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Monitoring the anaerobic digestion microbiome

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The anaerobic digestion (AD) microbiome

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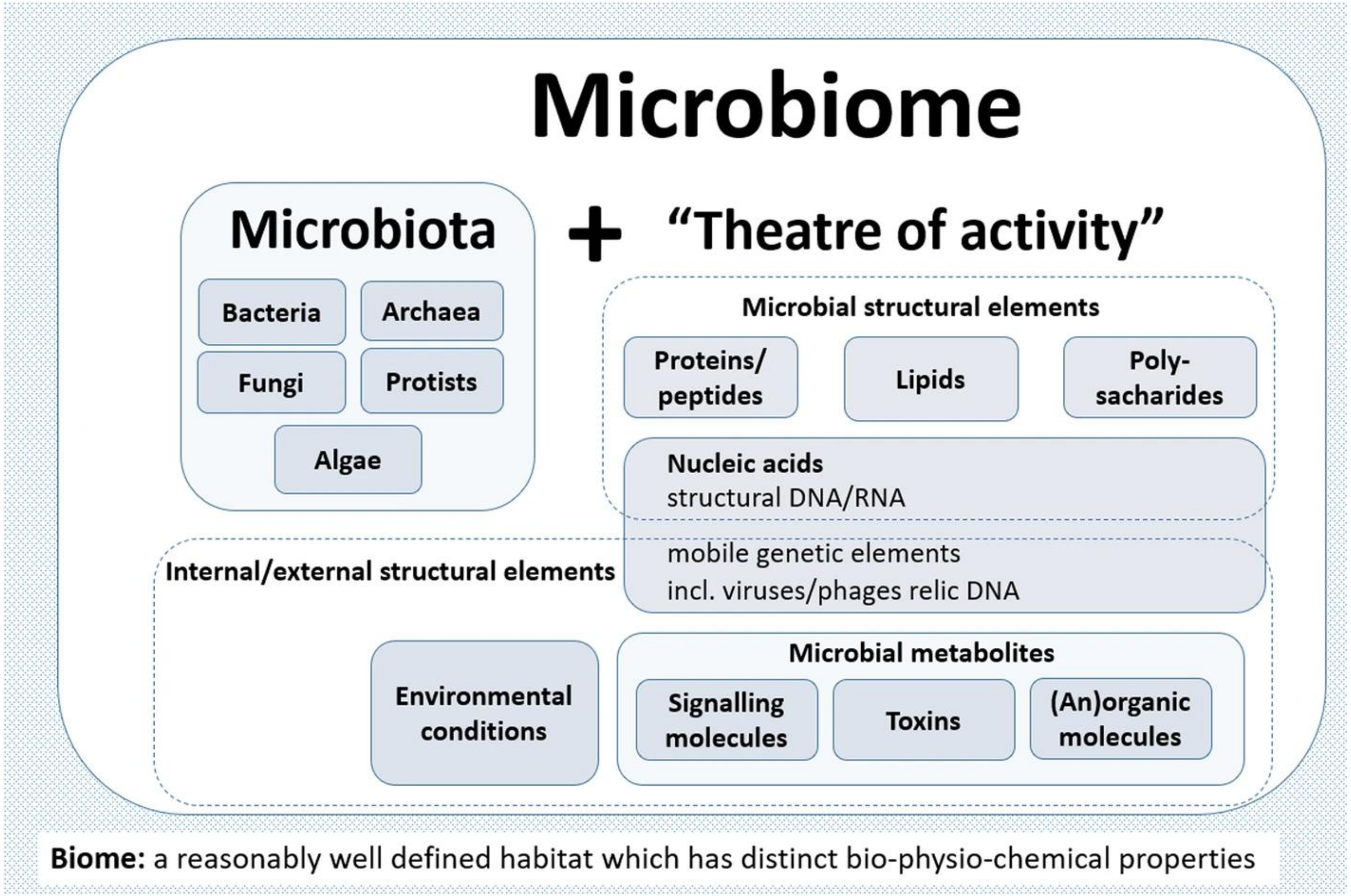
What is a 'microbiome'?



Microbiome

Microbiota + **"Theatre of activity"**

Microbiota
- Bacteria
- Archaea
- Fungi
- Protists
- Algae

Microbial structural elements
- Proteins/ peptides
- Lipids
- Poly-sacharides

Nucleic acids
structural DNA/RNA
mobile genetic elements
incl. viruses/phages relic DNA

Internal/external structural elements

Environmental conditions

Microbial metabolites
- Signalling molecules
- Toxins
- (An)organic molecules

Biome: a reasonably well defined habitat which has distinct bio-physio-chemical properties

