

From Microbial Ecology to Circular Economy

What are Future Challenges?

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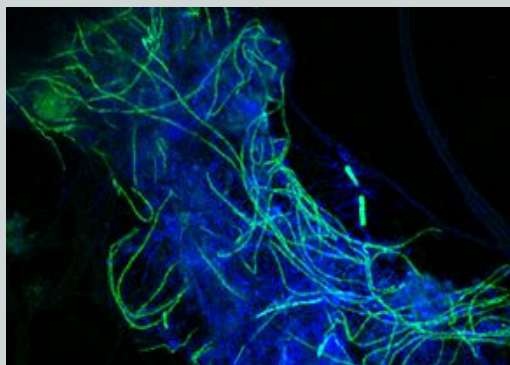


Research institute in different Biology topics:
Neuroscience, Ecology, Microbiology and Genetics



Microbial Ecology Laboratory

Research Topics



Microbial Ecology of
wastewater
treatment systems

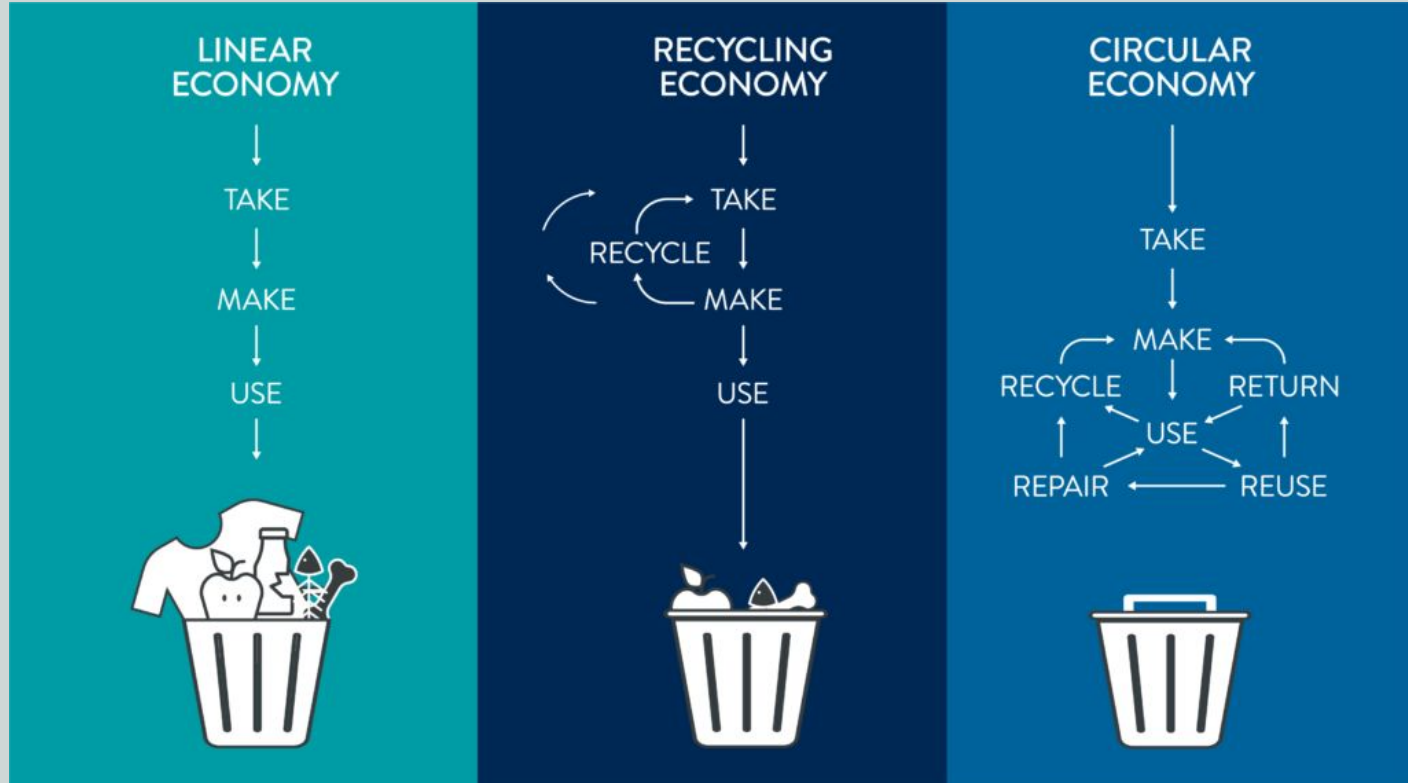


Valuable products
recovery from
wastewater

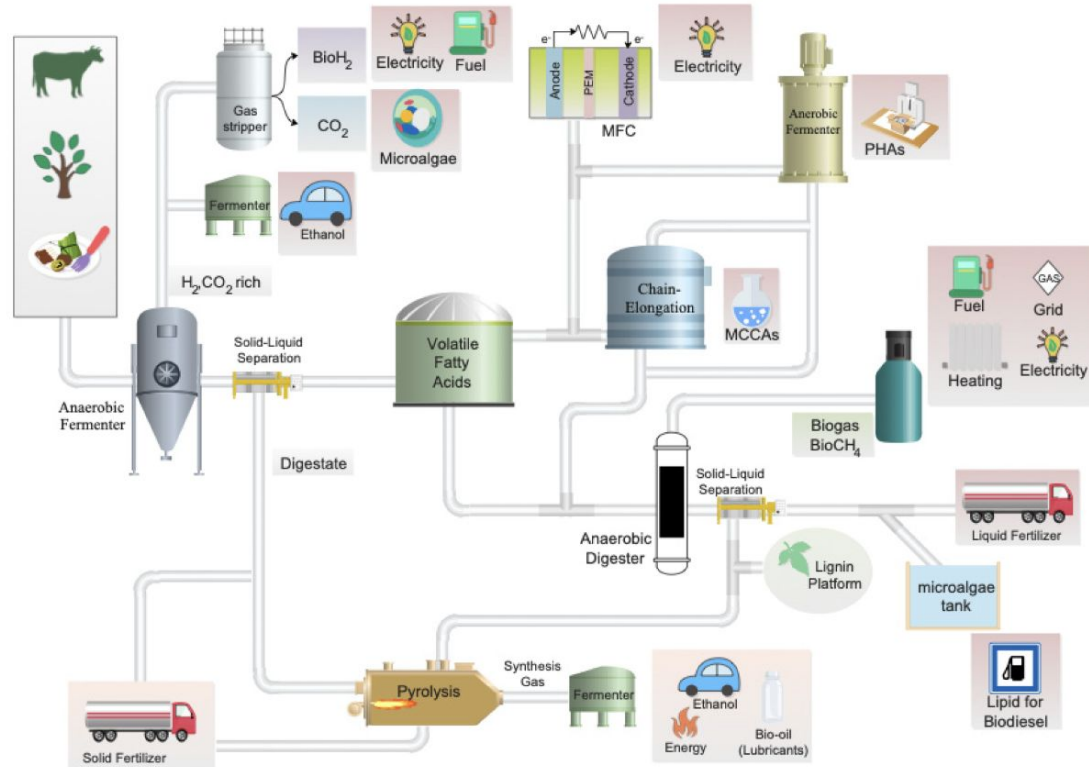


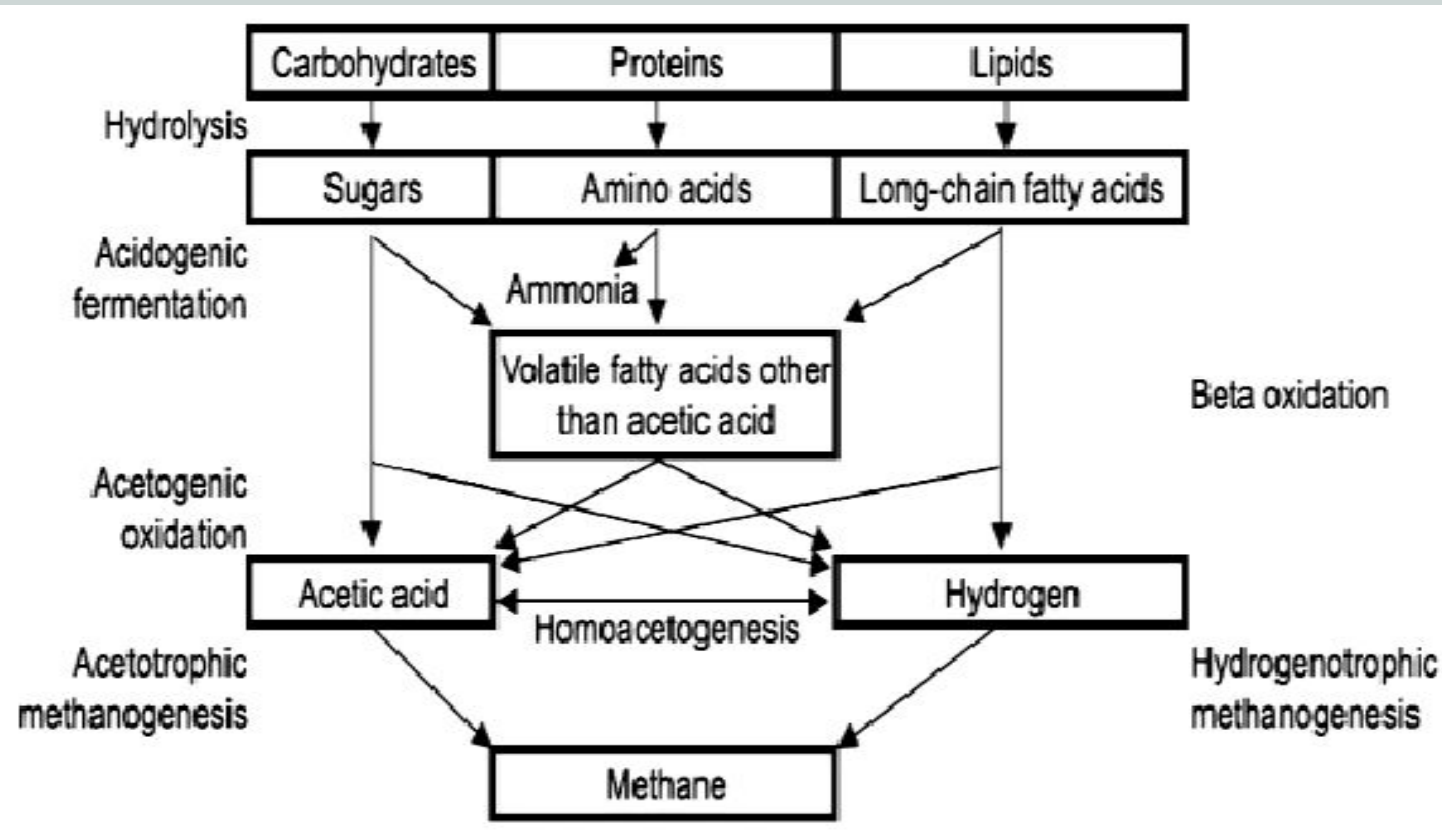
Greenhouse gases
emissions in cold
environments

Moving from linear economy to circular economy

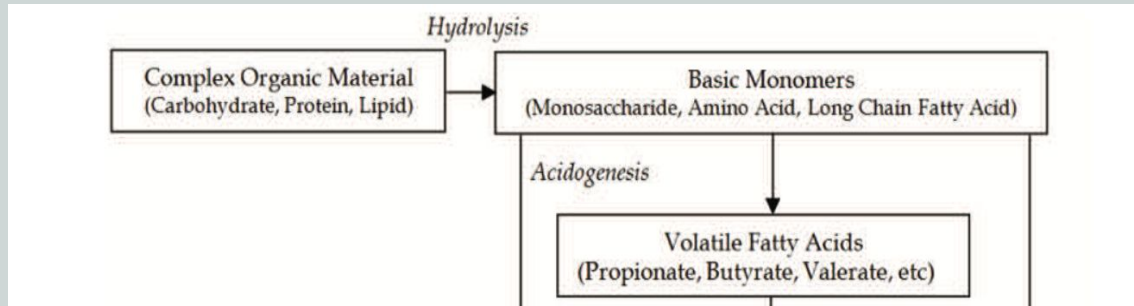


Anaerobic digestion biorefinery





Break the chain

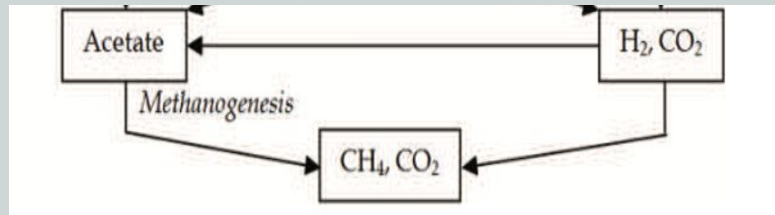


bioH₂

Organic acids

Bioplastics

Medium chain organic acids



CH₄ upgrade

CO₂ capture
to produce
acetate

Challenges



How can we select the microorganisms that carry out the process?

How can we prevent competing microorganisms?

How can we maintain the stability of the process?

