µ2gas: Enhancing Anaerobic Digestion Efficiency and unveiling the Microbial World of Anaerobic Digestion

> Manthos Panou Ph.D. Biologist Qlab Member

Innovative Biomethane Production Routes in Europe, 19 June 2024



Co-funded by the European Union





Manthos Panou

Ph.D. Biology focusing in characterisation of cyanobacteria from different habitats

Member of Qlab since May, 2023

- Analyst in microbiological and biological procedures
- Member of Qlab's research grants
- Next Generation Sequencing specialist, Aim to commercialize the NGS analysis for Qlab

Member of µ2Gas project

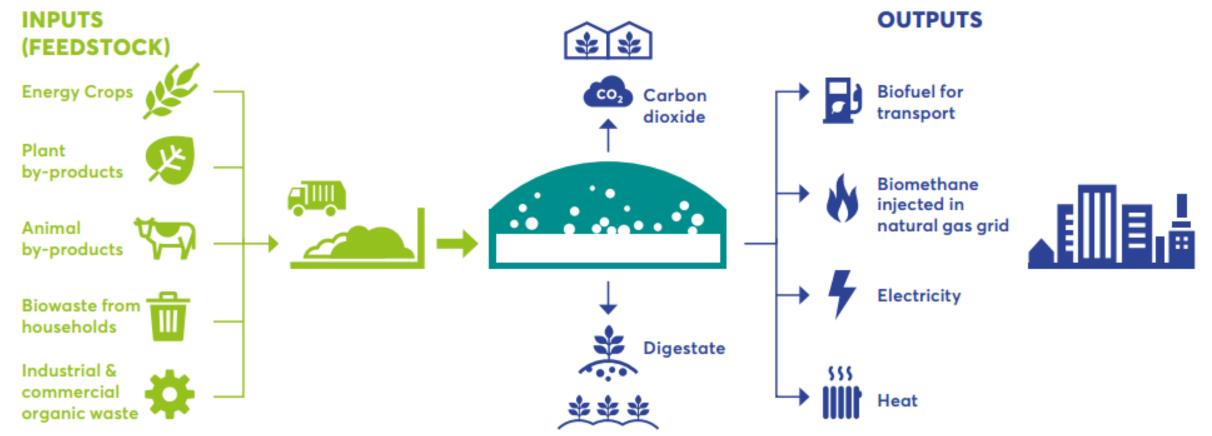
Responsibilities:

- Next generation sequencing for the microbiome of biogas plants
- Collection of data for the generation of databases
- CSTR experiments