Berg et al., 2020, Microbiome

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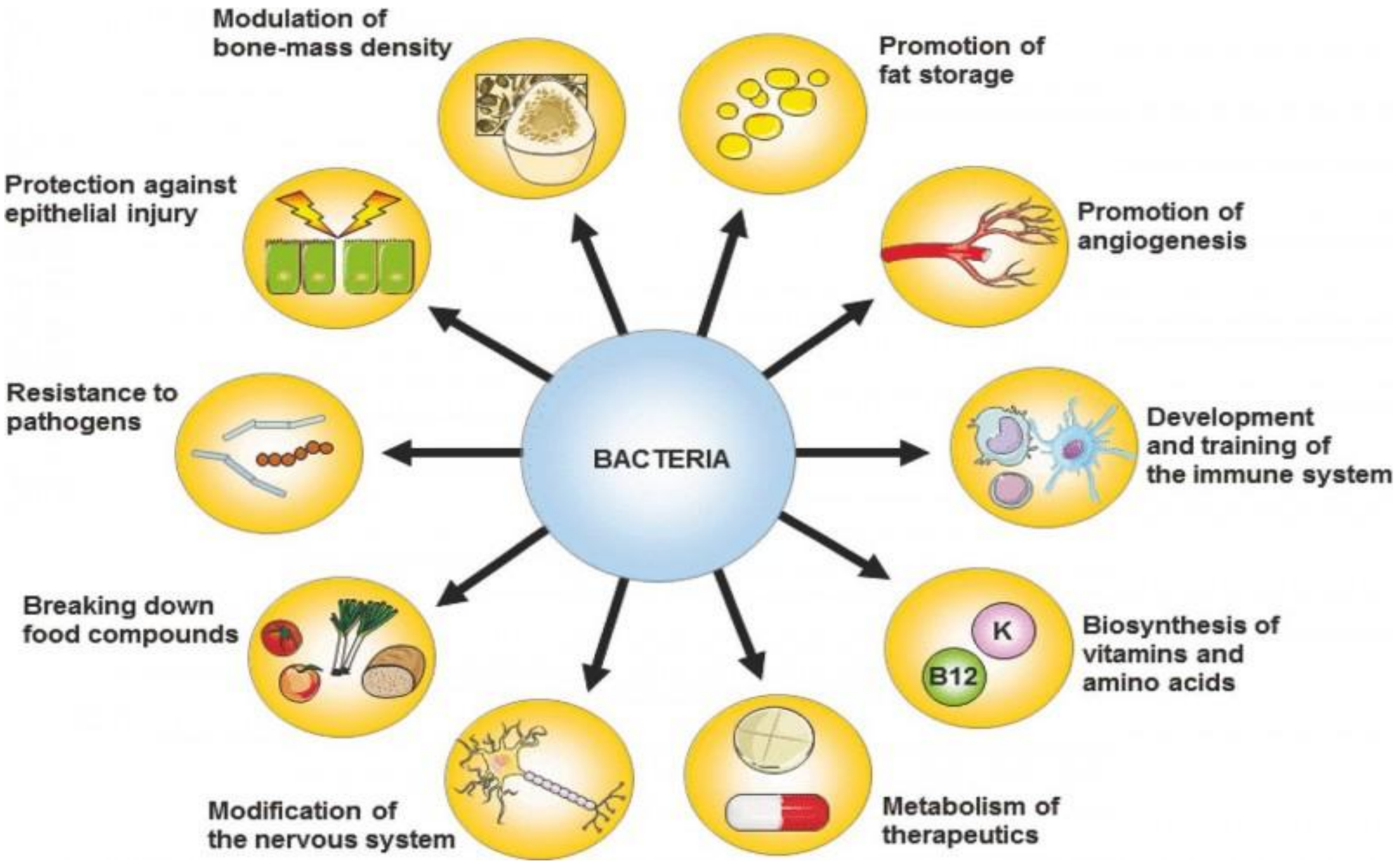
Human microbiome



Laukens et al., FEMS Microbiol. Rev, 2015

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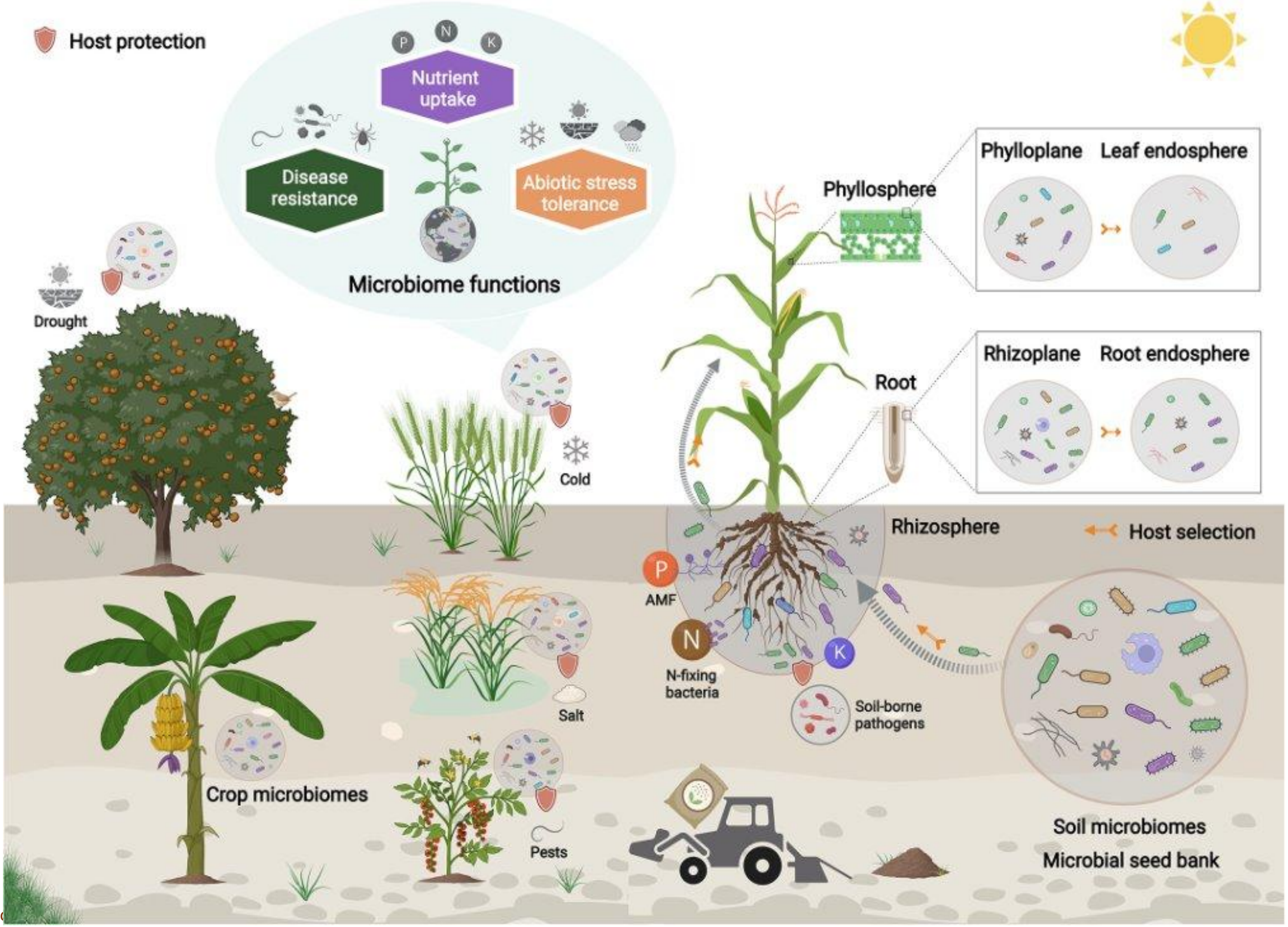
Soil microbiome