Workflow

Know the
microorganisms

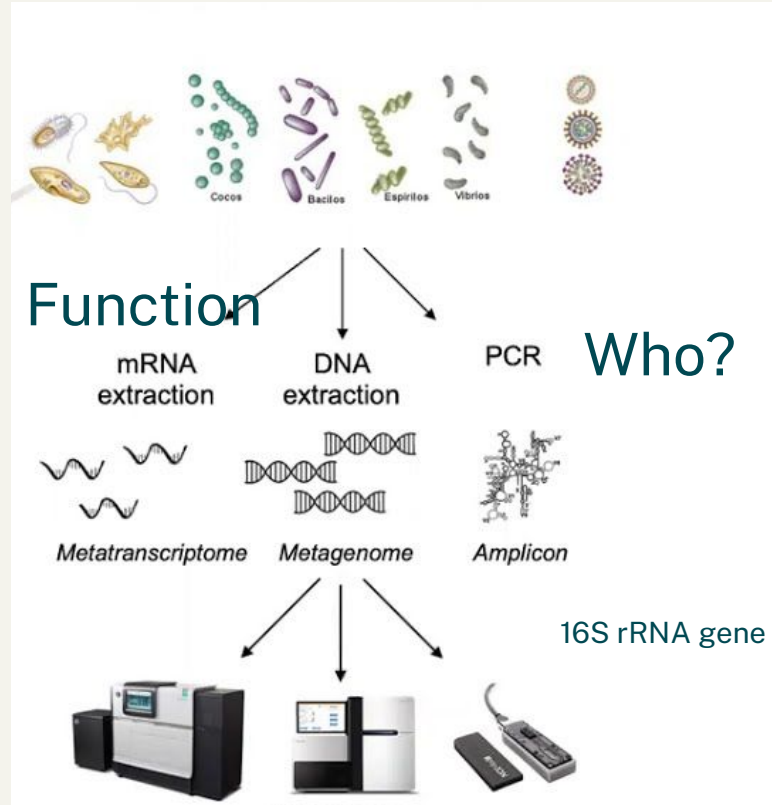
Lab scale
bioreactors



Predict their
function

Optimal conditions for the
selection of appropriate
microorganisms

Sequencing methods to study microbial communities



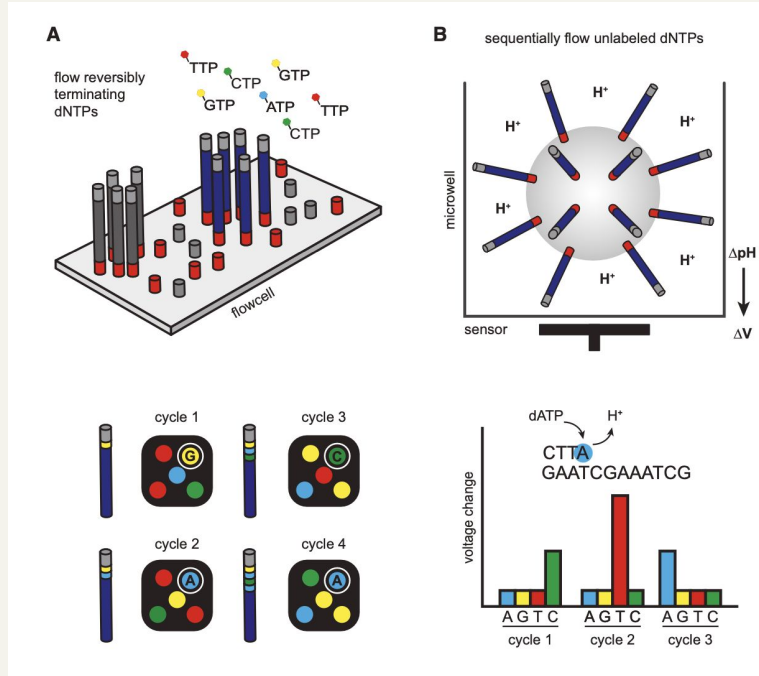
Sequencing methods

Illumina

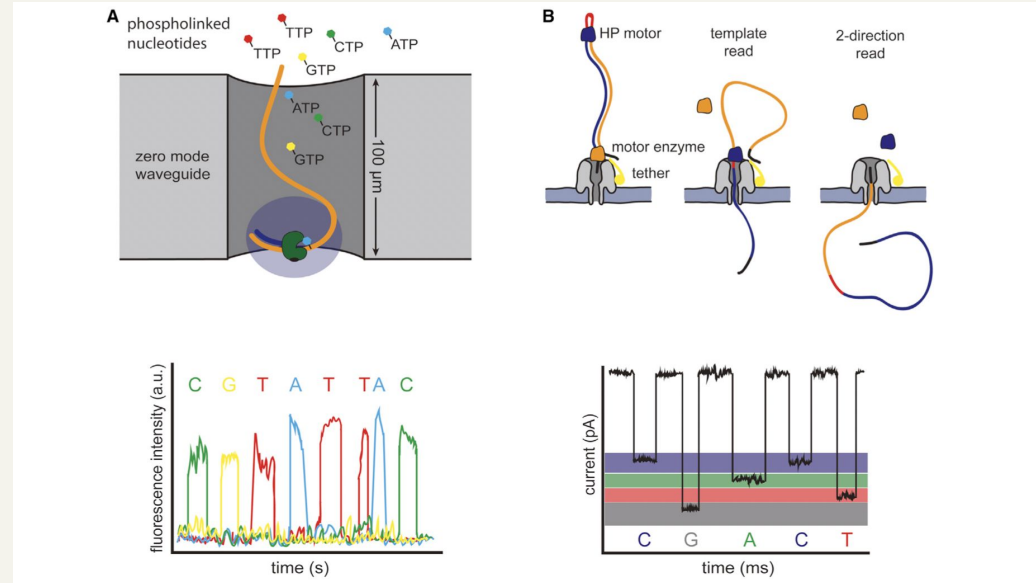
Ion Torrent

Pac.Bio.

Nanopore



Short reads

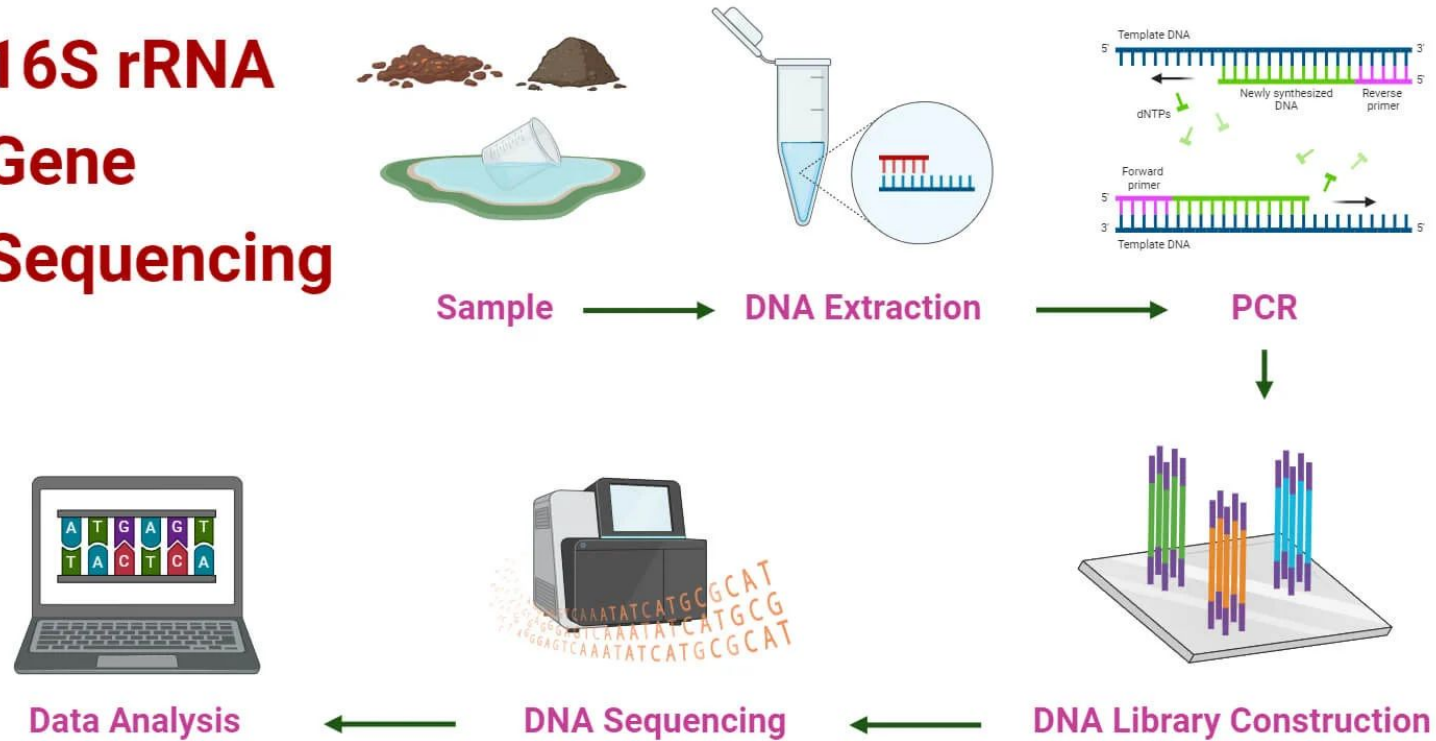


Long reads.

Third generation methods

Reuter et al., 2015¹¹

16S rRNA Gene Sequencing



Databases



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Release information: SILVA 138 SSU

Release information of the SILVA SSU database 138 as of December 16, 2019

	SSU 138	LSU	
Parc	9,469,656	(+ 3,396,475)	tbd
Ref	2,225,272	(+ 134,604)	tbd
Ref NR 99	510,984	(- 184,187)	tbd

Since release 115, for SSU only SSU Ref NR 99 contains a guide tree.

Former statistics:

- [SILVA 95](#), → [SILVA 96](#), → [SILVA 98](#), → [SILVA 100](#), → [SILVA 102](#),
- [SILVA 104](#), → [SILVA 106](#), → [SILVA 108](#), → [SILVA 111](#), → [SILVA 115](#),
- [SILVA 119](#), → [SILVA 123](#), → [SILVA 128](#), → [SILVA 132](#)

Statistics

SILVA Ref NR Changes (reduced size)

The SILVA 138 SSU Ref NR 99 contains fewer sequences compared to previous releases.

We decided to switch from an old version of UCLUST (1.0.50; reference to the UCLUST implementation in [USEARCH](#)) to a recent version of [VSEARCH](#) (2.13) which increases clustering performance and will, hopefully, allow us to provide a more stable Ref NR (in terms of reference sequences) in the future.

The reduced size of the Ref NR is caused by the two tools using a different definition of sequence/alignment identity. We cross-checked the clustering results by using a third tool ([CD-HIT](#) v4.8). Given the same identity threshold, VSEARCH (using USEARCH's as well as using CD-HIT's definition of sequence/alignment identity) and CD-HIT report similar clusters whereas the old version of UCLUST reports

ARNr de 16S all
microorganisms

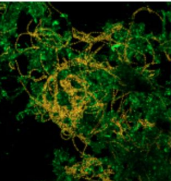
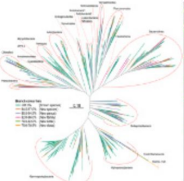
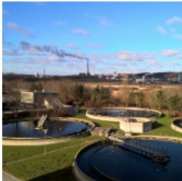


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[Global Abundance Activated Sludge](#) [Global Abundance Anaerobic Digesters](#) [Contact us](#)

