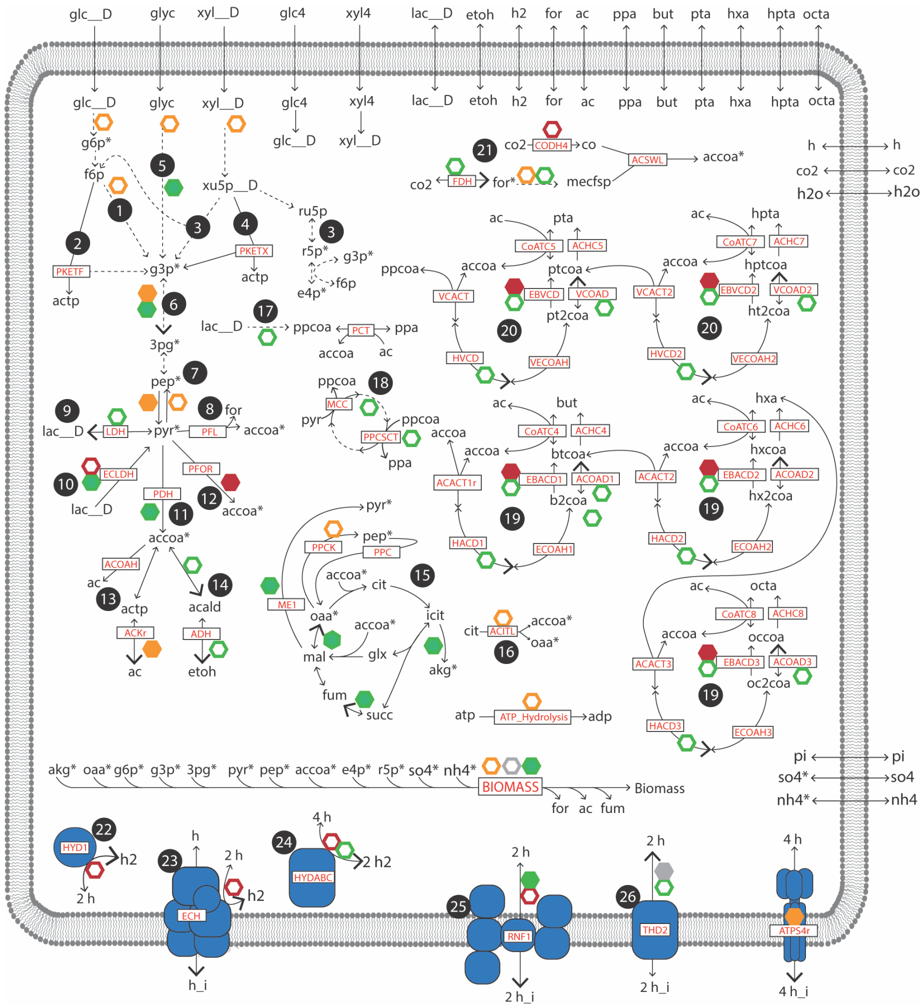
S

v1
v2
v3
v4
v5
v6
v7

v

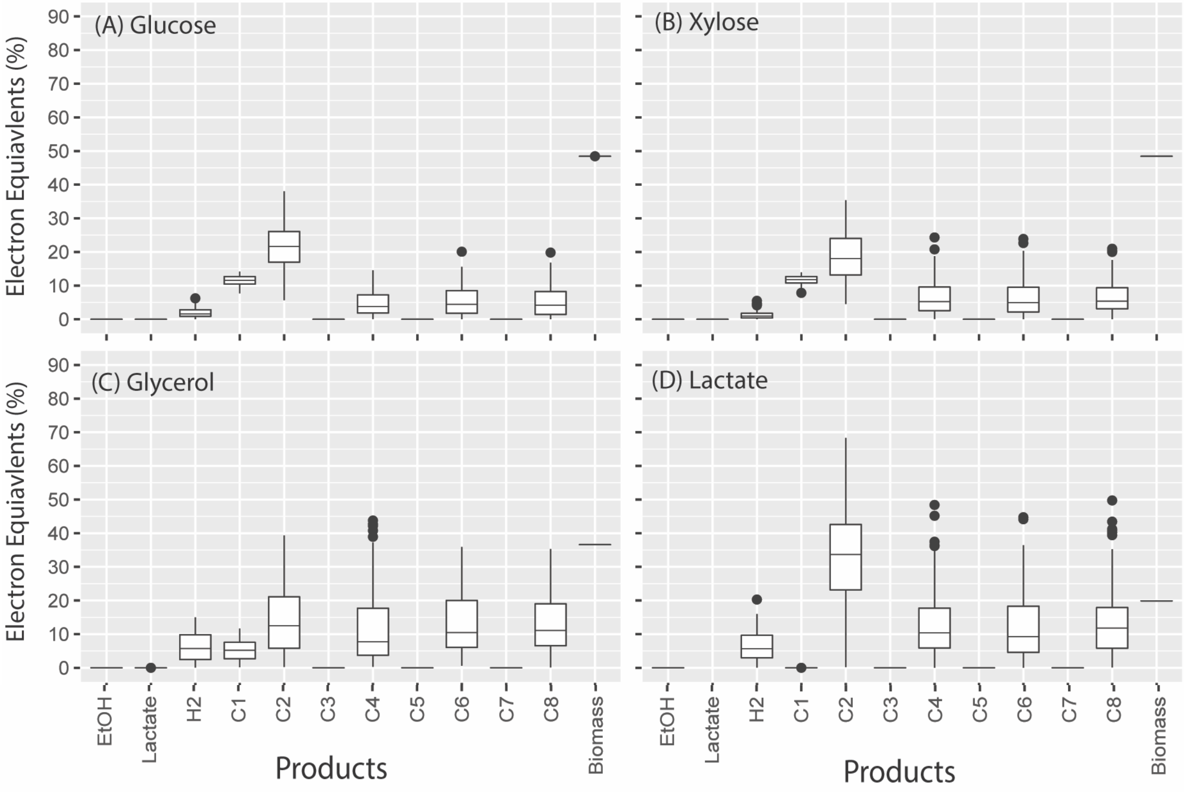
$$S \bullet v = 0$$

iFermCell215



Adapted from Scarborough, M, et. al. 