



# 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



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

# 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



Website: http://iwa-mia.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

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STR	STR	STR	STR
(Sept. 2012)	(Sept. 2014)	(2022)	(2022)

# **MIA SG: UPCOMING CONFERENCES**



## 8<sup>th</sup> Water Resource Recovery Modelling seminar (WRRmod2022+)

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

### 11<sup>th</sup> 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)

9<sup>th</sup> Water Resource Recovery Modelling seminar (WRRmod2024), Stowe, Vermont, USA





# FIND MIA SG ON SOCIAL MEDIA



Follow the Modelling and Integrated Assessment Specialist Group on:



https://iwa-connect.org/group/modellingand-integrated-assessment-mia/timeline



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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**







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





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Matthew Scarborough University of Vermont

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# Modeling Mixed Culture Fermentations

A short introduction

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



# Modeling *Wild* Fermentations

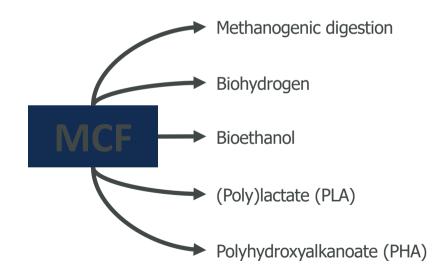
A short introduction

# WHY MIXED CULTURE FERMENTATION (MCF)?



#### Produce building blocks for other processes

**Substrate:** Carbohydrates, Glycerol... **Products:** Acetate, Propionate, Butyrate, Ethanol, Lactate, Hydrogen...



# 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?



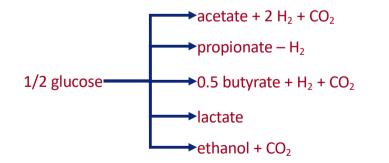
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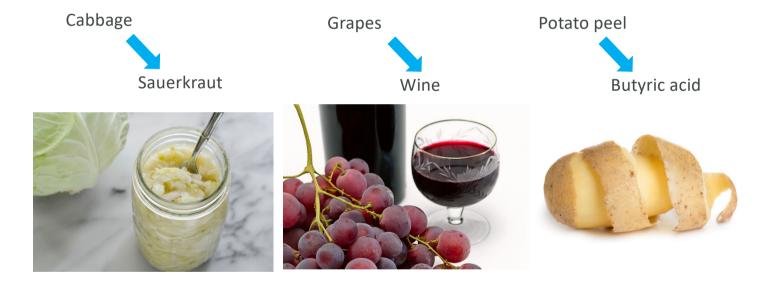
 $NH_4^{+1} + 2 \cdot O_2 \rightarrow NO_3^{-1} + 2 \cdot H^{+1} + H_2O$ 

But what determines the end-product of carbohydrate fermentations?









# **HISTORY OF MODELLING MCF**



Wat.Sci.Tech. Vol.15, Copenhagen, pp.209-232.0273-1223/83 \$0.00 + .50Printed in Great Britain.Copyright © 1983 IAWPRC/Pergamon Press Ltd.

#### 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**



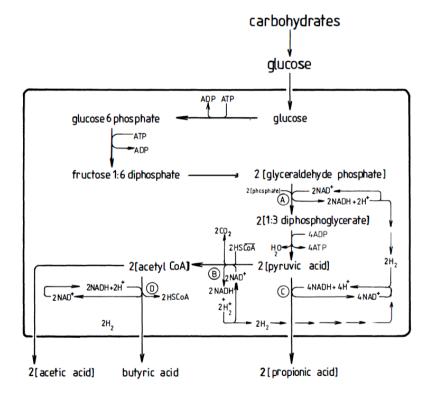


Fig. 2. Metabolic pathways inside acid-forming bacteria

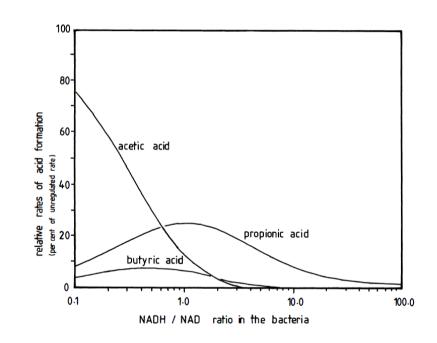


Fig. 3. Relative rates of acid-formation at different NAD<sup>+</sup>/NADH ratios as predicted by these rate equations

# **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**

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#### **Q&A Session Moderator:** Robbert

Kleerebezem (Delft University of Technology, The Netherlands)

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# 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

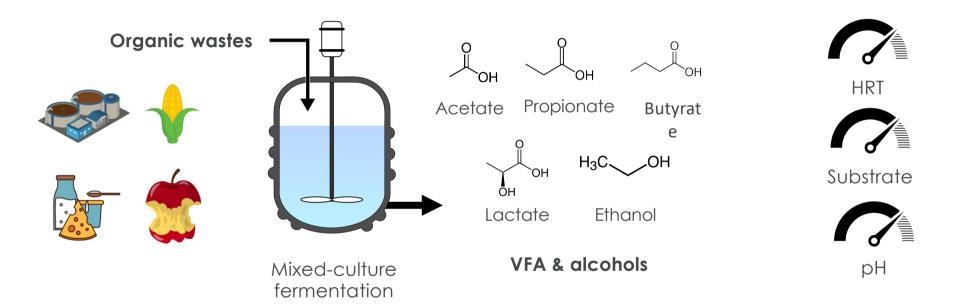
Alberte Regueira López







# **Anaerobic fermentation for VFA production**

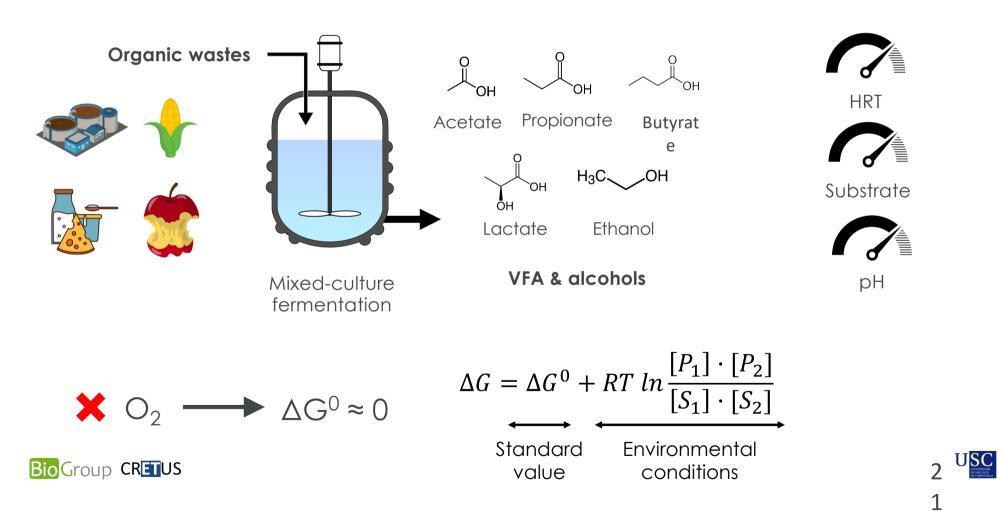








# **Anaerobic fermentation for VFA production**



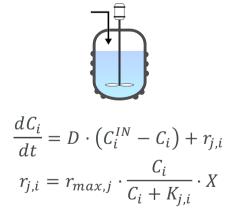


USC

# What are bioenergetic models?

### Kinetic unstructured models

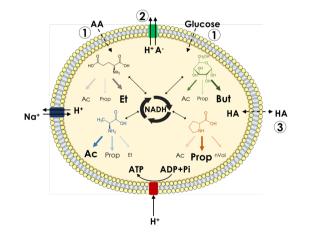
- ✓ Biomass is a black box
- ✓ Solve the macroscopic mass balances
- ✓ Variable selectivity is not addressed





## **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

<u>Reality</u>: **Multiple** species performing different or similar metabolic functions



<u>Model</u>: **One** virtual species is able of performing **all** the metabolic functions of the community

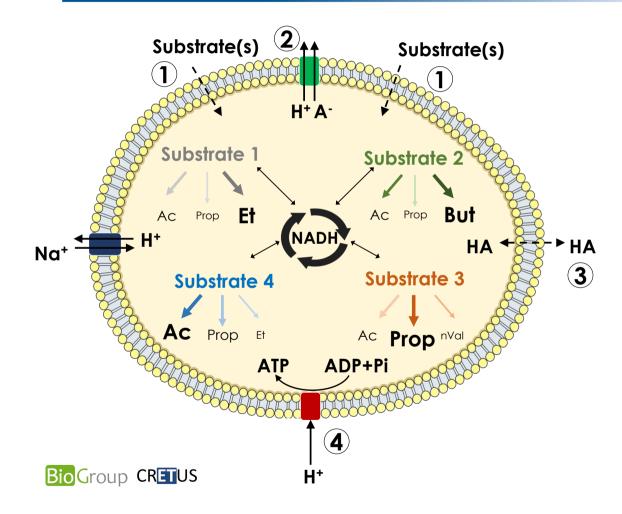








## One virtual microorganism does all the possible processes

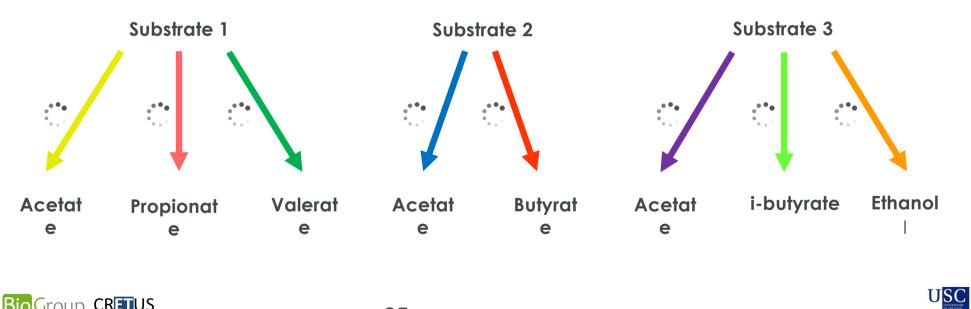


- 1. Substrate transport
- 2. Active transport of products
- 3. Passive transport of products
- 4. Energy conservation via proton morive force



## 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)





# Microorganisms behave in an efficient way

ATP production from the substrate is maximised



**Pathway selection**  $r_{ATP} = r_{TRANSPORT} + r_{PMF} + r_{CAT}$  $\rightarrow$ ∆V = -0.2 Lactate  $pH_{in} = 7$ Free difusion ATP 🔇 **`AcH** ↔ Ac<sup>-</sup> + H<sup>+</sup> Ac<sup>-</sup> + H<sup>+</sup> ↔ AcH  $H^+$ H+¢ Active Transport **ADP+Pi** nH nH+ NH4\*> Proton translocations Catabolic ATP Transport BioGroup CRETUS



# **Bioenergetic models are dynamic flux balance analysis models**

$$\frac{dC}{dt} = D \cdot (C^{in} - C) + r(z, C)$$
$$z(t) / \max_{z} r_{ATP}(C(t))$$
$$r_{NADH}(z) = 0$$
$$g(z) \le 0$$
$$h(z) = 0$$

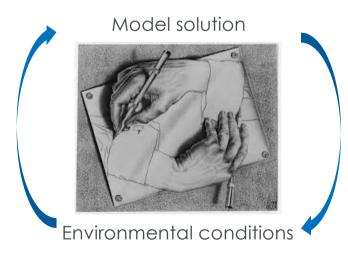
s.t.