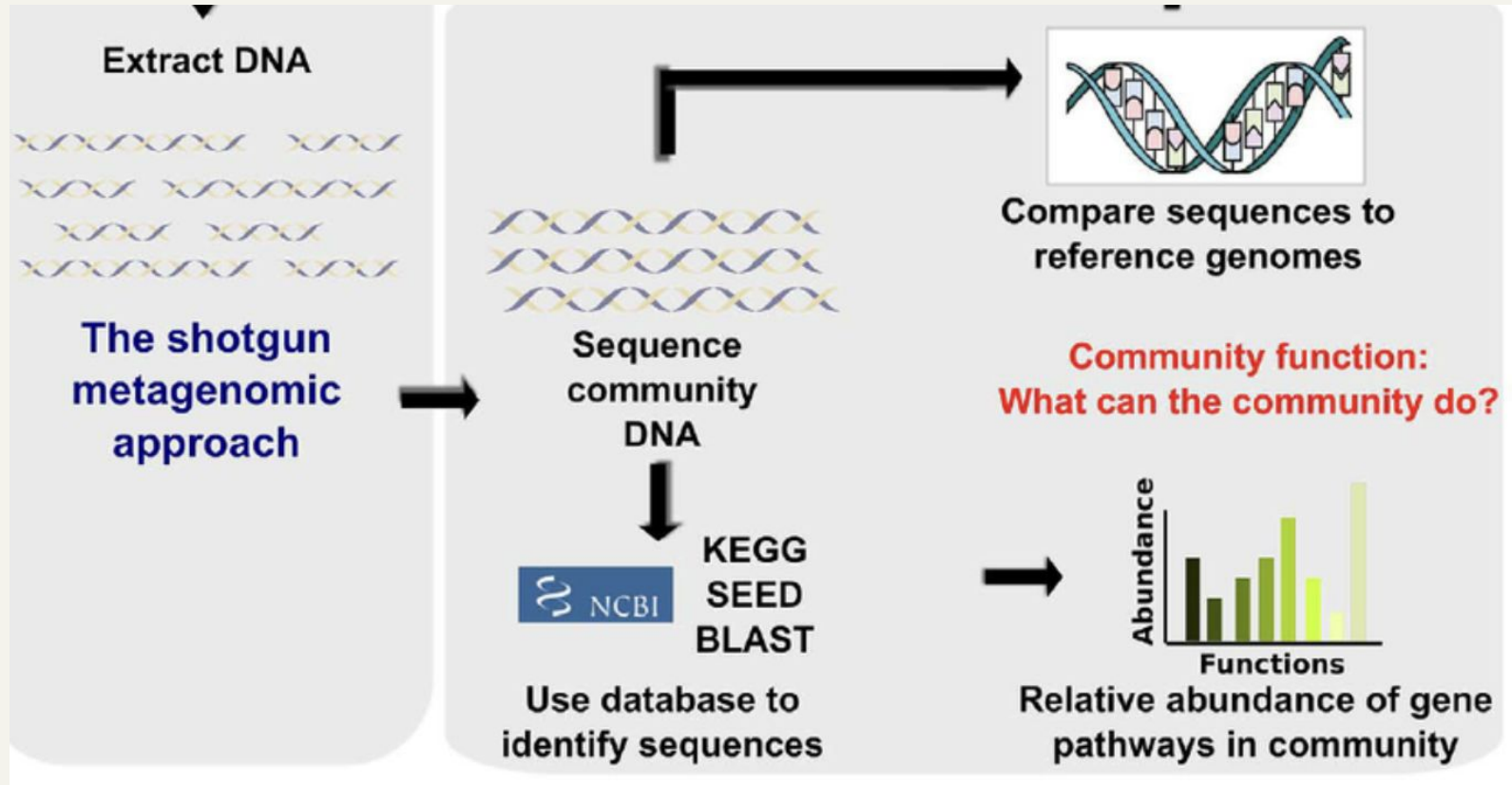
Field guide

MiDAS: Field Guide to the Microbes of Activated Sludge and Anaerobic Digesters

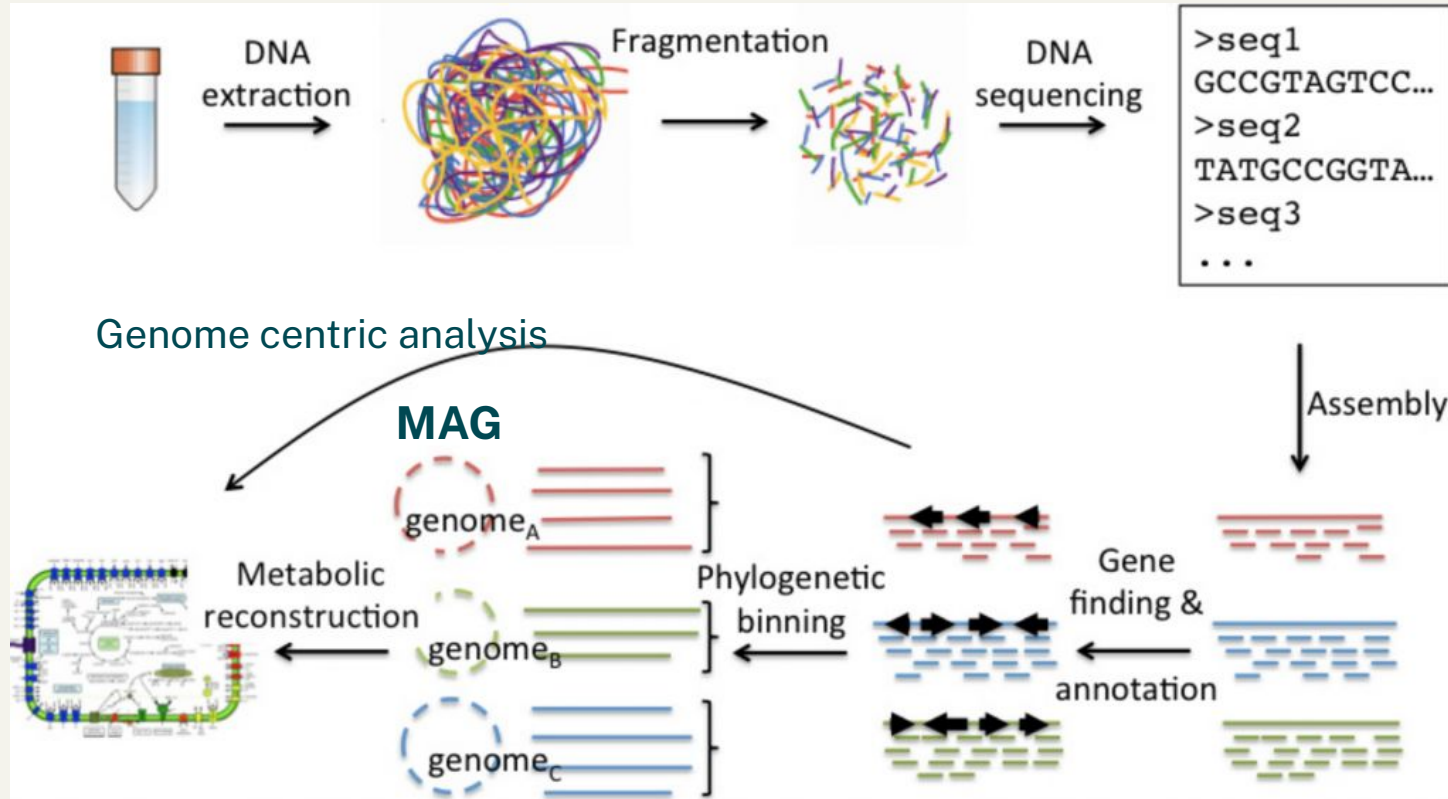


ARNr de 16S from wastewater
treatment plants

Metagenomic for function



Assembled genomes from metagenomic data



Sequencing strategies

16S rRNA gene

- Select primers
- Depth (reads by sample)
- Sequencing platform and reads length (Illumina, Ion Torrent, Mini-Ion)
- Database

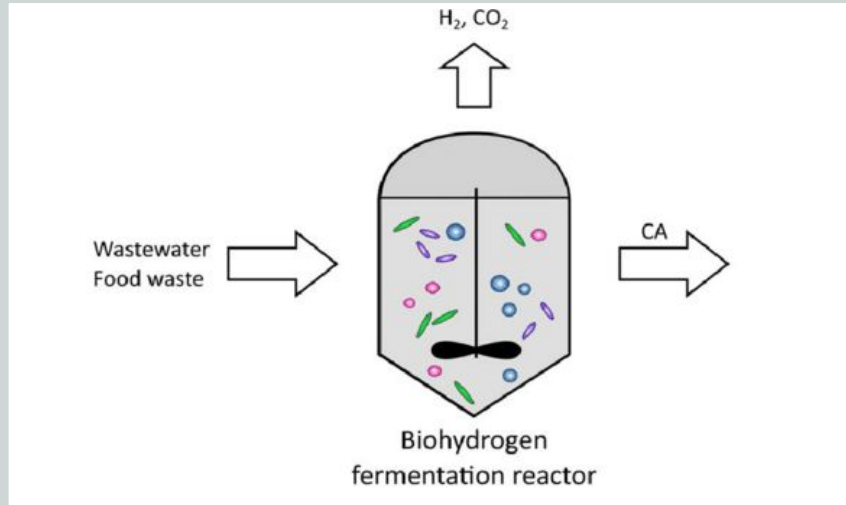
Metagenomic

- Replicates
- Depth and coverage
- Amount of DNA and quality
- Platform (Illumina)
- Analysis (functional, MAGs, taxonomy)

Metatranscriptomic

- Triplicates
- Depth and coverage
- Amount of RNA and quality
- Platform (Illumina)
- Analysis (functional, MAGs expression)

Examples



Valuable products recovery

- Biohydrogen production
- VFA/MCCA production

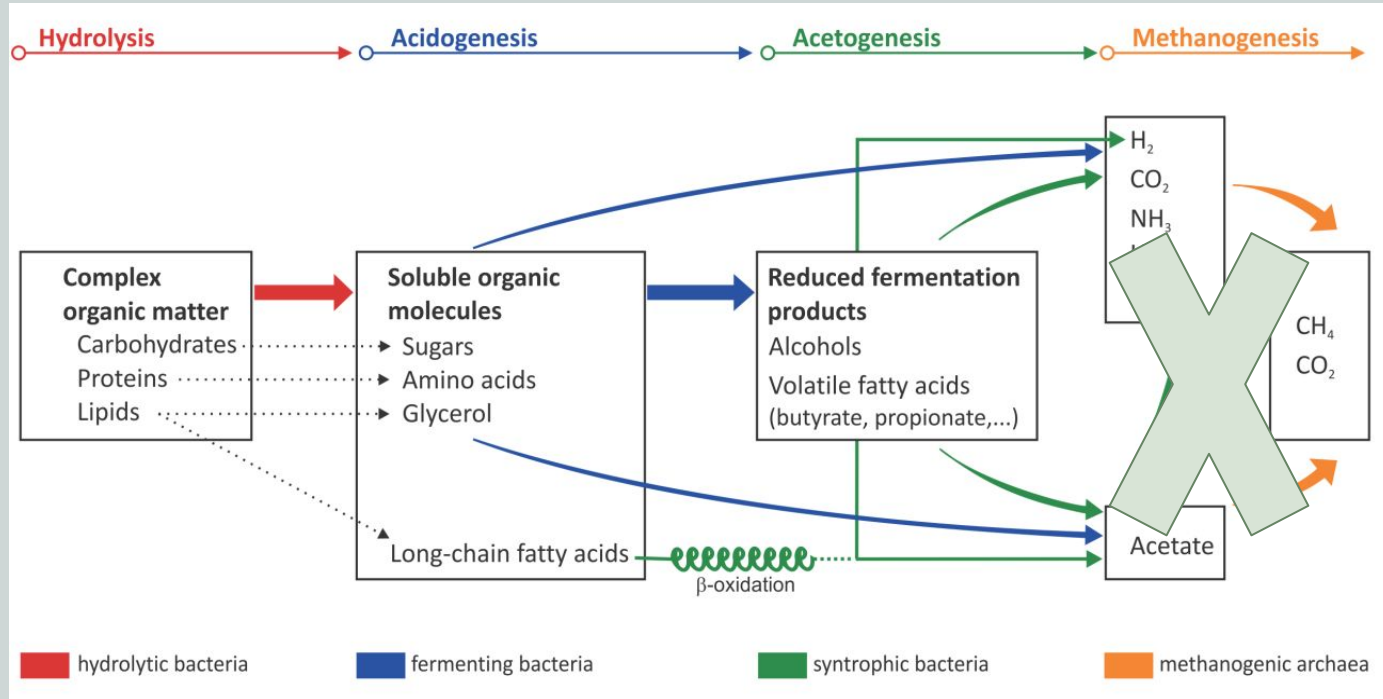


Microbiology of full scale methanogenic reactors

Unknown microorganisms: Chloroflexota

Verrucomicrobia

Biohydrogen production by dark fermentation



Microorganisms involved in H₂ production

BioH₂ producing microorganisms

Firmicutes

Clostridium

Ethanoligenens

Thermoanaerobacter

Delta Proteobacteria

Enterobacteriaceae

Thermotogales

Thermotoga

Kosmotoga

H₂ consumers

Methanogens

(hydrogenotrophic)

Homoacetogens

Competitors

Lactic acid bacteria

Propionic producing
bacteria

Workflow

Know the
microorganisms

Lab scale
bioreactors



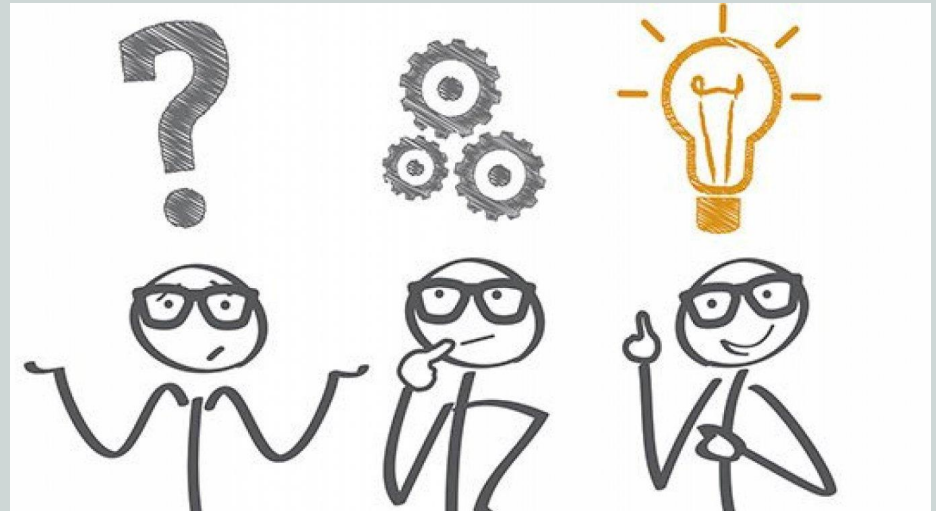
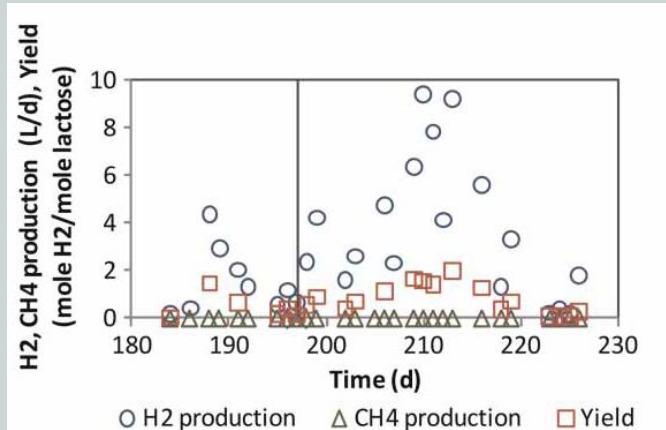
Predict their
function

