

9<sup>th</sup> International Conference on Sustainable Solid Waste Management, Corfu 15-18 June 2022

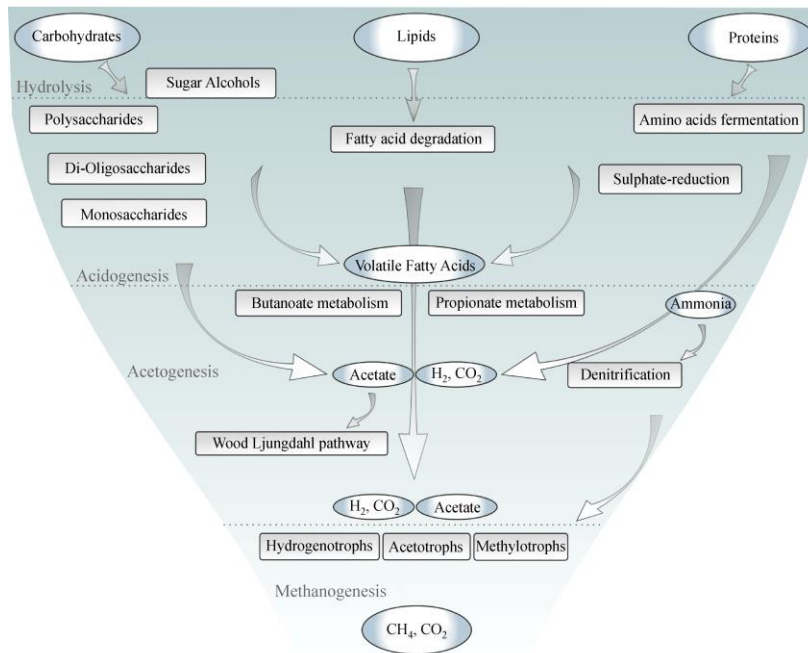
## **INHIBITORY EFFECT OF LONG CHAIN FATTY ACID ON BIOGAS PRODUCTION VIA A SINGLE PULSE: CHANGES IN MICROBIAL COMMUNITY DYNAMICS AND SIMULATION ASPECTS**

M. Gaspari<sup>a</sup>, M. Alvarado-Morales<sup>b</sup>, P. Tsapekos<sup>b</sup>, L. Treu<sup>c</sup>, S. Campanaro<sup>c</sup>, I. Angelidaki<sup>b</sup> & P.G. Kougias<sup>a</sup>

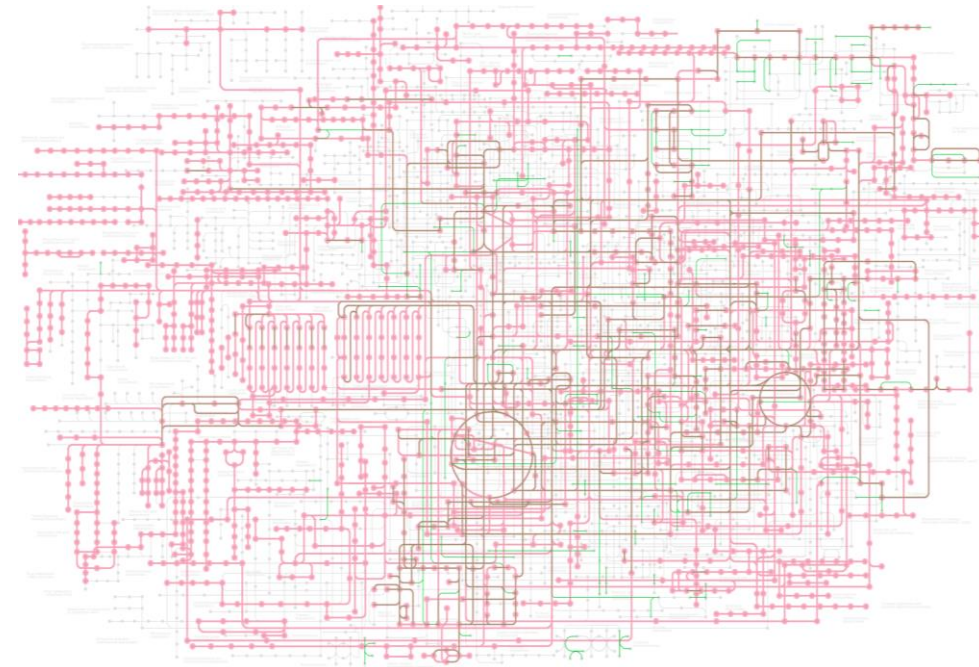
- a. Soil and Water Resources Institute, Hellenic Agricultural Organization Dimitra, Themi, Thessaloniki 57001, Greece
- b. Department of Chemical and Biochemical Engineering, Technical University of Denmark, Lyngby DK-2800, Denmark
- c. Department of Biology, University of Padova, Padova 35131, Italy

# Introduction – Anaerobic Digestion

Anaerobic digestion (AD) is a biological process, mediated by different groups of microorganisms, mainly bacteria and archaea, which follow diverse metabolic pathways to produce biogas (40-75% CH<sub>4</sub> and 25-60% CO<sub>2</sub>)



