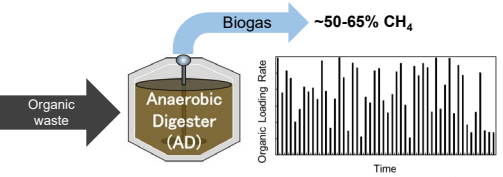
Microbial community and predicted biochemical pathways changes with organic load in anaerobic digestion

*Jericho Victor Mercado, Mitsuhiko Koyama, Kiyohiko Nakasaki
Tokyo Institute of Technology, School of Environment and Society

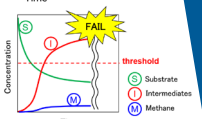
IC-3

Tokyo Tech

BACKGROUND



Practical-scale AD systems experience inherent **fluctuations in Organic Loading Rate (OLR)** due to demand variations leading to **unstable AD operations.**
There is still room to maneuver towards more robust AD systems that can handle OLR fluctuations by leveraging on **bioinformatics.**



Unstable Operations

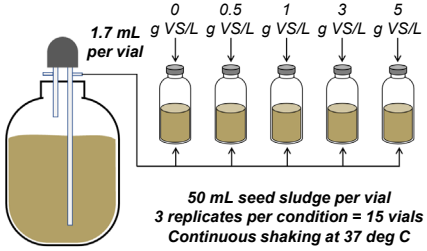
Objectives

To elucidate potential roles of microbial groups during high organic load conditions and to understand switches in biochemical pathways along with the contributing microbial groups

METHODOLOGY

Experimental Set-up

Input variable: Organic Load
Substrate: Same as acclimatization reactor



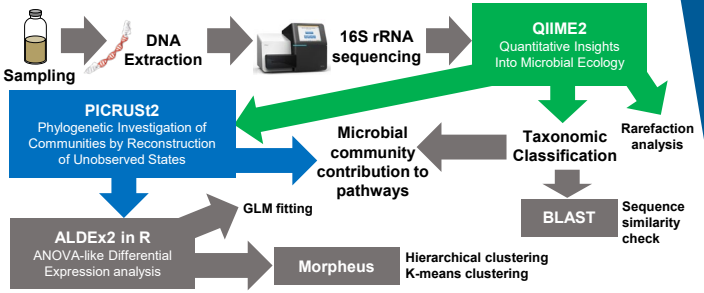
OLR: 1 g VS/L/d
Substrate:
Starch + Hipolypeptone
(21.2 : 2.8 by wt.)
+ Trace Elements & Vitamins

Response variables

- **AD Performance** — CH_4 yield, pH, VFA conc.
- **Microbial Community**
- **Biochemical Pathway Abundance**

Bioinformatics

Bioinformatics Workflow



RESULTS & DISCUSSION

1 Anaerobic Digestion Performance

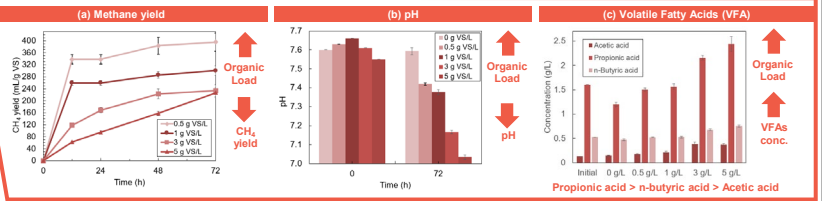


Figure 1. (a) Time course of methane yield in mL CH4 per g VS in substrate for each organic load condition; (b) VFA concentrations in g of corresponding VFAs per L of AD sludge; isobutyric acid was not detected in all of the samples; (c) Initial and final pH of AD sludge for each organic load condition