Maximise ATP production

NADH is conserved

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

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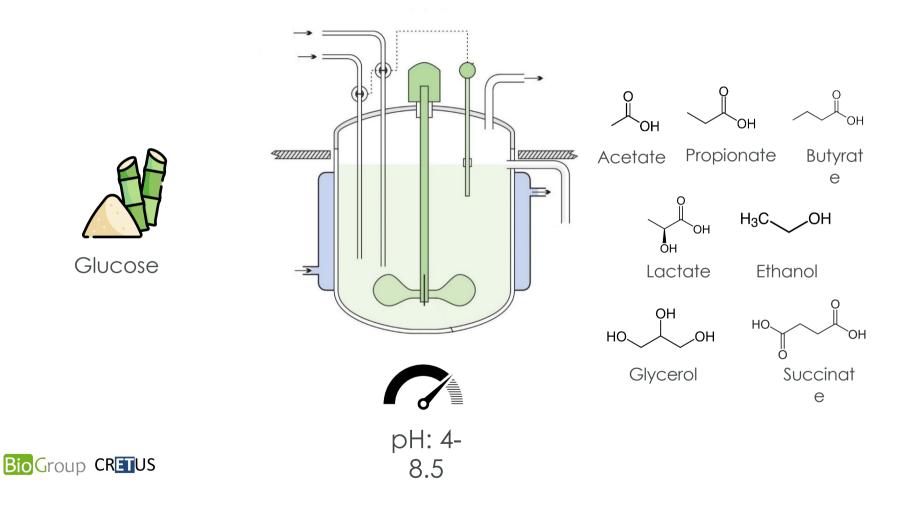
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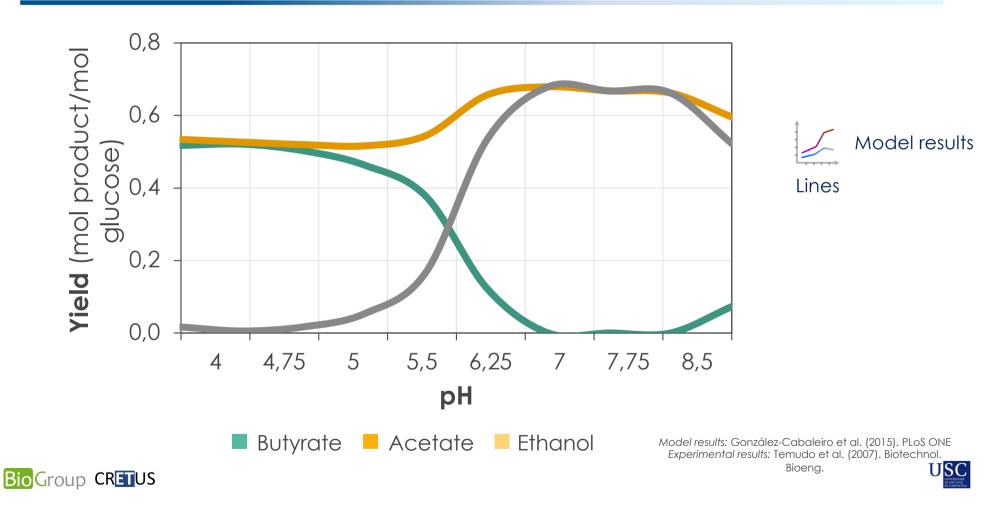
# **Bioenergetic model for glucose fermentation**





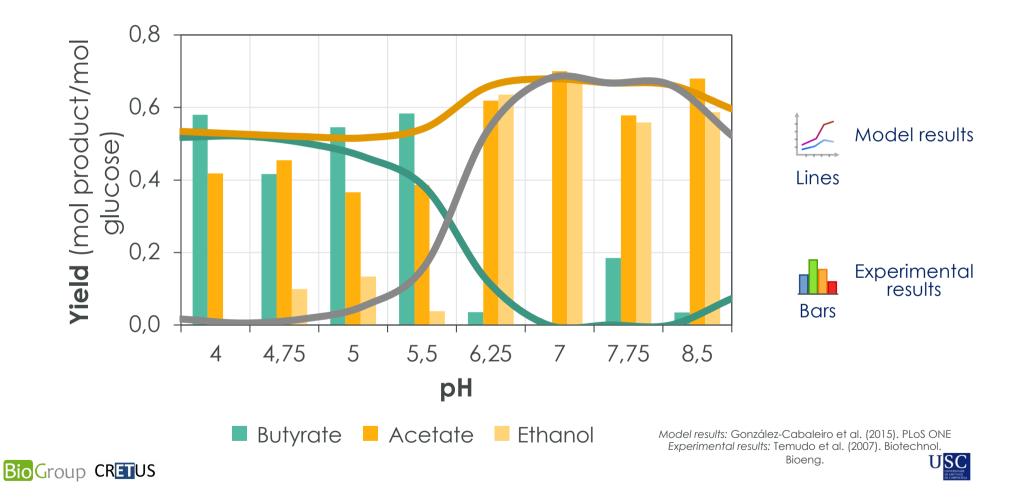


## The model predicts a shift with pH



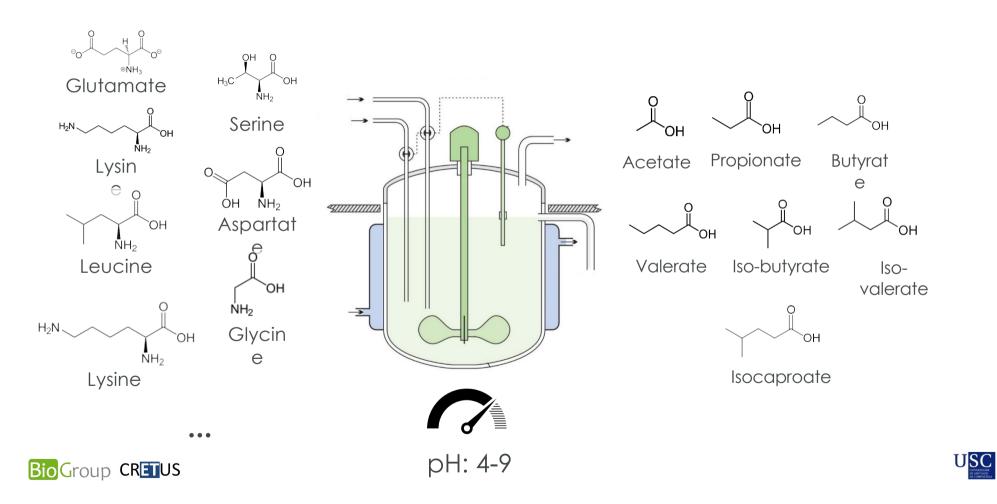


## The model was validated with experimental results





# **Bioenergetic model for protein fermentation**



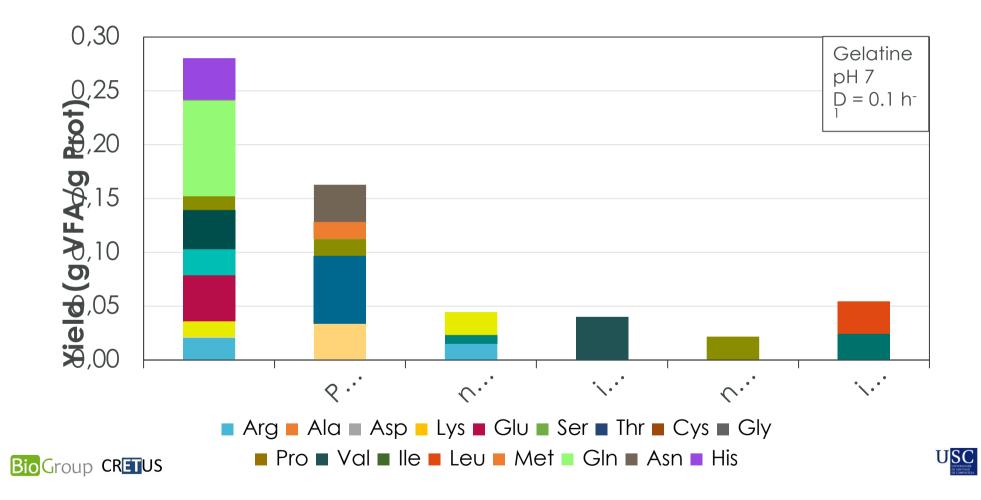


#### Enzyme soup approach: amino acids are converted ! - ! - 1 . -NADH CH<sub>3</sub> NH<sub>2</sub> NH<sub>2</sub> Glutamat Leucine е +AT Ac n-But Val ∑r<sub>NADH</sub> = NAD NAD Βι Н Н Н ·ŃΗ Proline 1⁄2 Ac + 1⁄2 Prop + 1⁄2 n-Val BioGroup CRETUS

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



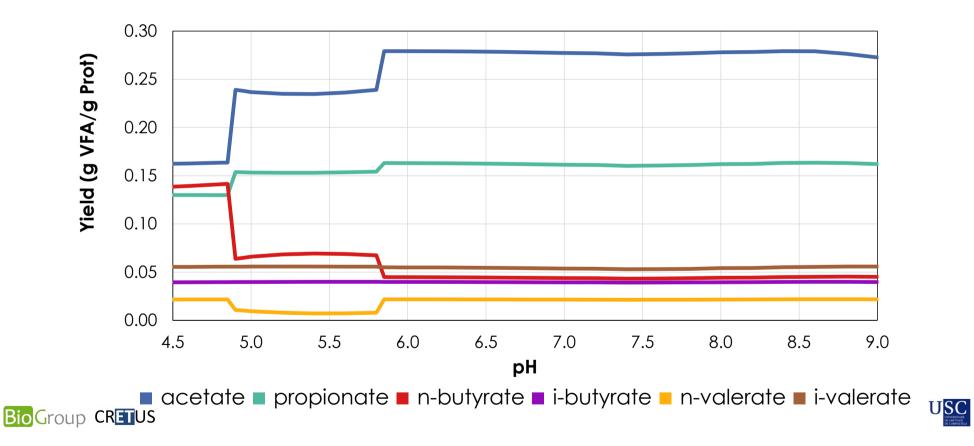
## Predicted VFA yields from gelatine fermentation at pH 7