2020. mSystems.

Simulating consumption of different substrates



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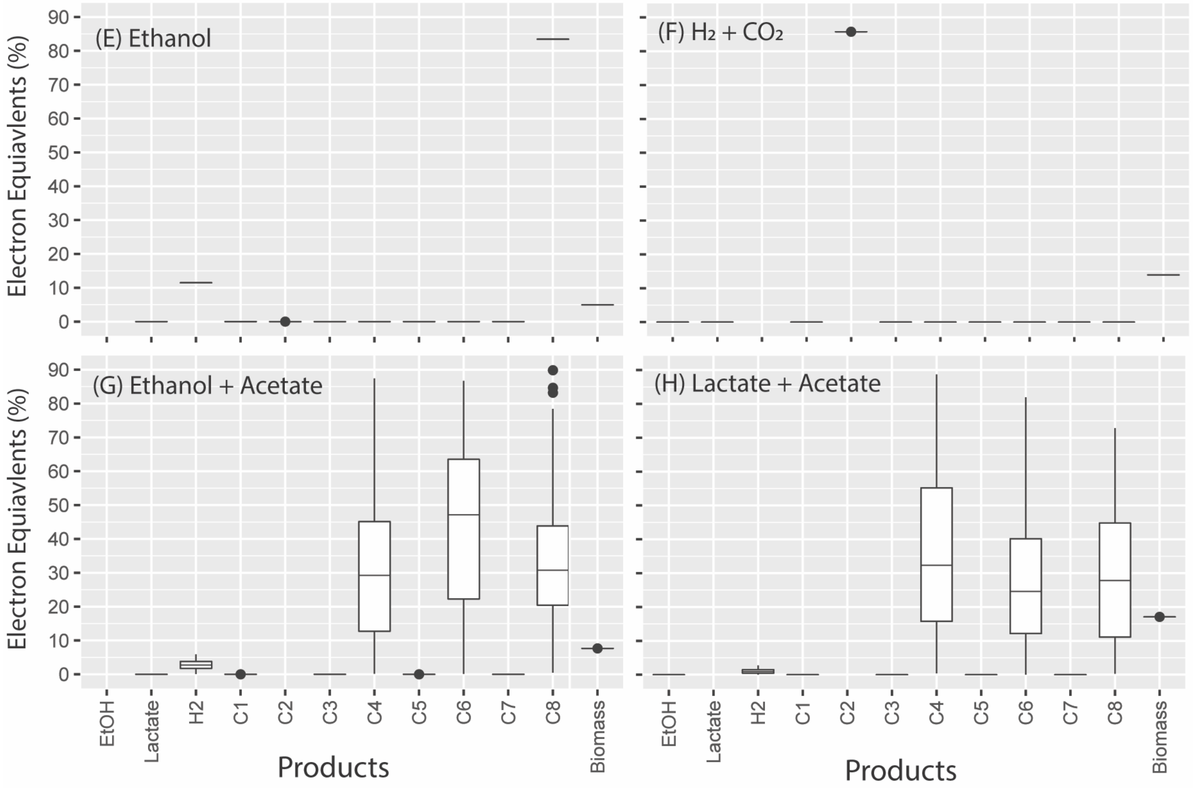

