DISENTANGLING MICROBIAL CHANGES UPON ORGANIC LOADING RATE DISTURBANCES IN ANAEROBIC REACTORS

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Developing Early Warning Systems for improved microalgae PROduction and Anaerobic DIGestIOn

1. Introduction

The anaerobic digestion (AD) of microalgal biomass for biogas production provides a smart alternative to fossil fuels. Anaerobic reactors are non-linear dynamical systems which often encounter process instability over time. Transient states make the system much more vulnerable to cope with additional perturbations, making the system unreliable to its use for large-scale production. For the next generation of renewable energy technologies to become a reality, the performance of AD needs to be ensured through failure prediction of the system and hence ideal countermeasures, for stable biogas flow production.

Here, we investigate the influence of microalgal organic loading rate (OLR) disturbances on the microbial community structure and their metabolic processes, in a search for clues to improve failure prediction.

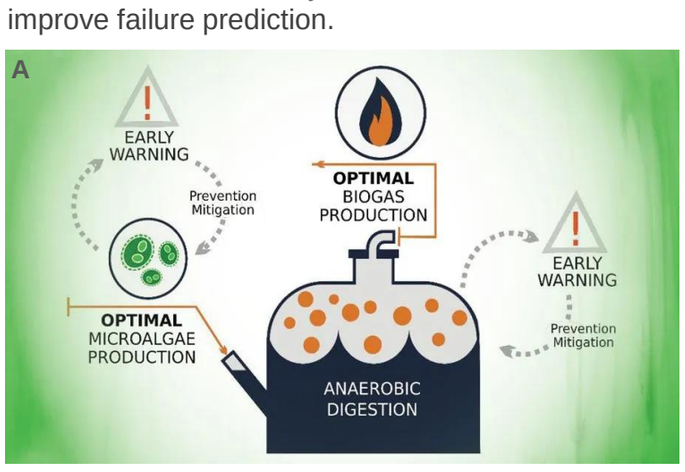

Fig 1. Project conceptualization. (A) Rationale. **(B)** *Scenedesmus*-dominant microalgae population growing in wastewater. **(C)** Anaerobic bioreactors.

2. Experimental Design

Microalgae biomass was produced in outdoor raceway reactors (IFAPA, Almeria, Spain) supplied with municipal wastewater. ADs fed with conventional OLR (1.5 g COD/Ld) where subjected to low (0.7 and 0 g COD/dL) and high (3 and 7 g COD/Ld) disturbances that each last 10 days. Two disturbances were applied after a monitored steady-state period. ADs were sampled for 16S rRNA amplicon and shotgun meta-omics sequencing.