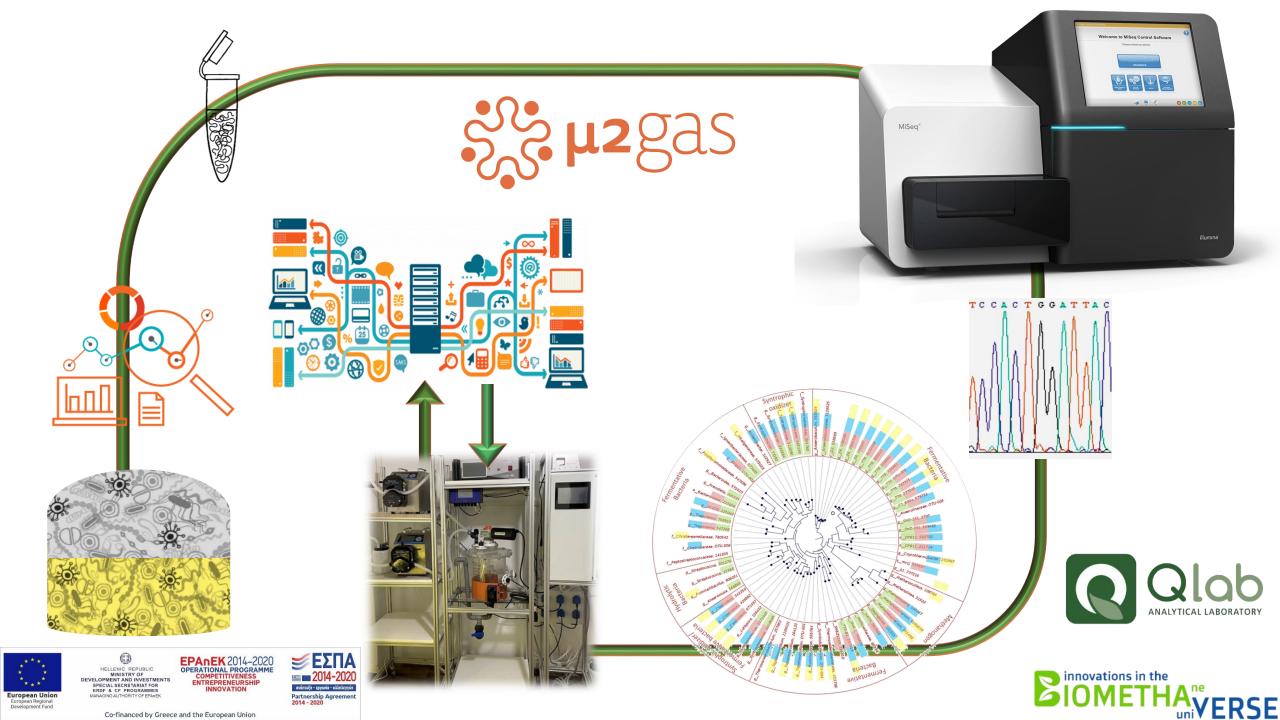






Source: EBA 2020. "Statistical Report of the European Biogas Association 2020."





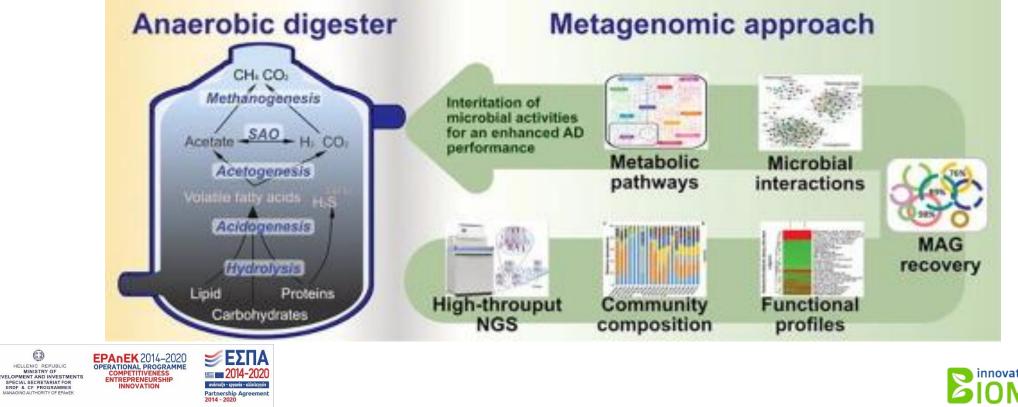


Co-financed by Greece and the European Union

Operational Management from data of 3 Biogas Plants:



- •Feedstock: Chemical Composition | Physical Characteristics | Quantity | Feedstock Microorganisms
- Process Design: Digester Technology | Digester Operation | Process Microorganisms | Diversity of Biogas Microbiome
- •Diversity of Biogas Microbiome: Taxonomic | Species Number and Distribution | Functional | Potential and Existing Species Functions | Process Stability and Efficiency | Ecological | Biotic and Abiotic Interactions







Sampling:

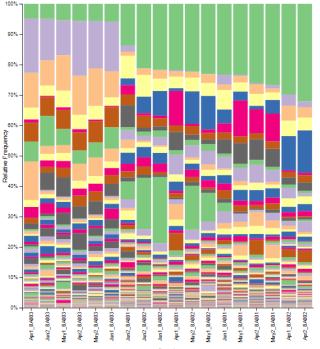
- •Collect samples from three different biogas plants
- •Ensure consistent sampling techniques for reliable comparison

Data Processing:

- •Quality control of raw sequence data from 16S rRNA
- •Filtering and trimming of low-quality reads
- •Clustering of sequences into Operational Taxonomic Units (OTUs)
- •Taxonomic assignment of OTUs

Analysis:

- •Alpha diversity analysis to assess microbial diversity within each biogas plant
- •Beta diversity analysis to compare microbial communities between biogas plants
- •Identification of core microbiome and key microbial species