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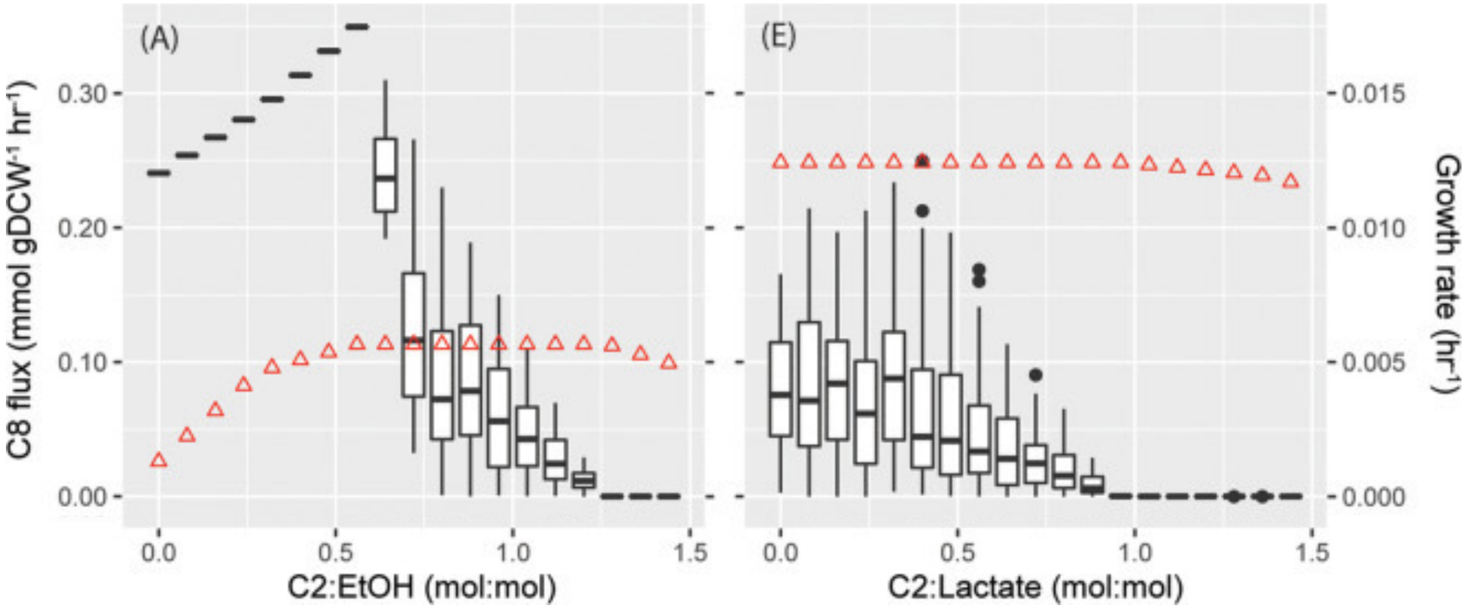
Simulating consumption of co-substrates