2 Microbial Community

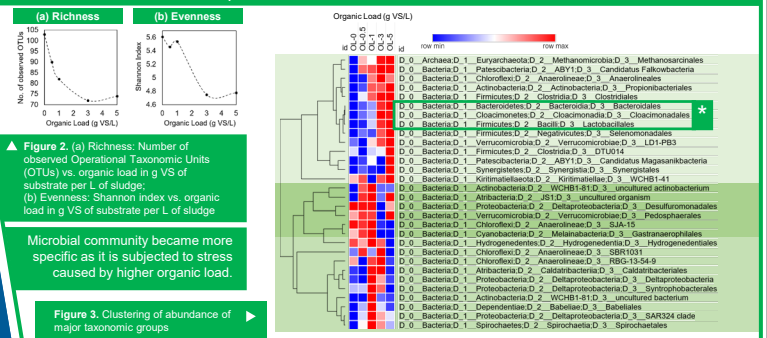


Figure 2. (a) Richness: Number of observed Operational Taxonomic Units (OTUs) vs. organic load in g VS of substrate per L of sludge; (b) Evenness: Shannon index vs. organic load in g VS of substrate per L of sludge

Microbial community became more specific as it is subjected to stress caused by higher organic load.

Figure 3. Clustering of abundance of major taxonomic groups

K-means clusters are identified by different shades of the background color of each taxonomic group.
★ Bacterial groups that are significantly increasing in relative abundance with increase in organic load as confirmed via GLM fitting (ALDEx2)

3 Biochemical Pathway Abundance

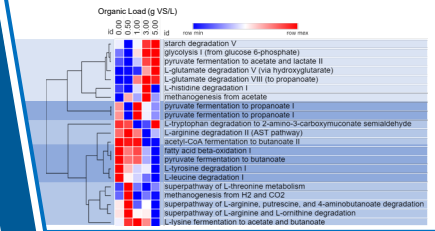


Figure 4. Clustering of predicted pathway abundances from PICRUSt2

The predicted pathway counts were normalized in terms of centered log ratio (clr) via the ALDEx2 package.

K-means clusters are identified by different shades of the background color of each pathway.

4

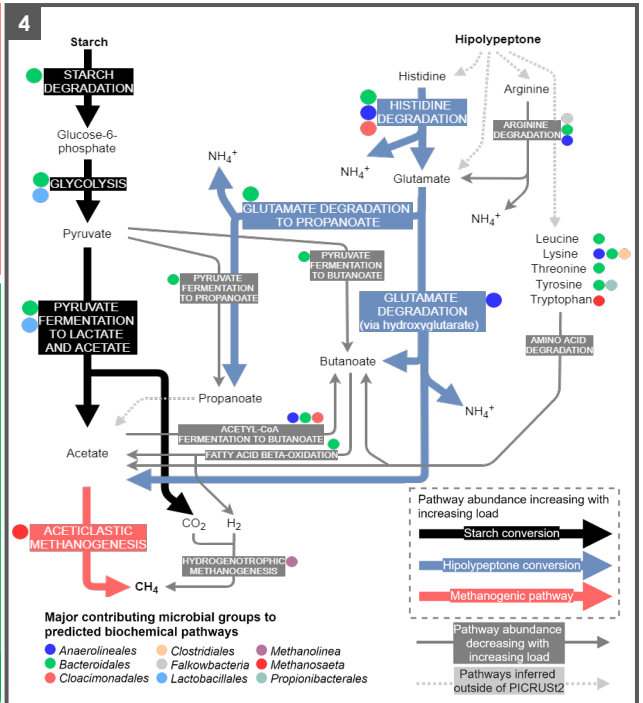


Figure 5. Proposed pathway of utilization of starch and hipolypeptone in AD inferred from the AD performance, microbial community, and biochemical pathway abundance data

- Lactobacillales was the key player in starch degradation leading to production of acetate.
- Fermentation with pyruvate to acetate vs. to butanoate is preferred at high OL as it allows faster consumption of starch and subsequent aceticlastic methanogenesis.
- With lower butanoate production, fatty acid beta-oxidation is impeded lowering H_2 production.
- With limited H_2, methanogens were forced to use acetate making Methanosaeta prevalent as Methanolinea cannot perform aceticlastic methanogenesis.

CONCLUSION

The **variations in the microbial community and the predicted biochemical pathways** were used to **explain the microbial interactions and mechanisms in AD** operating at high organic load with starch and hipolypeptone as substrate. Based on these, an overall pathway of utilization of the substrate was proposed. Knowledge obtained from this study supports further research on engineering of microbial consortium to manage carbohydrate-rich substrates.

31st Annual Conference of JSMCWM · September 2020