Biogas Upgrading Communities in High-Resolution 16S: Contrasting in situ and ex situ Setups

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Better Biogas - as biomethane becomes increasingly attractive biofuel, focus has shifted to enriching methane output of anaerobic biogas reactors.

**in situ** biological upgrading adds excess hydrogen to a standard biogas reactor, enabling methanogenic *Archaea* to convert biogas carbon dioxide (CO₂, 40-60% v/v) to methane (CH₄, 40-60% v/v).

**Ex situ** upgrading of biogas also converts CO₂ to CH₄ via methanogens, but occurs in an autotrophic reactor operated exclusively to facilitate methanogenesis without a fermentation feedstock (i.e. fed only CO₂ & H₂).

Risk of a Blow-Out: addition of H₂ severely disrupts aspects of the anaerobic community—in particular, low H₂ partial-pressure is of crucial for fermentation, the inhibition of which causes rapid acid accumulation. Additionally, the intense operation required for biogas upgrading can quickly overwhelm biogas communities, leading to reactor stall or failure.

Autotrophic biogas upgrading communities are extremely attractive, but the communities involved are poorly characterised, and their dependencies are unclear. To determine how feedstock, hydrogen, and CO₂ influence biomethanation, we applied high-throughput sequencing of prokaryote 16S amplicons to 23 samples taken from a series of 4 thermophilic upgrading reactors run in succession over 520 days, transitioning from in situ upgrading at low hydrogen flow rates (37L H₂/day), to extremely high hydrogen flow rates (400L H₂/day) ex situ [1].

**Highlights**

- Feedstock presence/absence determined dominant *Archaea* [methanogens] and Bacteria (fermenters)
- In **in situ** extremely sensitive to relatively low hydrogen flow rates (~37L/day), which lead to collapsing methanogenic/Thermotogae populations (ISA, ISB) and rapidly accumulating acids.
- In contrast, ex situ was highly robust to hydrogen addition up to >400L/day, greatly exceeded these levels with large Methanobacterium populations (20-30%) (CES)
- Enormous proportion (25-30%) are uncharacterised (Firmicutes: MBA03 Order, or have no known taxonomic placement [Domain: Unknown], underscoring the novelty of the community involved.
- In either upgrading setup, increased hydrogen disrupted fermenting and hydrolyzing populations, prior to disruption of methanogens.
- Instability due to H₂ supply coincides with increases in homoacetogens, demonstrating a pronounced competition effect with methanogens (ISB week 8&9)
- Reduction of hydrogen rates ex situ (258L/day) corresponds with proliferation of various syntrophic H₂-producing fermenters, possibly capitalising on the improved thermodynamics allowed by the large hydrogenotrophic population

**Methodology**

All extraction steps were carried out in three technical replicates before being combined. Biomass sampling, DNA extraction (E.Z.N.A., Qiagen), and library preparation (NEXTGEN, Illumina) followed by bioinformatics analysis and de novo assembly. Datasets were aligned to amplify sequence variants, tested for differential abundance and visualised, at a particular value were the packages phyloseq, DESeq2/DESNR, vegan, ggmix2 and ComplexHeatmap [2-9].

**References**

7. Jari Oksanen et al (2015). “Vegan: community ordination of differentiation between in situ and ex situ reactor communities. Each space bounded by a black box represents a population sharing an exact amplicon sequence variant (ASV, i.e. 100% sequence identity)

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