Bacteria:p Firmicutes:c Clostridia:o Clostridia:f Hungateiclostridiaceae Bacteria;p_Cloacimonadota;c_Cloacimonadia;o_Cloacimonadales;f_Cloacimonadaceae d_Bacteria;p_Firmicutes;c_Clostridia;o_Peptostreptococcales-Tissierellales;f_Sedimentibacteracea d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Dysgonomonadaceae _Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Propionibacteriales;f__Propionibacteriaceae Bacteria;p Firmicutes;c Clostridia;o Peptostreptococcales-Tissierellales;f Peptostreptococcales-Tissierella Bacteria:p Firmicutes;c Clostridia;o Lachnospirales;f Lachnospiraceae _Bacteria;p_Firmicutes;c_Clostridia;o_Peptostreptococcales-Tissierellales;f_Peptostreptococcac Bacteria:p Firmicutes:c Limnochordia:o MBA03:f MBA03 __Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae d Bacteria:p Firmicutes:c Bacilli:o Lactobacillales:f Streptococcaceae d_Bacteria;p_Firmicutes;c_Syntrophomonadia;o_Syntrophomonadales;f_Syntrophomonadacea Bacteria;p Firmicutes;c Bacilli;o Erysipelotrichales;f Erysipelotrichaceae Bacteria:p Firmicutes;c Clostridia;o Caldicoprobacterales;f Caldicoprobacteraceae d Archaea:p Halobacterota:c Methanosarcinia:o Methanosarciniales:f Methanosarcinacea Bacteria:p Bacteroidota:c Bacteroidia:o Bacteroidales:f Marinilabiliaceae Bacteria:p Actinobacteriota:c Actinobacteria:o Actinomycetales:f Actinomycetac __Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillacea __Bacteria;p__Firmicutes;c__Clostridia;o__Christensenellales;f__Christensenellaceae d__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae d_Bacteria;p_Synergistota;c_Synergistia;o_Synergistales;f_Synergistaceae __Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidales_UCG-001 Bacteria:p Cloacimonadota:c Cloacimonadia:o Cloacimonadales:f W27 Bacteria:p Planctomycetota:c Planctomycetes: : d Bacteria:p Firmicutes:c Clostridia:o Oscillospirales:f [Eubacterium] coprostanoligenes group __Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Anaerovoracaceae _____Bacteria;p___Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae Bacteria;p Actinobacteriota;c Actinobacteria;o Corynebacteriales;f Corynebacteriaceae d Bacteria;p Firmicutes;c Clostridia;o Oscillospirales;f Ruminococcaceae Bacteria;p Chloroflexi;c Anaerolineae;o Anaerolineales;f Anaerolineacea d__Archaea;p__Halobacterota;c__Methanosarcinia;o__Methanosarciniales;f__Methanosaetacea d Bacteria:p Firmicutes:c Incertae Sedis:o DTU014;f DTU014 Bacteria:p Verrucomicrobiota:c Verrucomicrobiae:o LD1-PB3:f LD1-PB3 __Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae d_Bacteria;p_Firmicutes;c_Desulfotomaculia;o_Desulfotomaculales;f_Desulfotomaculales d_Bacteria;p_Patescibacteria;c_Dojkabacteria;o_Dojkabacteria;f_Dojkabacteria

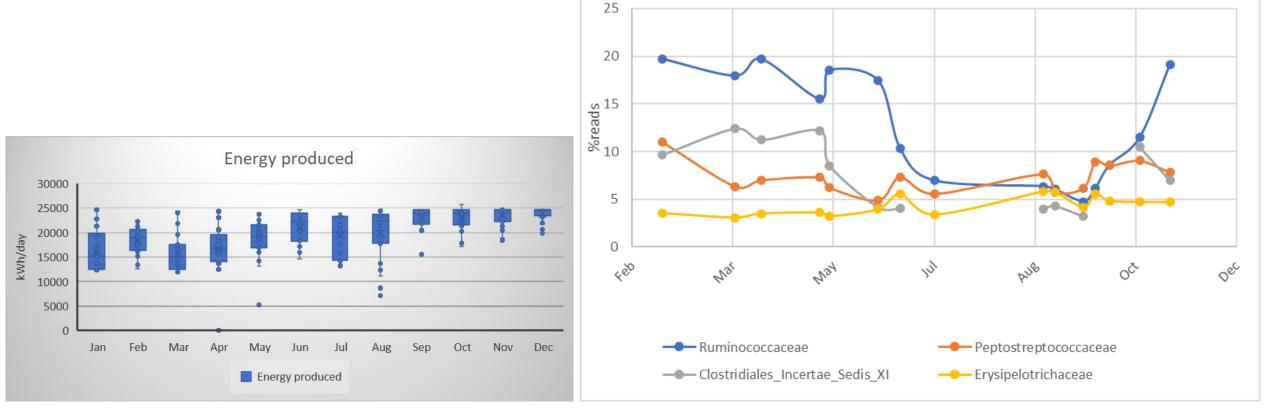
d_Bacteria;p_Firmicutes;c_Limnochordia;o_Limnochordia;f_Limnochordia





Microbiome-oriented data mining of operational monitoring of anaerobic digestion reactor during steady operation period, failure, and restoration.





Variation of abundance rates of the four main families during the monitoring period of the unit.









Goal: Verify correlation of functional changes at each stage of the process with microbiome composition and methane yields.