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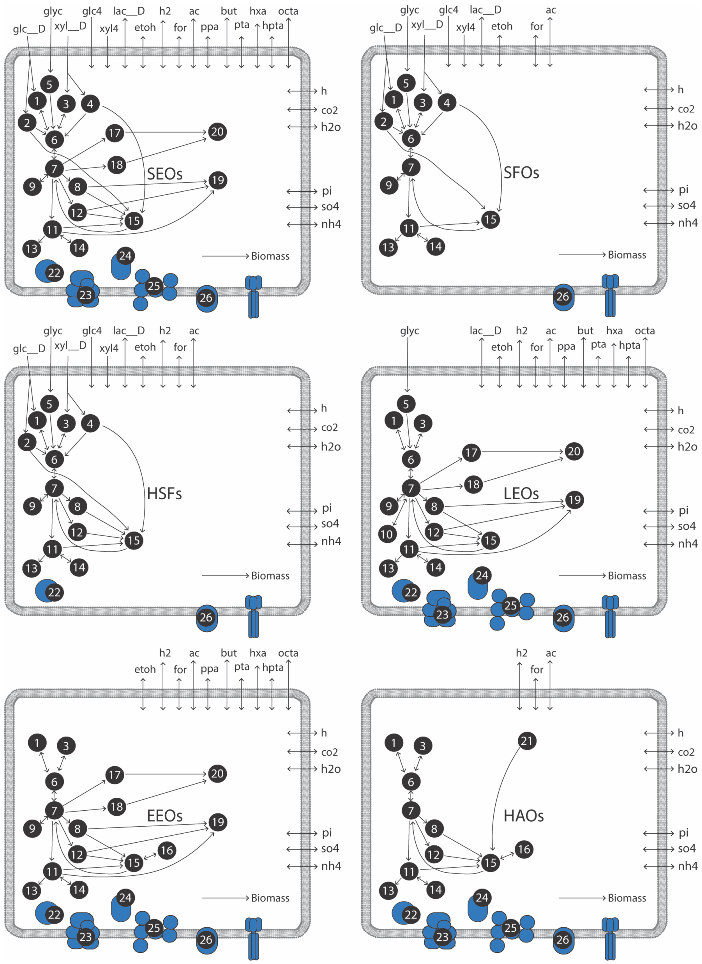
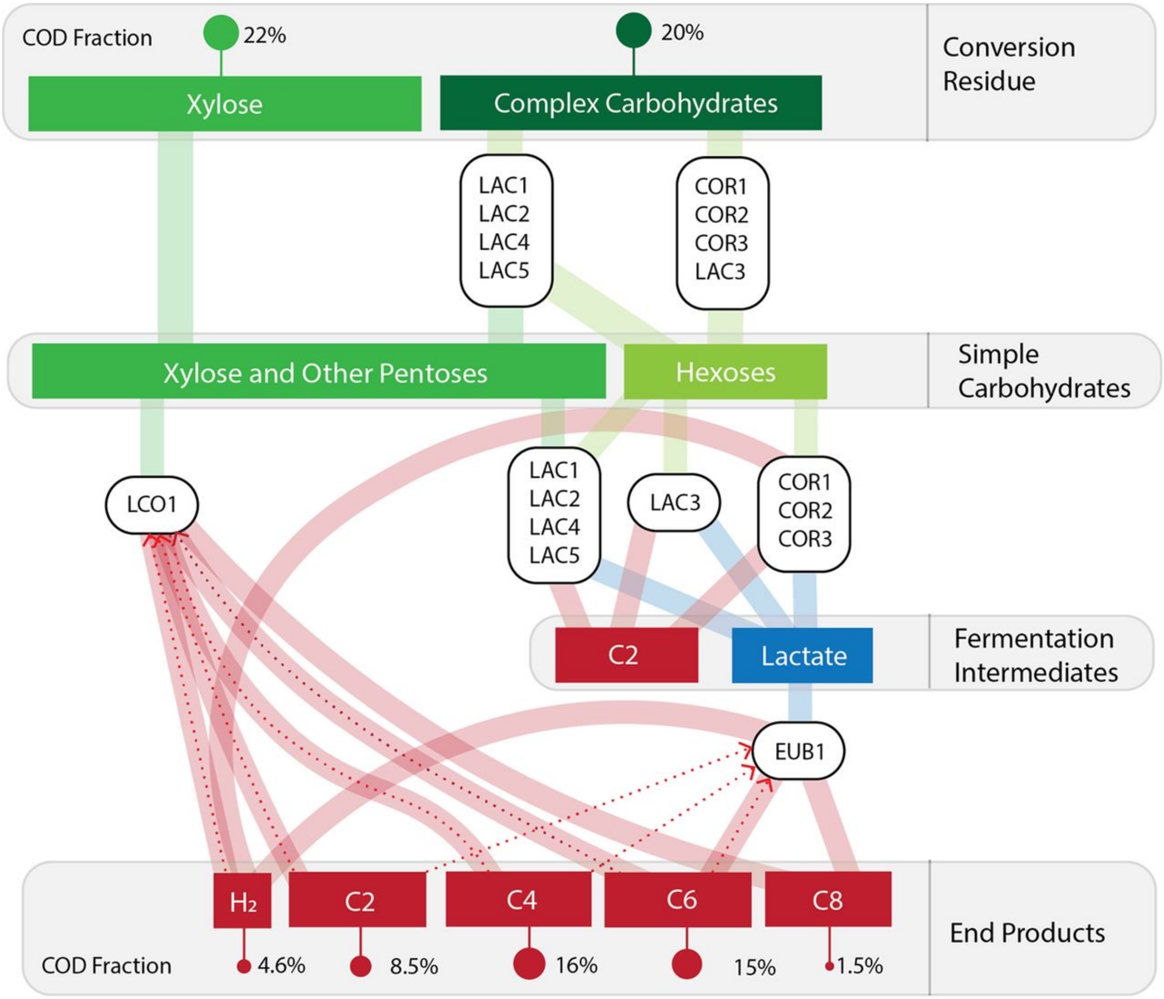


iFermGuilds789

Adapted from Scarborough, M, et. al. 2020. mSystems.