Optimal conditions for the
selection of appropriate
microorganisms

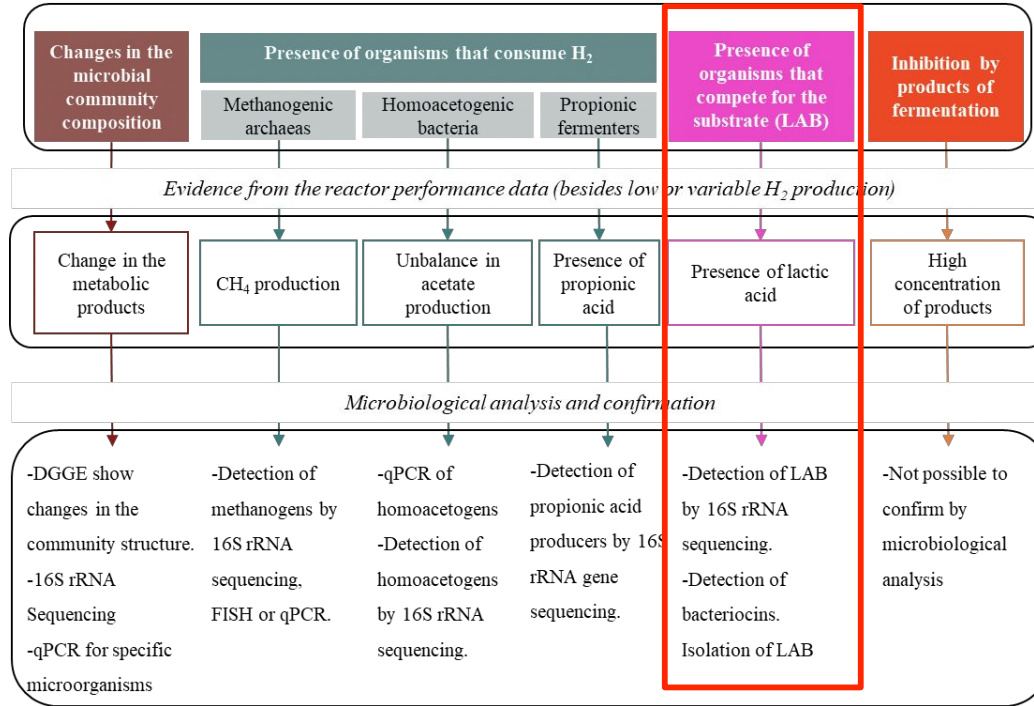
Problems

Low yields

Instability



Problems associated to the instability of H_2 production



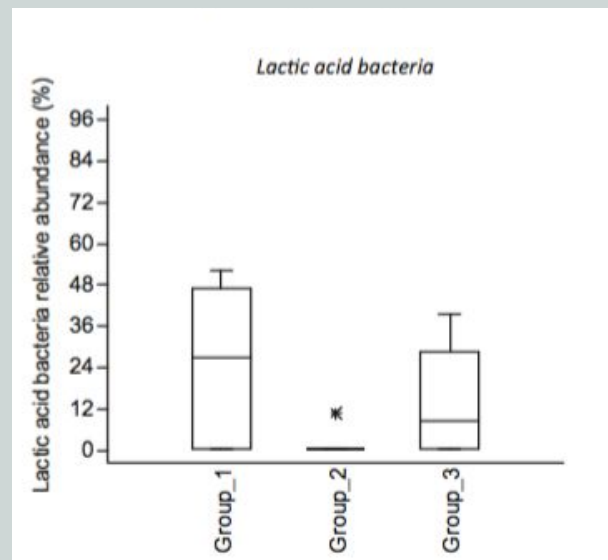
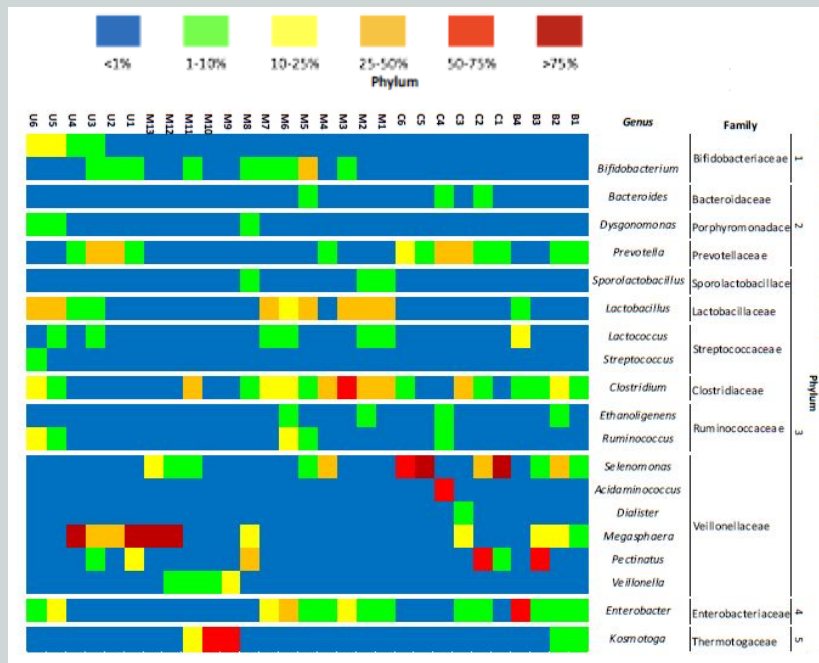
Stability problems in the hydrogen production by dark fermentation: Possible causes and solutions

Elena Castelló^{a,*}, Antonio Djalma Nunes Ferraz-Junior^b, Cristiane Andreani^c, Melida del Pilar Anzola-Rojas^d, Liliana Borzacconi^a, Germán Buitrón^e, Julián Carrillo-Reyes^e, Simone Damasceno Gomes^c, Sandra I. Maintinguer^{f,g}, Iván Moreno-Andrade^e, Rodolfo Palomo-Briones^h, Elías Razo-Flores^h, María Schiappacasse-Dasatiⁱ, Estela Tapia-Venegas^j, Ivania Valdez-Vazquez^c, Alejandra Vesga-Baron^l, Marcelo Zaiat^d, Claudia Etchebehere^b



Castelló et al., 2020

Microbial communities from 20 H₂-producing reactors using 16S rRNA genes pyrosequencing

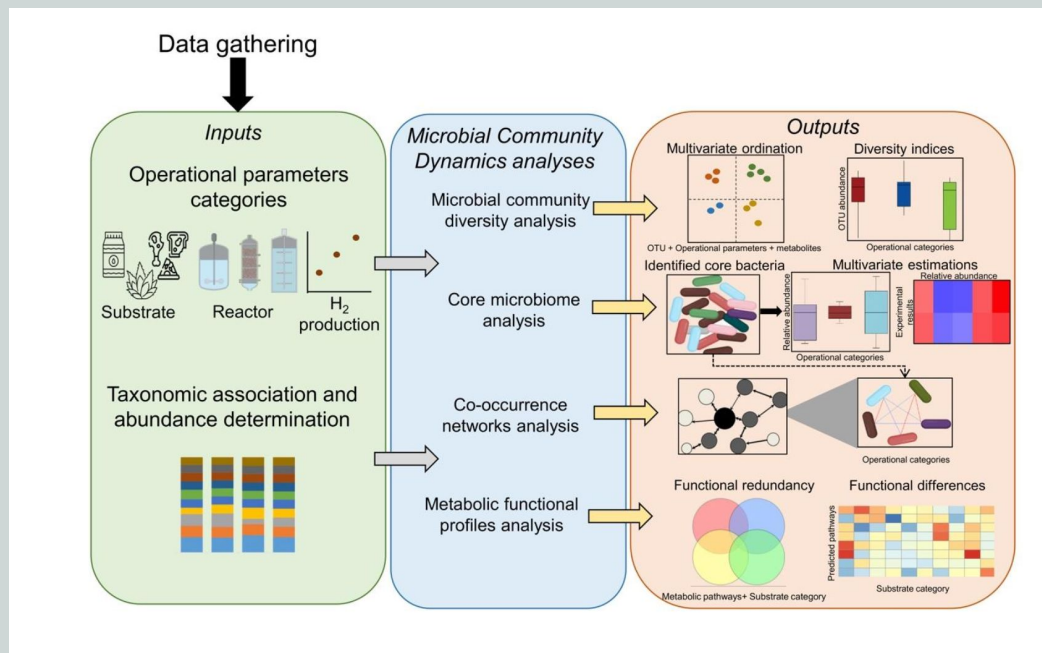


Group 1 samples with high production of H₂

Group 2 samples with medium production of H₂

Group 3 samples with low production of H₂

Meta analysis based in 16S rRNA gene sequences deposited in the databases

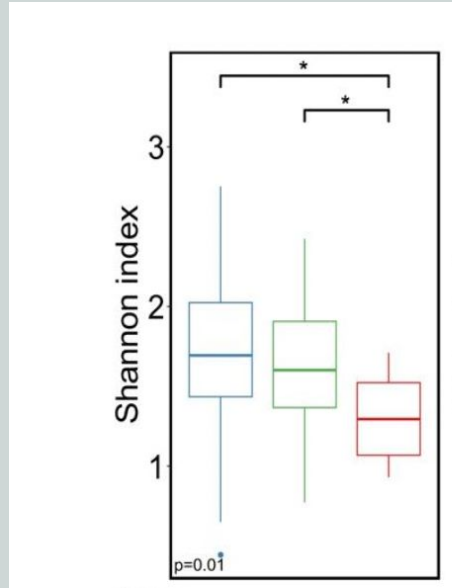


13 studies, 121 samples from H₂ producing bioreactors.

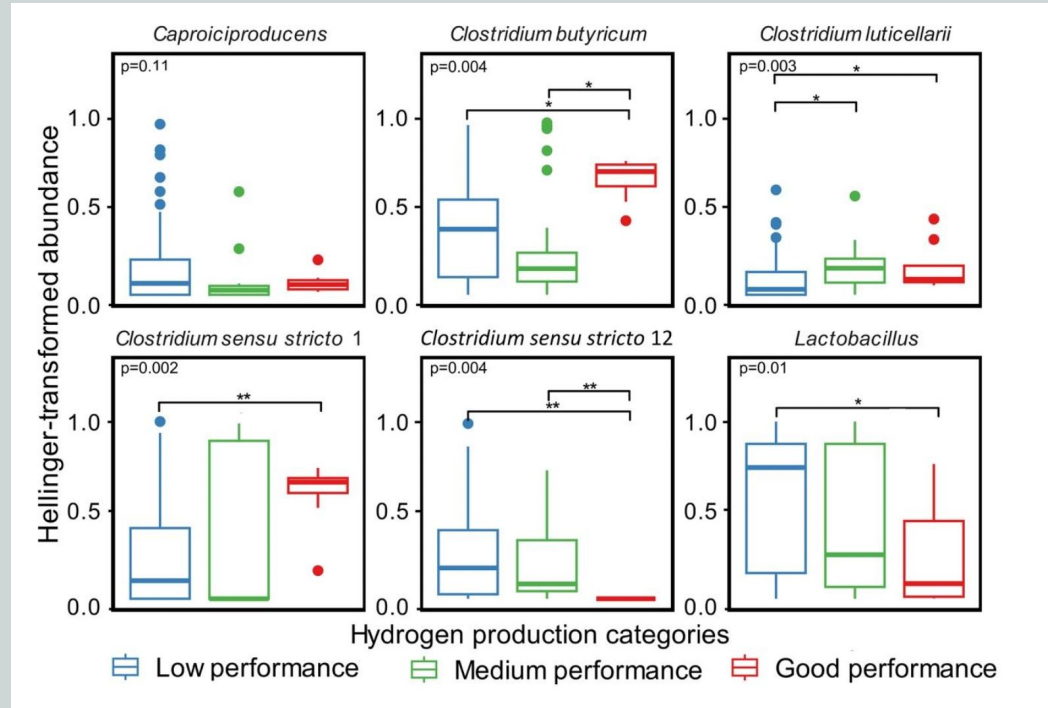
16S rRNA gene sequences deposited in the databases and metadata associated.

Different statistical analysis

Main results



Low diversity in reactors with good performance



Co dominance of Lactobacillus and Clostridium

Lactic acid bacteria in bioH2 reactors

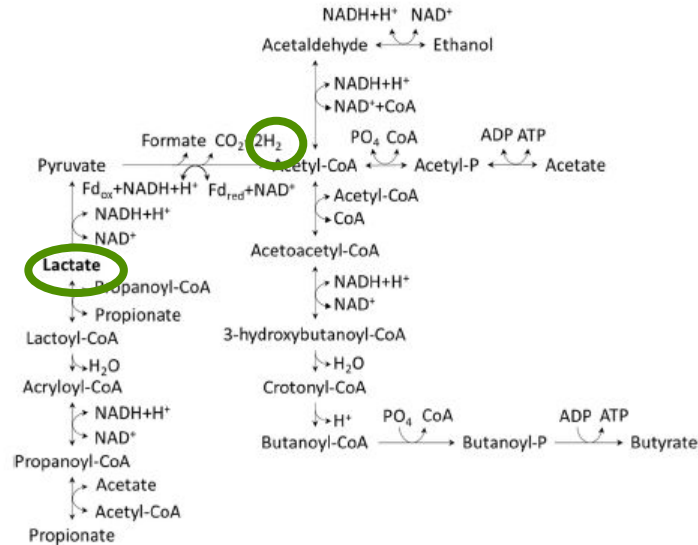


Fig. 1 – Metabolic pathway for Lactate fermentation for strict anaerobes as adapted from Refs. [29,33,34].

Chapter 21

Lactic Acid Bacteria in Hydrogen-Producing Consortia: On Purpose or by Coincidence?