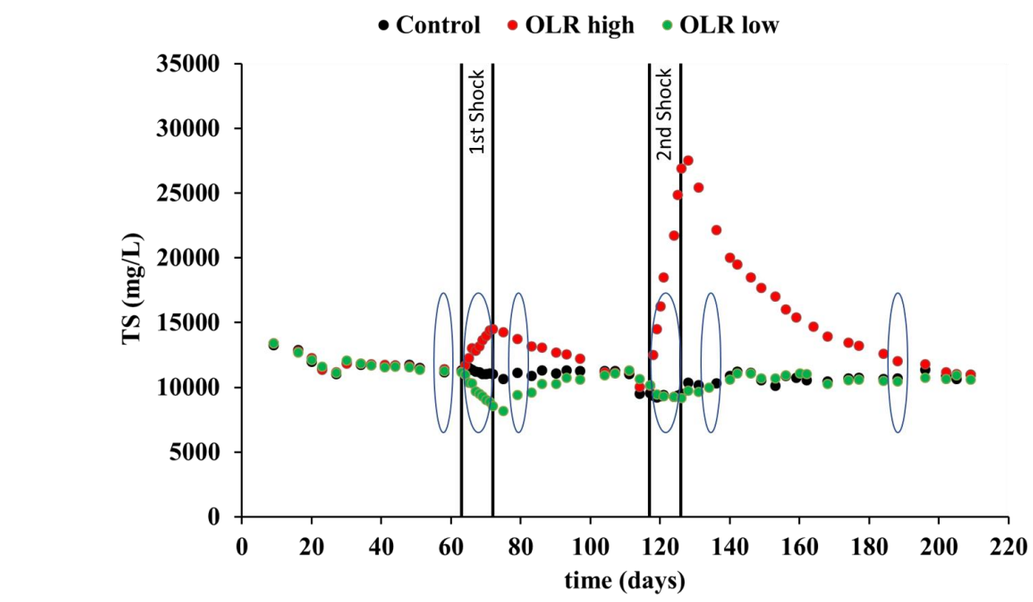

Fig 2. Overview of the experimental set-up. Sampling timeline for both 16S rRNA amplicon (n=35) and shotgun meta-omics sequencing (n=21). Time points included in the blue circles represent samples collected for meta-omics. The first and second disturbances are highlighted as 1st and 2nd shocks.

3. Data Aquisition and Analyses

16S rRNA and shotgun metagenomic sequencing were made using Illumina instrumentations. To identify proteins by mass spectrometry, the timsTOF Pro (Bruker Daltonik) instrument was used. Data were analyzed in R environment v. 4.1.3 using several packages, e.g., *Ampvis2* v2.7.8 (Albertsen et al., 2015), *phyloseq* (McMurdie and Holmes, 2013), *pheatmap* v1.0.12 (Kolde, 2019).

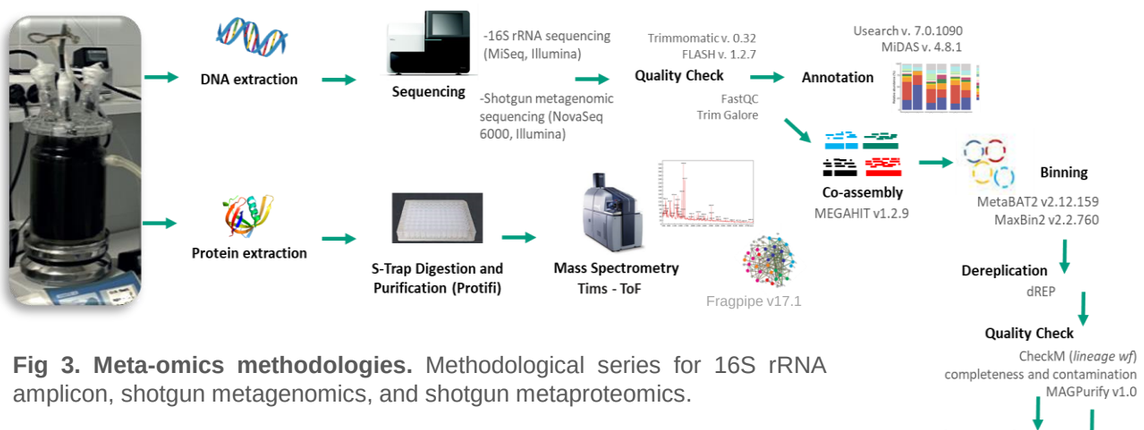

Fig 3. Meta-omics methodologies. Methodological series for 16S rRNA amplicon, shotgun metagenomics, and shotgun metaproteomics.

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High-throughput sequencing technologies allow deep investigations of the microbial populations, including interrelations predictions at the community level and interspecies interactions, besides their performance in response to different substrates and process parameters.

Group & Funding

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NAPI National network of Advanced Proteomics Infrastructure

The MEMO Group

4. Results and Discussion

The degradation of the organic matter in the AD occurs via an intertwined food web where known and unknown microbial species interact.