COD Fraction 22% 20%

Xylose

Complex Carbohydrates

Conversion Residue

LAC1
LAC2
LAC4
LAC5

COR1
COR2
COR3
LAC3

Xylose and Other Pentoses

Hexoses

Simple Carbohydrates

LCO1

LAC1
LAC2
LAC4
LAC5

LAC3

COR1
COR2
COR3

C2

Lactate

Fermentation Intermediates

EUB1

H_2

C2

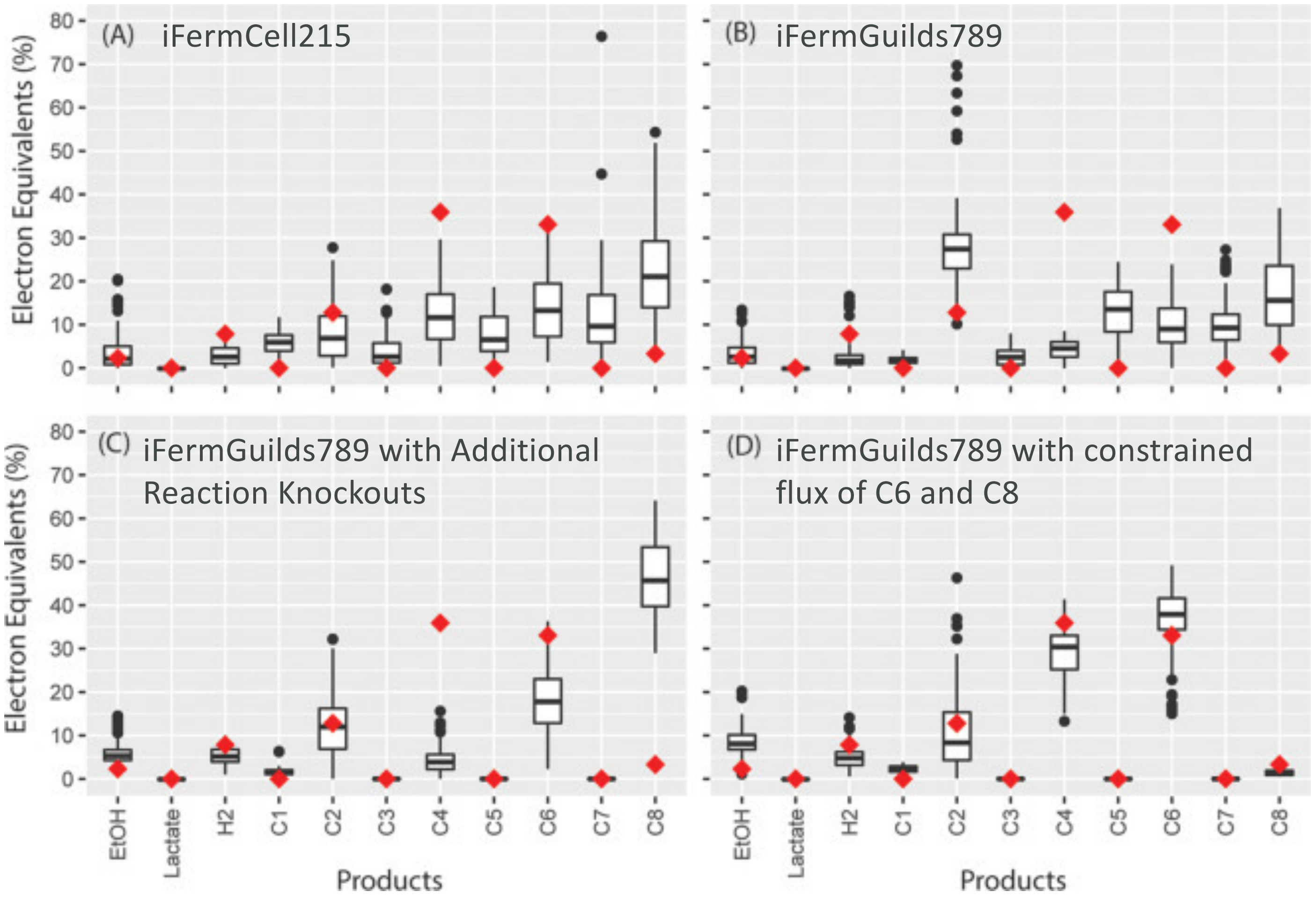
C4

C6

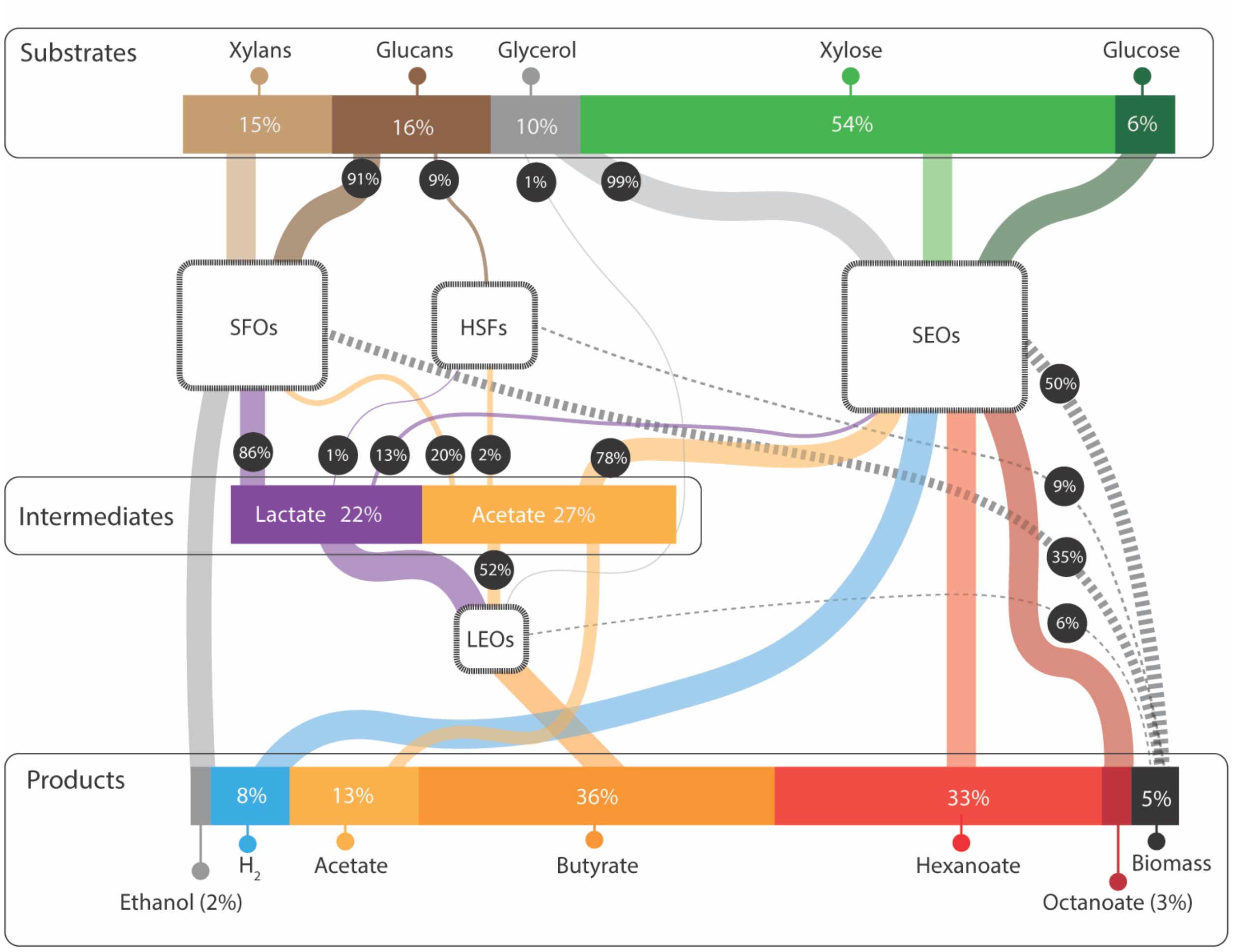
C8

End Products

COD Fraction 4.6% 8.5% 16% 15% 1.5%


(A) iFermCell215