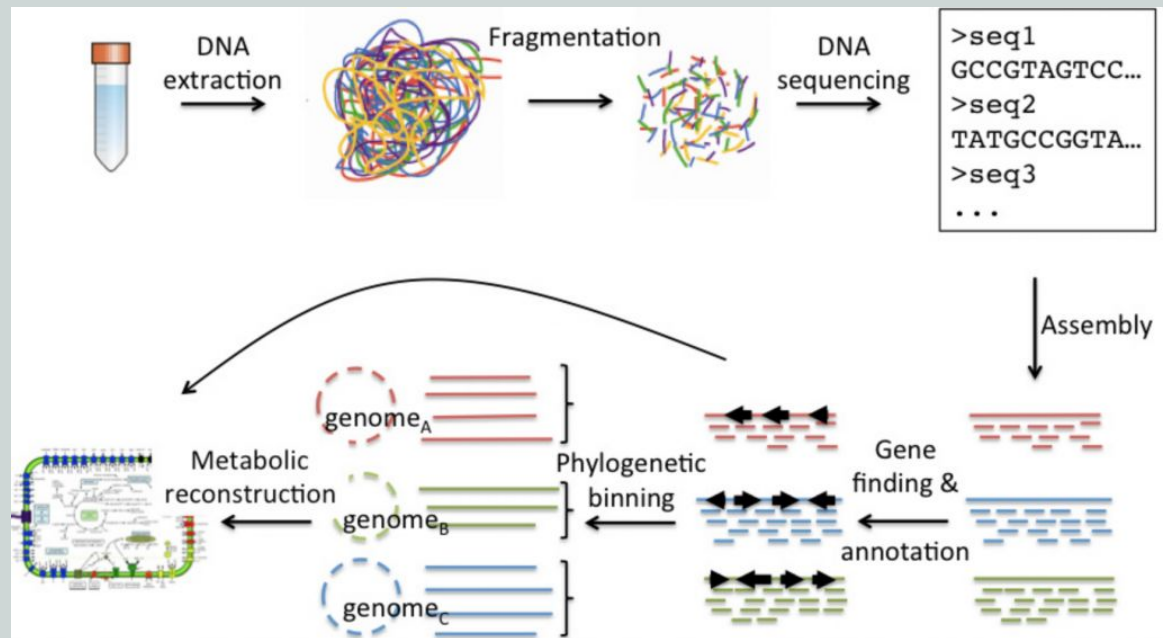
Anna Sikora, Mieczysław Błaszczyk,
Marcin Jurkowski and Urszula Zielenkiewicz

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/50364>

Metabolic pathways to produce hydrogen consuming lactic acid.

Metagenomic approach to bio-H₂ reactors

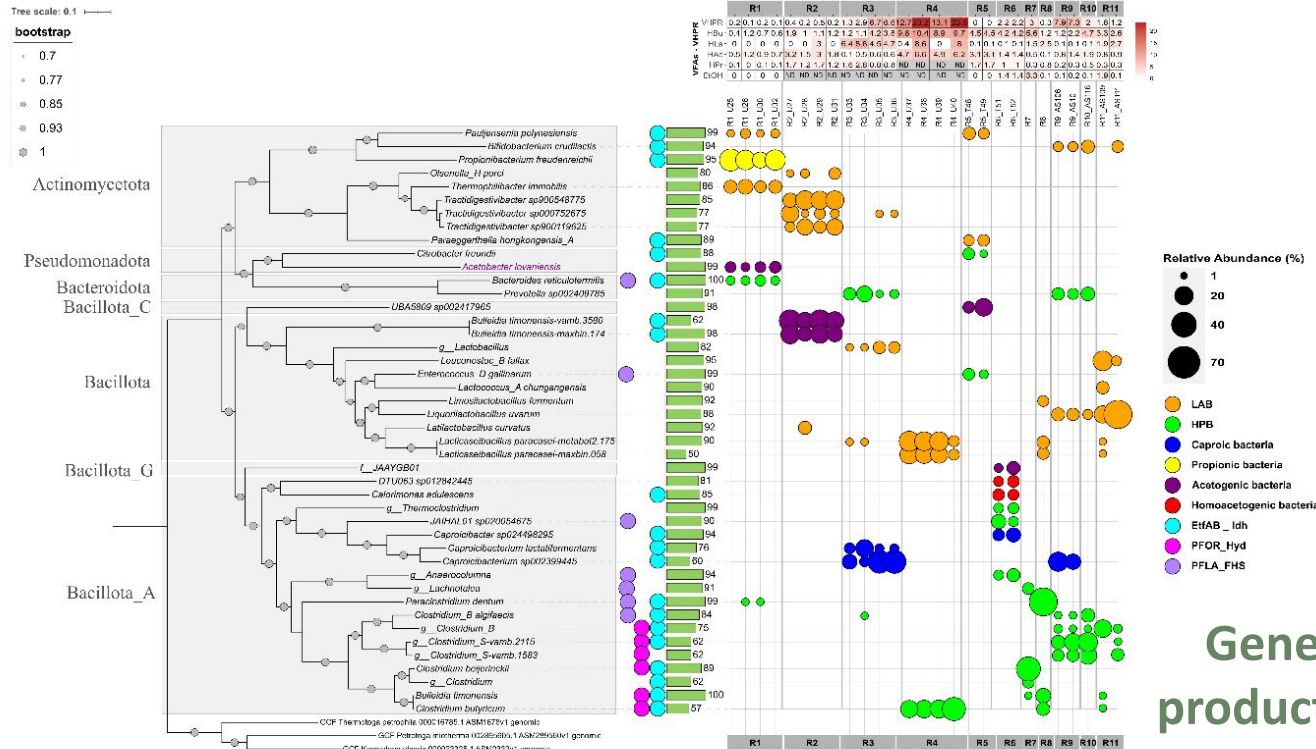


11 lab-scale reactors
producing H₂
Operated in Brazil and
Mexico.

27 samples



Results



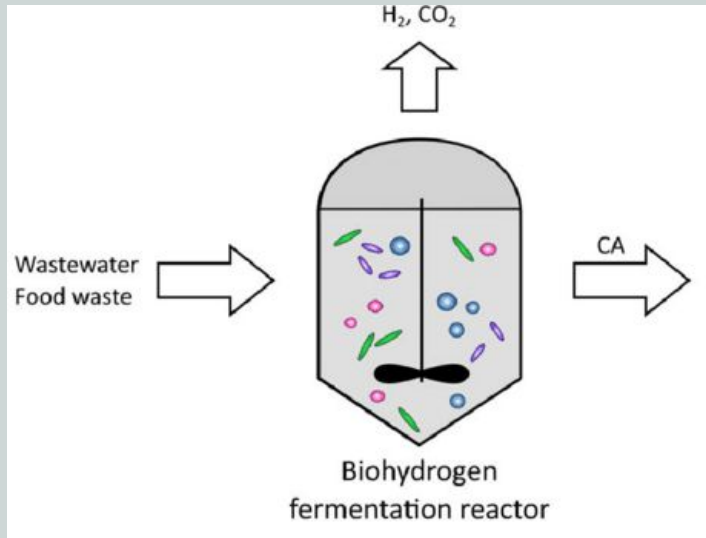
263 MAGs were assembled

Phylogenomic tree

Metabolic pathways reconstruction

Genes for H2 production using lactic acid

Lessons from bioH2 reactors microbiology studies



Low diversity, stability problems

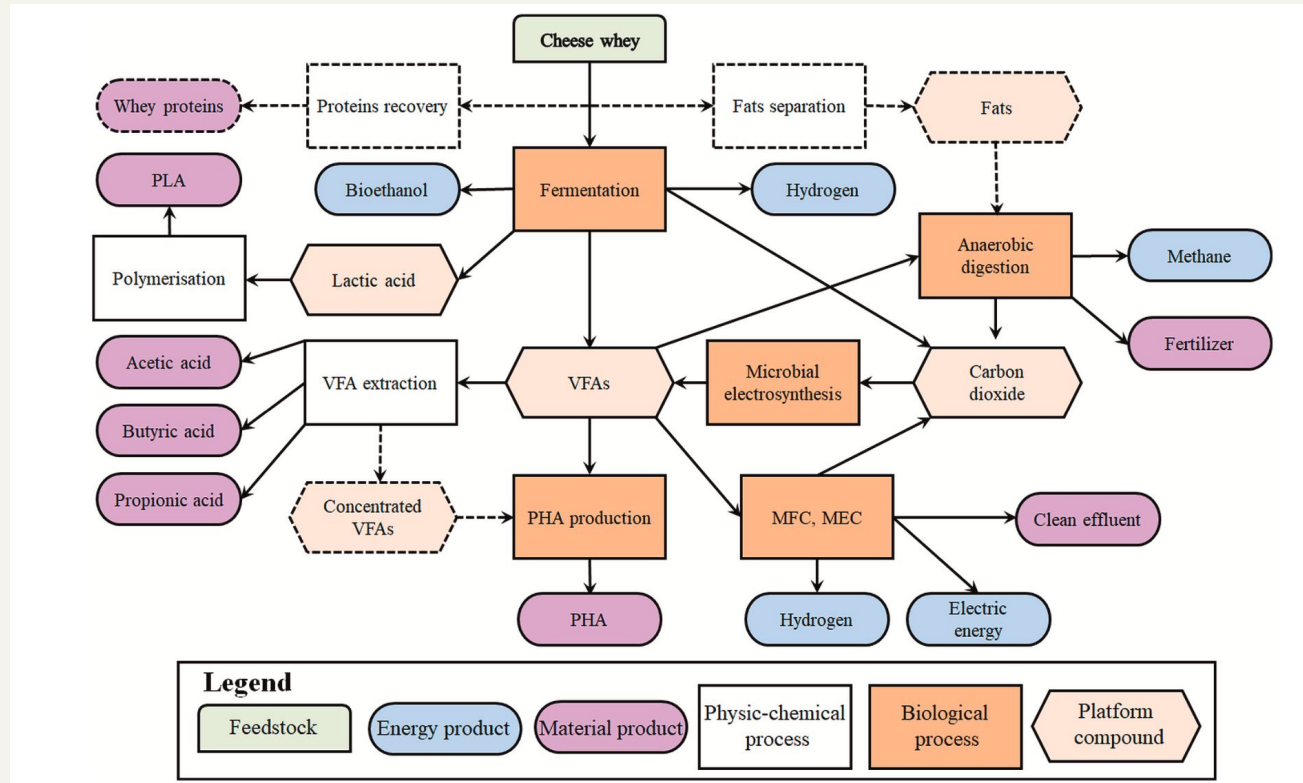
Interactions between microorganisms

Hidden metabolic pathways

It was easy to inhibit methanogenesis

It was difficult to control
homoacetogenesis

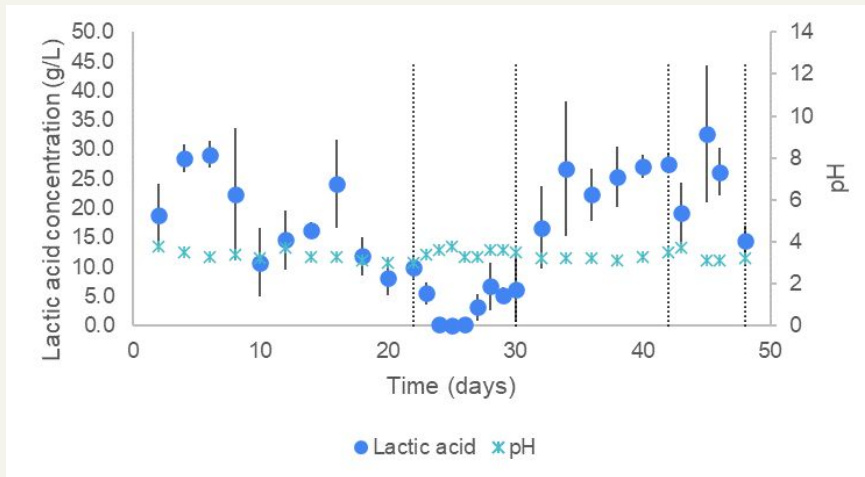
VFA as a central step in an anaerobic biorefinery



If you can't beat them, join them

Lactic acid production using cheese whey

Inés Etchelet thesis
Djalma Ferraz



	Inoculum	R2					
Lactobacillus-	41.6	98.2	32.4	97.9	99.9	98.4	99.2
Streptococcus-	2.9	0	48.5	0.4	0	0	0.2
Lactococcus-	38.1	0	16.5	0.2	0	0.1	0
Pseudomonas-	6.5	0	0.3	1	0	0.9	0.6
Lentilactobacillus-	0.1	0	0.1	0.4	0.1	0.2	0
uncultured	0.6	0	0	0	0	0	0
Aminicenantales-	2.2	0.2	0	0	0	0	0
Enterococcus-	0	0	0	0	0	0	0
Leuconostoc-	0.3	0.1	0	0	0	0.1	0
Remaining taxa (119)-	7.6	1.5	2.2	0	0	0.1	0
	Inoculum	16	26	29	34	40	46

Very low diversity

High selection of LAB

Depending on which microorganism predominates, the produced stereoisomer may differ.