Xiong & Lu, Environ. Microbiol. Rep., 2022

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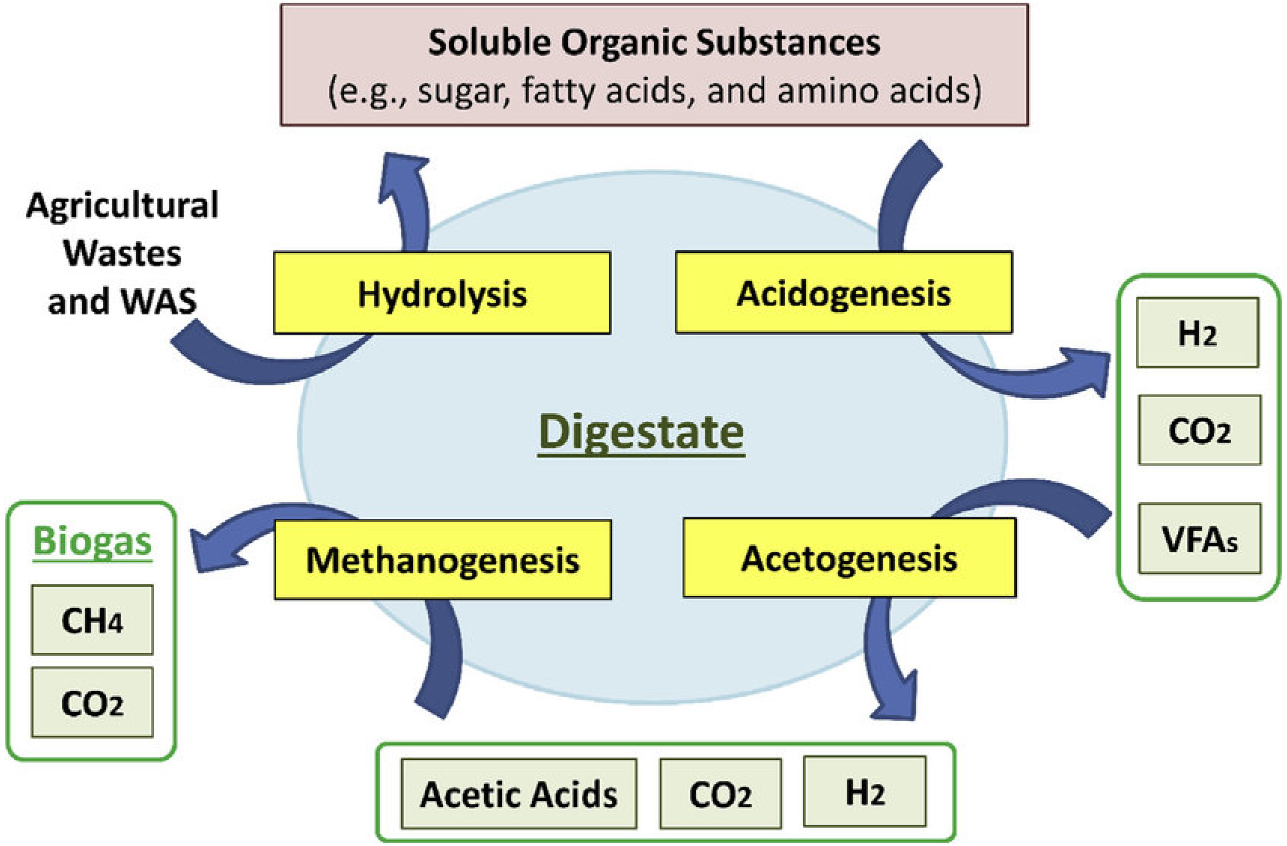
AD microbiome