(B) iFermGuilds789
(C) iFermGuilds789 with Additional Reaction Knockouts
(D) iFermGuilds789 with constrained flux of C6 and C8
Electron Equivalents (%)
0
10
20
30
40
50
60
70
80
EtOH
Lactate
H2
C1
C2
C3
C4
C5
C6
C7
C8
Products

Predicting the flow of carbon and electrons



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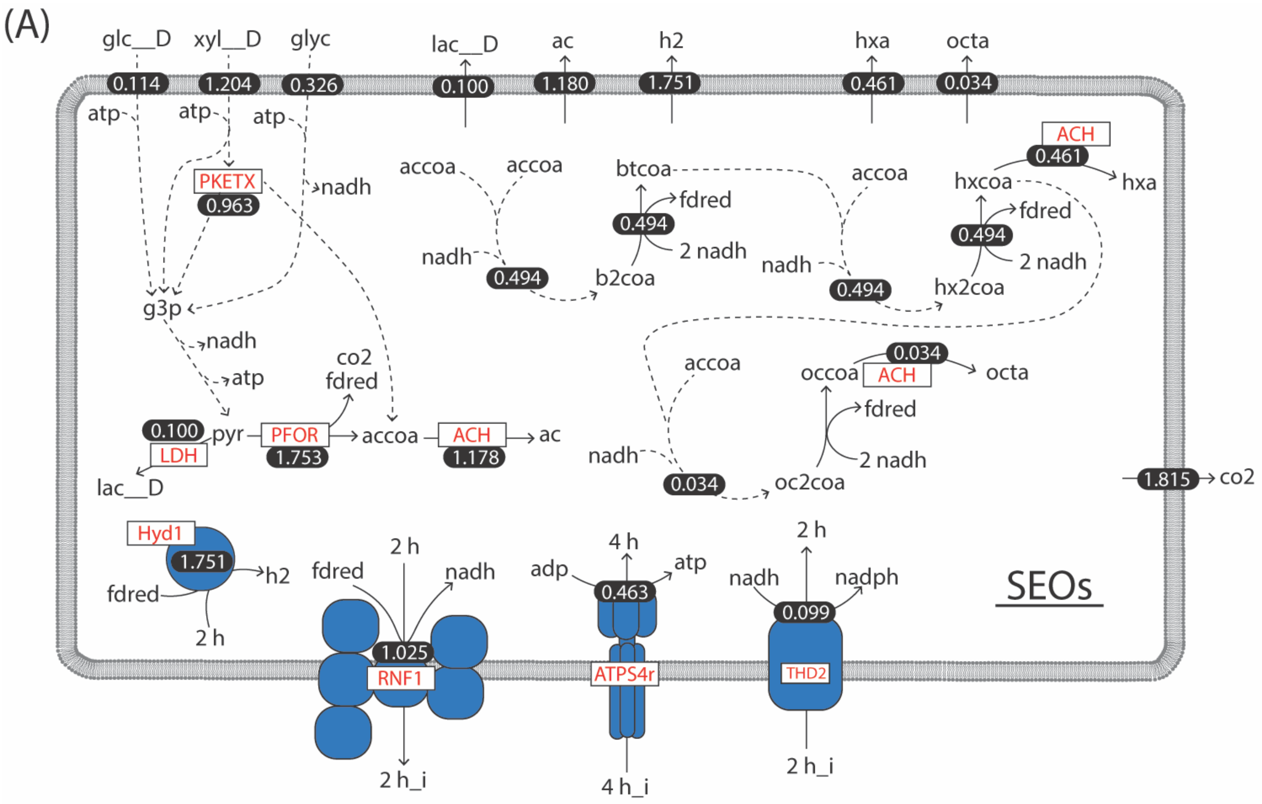
Predicted differences in sugar and lactate conversion