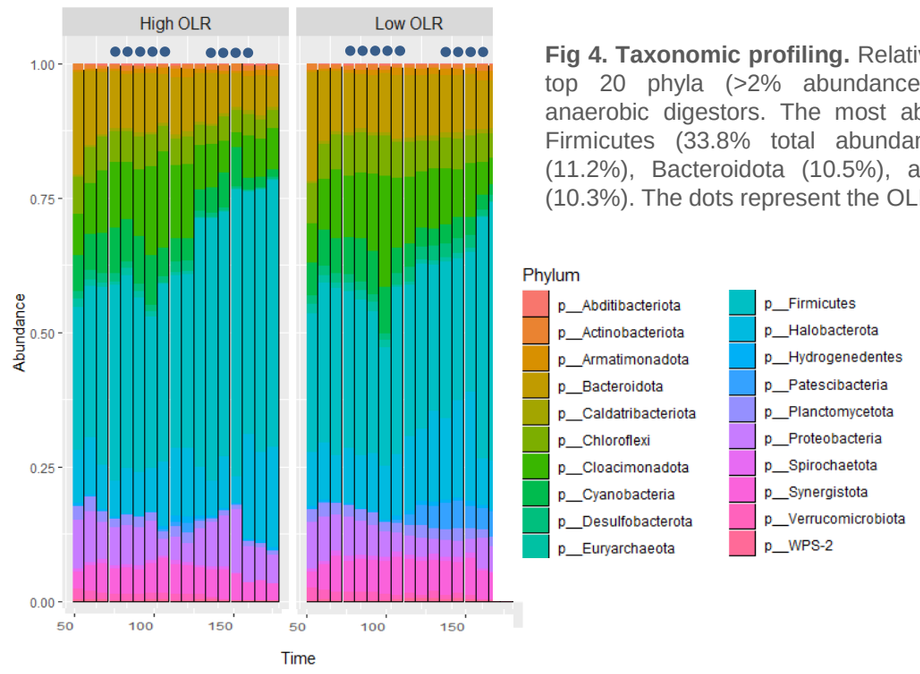

Fig 4. Taxonomic profiling. Relative abundance of the top 20 phyla (>2% abundance) detected in the anaerobic digestors. The most abundant phyla were Firmicutes (33.8% total abundance), Halobacterota (11.2%), Bacteroidota (10.5%), and Cloacimonadota (10.3%). The dots represent the OLR shocks.

The investigation of the most abundant genera that fluctuate in abundance over time may provide valuable information on the dynamics of microbial populations in the bioreactors and their role to the different steps of anaerobic digestion. Whereas *Methanothrix* dominate in more stable, steady-state systems, *Methanosarcina* prevail in high energy systems with higher OLR. Notably, rapid proliferation of *Petrimonas* population was observed after the 2nd high disturbance, indicating a faster fermentation pace and an important role under stress conditions.