# The product spectrum is sensitive to pH

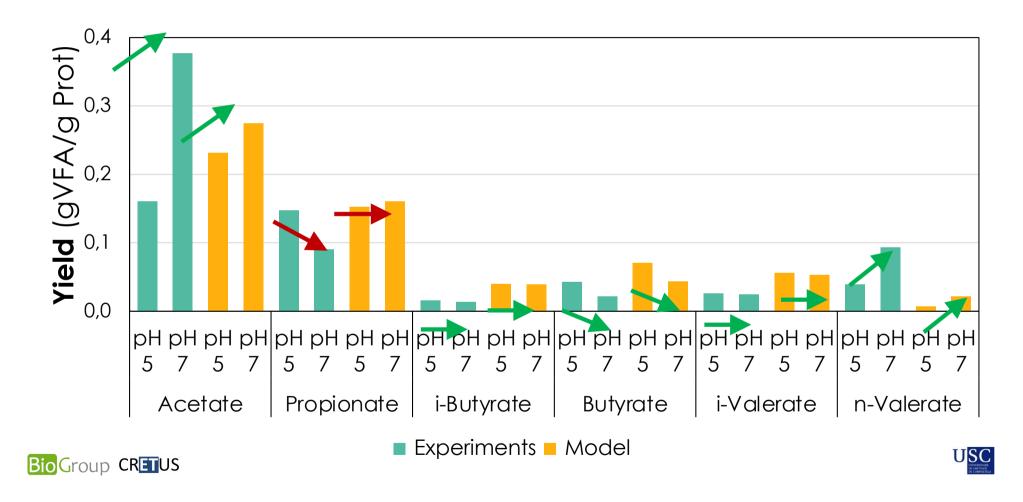
• Higher proton motive force energy available at low pH favours butyrate production





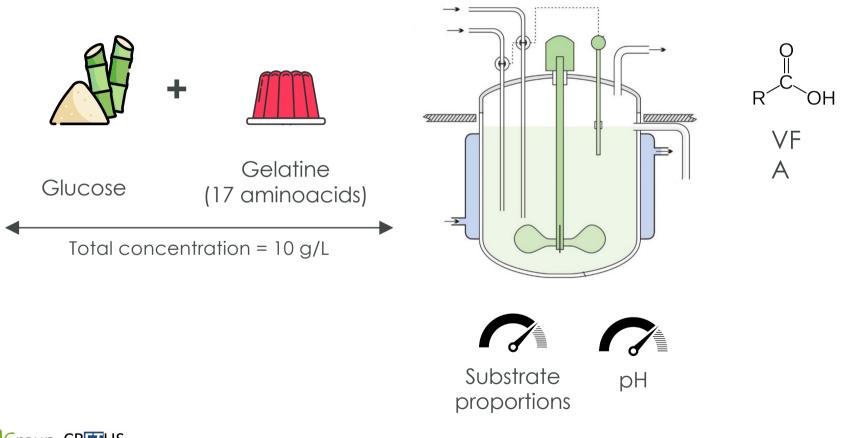


# The model captures the tendencies observed with pH



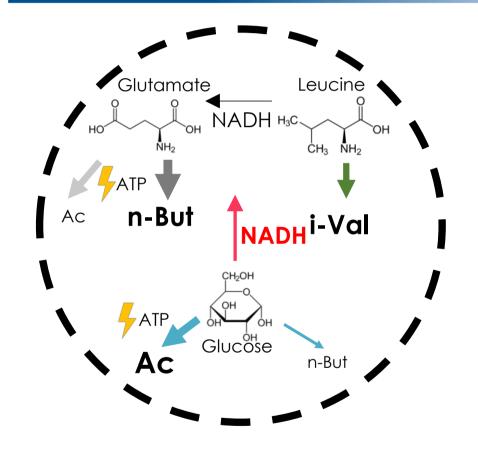


# Modelling the cofermentation of gelatine and glucose





# Cofermentation is not just adding two mono-fermentations



# Cofermentation

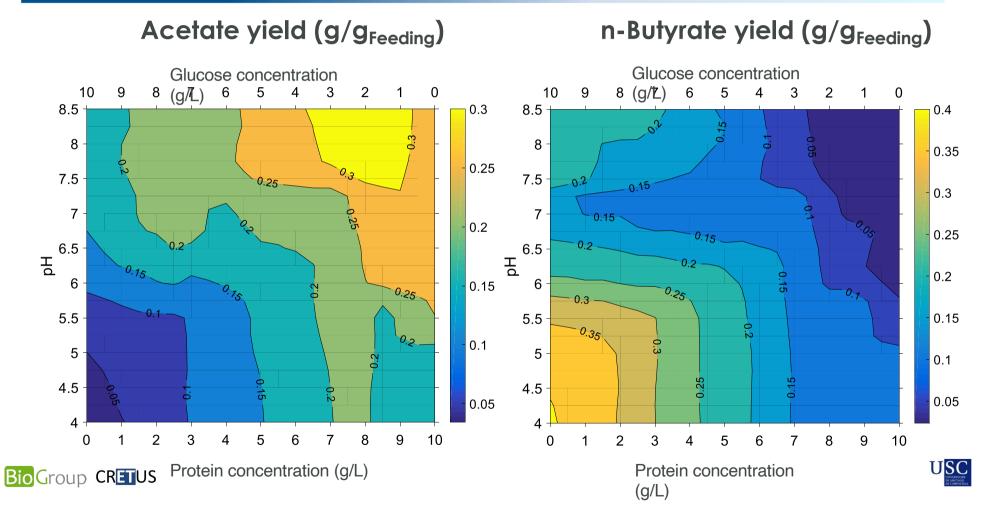
 $\begin{aligned} &Max(r_{ATP,Prot} + r_{ATP,Glucose}) \\ &\sum r_{NADH,Prot} + \sum r_{NADH,Glu} = 0 \end{aligned}$ 







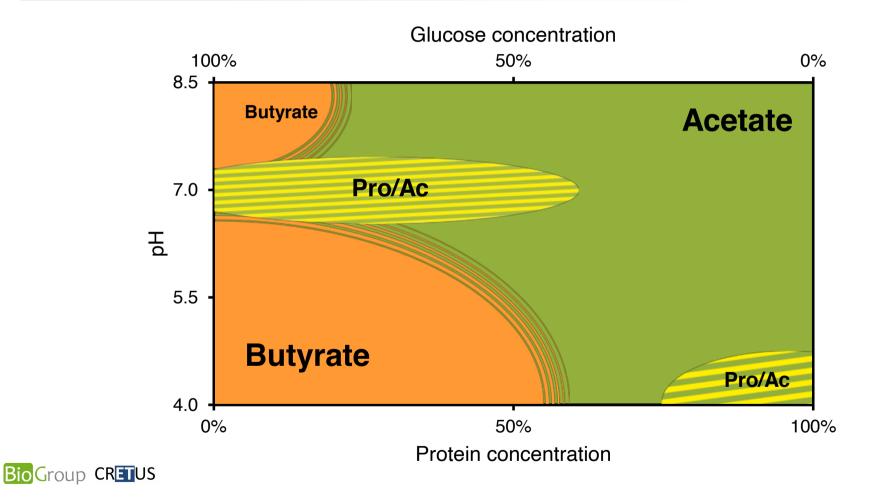
# **Changing operational conditions favour different VFA**



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# The model can explore the operational space





# 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.b



е

@Alberte Regueira













# **AGENDA AND HOUSEKEEPING**



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

**Speaker 2** *Adam Kovalovszki* (Technical University of Denmark)

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# INFERRING METABOLIC INTERACTIONS FROM GENOME-SCALE DATA: ANAEROBIC DIGESTION 'UNDER THE MICROSCOPE'

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

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



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U Who's who in the AD microbiome



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Who's who in the AD microbiome
 AF

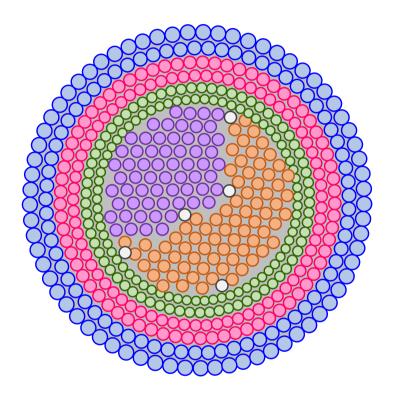


□ What is genome-scale metabolic modeling (GSMM)?

U Who's who in the AD microbiome

 $\hfill\square$  Microbial interactions in AD

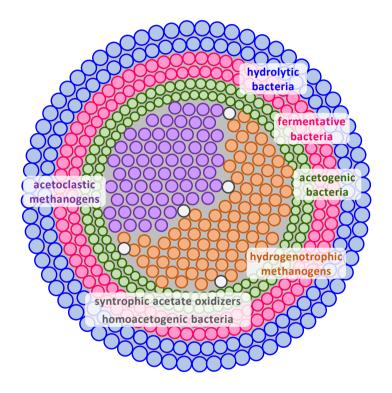




METHANOGENIC GRANULE STRUCTURE

(theoretical)

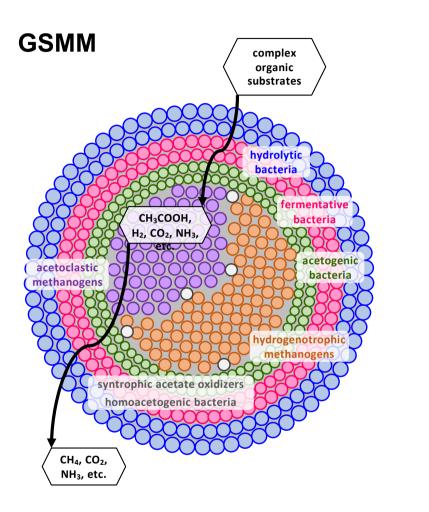




METHANOGENIC
GRANULE
STRUCTURE

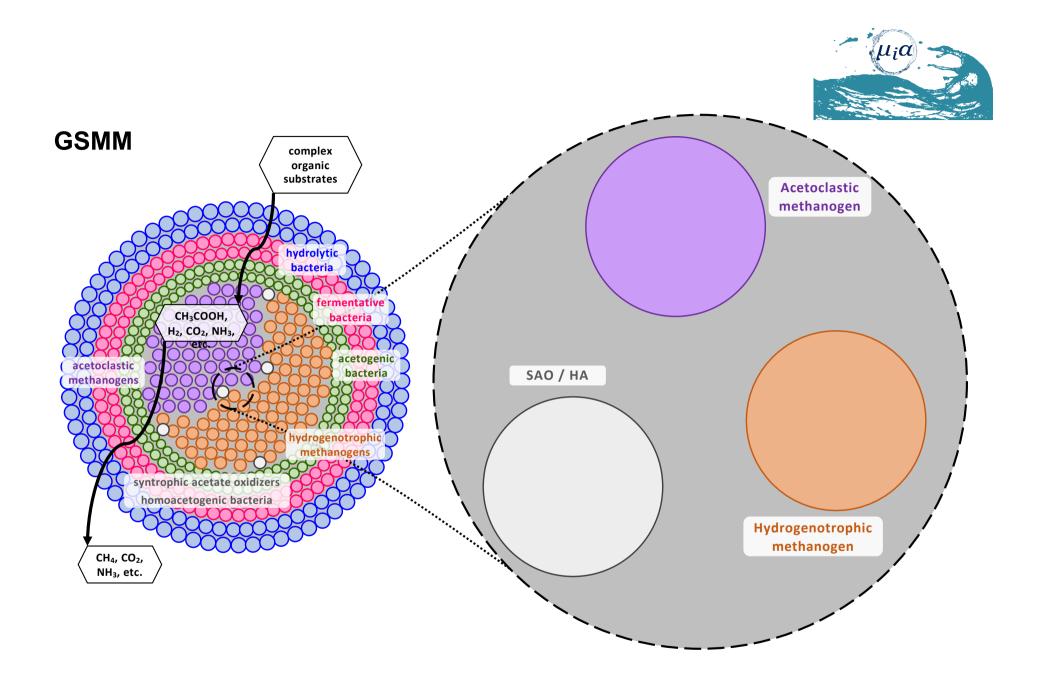
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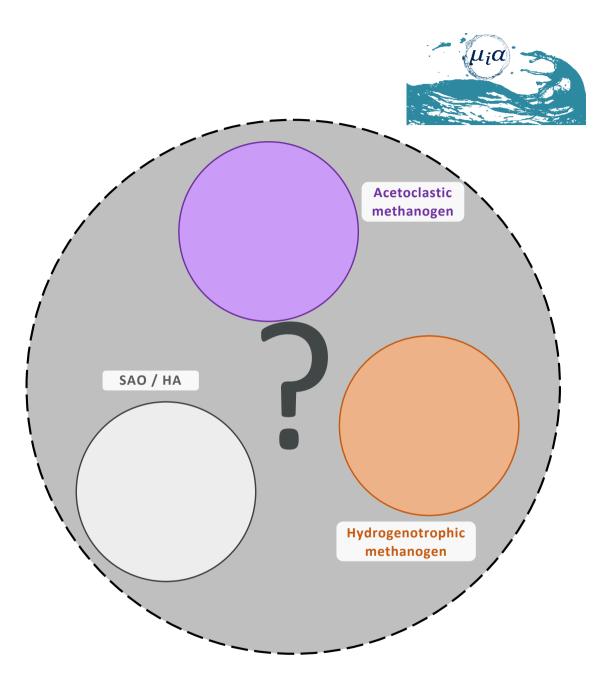