Pan et al., iScience, 2021

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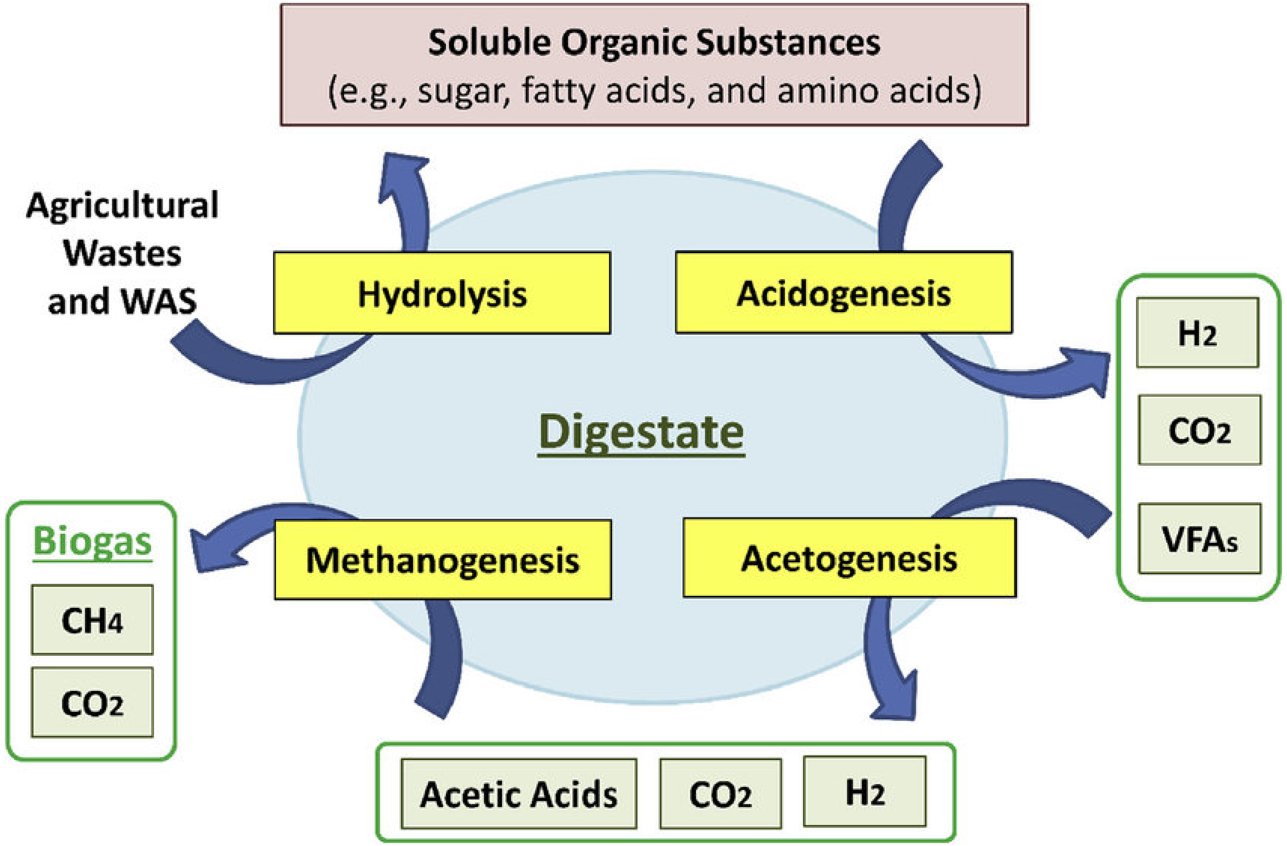
AD microbiome



Soluble Organic Substances
(e.g., sugar, fatty acids, and amino acids)

Agricultural Wastes and WAS

Hydrolysis

Acidogenesis

H_2

CO_2

VFAs

Digestate

Biogas

CH_4

CO_2

Methanogenesis

Acetogenesis

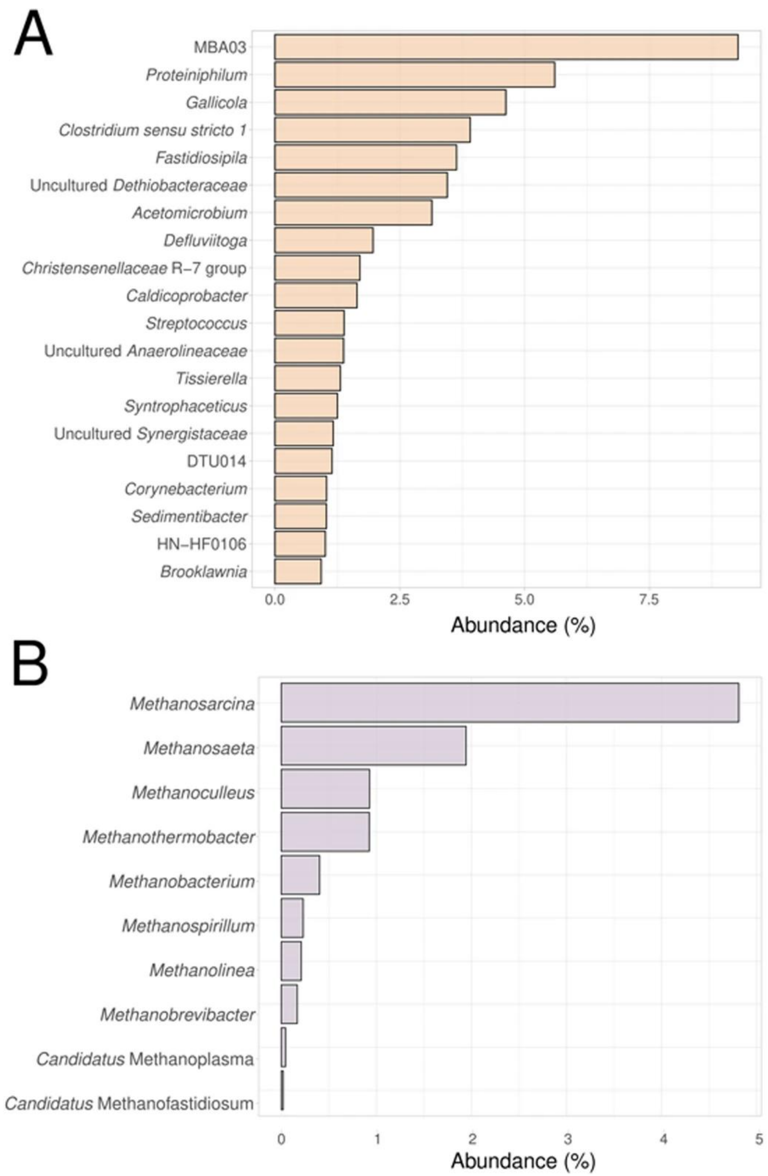
Acetic Acids

CO_2

H_2

Pan et al., iScience, 2021

AD microbiomes are very complex



Importance: it reflects the 'health' of anaerobic digestion

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How to monitor the AD microbiome