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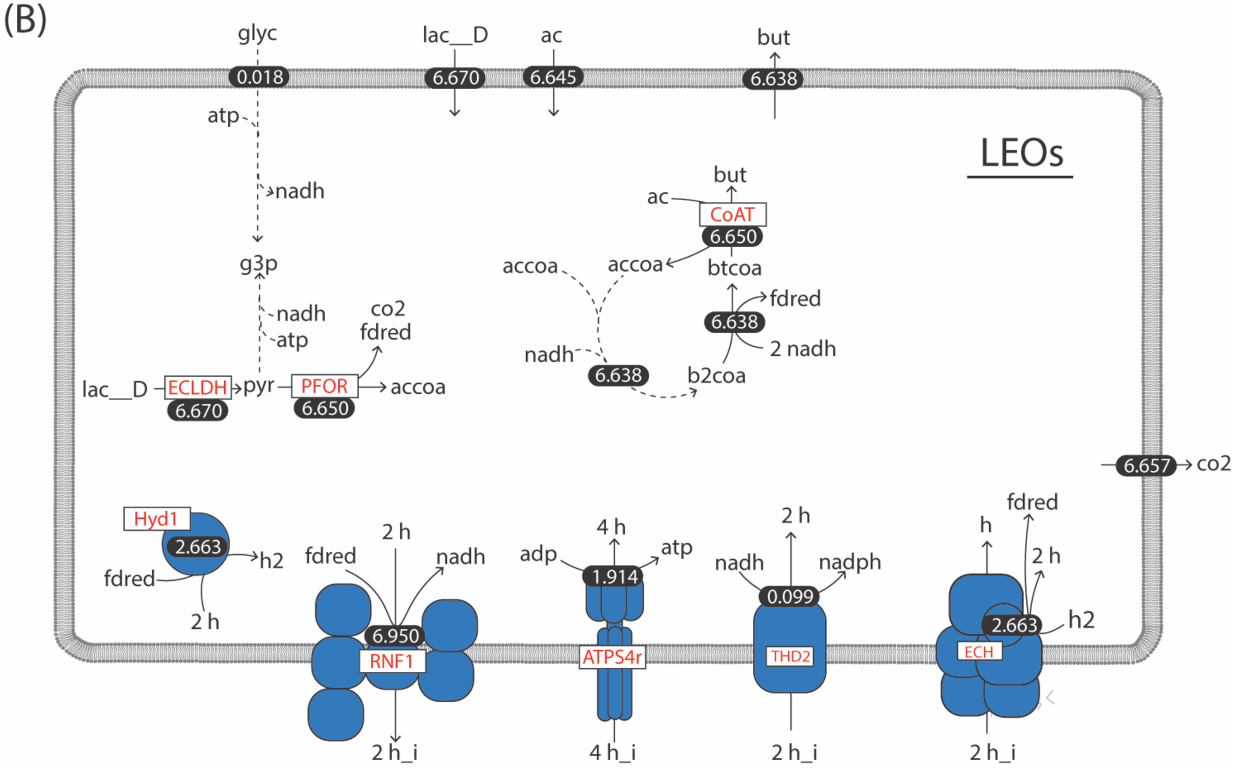