*Kougias & Angelidaki, 2018. Biogas and its opportunities – A review*



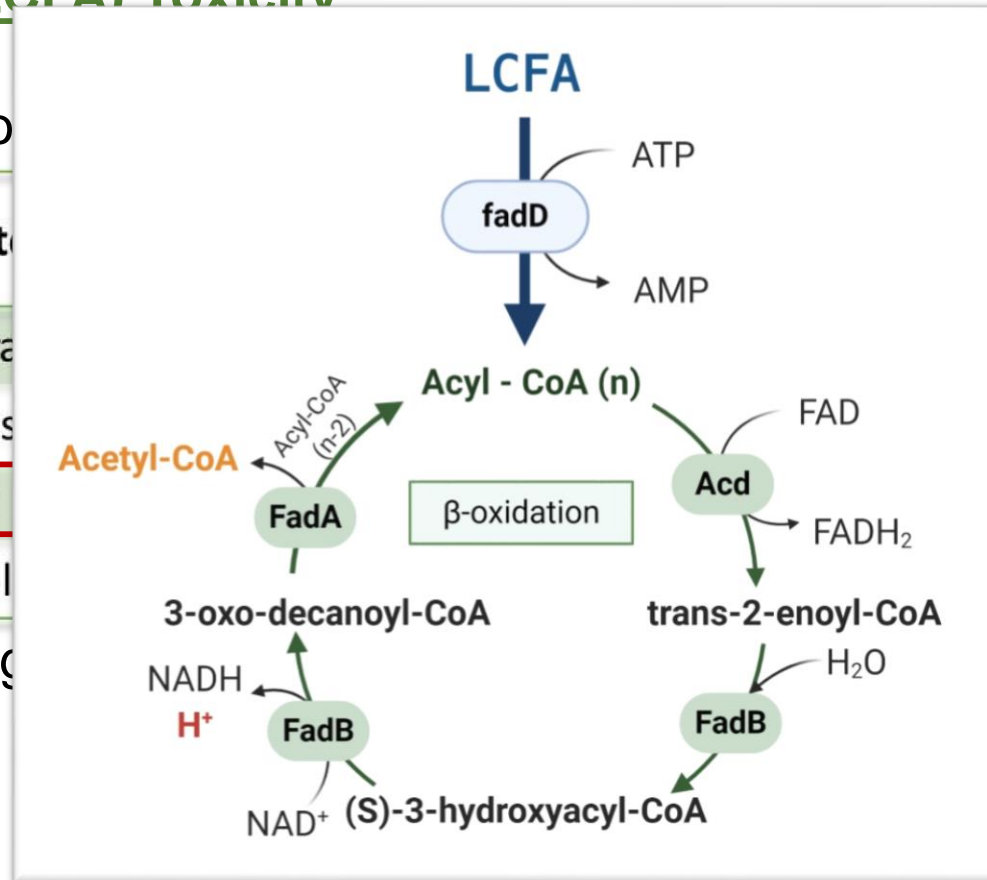
*KEGG, Metabolic Pathways during Anaerobic Digestion*

# Problem Statement

## Long Chain Fatty Acids (LCFA) Toxicity

- Lipids are especially important
- LCFA are
- The anaerobic
- $\beta$ -oxidation
- bacteria are
- The inhibition
- thermodynamically-limiting

Substrates
Carbohydrates
Proteins
Lipids
Ethanol



Composition (%)	Effect
50	cellular lipases
50	released through
70	oxidizing
75	accumulate to
	oxidized

# Aim & Objectives



## Process Monitoring

Replicate reactor operation,  
Biochemical parameters

## Molecular Techniques

Shotgun sequencing,  
Metagenomic analysis

## Bioinformatic Pipeline

Dedicated pipeline for data analyses

## Enlighten responses

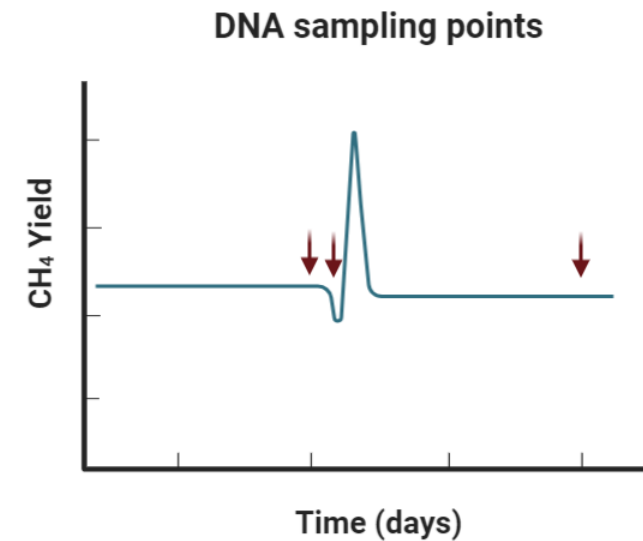
Identify microbes enhancing or constraining efficiency