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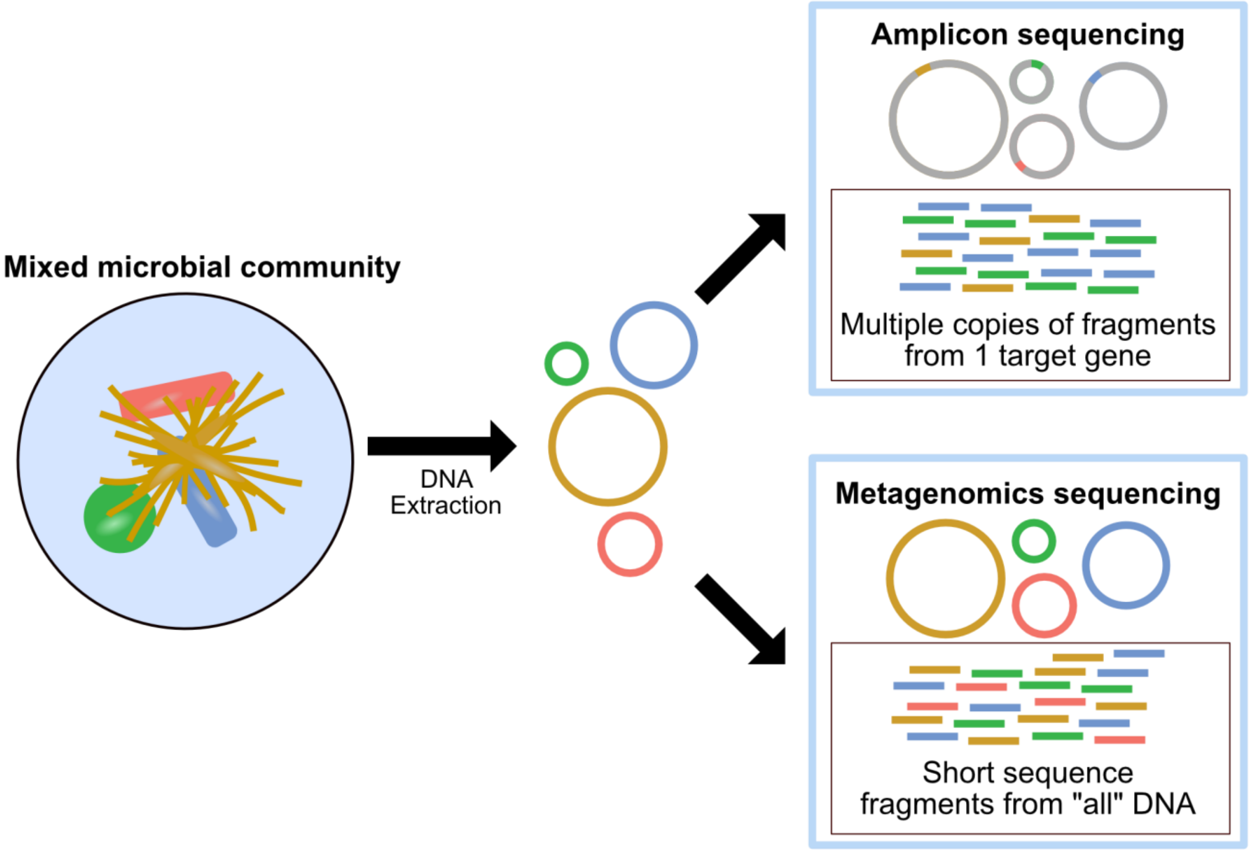
DNA sequencing

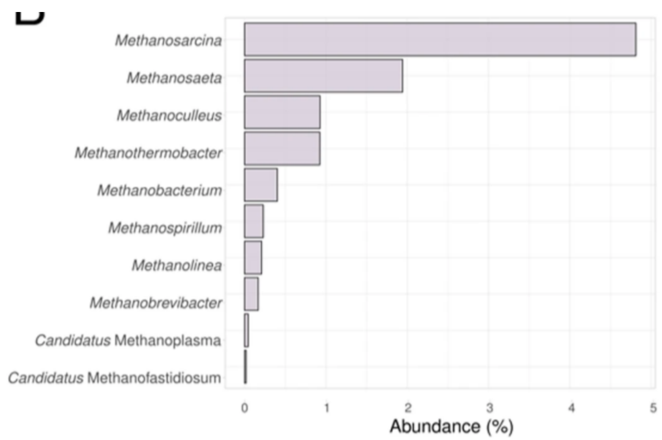
What are we looking for?
Detecting the abundance of key microorganisms (e.g. archaea) or key functions (e.g. carbohydrate degradation pathways).