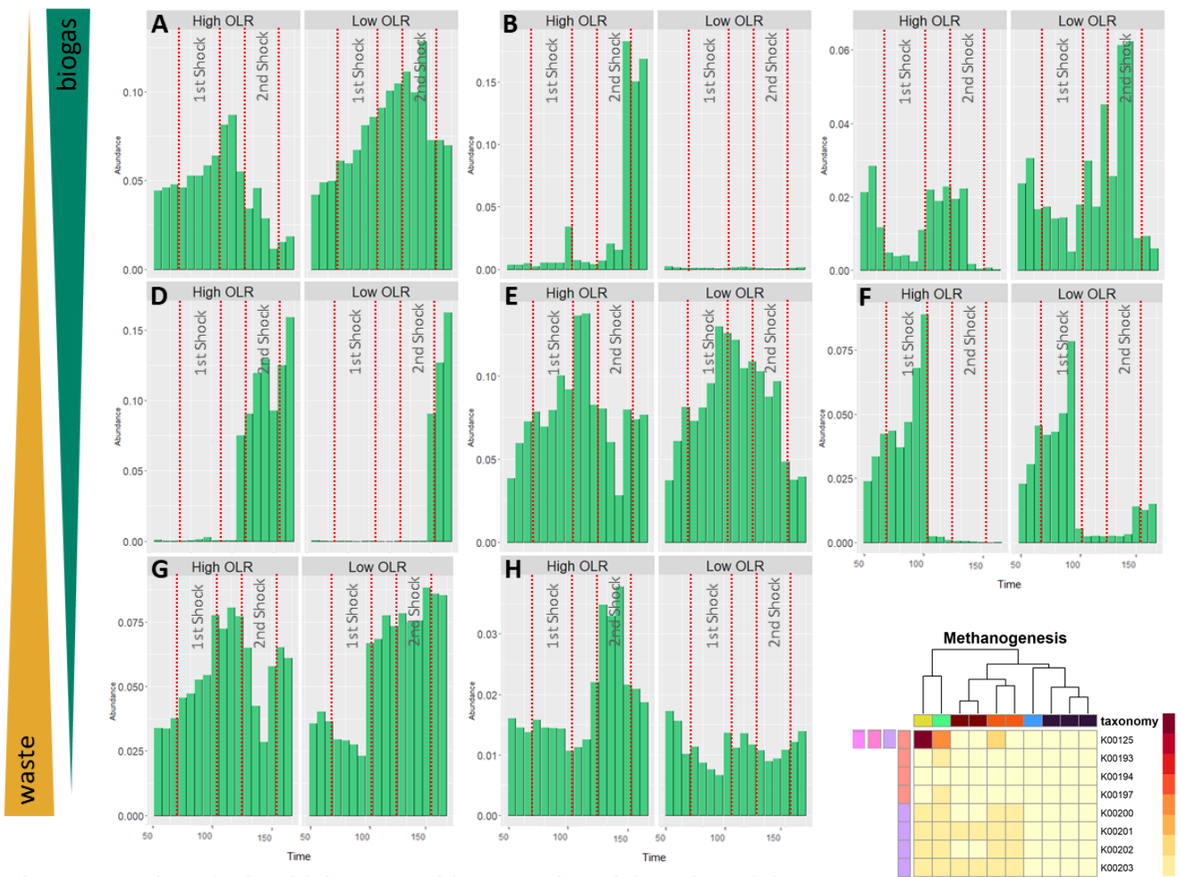

Fig 5. Dynamics of microbial communities over time. (A) *Methanothrix*; **(B)** *Methanosarcina*; **(C)** *Methanospirillum*; **(D)** *Paraclostridium*; **(E)** g__W5; **(F)** *Ca. Cloacimonas*; **(G)** *Syntrophomonas*; **(H)** *Petrimonas*. Genera **A-C** are methanogenic archaea; and genera **D-H** present syntrophic lifestyle, with propionate oxidation, and many are able to degrade microalgae cell-wall components.

After dereplication and refinement, a total of 236 high- and medium-quality metagenome-assembled genomes (MAGs) were obtained, and its metabolic reconstruction revealed the major dynamics under the OLR disturbances that explain the biogas production measured. In the example of methanogens, the heatmap revealed key genes related to the enzymatic conversion of methane present in the biogas released.