# Bioreactors' Operation – CSTR Set-Up

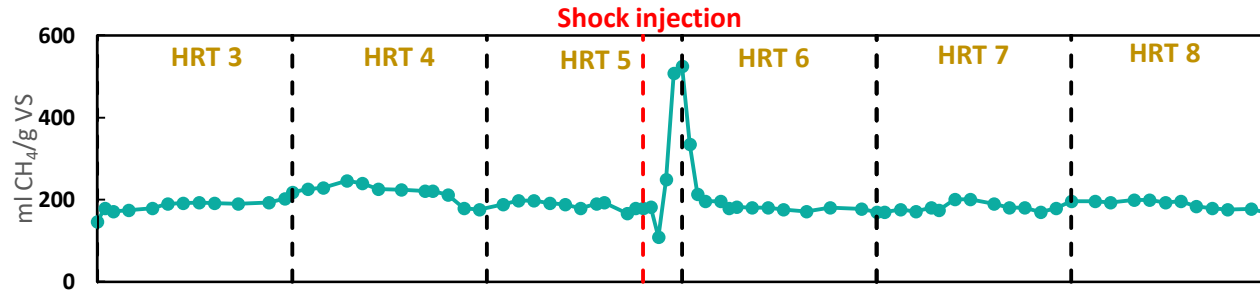


- **3 Reactors – Biological Replicates**
- **Mesophilic Conditions ( $37 \pm 1^\circ\text{C}$ )**
- **Cattle manure as Feedstock**
- **Single inhibitory shock load of 3g Na-Oleate/ $L_R$**
- **3 time points for DNA**