From: https://astrobiomike.github.io/

Microbial profiles
(16S, ITS, 18S…)

Taxonomic and functional profiles



Pro: can detect all the microbiome
Con: relative abundances

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DNA sequencing

	1st Generation (Sanger)	2nd Generation (Illumina)	3rd Generation (PacBio & ONT)
Read length	400-900 bp	75-300 pb	>10 Kbp
Accuracy	>99.9%	99.5 - 99.9%	87 – 99.9%
Yield	~96 Kbp	1.2 Gbp – 6 Tbp	2.8 Gbp – 14 Tbp

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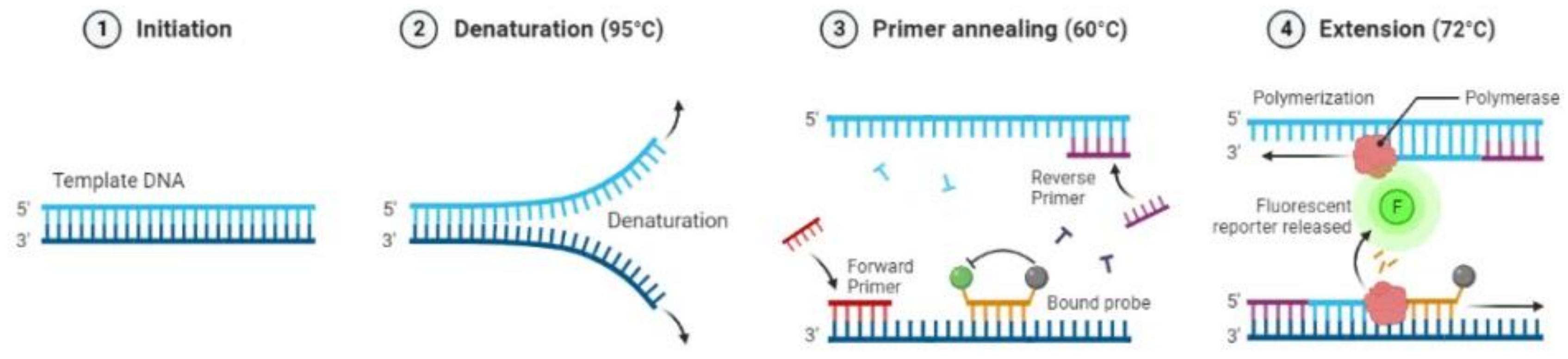
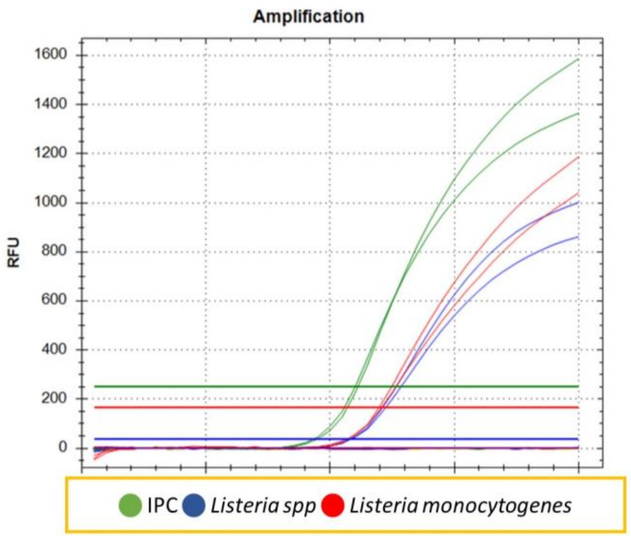
Quantitative PCR



From: https://clinicalsci.info/real-time-pcr/



Pro: absolute abundances
Con: only few targets per reaction

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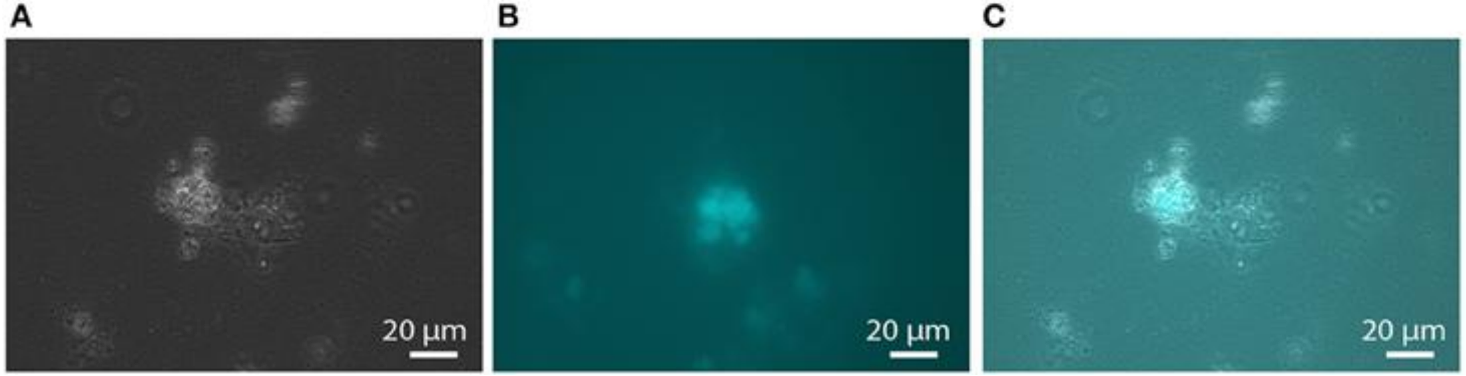
Fluorescence



Arshad et al., Front. Microbiol., 2015

- Coenzyme F420 is a fluorescent cofactor found almost exclusively in methanogenic archaea.
- F420 exhibits strong fluorescence, with excitation and emission wavelengths typically around 420 nm and 470 nm, respectively

Pro: easy, versy specific
Con: valid only for methanogens, difficult to distinguish the species involved

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Other methods

Metatranscriptomics: RNA sequencing

Which genes are being expressed?

Metaproteomics: protein detection

Which proteins are being produced?