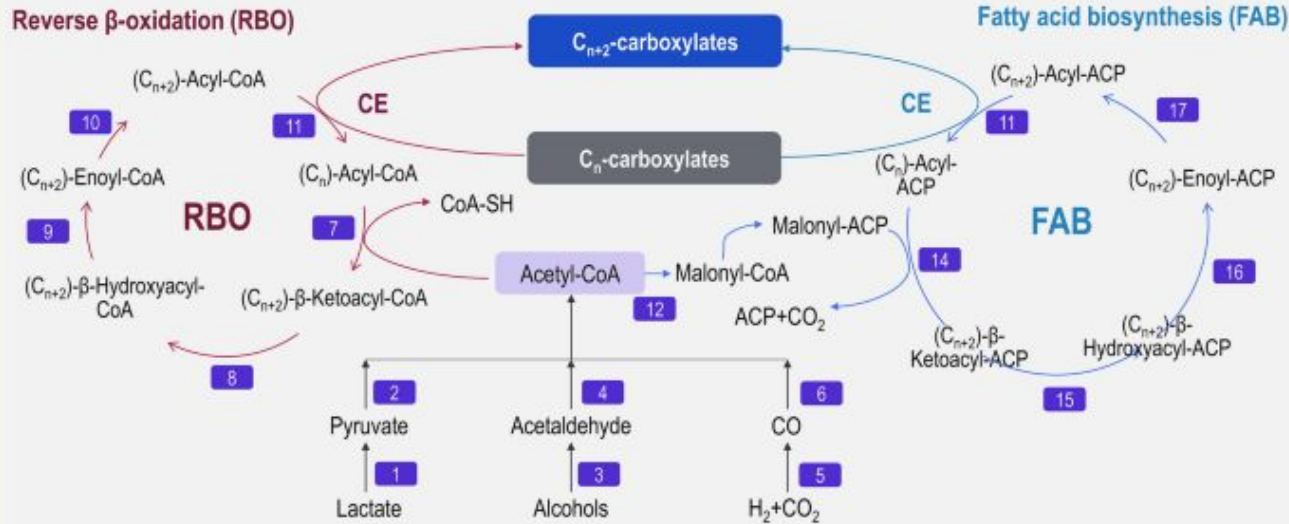
Fed batch reactor

Low pH favour the lactic acid production (pH 4)

High selectivity (97%)

High optical purity (98%)

Chain elongation



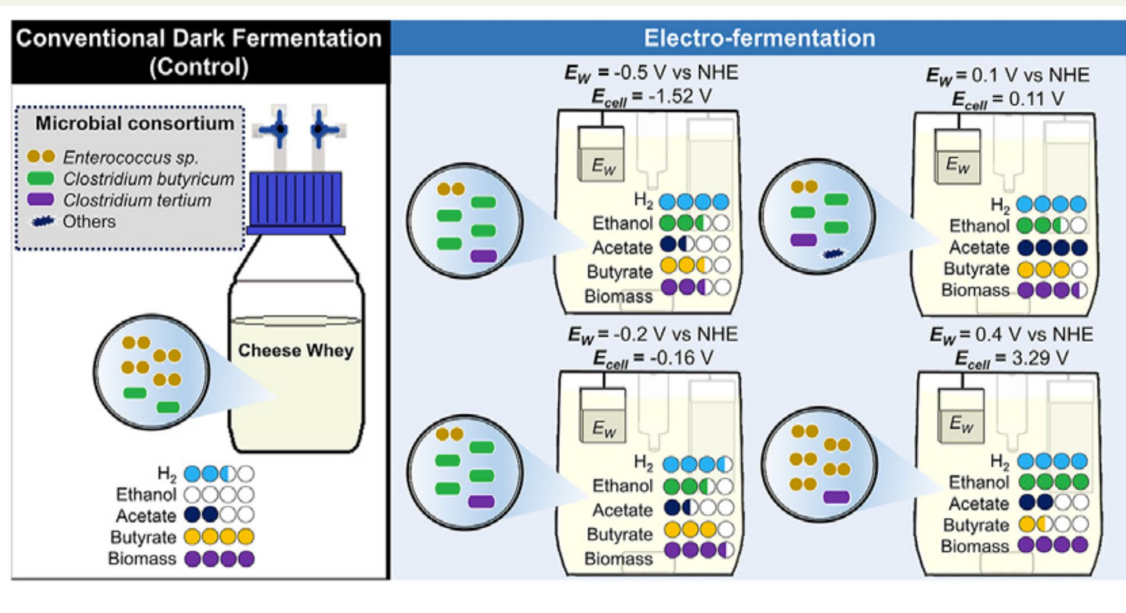
Two main pathways

Who are the microorganisms involved?

Which are the best conditions?

Culture strategy

Electro-fermentation



The application of an electric current enables control over the distribution of fermentation products.

Cardena et al., 2024

Understanding AD microbiology using metagenomics



Meegoda et al., 2025

Using data from databases to understand the process.

Metagenomics and metatranscriptomic approach.

Answer general questions

Know the role of unknown microorganisms

Discover new interaction within microorganisms

Discover new functions

MiDAS database

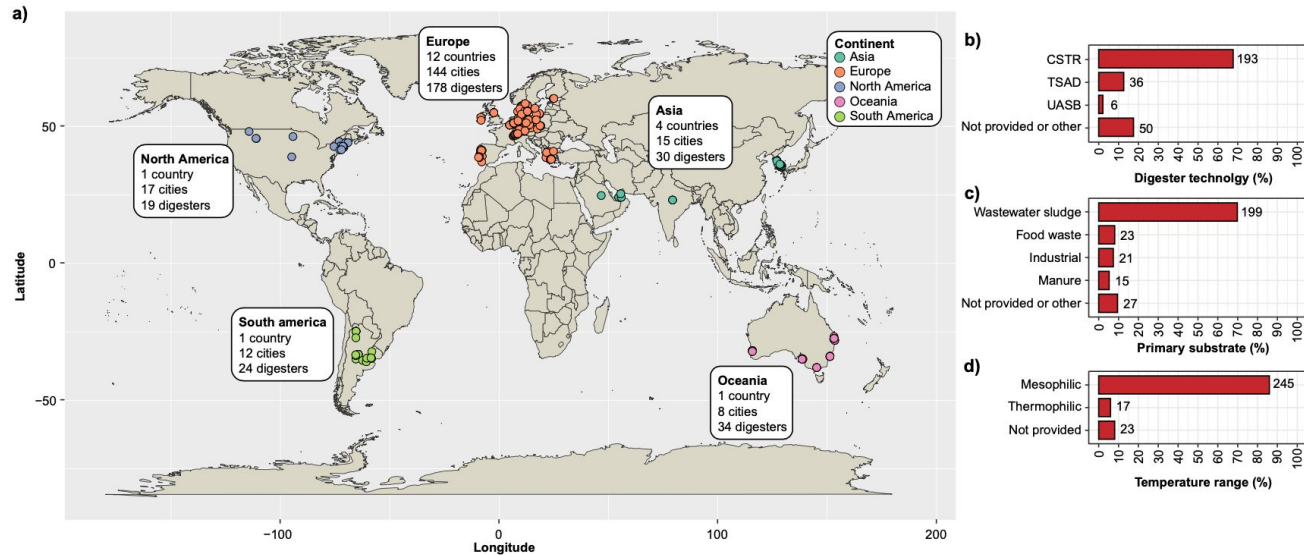


Fig. 1 | Sampling of anaerobic digesters (ADs) across the world. a Geographical distribution of ADs included. **b** Distribution of digester technologies. CSTR Continuous stirred-tank reactor; TSAD Two-stage anaerobic digestion, UASB Upflow

anaerobic sludge blanket. **c** Distribution of primary substrates. **d** Distribution of digester temperatures. The values next to the bars are the number of ADs in each group.

Full-length 16S rRNA gene sequencing of 285 full-scale anaerobic digesters (ADs) (Dueholm et al., 2024)

Browse taxonomy



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Field guide -> [Bacteria](#) -> [Chloroflexi](#) -> [Anaerolineae](#) -> [Anaerolineales](#) -> [Anaerolineaceae](#) -> [Anaerolinea](#)

Genus: *Anaerolinea*

Alternative names: n/a

Source: Published

NCBI Taxonomy ID: [233189](#)

Genome: Yes ¹ [NCBI](#)

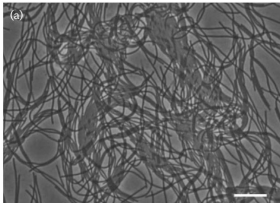
16S rRNA Gene Copy Number: 2

MIDAS 5.3 Taxonomy

- Kingdom [Bacteria](#)
- Phylum [Chloroflexi](#)
- Class [Anaerolineae](#)
- Order [Anaerolineales](#)
- Family [Anaerolineaceae](#)
- Genus [Anaerolinea](#)

Species (110)

[midas_s_8221](#), [midas_s_9806](#), [midas_s_10552](#), [midas_s_14421](#), [midas_s_15594](#), [midas_s_17041](#), [midas_s_17062](#), [Have a s](#)

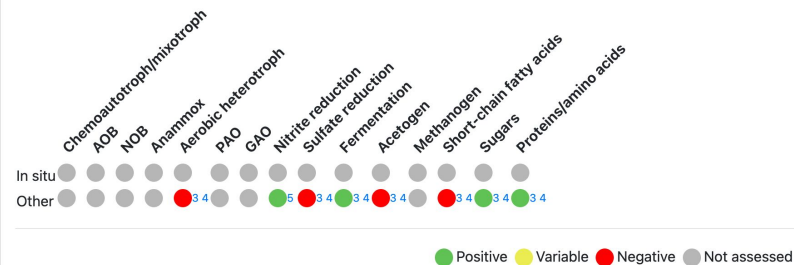


(a)

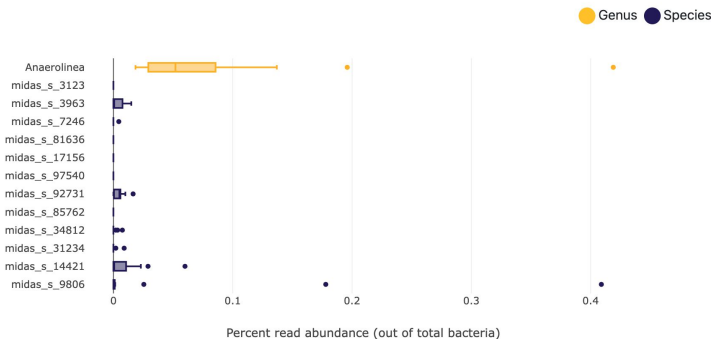
Phase-contrast photomicrograph of strain IMO-1T, grown under optimal conditions. Bar= 10 microns³

It gives a number for unknown genera and species

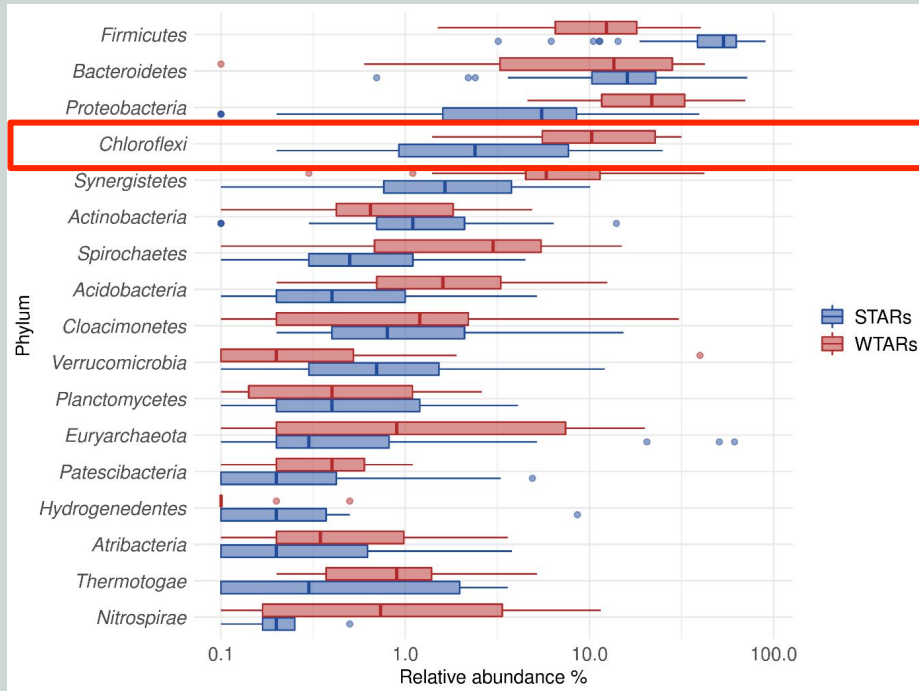
Metabolism



All countries - Wastewater sludge (Mesophilic)



Unknown microorganisms in full scale anaerobic reactors

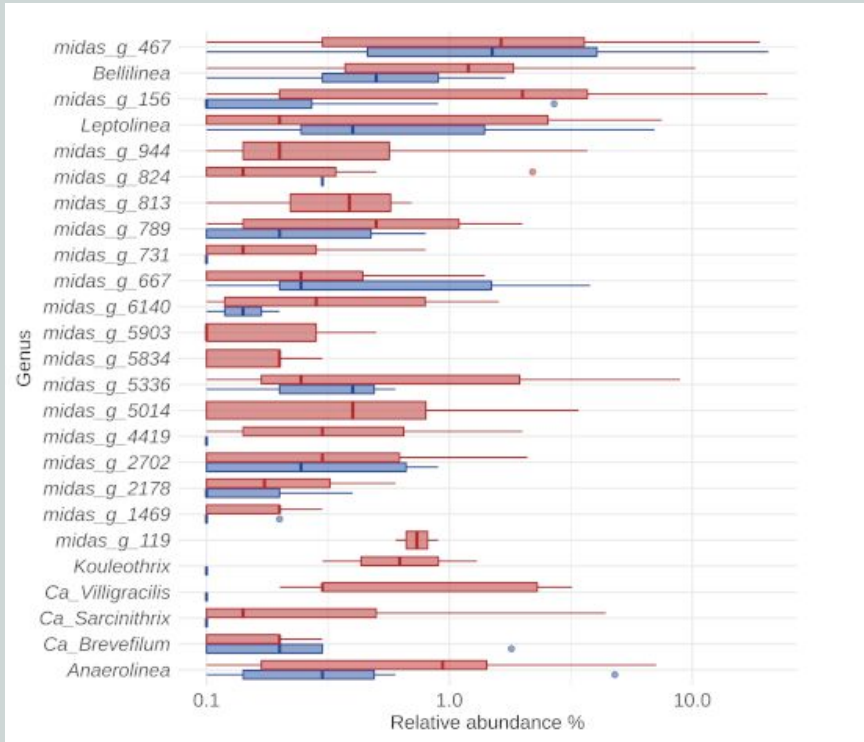


Many important phyla from the AD microbiota are composed by unknown microorganisms.