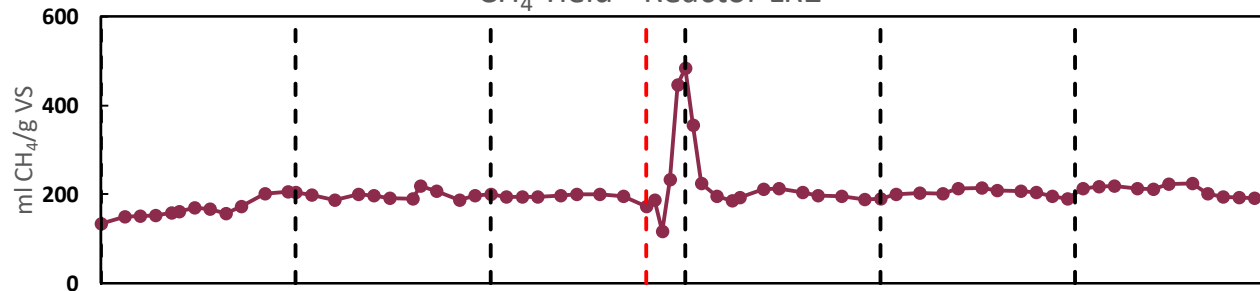


# Biochemical Results

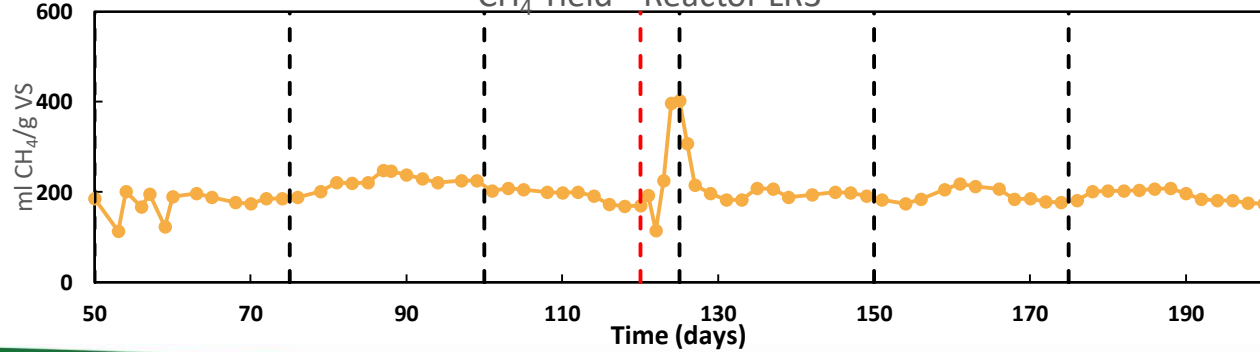
CH<sub>4</sub> Yield - Reactor LR1



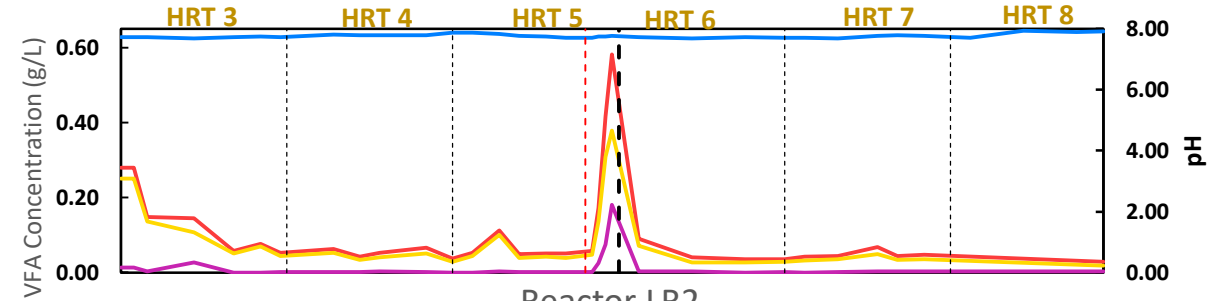
CH<sub>4</sub> Yield - Reactor LR2



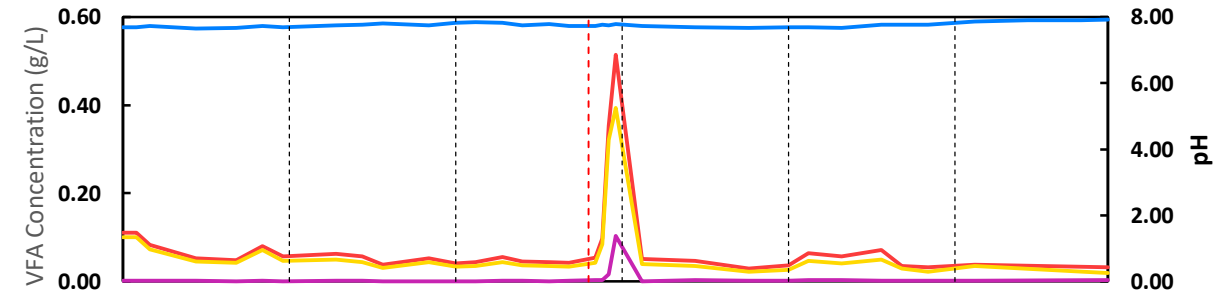