Metabolomics: metabolite detection

Which metabolites are being produced?

+ informative
+ complex

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Practical application

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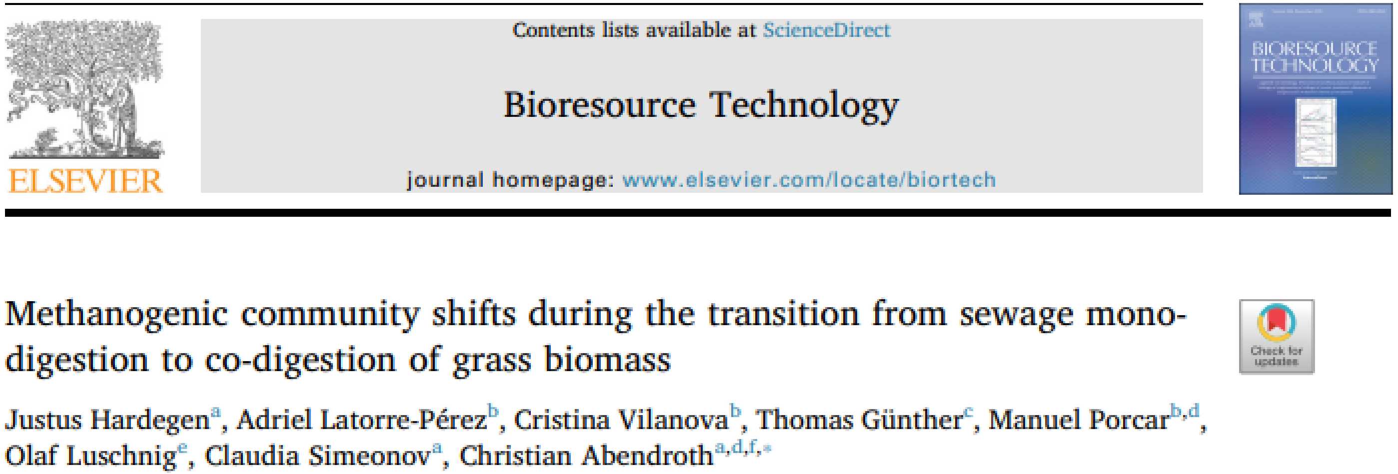
Practical application



Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Methanogenic community shifts during the transition from sewage mono-digestion to co-digestion of grass biomass

Justus Hardegen[a], Adriel Latorre-Pérez[b], Cristina Vilanova[b], Thomas Günther[c], Manuel Porcar[b,d], Olaf Luschnig[e], Claudia Simeonov[a], Christian Abendroth[a,d,f,*]

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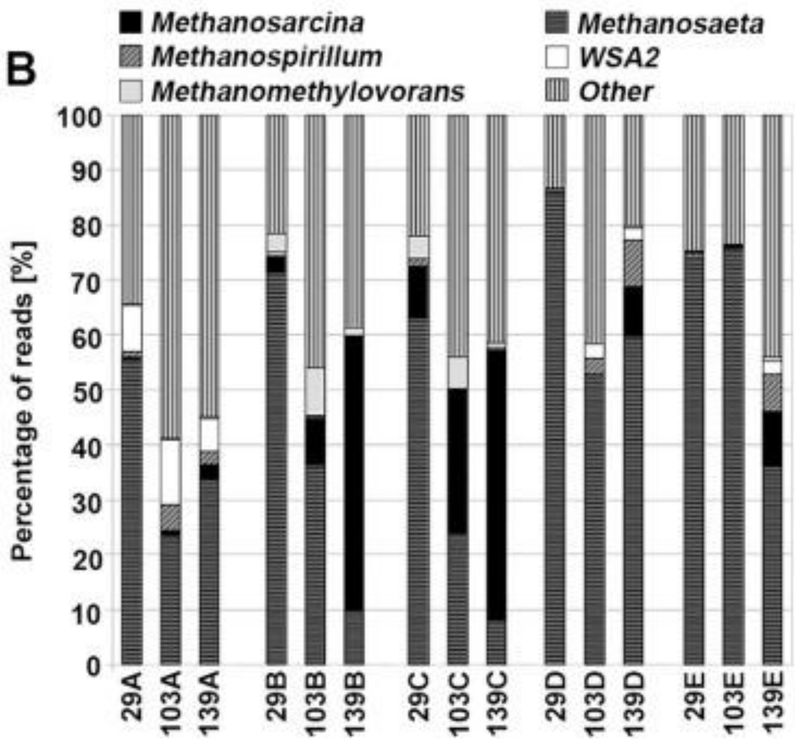
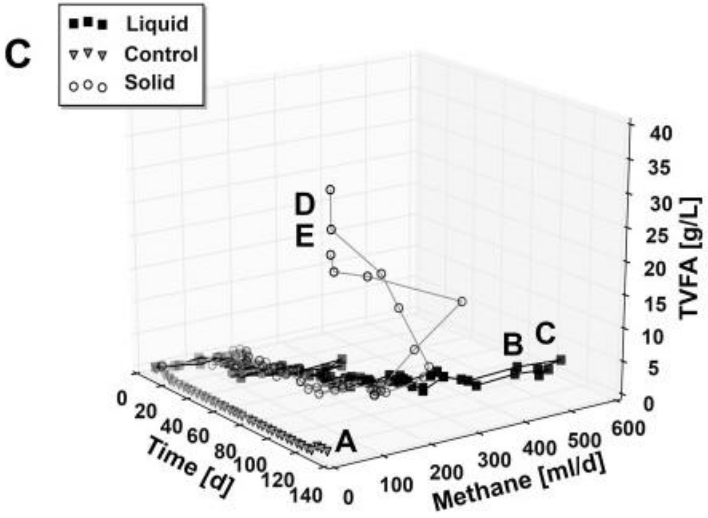
Practical application

Archaeal community



CH$_4$ and TVFAs production



Microbiome monitoring was combined with methane and TVFAs quantification in 5 different reactors.