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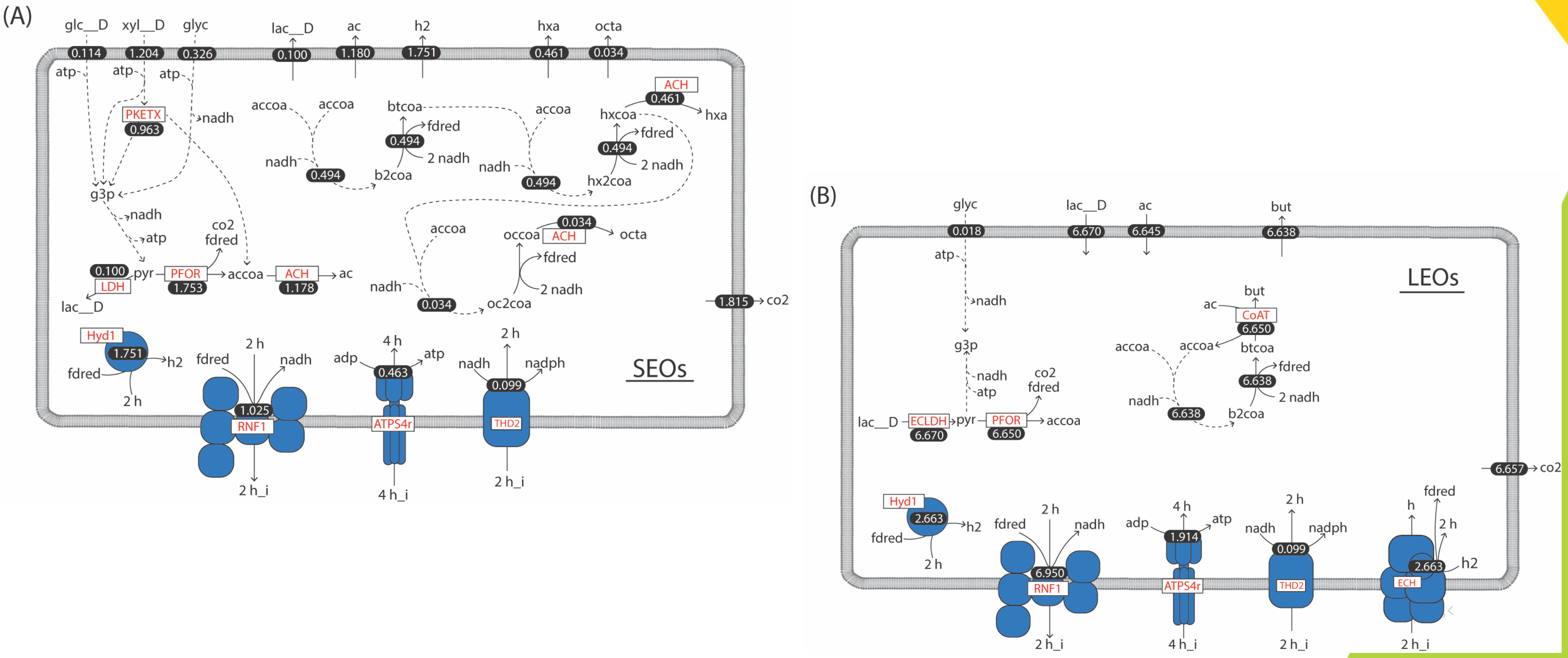

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Hypotheses from modeling

- MCFAs are produced from sugars but not lactate
- Lactate and acetate are used to produce butyrate
- Lactate consumption occurs via an electron-confurcating lactate dehydrogenase
- SEOs and LEOs vary in their routes of hydrogen production
- SEOs and LEOs vary in terminal enzymes for chain elongation

Conclusions

- Metabolic models are…
    - A useful tool to augment multi-omic techniques
    - Diagnostic tools to refine hypotheses
    - A great approach for thinking about chain elongation
- Predictive models will likely require…
    - Further understanding of chain elongator metabolism
    - Quantification of toxic effects of end-products
    - Enzyme-level kinetic analyses of different product lengths

Thanks!

mscarbor@uvm.edu

github.com/mscarbor/Mixed-Culture-Fermentation-Models

AGENDA AND HOUSEKEEPING

Speaker 1
Alberte Regueira (Universidade de Santiago de Compostela, Spain)

Speaker 2
Adam Kovalovszki (Technical University of Denmark)

Speaker 3
Matthew Scarborough (University of Vermont, USA)

Q&A Session Moderator: *Robbert Kleerebezem (Delft University of Technology, The Netherlands)*

- This session is being recorded;
- Microphones and cameras have been disabled due to the large number of attendees;
- The normal chat function is disabled;
- Please put any questions and comments you may have in the Q&A (icon to the low right in Zoom) and we will do our best to answer them during the session (in writing or orally).

CLOSING REMARKS

Great thanks to all presenters for a wonderful show!

Look out for MIA’s NEXT webinar in May 2022:

“Topic to be decided”

If you have ideas for your own future webinar then contact MIA MC and we will help you make it happen!

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