CH<sub>4</sub> Yield - Reactor LR3



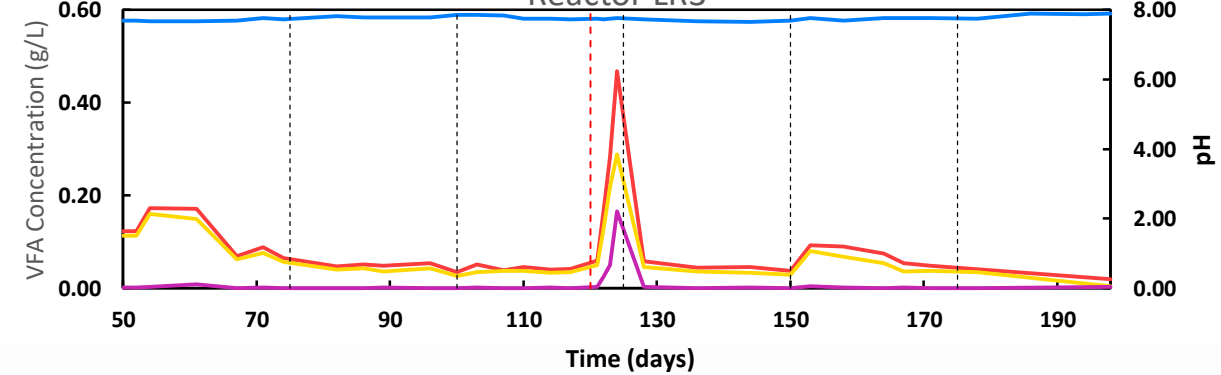
VFA Concentration- Reactor LR1



Reactor LR2

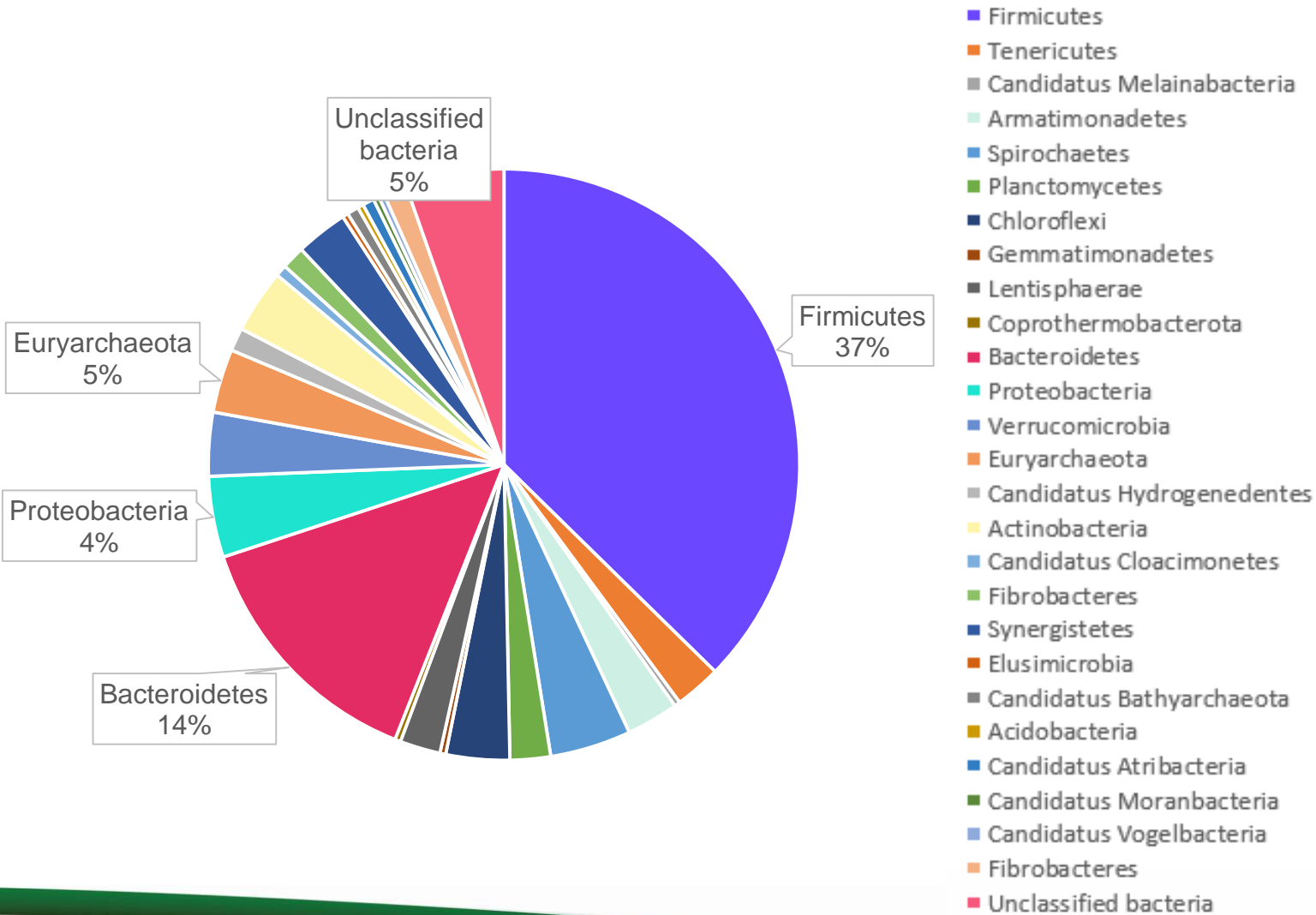


Reactor LR3



— TVFA — Acetic Acid — Propionic acid — pH

# Microbial community overview



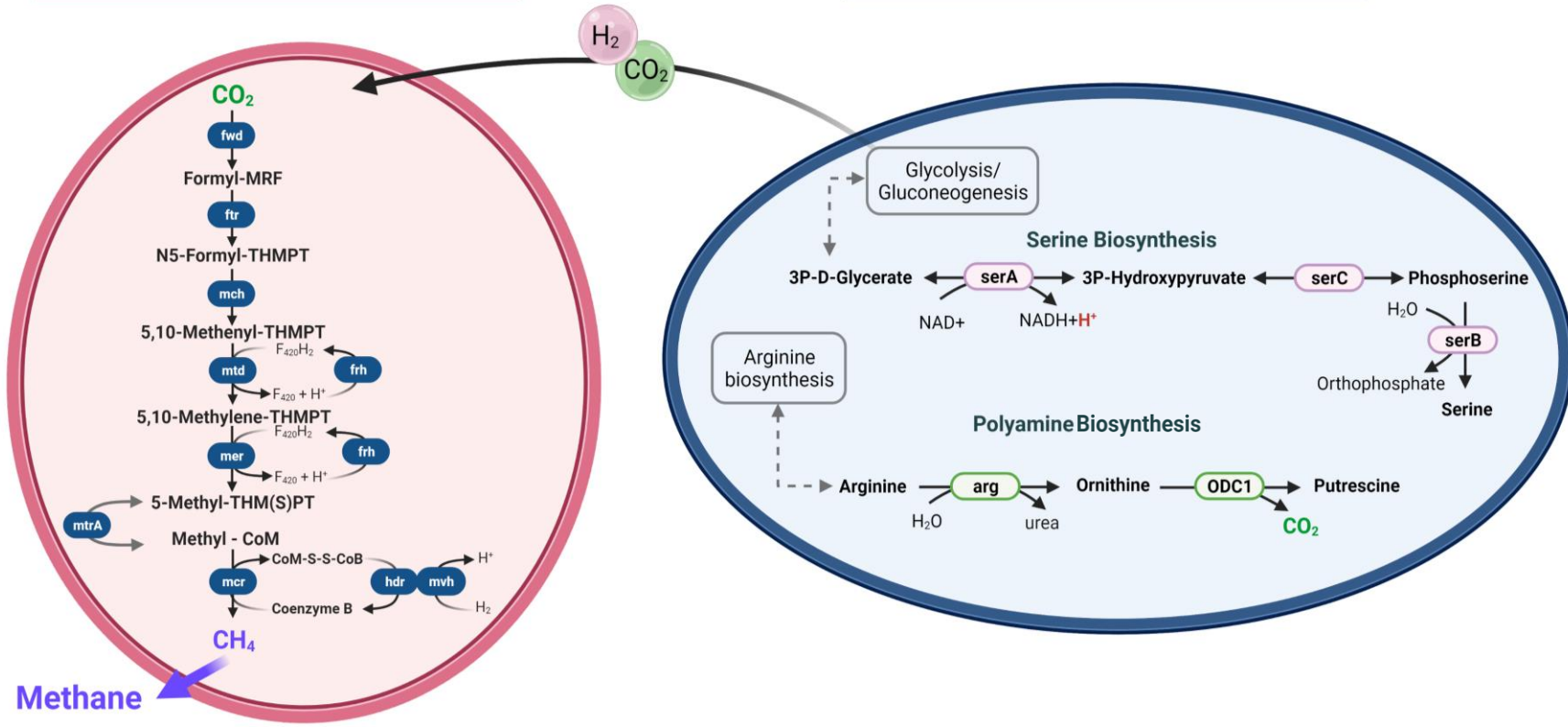
Parameter	Compl. (%)	Cont. (%)	No. MAGs
<b>High Quality</b>	≥90	≤5	<b>214</b>
<b>Medium-High Quality</b>	90>Cp ≥70	<10	<b>102</b>
<b>Medium Quality</b>	70>Cp ≥50	<10	<b>48</b>
<b>Low Quality</b>	<50	≥10	<b>73</b>
<b>TOTAL</b>			<b>437</b>