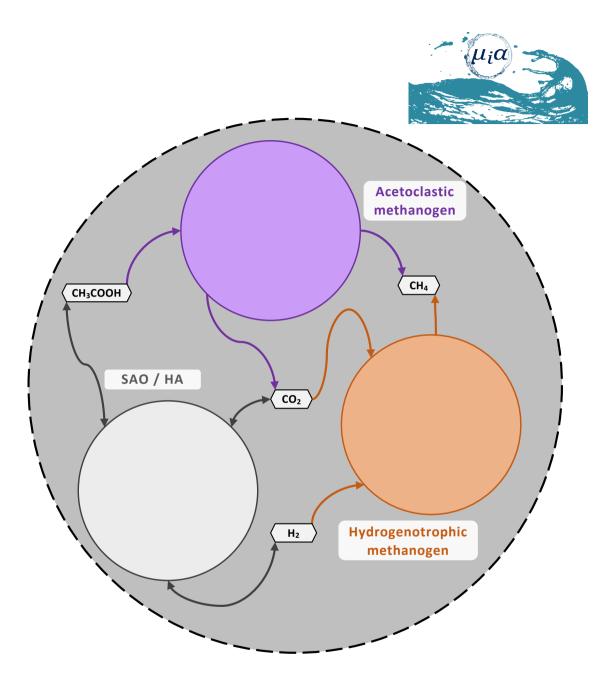


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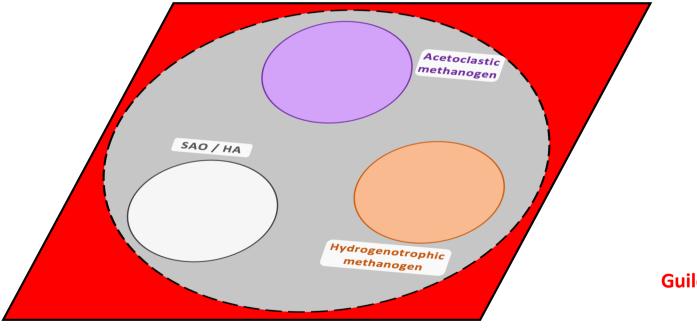






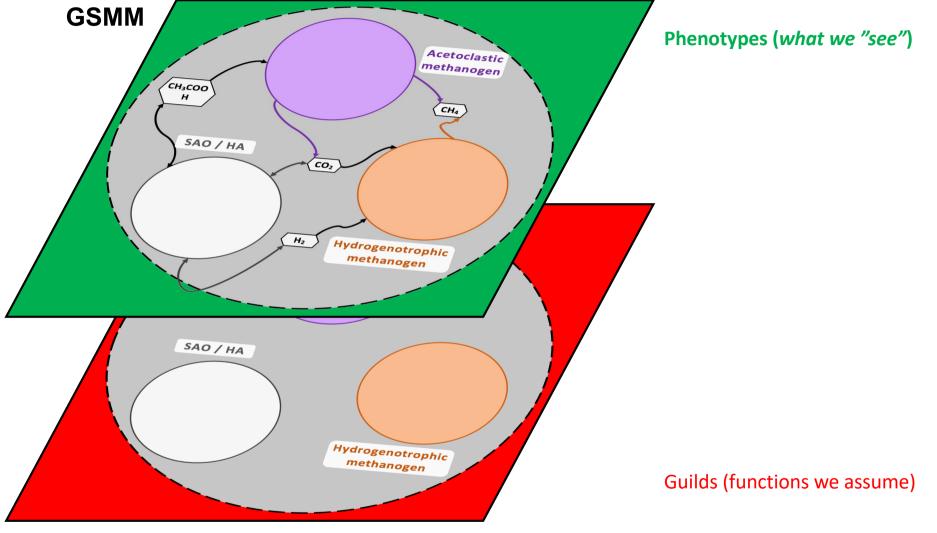




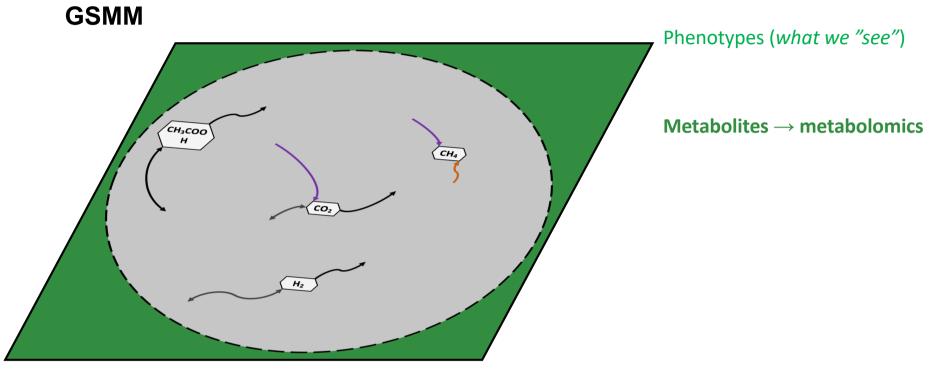


### Guilds (functions we assume)





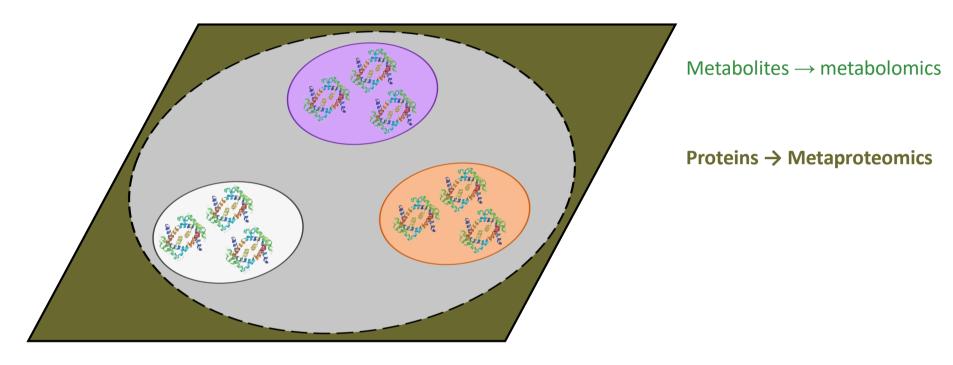




Guilds (functions we assume)



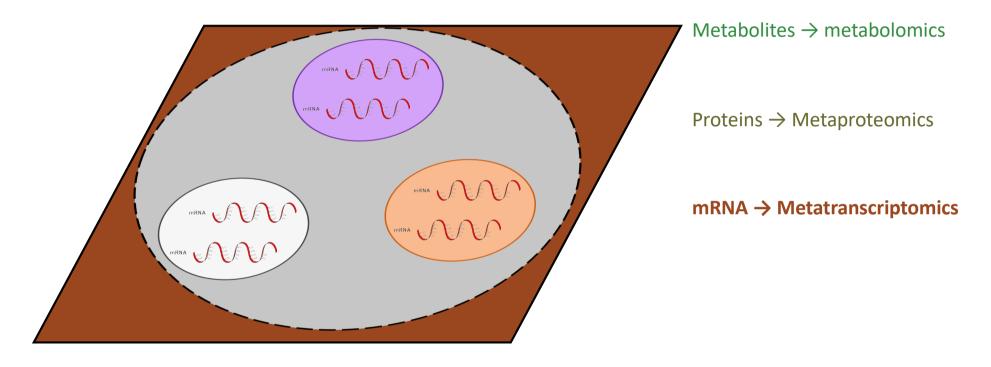
### Phenotypes (what we "see")



Guilds (functions we assume)



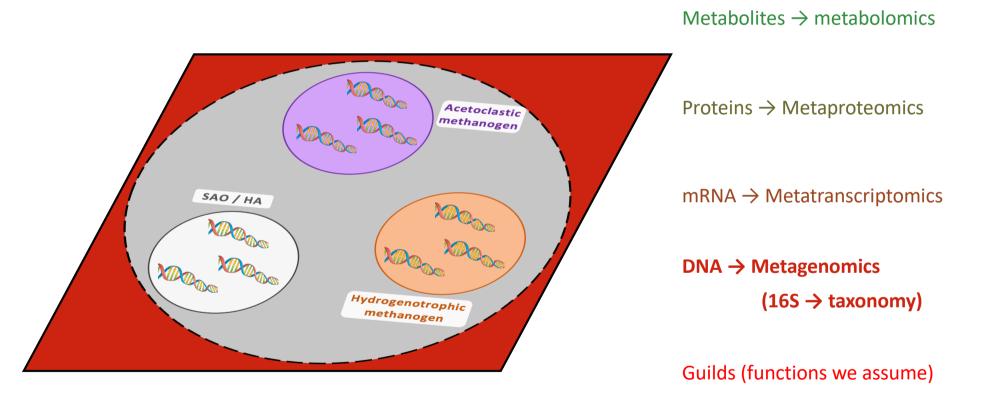
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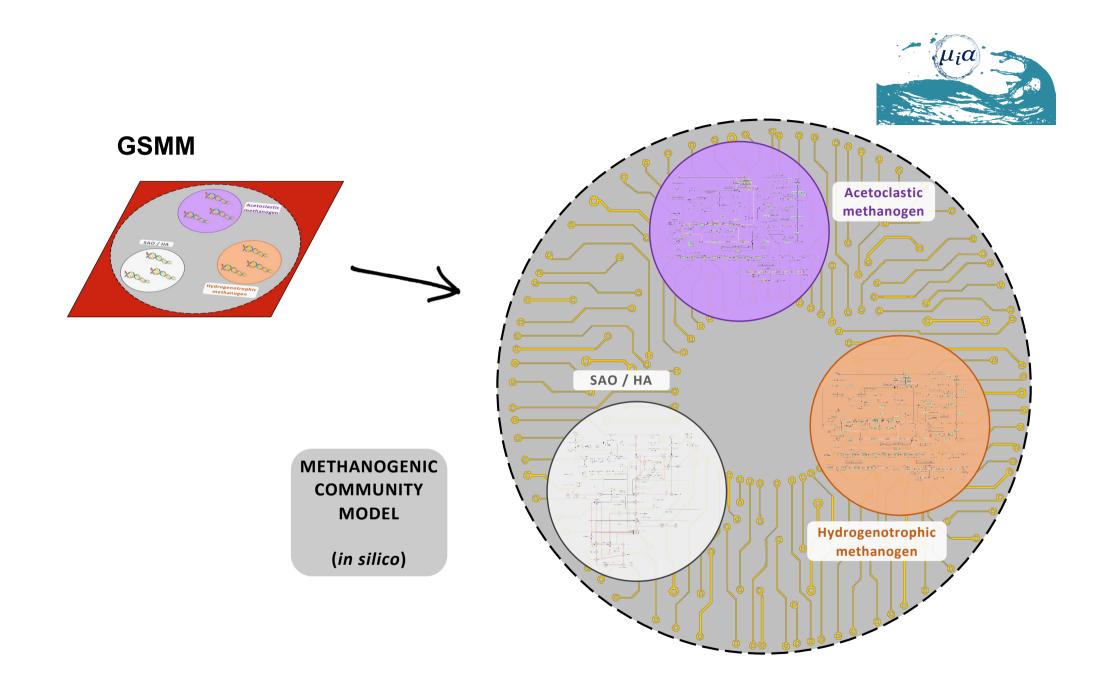