Outcome:

- •Develop a commercial service offering next generation sequencing and bioinformatics support to AD system practitioners
- •Identify OTUs and key microorganisms in anaerobic digestion
- •Correlate key microorganisms with operational interventions
- •Develop rapid molecular protocols for detecting key microorganisms for diagnosing interference in biogas plants





Investigations Include:

- Raw materials
- Operating temperature
- pH
- Free ammonia
- Hydrogen sulphide percentages

Sampling and Sequencing:

- At defined chemical accumulation values
- When an increase or decrease in yields is observed

Further Experiment Design:

- Based on initial knowledge from the Database **Work Module Aim**:
- Find efficient corrective actions in the operation of the anaerobic digestion reactor
- Verify remedial actions in laboratory conditions
- Investigate and identify the relationship between the microbiome and high methane yields

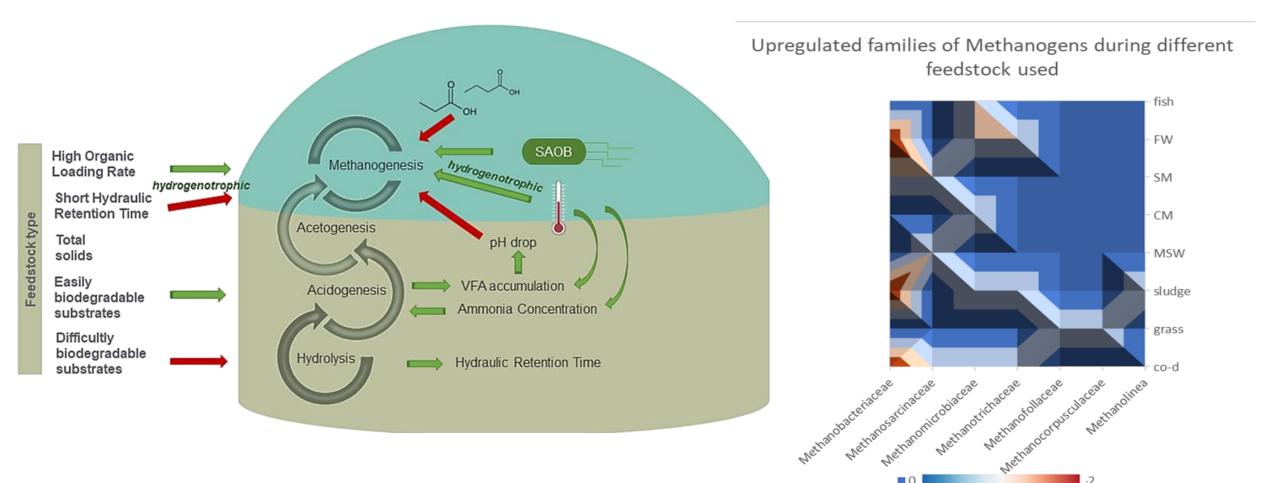






Environmental Factors Affecting Microbiome Composition and Function (under

review)



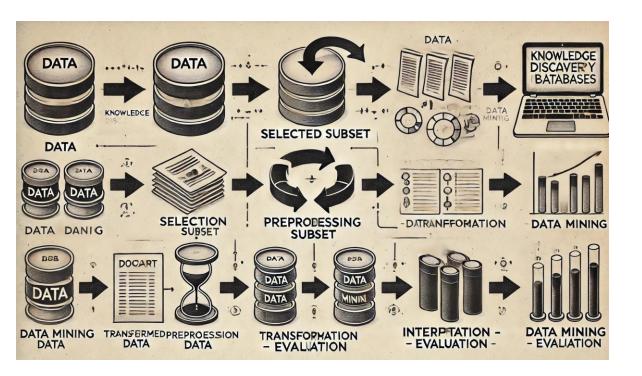


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Microbiome-oriented data mining of operational monitoring of anaerobic digestion reactor during steady operation period, failure, and restoration.



Knowledge Discovery in Databases (KDD):

- Process of extracting interesting, previously unknown, and potentially useful information or patterns from data in large databases.
 Data Mining:
- Most important step in the KDD process
- Application of data analysis and discovery of algorithms to produce a specific enumeration of patterns in the data **Stages of Knowledge Discovery:**
- Data Selection

Data Preprocessing:

- Handling incomplete data, empty fields, and other anomalies
- Also known as the data cleaning stage

Selection of an Algorithm and Application:

- Determining the type of knowledge to be sought
- Choosing the appropriate category of algorithm
- Derivatives of the knowledge discovery process include information patterns informative patterns









Thank You



the European Union