Bovio-Winkler et al., 2021. Database mining to unravel the ecology of the phylum Chloroflexi in anaerobic full scale bioreactors.

Meta analysis performed with 16S rRNA gene sequences from 62 methanogenic reactors:
27 reactors treating wastewater (WTAR) and 35 reactors treating solid waste (STAR).

Most abundant genera within Chloroflexota



Anaerolineae family precominate

Some genera are shared within many reactors.

Many un-described genera (midas_g)

frontiers
in Microbiology

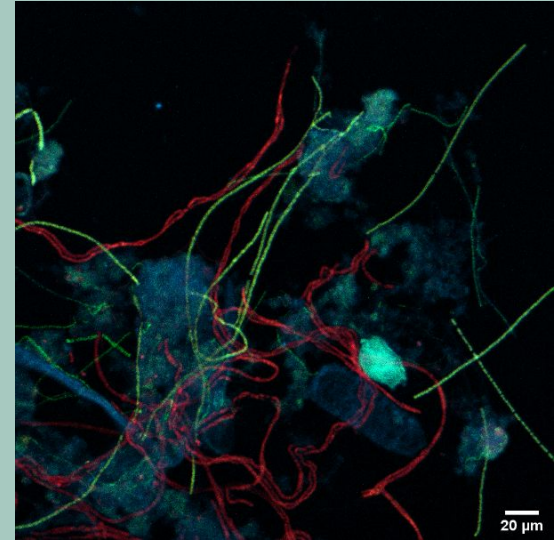
ORIGINAL RESEARCH
published: 20 January 2021
doi: 10.3389/fmicb.2020.00524

Database Mining to Unravel the Ecology of the Phylum Chloroflexi in Methanogenic Full Scale Bioreactors

Patricia Bovio-Winkler¹, Angela Cabezas² and Claudia Etcheberry^{3*}

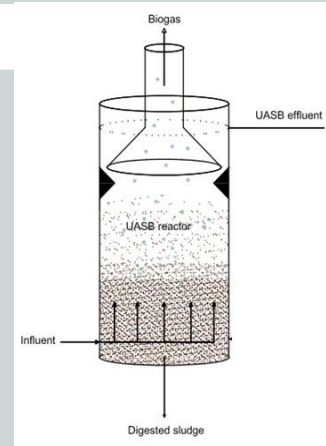
Why Chloroflexota?

- They are always present in the different wastewater systems
- They are filaments
- They can produce bulking problems
- They can be important for the aggregation of the biomass

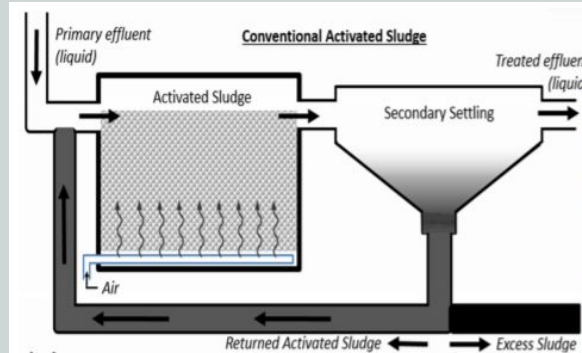


Chloroflexota in three different wwt systems

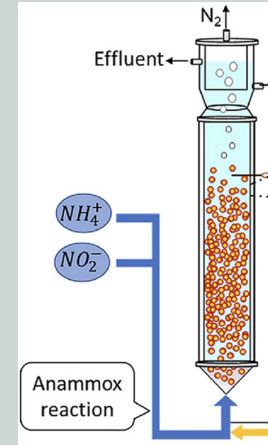
Do Chloroflexota have the same role in the different systems?



Full scale
Methanogenic
reactor
Anaerobic
COD to CH₄



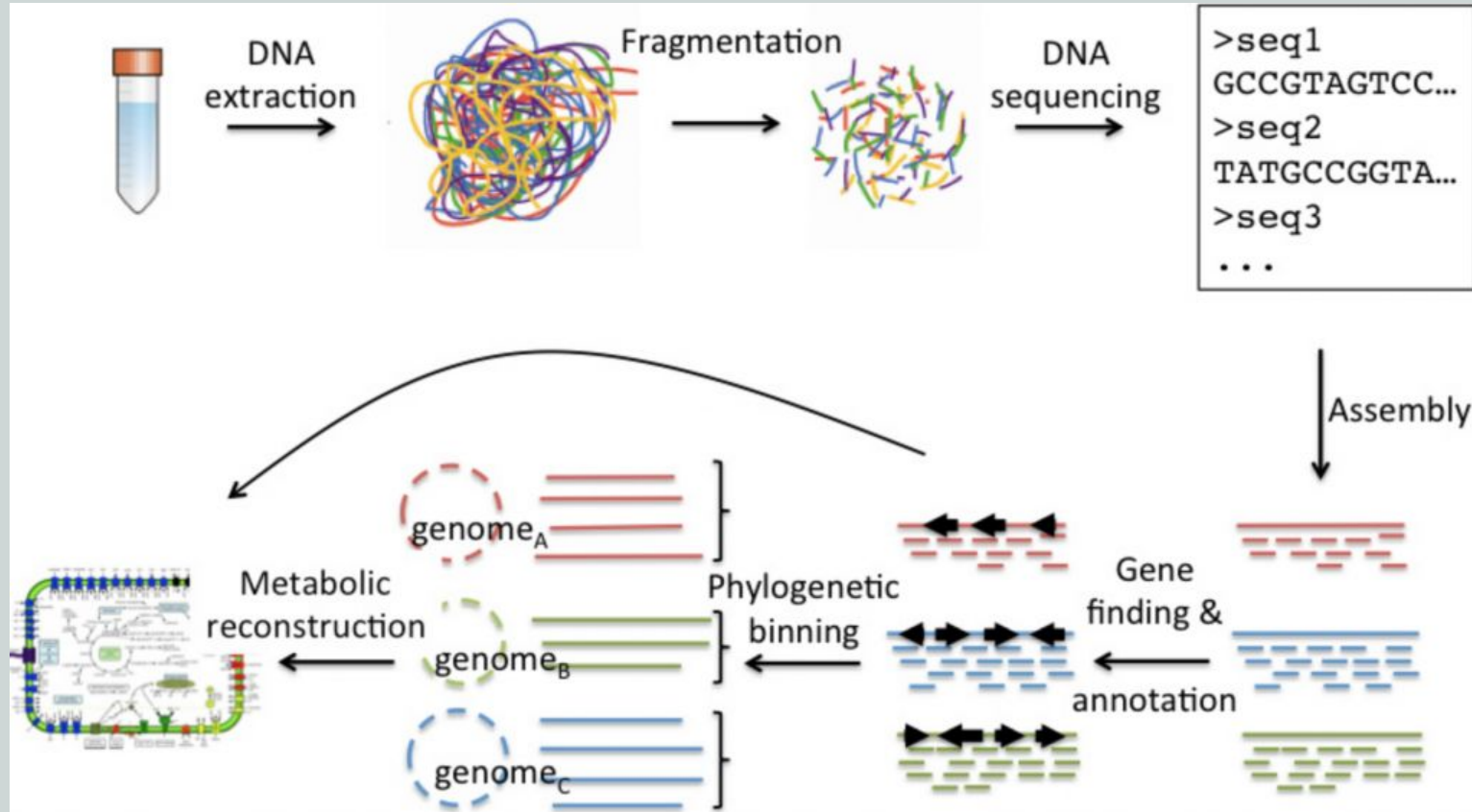
Full scale
Activated sludge
Aerobic
COD to CO₂ and biomass



Lab scale
Anammox reactor
Anaerobic
NH₄⁺ + NO₂⁻ to N₂

RED CYTED TRITON

Metagenomics



RESEARCH

Open Access

Genome-centric metagenomic insights into the role of Chloroflexi in anammox, activated sludge and methanogenic reactors

Patricia Bovio-Winkler¹, Leandro D. Guerrero², Leonardo Erijman², Pia Oyarzúa³, María Eugenia Suárez-Ojeda³, Angela Cabezas⁴ and Claudia Etchebehere^{1*}



Chloroflexi are present in the three reactors with high abundance

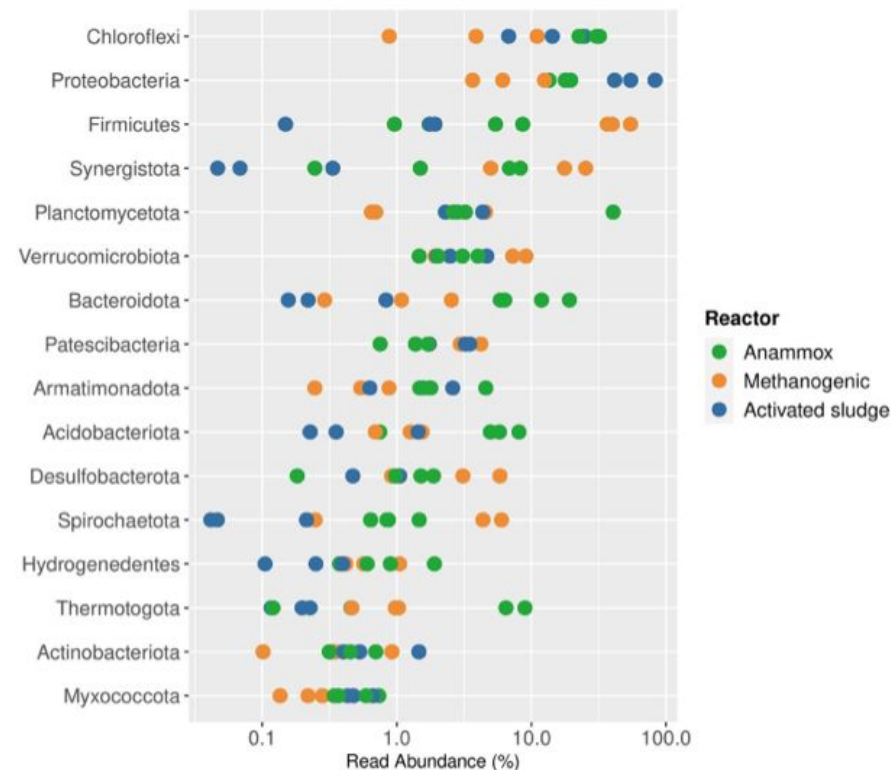


Fig. 1 Boxplots showing the relative abundance of the 16 most abundant phyla in the total community according to the 16S rRNA gene amplicon sequence in each reactor type

Function

Respiration

	R121	R138	R143	R152	M016	M053	M066	M0118	AM09	AM014	AM015	AM039	AM047	AM055	AM056	AM057	AM068
RESPIRATION																	
NADH:quinone oxidoreductase	■			■	■		■			■	■	■		■			■
F-type ATPase			■	■			■						■		■		
Cytochrome c oxidase			■						■								■
Superoxide dismutase	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Catalase peroxidase			■	■		■							■				
Catalase			■					■									
Glutathione peroxidase								■									
HYDROLISIS																	
Oligosaccharide-degrading enzymes	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Extracellular peptidases	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Cellulases	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Endochemicellulases	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Amylases	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Amino sugar-degrading enzymes	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Debranching enzymes	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

Hydrolyti
C
enzymes

Chloroflexota in wastewater treatment reactors a metagenomic approach



Unveiling the hidden diversity and functional role of Chloroflexota in full-scale wastewater treatment plants through genome-centric analyses