#### Guilds (functions we assume)



Phenotypes (what we "see")







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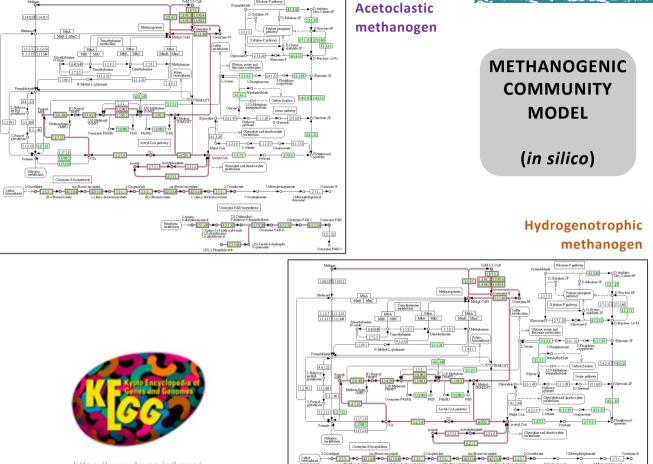
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Acetyl-CoA pathway

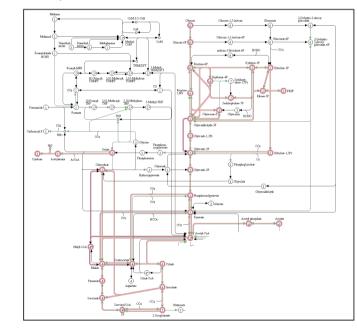
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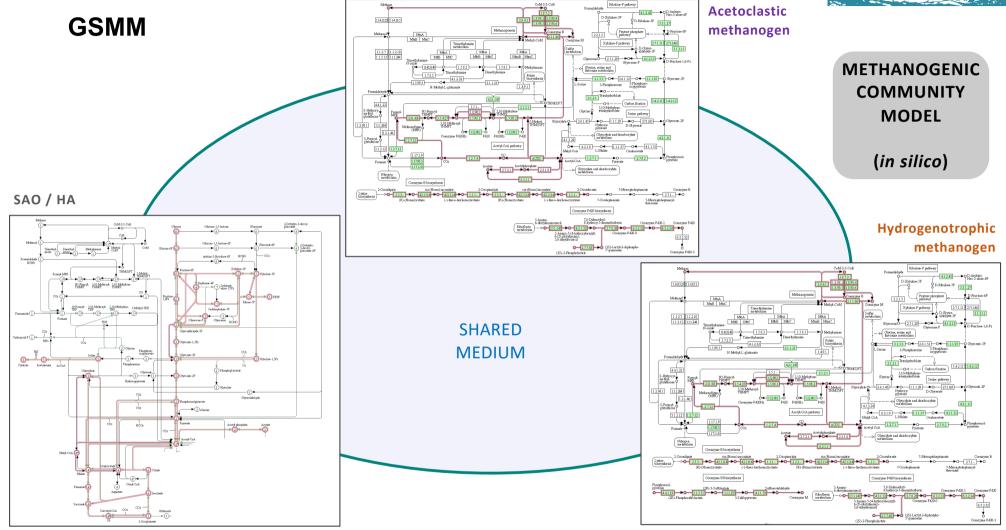
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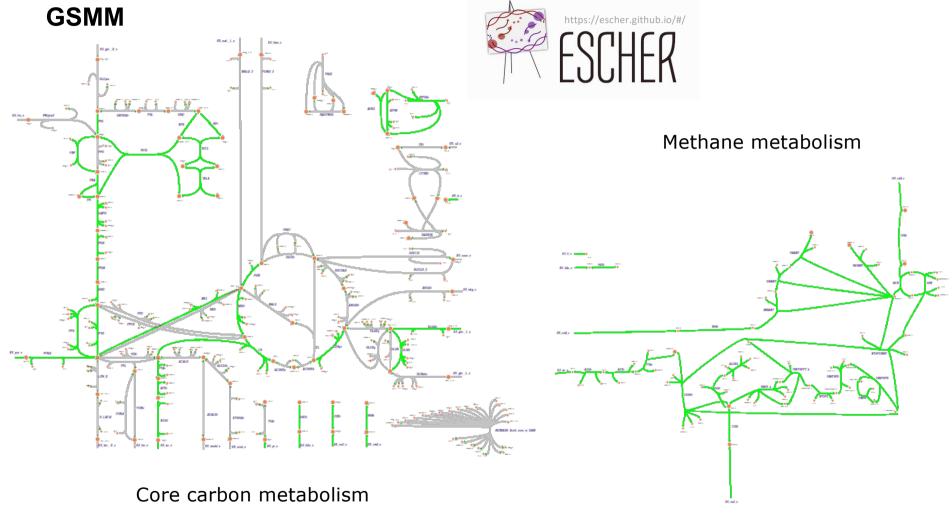




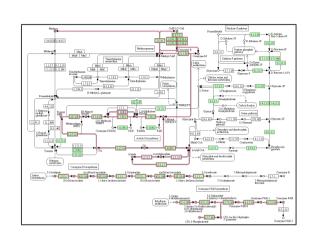


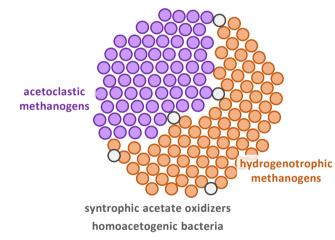


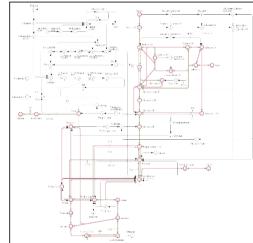


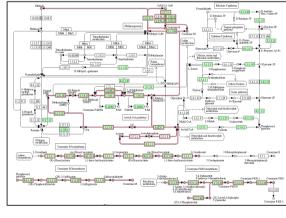




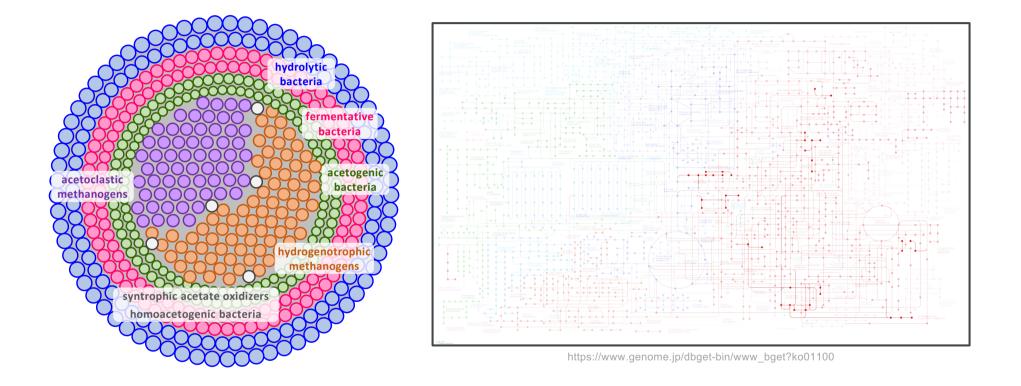














**Open Access** 

#### Who's who in AD

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

**Biotechnology for Biofuels** 

#### RESEARCH

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

Stefano Campanaro<sup>1,3</sup>, Laura Treu<sup>1,2\*</sup><sup>(0)</sup>, Luis M. Rodriguez-R<sup>4</sup>, Adam Kovalovszki<sup>2</sup>, Ryan M. Ziels<sup>5</sup>, Irena Maus<sup>6</sup>, Xinyu Zhu<sup>2</sup>, Panagiotis G. Kougias<sup>7</sup>, Arianna Basile<sup>1</sup>, Gang Luo<sup>8</sup>, Andreas Schlüter<sup>7</sup>, Konstantinos T. Konstantinidis<sup>4</sup> and Irini Angelidaki<sup>2</sup>

#### 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  $\geq$  90% complete with  $\leq$  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.

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

**Biotechnology for Biofuels** 

#### RESEARCH

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

Stefano Campanaro<sup>1,3</sup>, Laura Treu<sup>1,2\*</sup><sup>(0)</sup>, Luis M. Rodriguez-R<sup>4</sup>, Adam Kovalovszki<sup>2</sup>, Ryan M. Ziels<sup>5</sup>, Irena Maus<sup>6</sup>, Xinyu Zhu<sup>2</sup>, Panagiotis G. Kougias<sup>7</sup>, Arianna Basile<sup>1</sup>, Gang Luo<sup>8</sup>, Andreas Schlüter<sup>7</sup>, Konstantinos T. Konstantinidis<sup>4</sup> and Irini Angelidaki<sup>2</sup>

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 Ca. Kuenenbacteria • Ca. Magasanikbacteria

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Ca. Moranbacteria

Ca. Nomurabacteria

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Ca. Saccharibacteria

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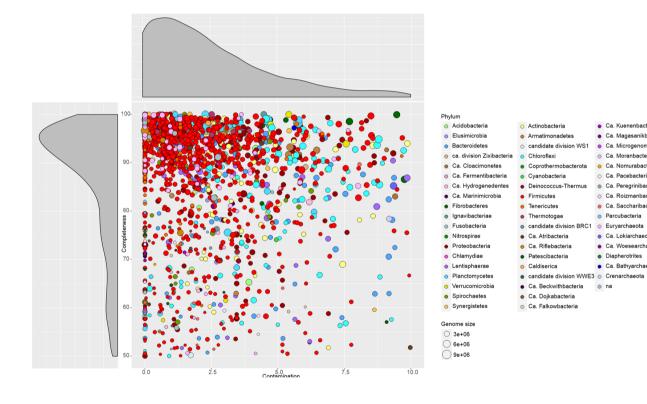
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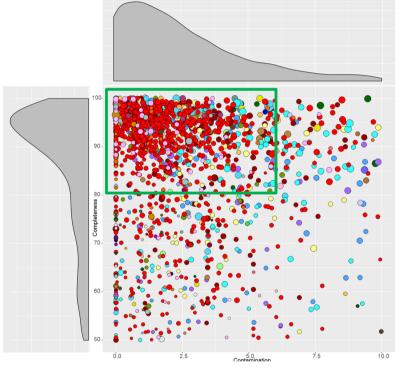
#### Who's who in AD