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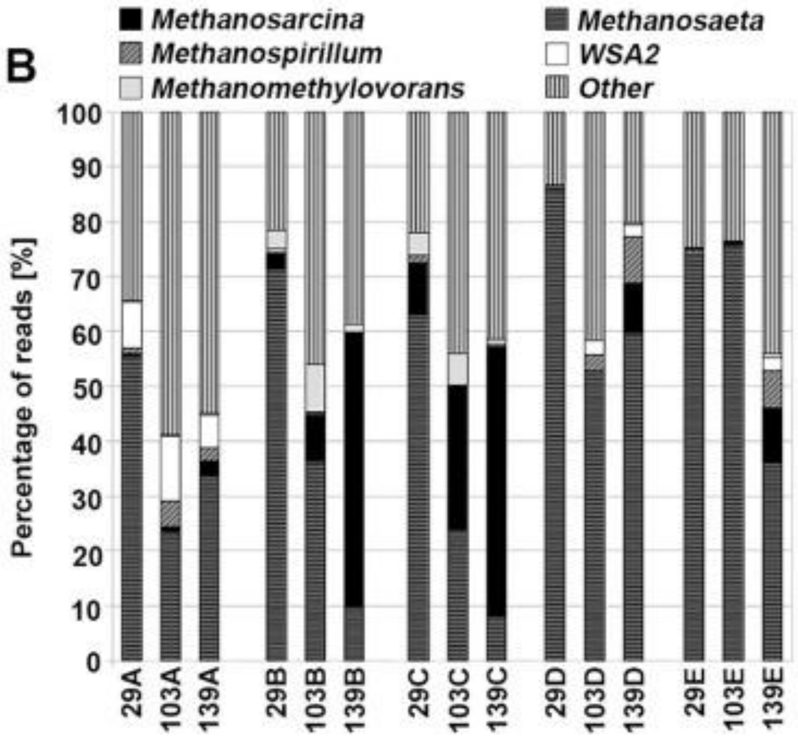
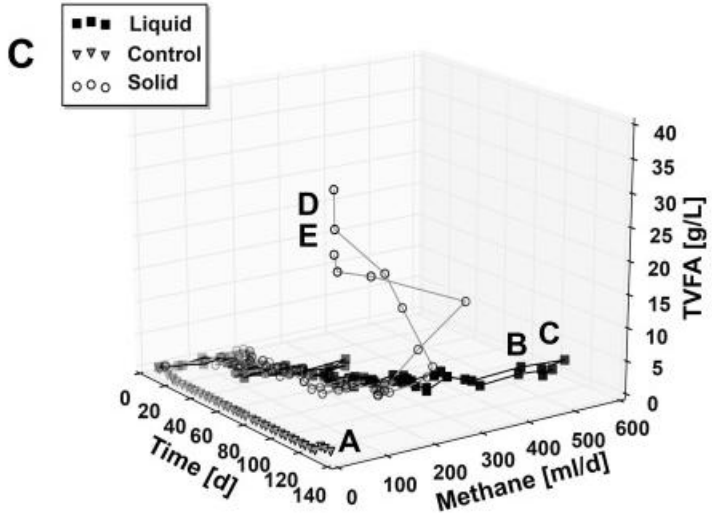
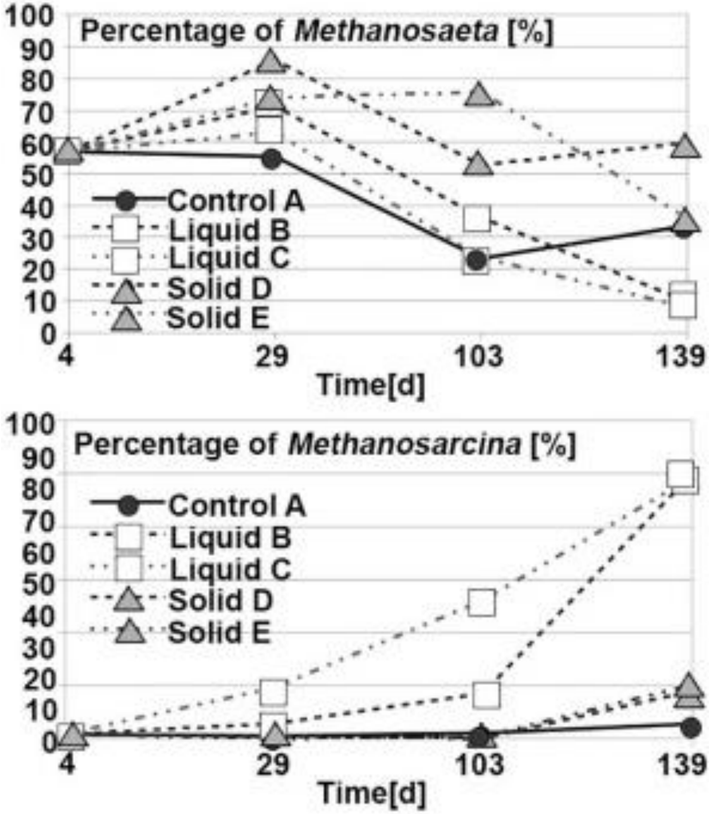
Practical application

Archaeal community



CH$_4$ and TVFAs production





Microbiome monitoring was combined with methane and TVFAs quantification in 5 different reactors.
The **increase in methane production** matched the **increase in the abundance of *Methanosarcina***.

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