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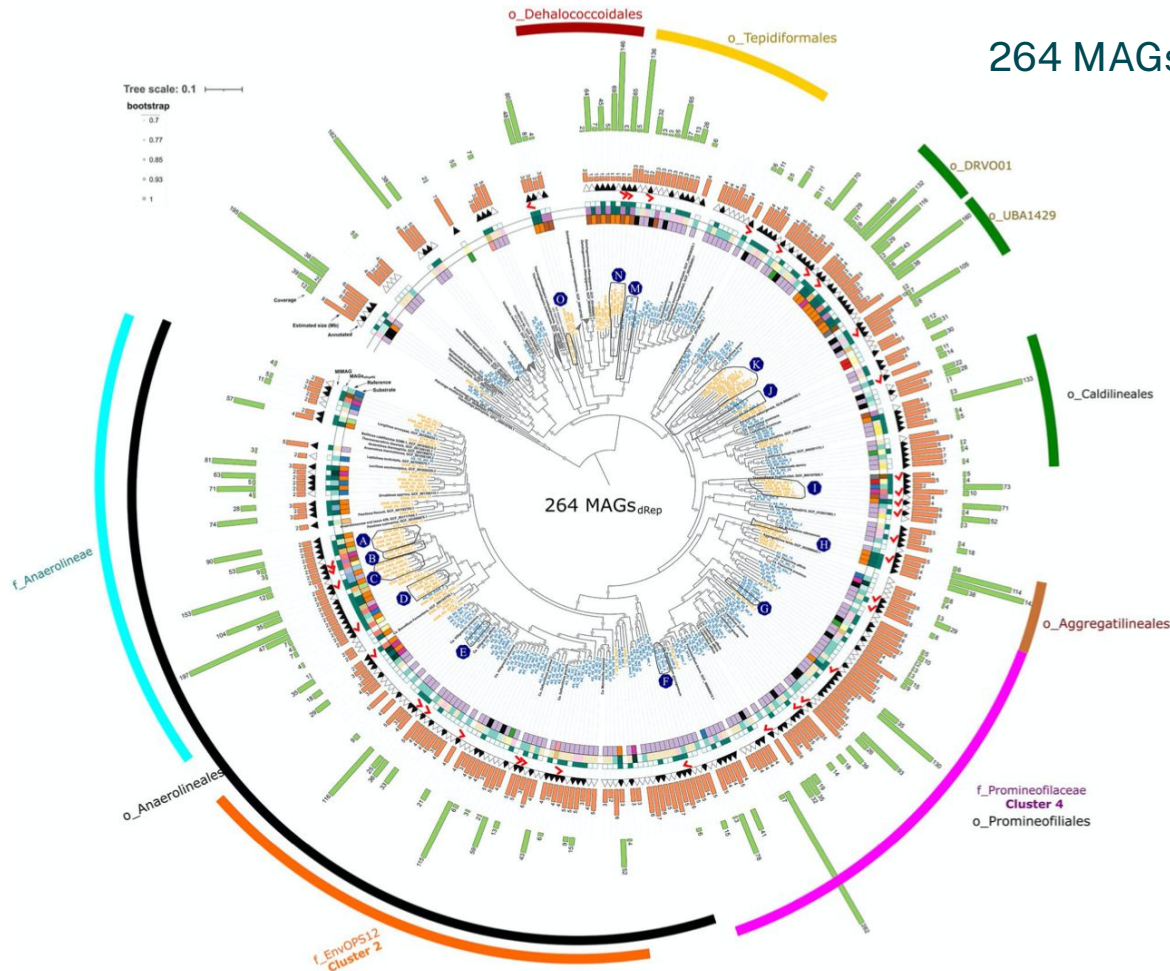
Original Article

18 published papers and data from the present work, which used shotgun
87 full-scale reactors distributed in 15 countries, comprising 45 AS and 42
MET reactors

A

Anaerolineae -	81	84
Dehalococcoidia -	10.2	13.4
Chloroflexia -	6.8	0.8
Limnocylinidia -	2	0
FW602-bin22 -	0	0.8
UBA6077 -	0	0.8
	AS	MET

264 MAGs



Orange: MAGs from activated sludge

Blue: MAGs from anaerobic reactors

Most MAGs are not close to any cultivated representative

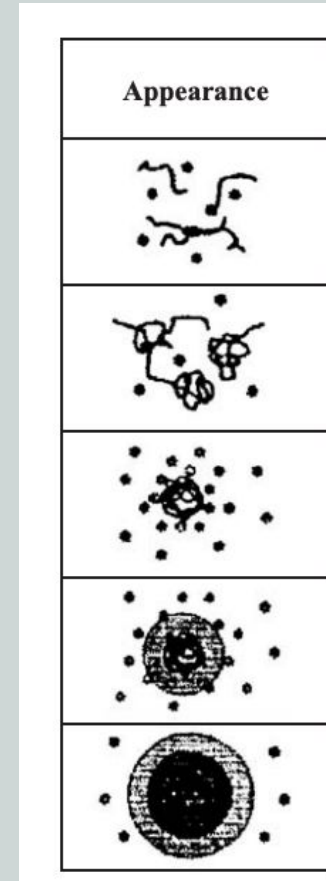
MAGs from activated sludge with aerobic respiration genes

They share a large number of hydrolytic enzyme and adhesion genes.

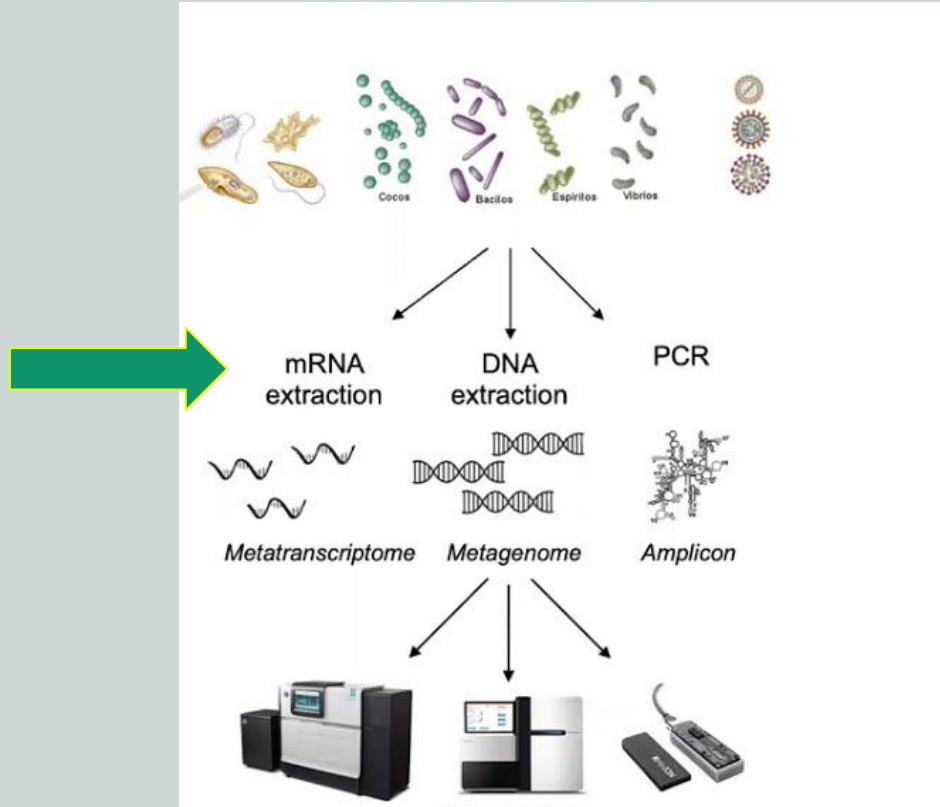
Figure 3. Phylogenomic tree of Chloroflexota MAGs_{dRep} (n = 264) and reference genomes retrieved from NCBI (n = 96). Letters from A to O indicate clusters containing high-quality (HQ) draft MAGs with no representative cultures. Genomes from the phylum Thermotogota were used as outgroup to root the tree.

Chloroflexota role in wwt reactors

- Present in systems with a high cell content and long cell residence time.
- They grow from complex organic polymers as exopolymers or cell debris.
- They have different ways of obtaining energy depending on the reactor operation.
- Slow growth.
- Filamentous morphology is essential for forming granules and flocs.



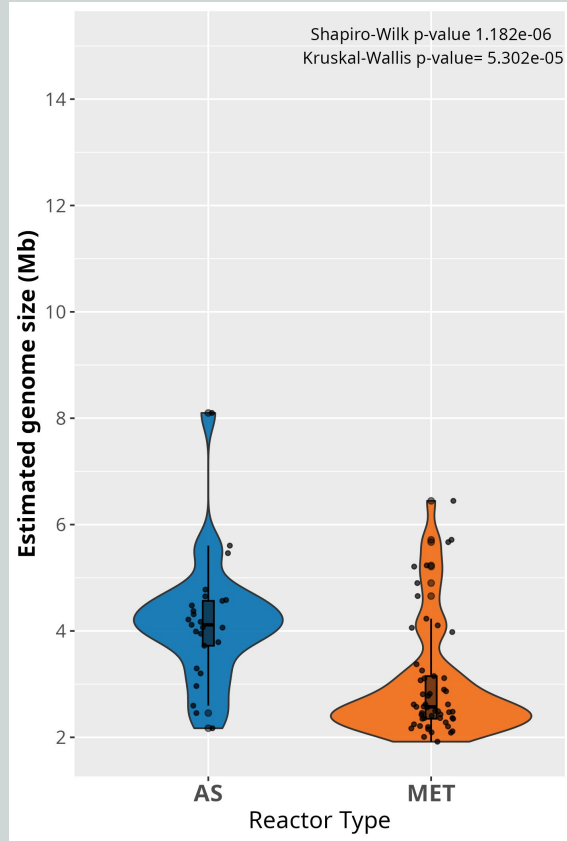
Which genes are active in the reactors?



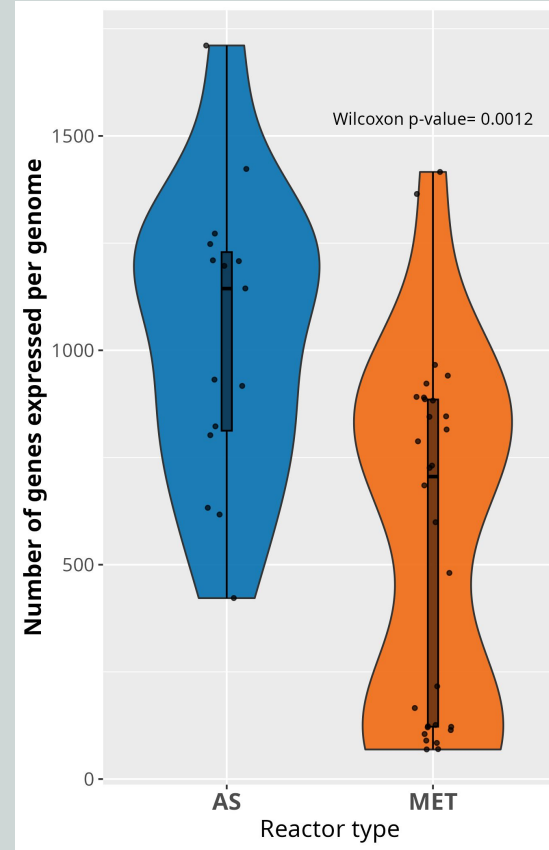
Metatranscriptomic data

Reference	Category	Scale	Reactor type	Reactor name	Country	MAGs with expression	Substrate
Hao et al. 2020	MET (53)	Full-scale	CSTR	Fred	Denmark	2	Surplus AS pretreated by thermal hydrolysis process
				RandGcluCo1		1	Acetate/butyrate/or/propionate
				RandGcluCo2		2	Acetate/butyrate/or/propionate
		Lab-scale	Batch	RandCo1		2	Acetate/butyrate/or/propionate
				RandS7		1	Acetate/butyrate/or/propionate
				RandS10		1	Acetate/butyrate/or/propionate
Mei et al. 2022		Full-scale	CSTR	UR	USA	5	Wasted activated sludge
Wirth et al. 2023				WT	Hungary	2	Pig slurry-maize silage-sweet sorghum
Yan et al. 2024		Lab-scale	AnMBR	YN	China	31	Leachate
This work*				IC	Uruguay	2	Brewery WW
			UASB	MO		3	Malt WW
			CO	1		Oil and grease	
			RH	2		Hospital WW	
	AS (24 MAGs)	Full-scale	AS	BL		10	Landfill (86%) + condensate buried (5%) + humus buried (9%)
				LK		1	Municipal WW
				Liu et al. 2019*	WS	Taiwan	4

Data from 16 reactors, 9 methanogenic (anaerobic), 7 activated sludge (aerobic) from Uruguay and other countries.



Genome size



Genes expressed by genome

Hydrolyases

ABC transporters

Electron transport chain

I	2%
II	6%
III	18%
IV	6%
NADH	5%
Succ	14%
Cyt C	18%
ATP syn	18%

Alternative electron transport chain

NDH-2	4%	64%
cydAB	17%	36%
menA	8%	68%

Carbon metabolism

PPP

PPP non-ox	10%	41%
PPP ox	33%	50%
PPP ox	55%	10%

Glycolysis

50%	59%
-----	-----

Pyruvate

58%	86%
-----	-----

Acetyl-coA

10%	82%
-----	-----

TCA

TCA cycle 1	0%	0%
TCA cycle 2	8%	41%
Glyoxylate cycle	0%	0%
Glyoxylate cycle	0%	18%

Fermentation

Lactate	13%	18%
Acetoin	8%	41%
Formate	2%	32%
Acetate	0%	33%
Acetate	2%	0%

Nitrogen metabolism

narGHI

4%	27%
----	-----

nrfAH

8%	23%
----	-----

DNRA

2%	18%
----	-----

nirK

0%	32%
----	-----

nirS

2%	14%
----	-----

nosZ

0%	41%
----	-----

Oxidative stress

63%	68%
-----	-----

Nitrosative stress

5%	37%
----	-----

Formate

23%	41%
-----	-----

CO2 + H2

13%	55%
-----	-----

Pili

2%	5%
----	----

Polyphosphate accumulation

2%	5%
----	----

AA degradation

Triptolan *tnaA*

42%	45%
-----	-----

Alanine *ald*

12%	55%
-----	-----

Arginine *arg*

0%	50%
----	-----

Histidine *hutHUL*

29%	50%
-----	-----

Beta-oxidation

LCFA

fadD	0%
pcd	45%
paof	0%
fadI/fadB	0%
fadA	0%

Acetate *acsA*

0%	82%
----	-----

Methylmalonyl-coA

46%	77%
-----	-----

Multiple sugar

54%	64%
-----	-----

General L-AA

2%	23%
----	-----

Branched-chain AA

33%	50%
-----	-----

Polar AA

38%	41%
-----	-----

Peptides/Nickel

40%	68%
-----	-----

Lipopolysaccharide

52%	59%
-----	-----

α-glucoside

29%	32%
-----	-----

Putative ABC

60%	77%
-----	-----

ABC type

54%	64%
-----	-----



Challenges

- Use data from database to answer specific questions.
- Create specific sequences databases with metadata of reactors performances.
- Understand the microbiology of new process (chain elongation, electromethanogenesis)
- Design strategies to improve process based on metagenomics data.
- Modelling and process control based on genomic data

Promote collaborative projects



Thank You!

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Uruguay, october 2027



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