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#### Who's who in AD



0	Acidobacteria	0	Actinobacteria		Ca. Kuenenbacteria
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0		•	Armatimonadetes	•	Ca. Magasanikbacte
0	Bacteroidetes	0	candidate division WS1	•	Ca. Microgenomates
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•	Verrucomicrobia	٠	Ca. Beckwithbacteria	۲	na
•	Spirochaetes	٠	Ca. Dojkabacteria		
0	Synergistetes	0	Ca. Falkowbacteria		

Genome size 3e+06 6e+06 9e+06

#### Majority of MAGs with

- High level of completeness
- Low contamination

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Original Research Article		tion in the anaerobic digestion
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Arianna Basile <sup>a</sup> , Stefano Cam	2	multi Conida Zamaini ed
Alessandro Rossi <sup>a</sup> , Irini Ange		
<sup>6</sup> Department of Biology, University of Padawa, Via <sup>b</sup> CRIBI Biotechnology Center, University of Padawa	<ol> <li>Bassi 58/b, 35727, Padon, Huly</li> <li>35131, Padon, Italy</li> </ol>	
<sup>1</sup> Department of Environmental Engineering, Technic <sup>1</sup> Department of Computer Science and Information	ai University of Denmark, 2800, Kgs. Lyngh	y, Denmark United Kingdom
ARTICLE INFO	A B S T R A C T	biological process for renewable energy, yet the mechanistic knowledge on i
genome scale metabolic modeli/microbiol in teractium/renewable energy	changes. To this goal, a nov functional capabilities of bac- microbes were shown to prefi alism and competition, and ar Additionally, external hydrog	full characterization of pairwise interactions and the associated metabolice of el collection of 50% genome-scale metabolic models was built to represent it teria and archaes species detived fram genome-centric mergenomics. Demina en mutualitier, parantici and commensitutie interactions over neuralitien, any re more likely to behave as metabolite importers and profiteers of the coexistence.
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Microorganisms play an important relevance, ranging from human heat biorchenology (herbunn et al., 2015). In may have various responsibilities, from applied processes (e.g. biogas product synthesis of polymaric substances (the works, however, are still poorly un isolating most of the microbial species are of heir interactions (Muller et al., 2018 microbione might reveal which species process (Faih), 2015), the rule of rar clarified (Jousset et al., 2017). Micro important for environmental niche odi	could by an incomplete mic conditions for the microbes, principle, this apprends could microbioms, to allow a bread the second second second second second the (Clemente et al., 2012) to particular, diverse microbiomes counsing diseases to influencing ion) (Chu et al., 2020) or the we et al., 2030). Microbial net- lerisond due to difficulties in and to the heirergeneois nature are the key playners for a specific emembers still remains to be bial cooperation is extremely onization and completing second not which single species could not addr (S. Campanamo).	By exchanges of ghatogenic animo acids were shown to overcome assocrable and animovies and cycle. Can need varages periodical the and forwards gauge overall suggesting stategies to increasing the bioga protection efficiency. Impection of the microbial interploys perform independently (Day et al., 2004). This is the case is an annexed digension (Ab), biogas productions, when accolated and animative perform independently (Day et al., 2004). This is the case is an annexed digension (Ab), biogas productions, when accolated and the perform independently (Day et al., 2004). This is the case is an annexed perform independently (Day et al., 2004), when accolated and the perform independently (Day et al., 2004), when accolated and the state of the interploy of the state of the state of the perform independently (Day et al., 2004), when accolated and the state of the state of the state of the state of the state of the heat of the interploy of the state of the state of the state of the state of the state of the state of the state of the heat of the state of the state of the state of the state of the heat of the state of the state of the state of the state of the heat of the state of the state of the state of the state of the heat of the state of the state of the state of the state of the heat of the state of the state of the state of the state of the heat of the state of the state of the state of the state of the heat of the state of the state of the state of the state of the heat of the state of the state of the state of the state of the state of the state of the state of the state of the state of the state of the state of the state of the state of the state of the state of th

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BSTRACT	
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hugust 2020; Accepted 24 A ng Society. Published by Ek	uugust 2020 sevier Ine. All rights reserved.
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- 836 GSMMs
- 349k+ pair-wise interactions studied

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Arianna Basile <sup>a</sup> , Stefano Camp Alessandro Rossi <sup>a</sup> , Irini Angelia			
<sup>6</sup> Department of Biology, University of Padawa, Via U. <sup>b</sup> CRIBI Biotechnology Center, University of Padawa, 3 <sup>c</sup> Department of Knvirusmental Kngiaeering, Technical <sup>c</sup> Department of Computer Science and Information Sy	5131, Pedue, Italy University of Denmark, 2800, Kgs. Lyngh	y, Domuark United Kingdom	
ARTICLEINFO	ABSTRACT		
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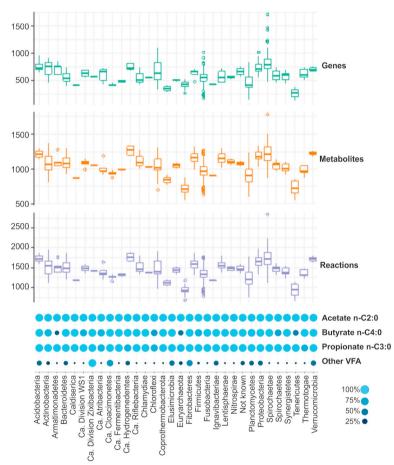


#### • 836 GSMMs

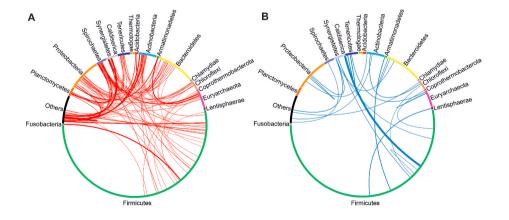
- 349k+ pair-wise interactions studied
- Dominant species
  - Mutualistic (+/+)
  - Parasitic (+/-)
  - Commensalistic (+/0)

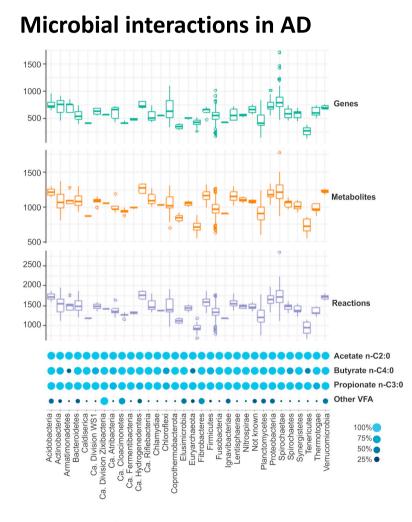
		Metabolic Engineerir	g on (analy) the add	
			able at ScienceDirect Engineering	METADO INCINEER
ELSEVIER		journal homepage: www.	elsevier.com/locate/meteng	
Original Research A	ticle			
Revealing meta microbiome by			tion in the anaerobic digestion	Glowsh for updates
Arianna Basile <sup>a</sup> , St Alessandro Rossi <sup>a</sup> ,	efano Campa Irini Angelida	naro <sup>a,b,*</sup> , Adam Kovale aki <sup>c</sup> , Giorgio Valle <sup>a,1</sup> ,	ovszki <sup>c</sup> , Guido Zampieri <sup>a,d</sup> , Laura Treu <sup>a, 1</sup>	
<sup>6</sup> Department of Biology, Univers <sup>b</sup> CRIBI Biotechnology Center, U <sup>c</sup> Department of Environmental E <sup>c</sup> Department of Computer Science	niversity of Padeve, 351 agineering, Technical (A	assi 587b, 35727, Padan, Italy 131, Padan, Italy niversity of Desmark, 2800, Kgs. Lyngh mu, Teasside University, Middlasbrough	y, Domaark United Kingdom	
ARTICLE INFO		ABSTRACT		
Keywords: Anacrobic digestion/flux balan genome scale metabolic model tenetims/renewable energy		hidden microbial dynamics is	biological process for renewable energy, yet the mechan still limited. The present work charted the interaction net full characterization of pairwise interactions and the ass	twork in the anaero
		changes. To this goal, a nov functional capabilities of bac microbes were shown to pref alism and competition, and at Additionally, caternal hydrog salism over amensalism. Fina caused by an incomplete tri- conditions for the microbes, principle, this approach could	And traincontraction or painway immediations and the av- ily indication of System and the second second second environment of the second second second second second environment, paratile and commendiation temerations or non links to behave an analytic languagest rand position can links on pain-help influences and modeling and the second second second second second second second paraticles and cycle. Can novel average paradiment the automatics is in constant to bioaxedical imped- ation be applied to microbial papulations of bioaxedical impe- tation be applied to microbial papulations of bioaxedical impe- tant behaviored and the microbial interplays.	built to represent tragenomics. Domin ver neutralism, ame cers of the coexisten by pronoting comm promoting common swercome auxotroph not favourable grou- oduction efficiency.
<ol> <li>Introduction Microarganism splay relevance, ranging from biotechnology (Leibun any have variatious respon applied processes (seg.) synthesis of polymetic is works, however, are s ynthesis of polymetic is processed (range), and any polymetic processes (range), 2015), 1 clarified (Journet et al., 2015), 1 clarified (Journet et al., 2015), 1 clarified (Journet et al., 2015), 1 </li> </ol>	t human health ( t al., 2015). In part sibilities, from car ologas production, ubstances (Chow of ill poorly unders) robial species and ler et al., 2018). W which species are he role of rare m , 2017). Microbia nutal niche coloniz	changes. To this goal, a nov functional capabilities of bac microbes were shown to pref alism and competition, and at Additionally, caternal hydrog salism over amensalism. Fina caused by an incomplete tri- conditions for the microbes, principle, this approach could	el collection of 85% genome-scale methodic models was treats and archarsa specific derived from genome-entritic er numulatilitier, paratalitic and commensatistici interactions or to none likely to behave as metabolitie importers and profili- ier, nitection pathicely influences mitrobiome dynamics in Jily, eachanges of glucogatic amino acids were shown to e antrobyin and cyto. Caur novel transpy predicted them overall stogescing, strategies to increasing the hiogas pri also be applied to uncholal populations of biometecial interpre- sent strategies and the strategies of biometecial interpre- table higher than the strategies of biometecial meta- tion of the strategies of biometecial meta- tions.	built to regressent regressions. Compression of the compression of the regression of the coasilies of the co
<ol> <li>Introduction Microarganism splay relevance, ranging from biotechnology (Leibun any have variatious respon applied processes (seg.) synthesis of polymetic is works, however, are s ynthesis of polymetic is processed (range), and any polymetic processes (range), 2015), 1 clarified (Journet et al., 2015), 1 clarified (Journet et al., 2015), 1 clarified (Journet et al., 2015), 1 </li> </ol>	h human health ( t al., 2015). In part stillittes, from can ologas production) bistances (chow of all poorly unders) robial species and her et al., 2018). W which species are he role of rare m 2017). Microbia ratal niche colonia et al., 2007), whis	changes. To this geal, a nor functional capabilities of bac- microbes were shown to per- microbes were shown to per- ander the second second second second additionally, carcinal hydrog addition over amenadian. The conditions for the microbes, microbiome, to allow a lesses principle, this approach could microbiome, to allow a lesses principle, this approach could microbiome, to allow a lesses of the second second second second microbiome, and the second microbiome, and the second microbiome, and the second microbiome and the second second microbiome and the second second microbiome and the second microbiome and the second second problem and the second second problem and the second second problem and the second second microbiome and second second second second microbiome and second second second second second second second second microbiome and second seco	et al. callection of 856 genome-solare methods: models was then and archaes append selversh fram genome-entric me remarkation, generative and commensuintic inservations on instruction solarities, plantatic and commensuintic inservations on instruction solarities, binducers and crobing or synamic to bits, exclusions of plantatic and commensuintic and the solar solarities and commensuintic inservations of an exclusion solarities, and an exclusion of the solarities of an exclusion solarities in the solarities of the solarities inservation (AD), which is a holtechnological proc diagestion (AD), which is a holtechnological pro- truction (AD), which is a holtechnological pro- truction (AD), which is a holtechnological pro- ceeding and the minimum solarities of the solarities of archaes are inhibited, a plyrotal role is played by hydrogen-milling mechanogan defined in cortise out have et al., 2016) (SAOB). An example is the mu entruloxygenic archaes and syntrophysical incretes out formation or to Dig and carbon distributed (and the (CL)). The bits formation or to Dig and carbon distributed (and the CL), proceeding and an example is the mu entrumological archaes and syntrophysical incretes out formation or to Dig and carbon distributed (and the CL). The bits are an extending the common solar bits of the solarities of the solarities of the minimum solarities of the solarities of th	built to regressent regressions. Compression of the compression of the regression of the coasilies of the co