# Syntrophies between uncharacterized bacteria and archaea

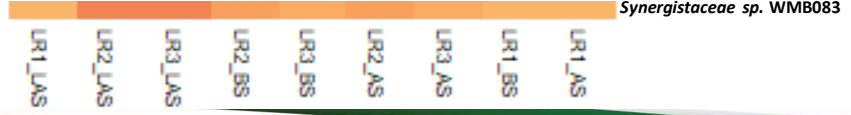


*Methanoculleus bourgensis* WMB155

Candidatus *Cloacimonetes* sp. WMB078



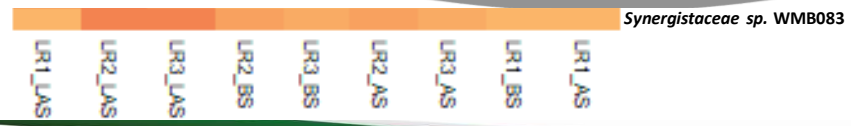
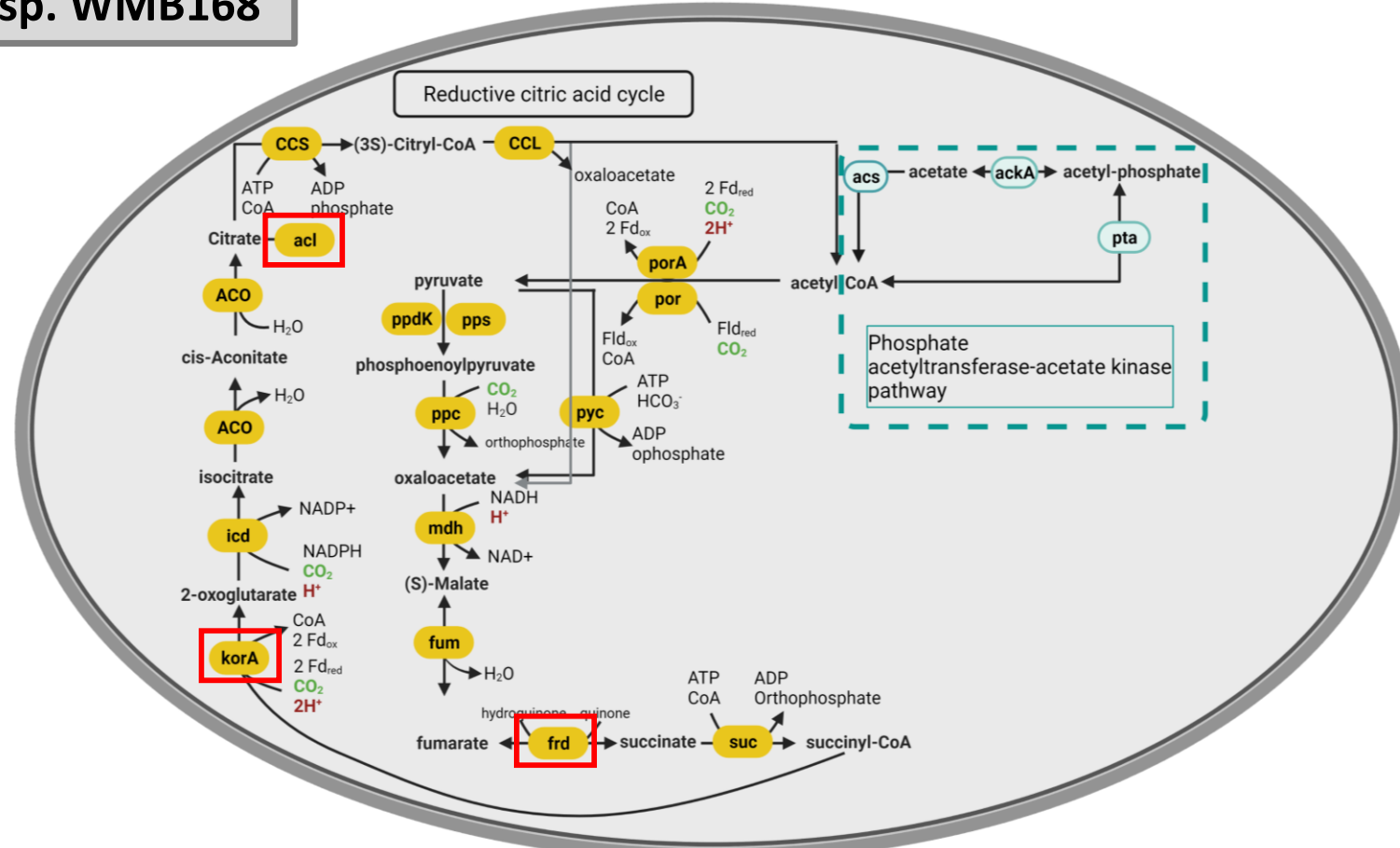
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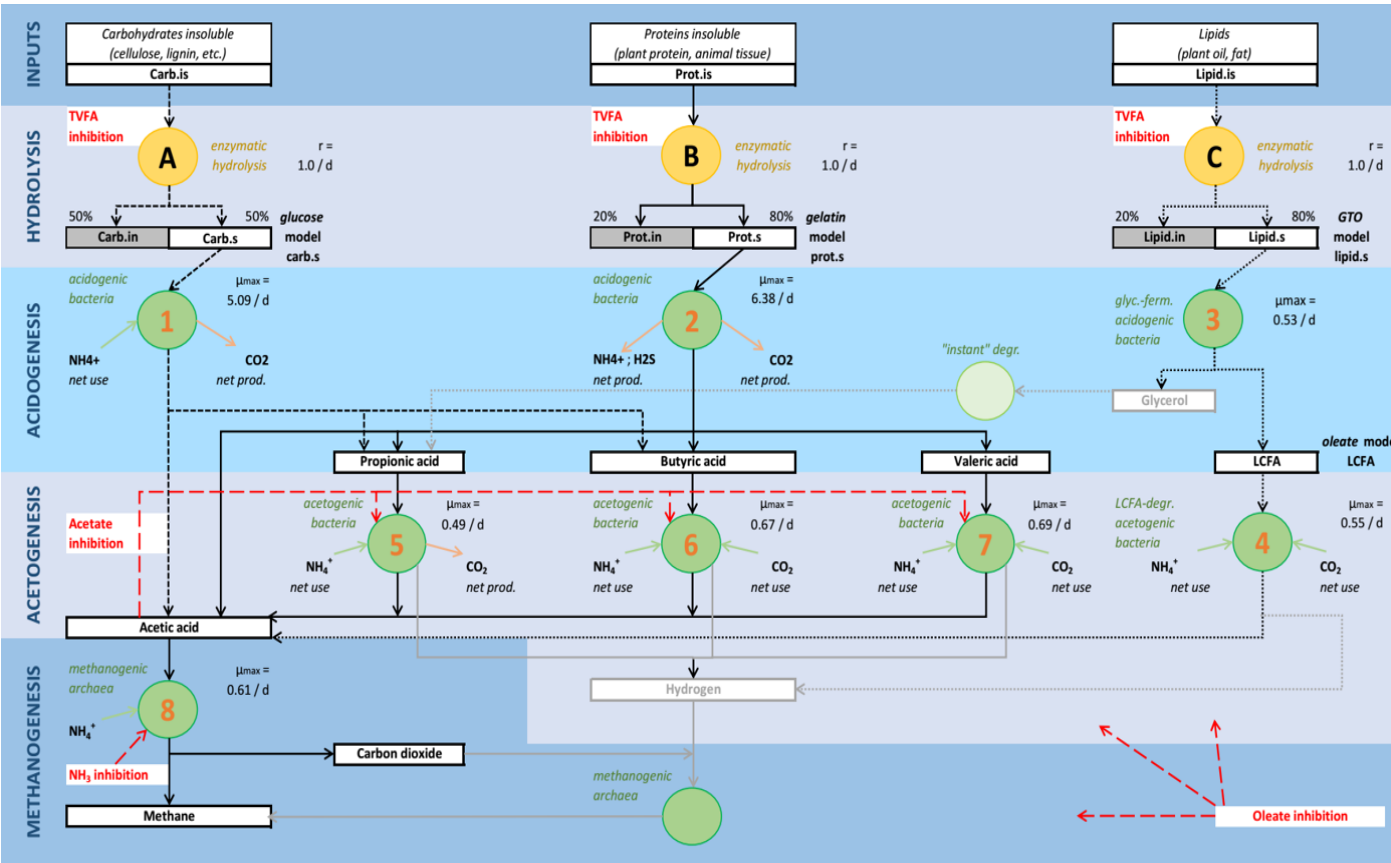