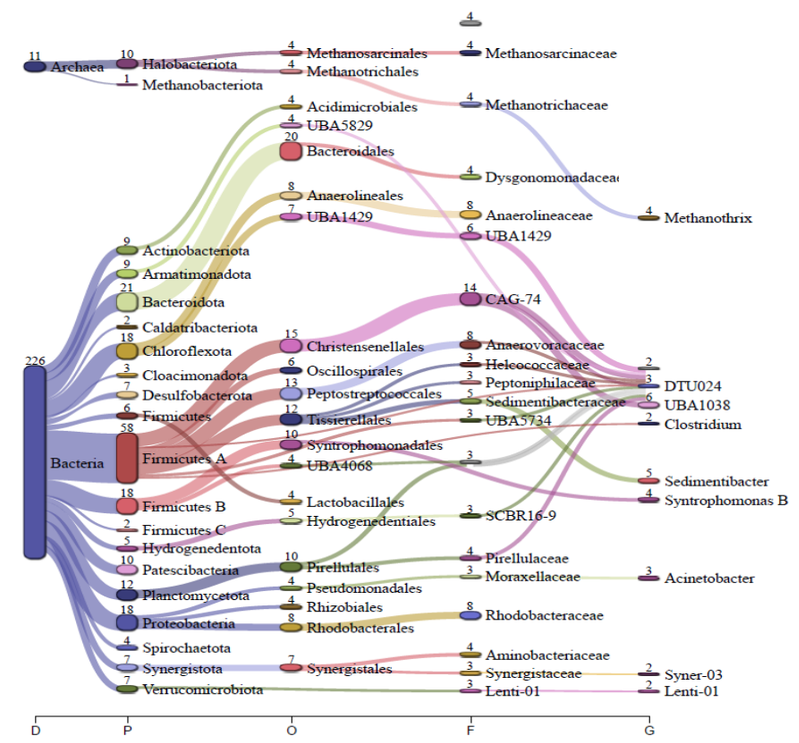

Fig 6. Abundance of MAGs across taxonomic levels.

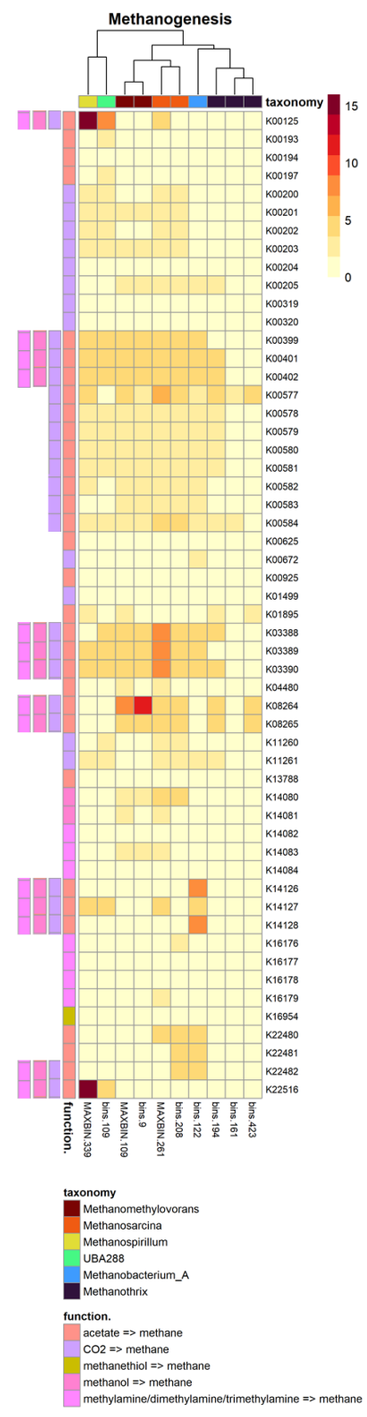

Fig 7. Methanogenesis. Archaea MAGs display key-genes for methane production.

5. Future Directions

A MAG-centric proteomics analysis resulted in **69%** of all MS-scans identified and 4,005 proteins quantified and belonging to 224 MAGs, allowing us to deduce metabolic patterns in the bioreactors.

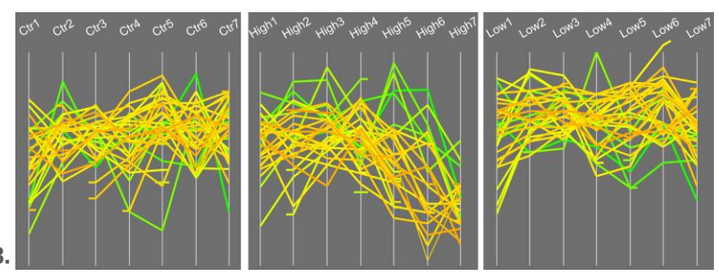

Fig 8.