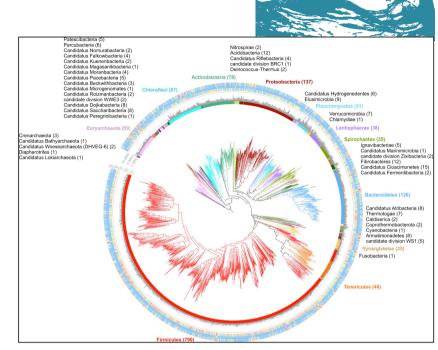




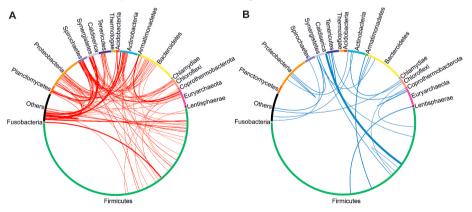




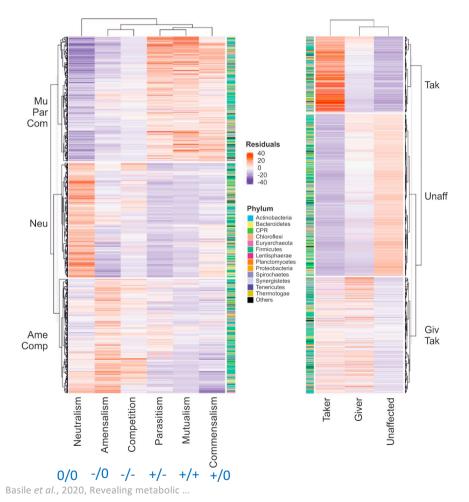
Basile et al., 2020, Revealing metabolic ...



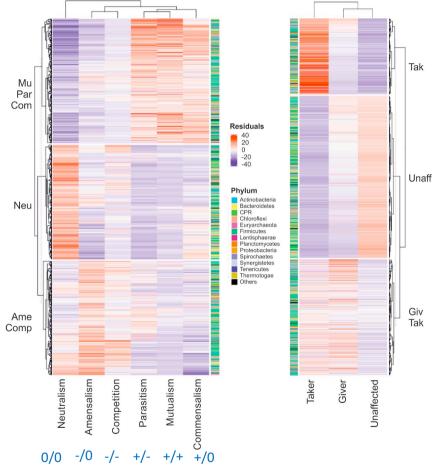
Campanaro et al., 2020, New insights ...

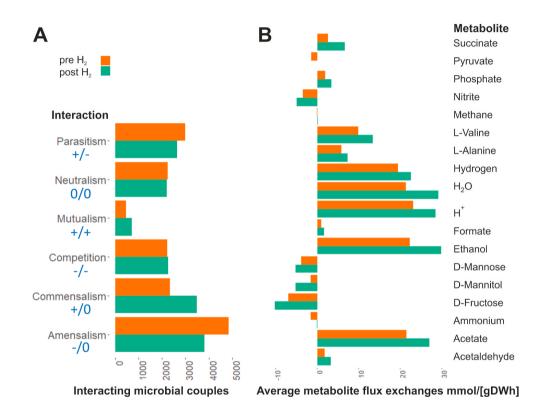






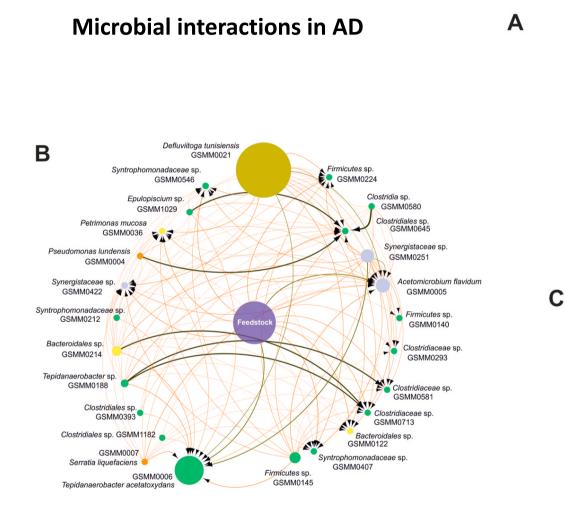


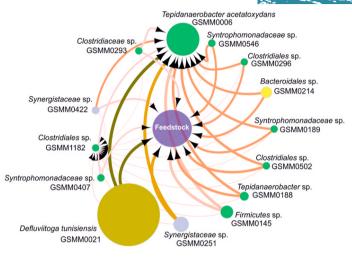


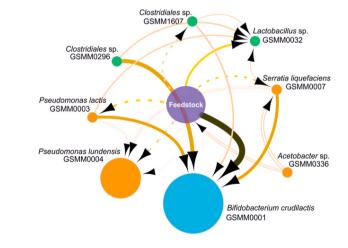


Basile et al., 2020, Revealing metabolic ...









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





GSMM a holy grail?



GSMM a holy grail?  $\rightarrow$  not quite



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- Experimenting is computationally and time-wise intensive (constraints, futile cycles, sinks)



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- GSMM: what <u>could be</u> and NOT what <u>is</u>  $\rightarrow$  other omics to corroborate
- Productivity vs survival  $\rightarrow$  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

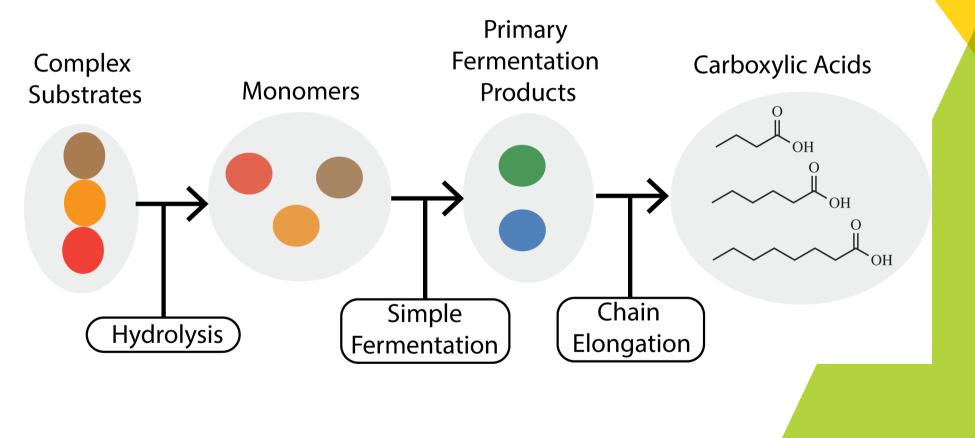
Matthew Scarborough (Matthew.Scarborough@uvm.edu) University of Vermont



Guild-based metabolic modeling for deciphering anaerobic bioprocesses

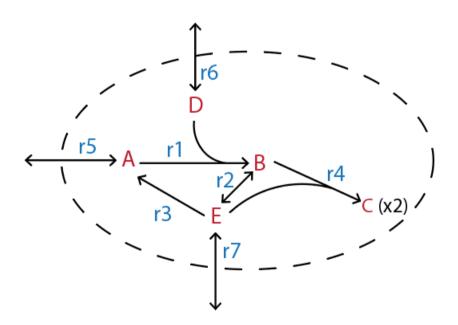
Matthew Scarborough, P.E., Ph.D. Department of Civil and Environmental Engineering College of Engineering and Mathematical Sciences The University of Vermont

## Chain elongation from complex organic wastes



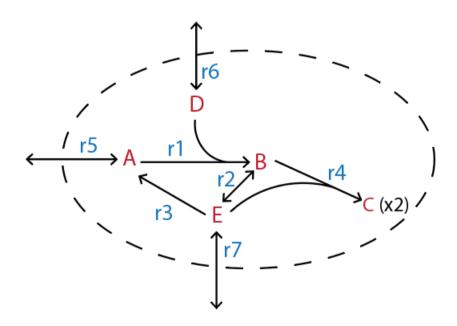
## A metabolic modeling primer

Metabolic Network Representation

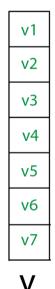


## A metabolic modeling primer

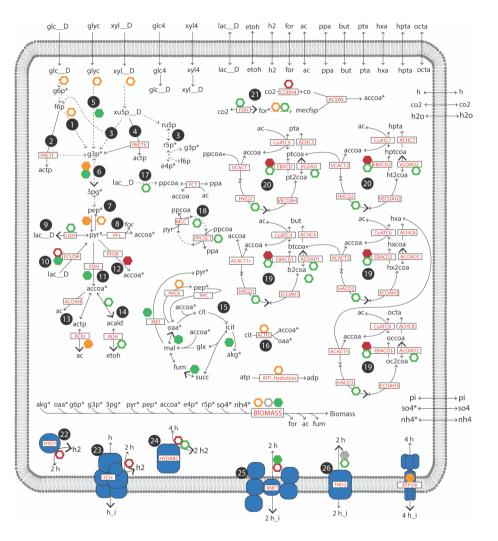
Metabolic Network Representation



	r1	r2	r3	r4	r5	rб	r7
Α	-1	0	1	0	1	0	0
В	1	-2	0	-1	0	0	0
С	0	0	0	2	0	0	0
D	-1	0	0	0	0	1	0
E	0	2	-1	1	0	0	1
S							