# Novel microbe in Anaerobic Digestion food chain

***Nigerium* sp. WMB168**



# Bioconversion Model – “BioModel”

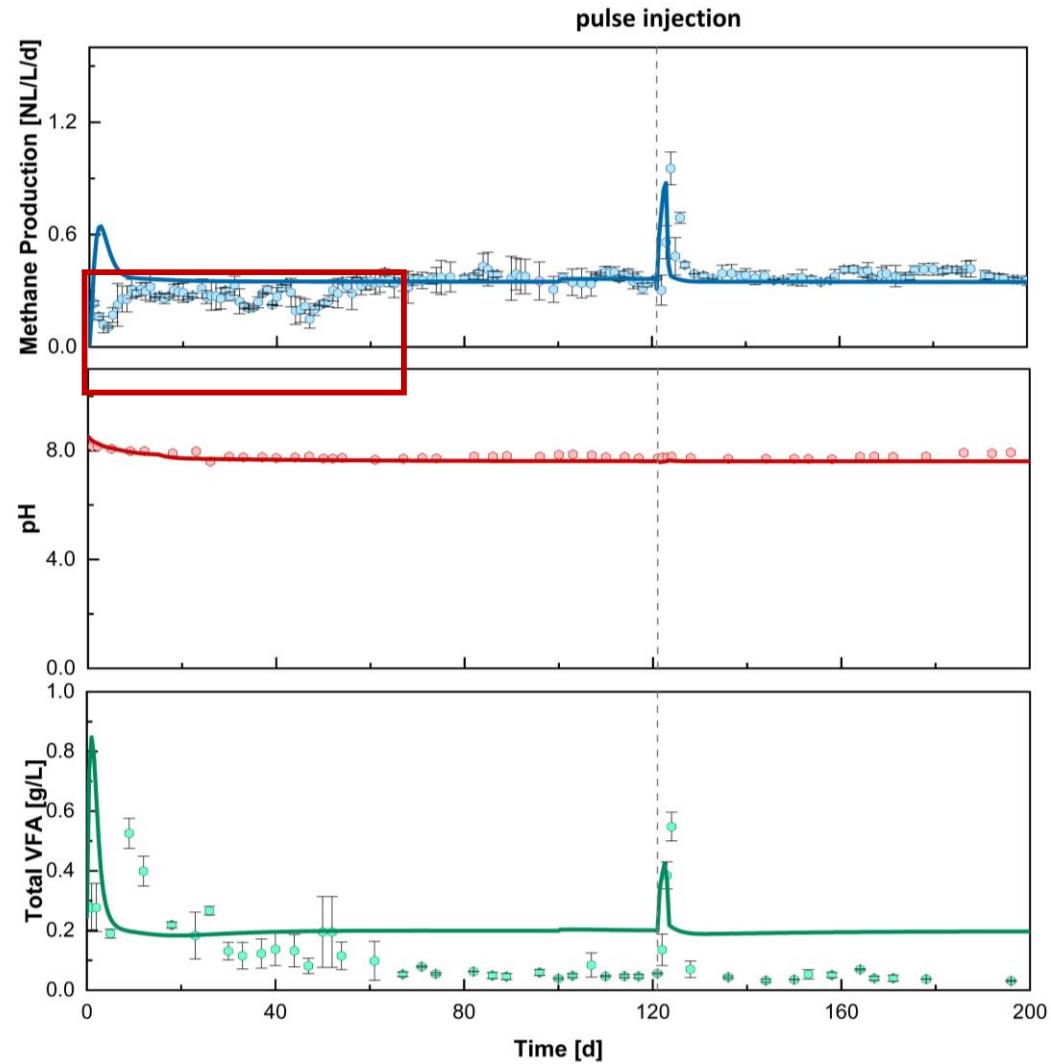


- Anaerobic degradation of complex material, and co-digestion of different types of wastes
- Manure-based anaerobic digestion systems
- Mass-based unit system
- Model includes inhibition from LCFA

STAGE	INHIBITOR	INHIBITION TERM
Acidogenic glucose degradation	LCFA	$\frac{1}{1 + \frac{[LCFA]}{K_{iLCFA}}}$
Lipolysis	LCFA	$\frac{1}{1 + \frac{K_s}{[LCFA]} + \frac{[LCFA]}{K_{iLCFA}}}$
Acetogenic LCFA degradation	LCFA	$\frac{1}{1 + \frac{[LCFA]}{K_{iLCFA}}} \times \frac{1}{1 + \frac{[HAc]}{K_i}}$
Acetogenic VFA degradation	LCFA Acetic Acid	$\frac{1}{1 + \frac{[LCFA]}{K_{iLCFA}}} \times \frac{1}{1 + \frac{[NH_3]}{K_i}}$
Acetoclastic methanogenesis	LCFA Free Ammonia	$\frac{1}{1 + \frac{[LCFA]}{K_{iLCFA}}} \times \frac{1}{1 + \frac{[NH_3]}{K_i}}$

Tsapekos et al., 2018. Energy recovery from wastewater microalgae through anaerobic digestion process: Methane potential, continuous reactor operation and modelling aspects

# Simulations



\*Points indicate the experimental data and continuous lines indicate the BioModel simulation. Dashed vertical line mark the shock load injection. Note: Each point is the mean value of the three reactors, therefore there is a standard deviation.

# Conclusions

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- Syntrophic interactions between uncharacterized bacteria and archaea was recorded.
- Strong evidences for alternative pathways for carbon fixation (rTCA) from novel bacteria.
- BioModel was capable to predict the process indicators “methane” and “pH” during lipid load.
- BioModel underestimated the VFA degradation but forecasted the alternation trend over time.



# Acknowledgement

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This project has received funding from the Hellenic Foundation for Research and Innovation (HFRI) and the General Secretariat for Research and Innovation (GSRI), under grant agreement No580



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REGION OF CENTRAL MACEDONIA  
MANAGING AUTHORITY  
O.P. Region of Central Macedonia



**Thank you for your attention!**

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@WMBlab



@wmb\_lab



Themi-Thessaloniki, GR