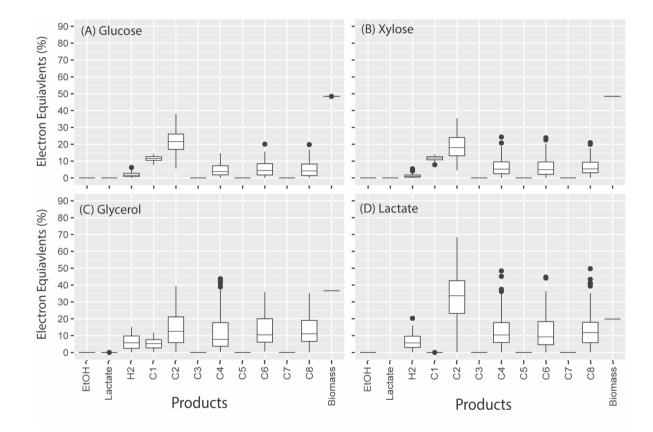
 $S \bullet v = 0$ 



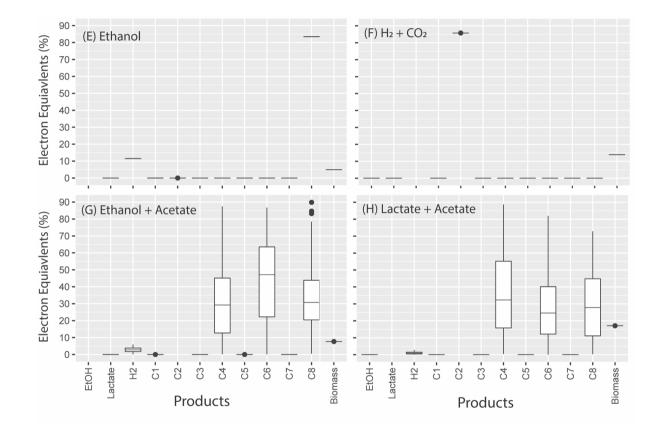
Adapted from Scarborough, M, et. al. 2020. mSystems.

## iFermCell215

## Simulating consumption of different substrates

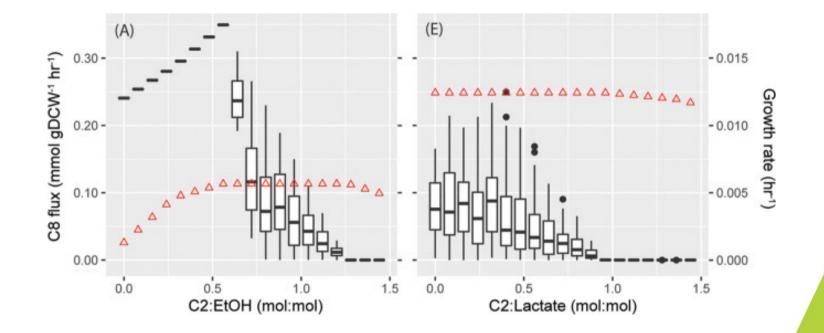


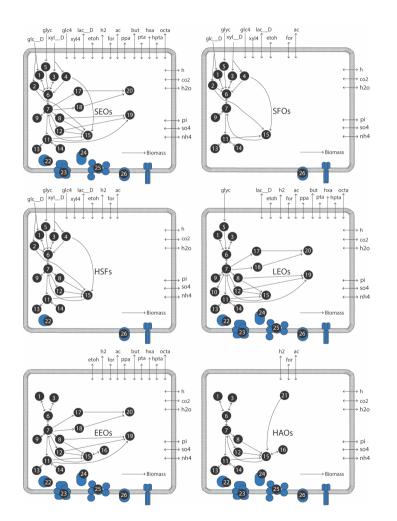
## Simulating consumption of different substrates





## Simulating consumption of co-substrates

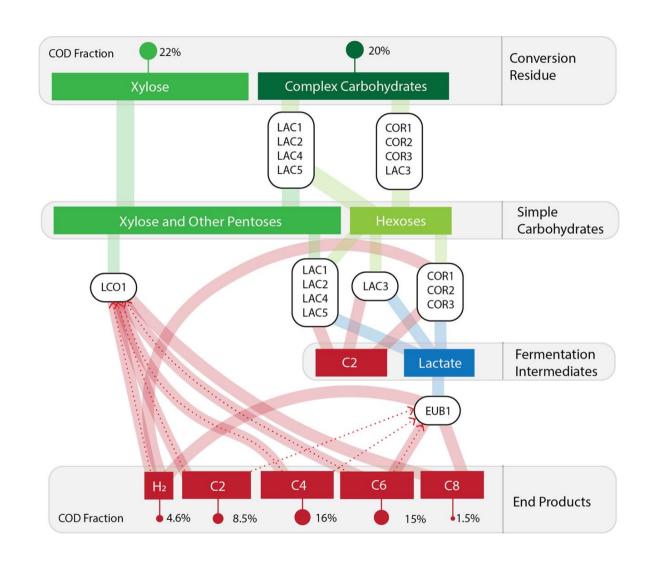


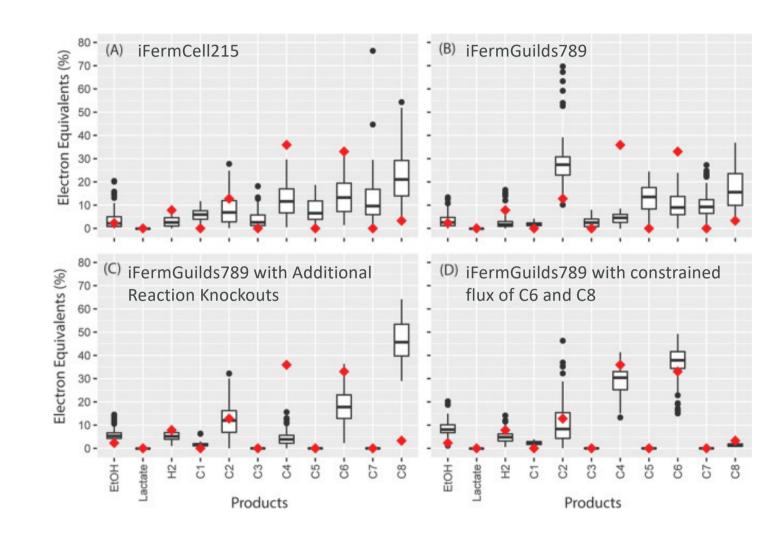


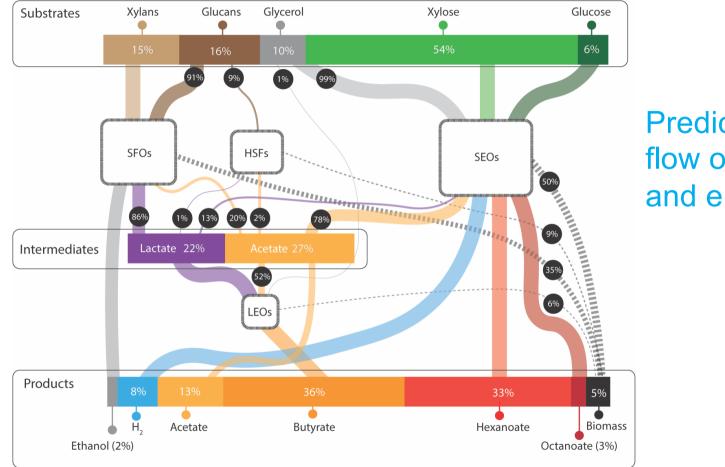
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## iFermGuilds789





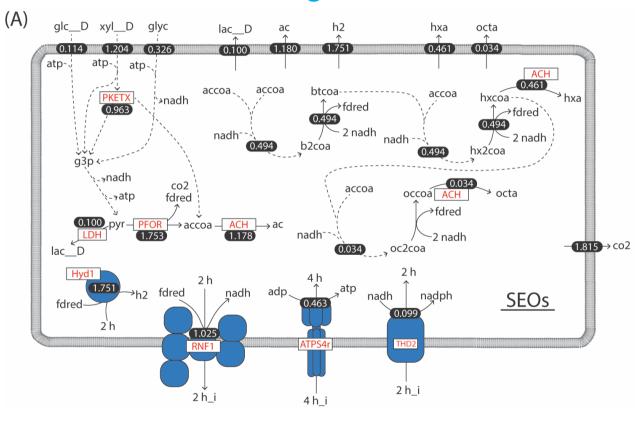




Adapted from Scarborough, M, et. al. 2020. mSystems.

# Predicting the flow of carbon and electrons

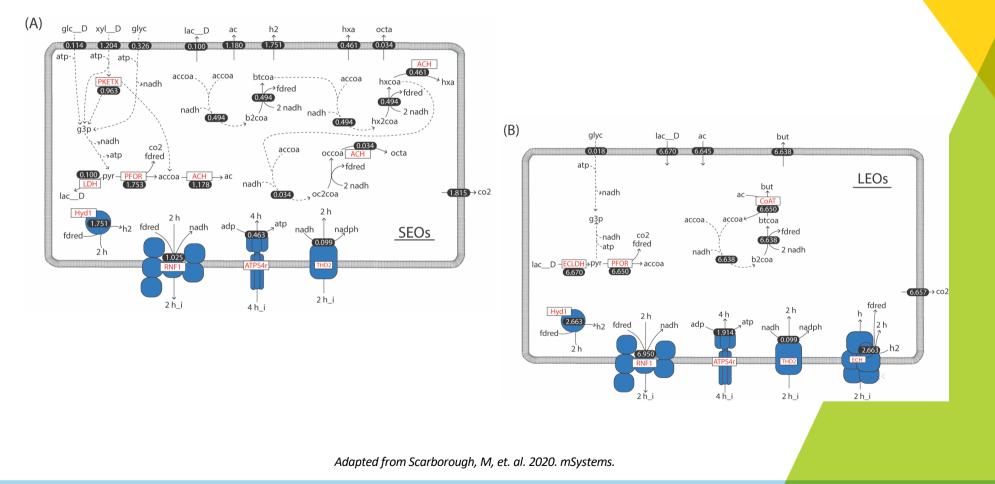
## Predicted differences in sugar and lactate conversion



#### (B) glyc lac\_\_D but ac $\mathbf{T}$ 6.638 0.018 (6.670) 6.645 atp-、 LEOs but `∍nadh CoAT 6.650 g3p btcoa accoa、 accoa\* 1 → fdred co2 -nadh 6.638 fdred atp 2 nadh nadh<sup>-</sup> 6.638 b2coa lac\_\_D –<mark>ECLDH</mark>→pyr – <mark>PFOR</mark> 6.670 6.650 →accoa (6.650) -6.657 → co2 fdred 2 h Hyd1 4 h ↑ h 2 h 2.663 \_atp ⇒h2 fdred nadh adp. 2 h nadph nadh fdred-1.914 0.099 ∠h2 2 h 2.663 6.950 ECH THD2 RNF1 2 ĥ\_i 2 h\_i 4 h\_i 2 h\_i

## Predicted differences in sugar and lactate conversion

## Predicted differences in sugar and lactate conversion



# 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).





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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