Long-term preserved composite methanogenic inocula for bioaugmentation of unwanted biomasses

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Anaerobic digestion (AD) has been identified as a sustainable technology for the conversion of organic waste into bioenergy (Dhanya et al., 2020). However, the efficacy of AD is frequently impeded by the presence of lignocellulose- and ammonia-rich substrates, which restrict hydrolysis and disrupt methanogenic activity, resulting in methane yield losses of up to 30% (Fotidis et al., 2017). Acclimating ammonia-tolerant methanogens and hydrolytic bacteria is essential for their survival, efficiency in high-ammonia environments and high concentrations of lignocellulosic material respectively. Consequently, since methanogens grow slowly, it is vital to create a robust and easy way to preserve ammonia-tolerant methanogenic consortia, in a ready-to-use product for a cost-efficient commercial application (Fotidis et al., 2014). This study explores the potential of bioaugmentation with lyophilized hydrolytic bacteria and ammonia-tolerant methanogens to enhance AD performance in continuous stirred tank reactors (CSTR).

Ammonia-tolerant methanogenic consortia were identified and cultivated, which mainly include *Methanoculleus bourgensis*, to withstand extreme levels of ammonia (>5 g NH_4^+–N/L). At the same time, key strains of hydrolytic bacteria, including *Ruminococcus Albus* and *Bacteroides Cellulosolvens*, were purchased (DSMZ, Germany) for their ability to degrade efficiently lignocellulosic material and then acclimatised at concentrations up to 4 g VS L^{-1} of wheat straw. Following centrifugation at 1500 rpm, the specialised microbial consortia were lyophilised at -80°C for 15 h using a novel lyophilisation process that ensures high revival rates (>80%) and prolonged viability (data not shown). After lyophilization, the consortia were stored at room temperature for more than 150 days (Figure 1), before used into the batch reactor bioaugmentation experiment in equal (w/w) quantities.

Figure 1. Long-term preserved composite methanogenic inocula for bioaugmentation of unwanted biomasses

Two sets (in triplicates) of batch reactors (R_{Ctrl} and R_{BioAug}) were configured with 150 ml and 320 ml working and total volume, respectively. These were filled with 50 ml of active unacclimatised inoculum, from a full-scale biogas plant, 100 ml of BA medium (Angelidaki & Sanders, 2004), 5 g NH_4^+-N L^{-1} of NH_4Cl was used as ammonia source and 4 g VS L^{-1} of wheat powdered straw was used as lignocellulosic source. Bioaugmentation was performed in the R_{BioAug} reactor by transferring 2.5 g L^{-1} of composite lyophilised inoculum to the reactors. At the same time, 2.5 g L^{-1} of inactivated (autoclaved at 121°C) lyophilized inoculum was also introduced into the R_{Ctrl} reactor, to reproduce the same chemical, organic loading and hydraulic effects (abiotic augmentation) that the lyophilised inoculum had in the R_{BioAug} reactor. The wheat's biochemical methane potential (BMP) was 307.9 ±12.7 mL CH_4, and was evaluated according to (Holliger et al., 2016), in three batch reactors filled with 100 mL of inoculum, 50 mL BA medium and 1 g VS L^{-1} of wheat straw. Also, three more reactors were used to measure methane production from inoculum (background methane production). Reactors' performance was then assessed based on methane yield, maximum growth rate (μ_{max}) and process stability.

The results demonstrated that the R_{BioAug} demonstrated a methane recovery efficiency of more than 85% compared to the BMP results, while the R_{Ctrl} reactors achieved only 63% of the BMP (Figure 2a). Furthermore, it was found that bioaugmentation of methanogens improved methane yield by at least 30%, compared to the non-augmented control reactors. Additionally, bioaugmentation with lyophilized composite methanogenic communities accelerated the degradation of lignocellulose and ammonia-rich substrates with the μ_{max} of R_{BioAug} was 18,2 % higher than the μ_{max} of R_{Ctrl} (Figure 2b). Finally, the R_{Ctrl} exhibited a lag phase that lasted approx. five days, contrary to R_{BioAug}, which entered the exponential production phase after only two days.

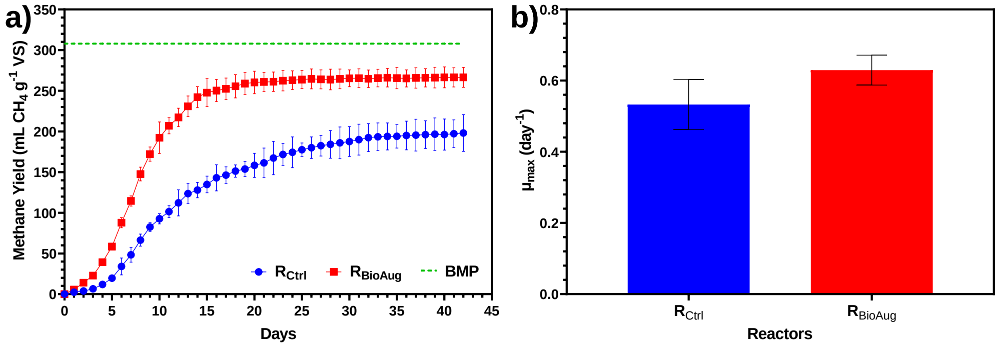

Figure 2. a) methane production yield and b) maximum growth rate (μ_{max})

Furthermore, microbial community analyses confirmed that *Methanothermobacter* sp. and *M. bourgensis* became the dominant methanogens in then bioaugmented batch reactors, verifying their successful establishment. The bioaugmentation process, facilitated a rapid microbial succession, known as the "microbiological domino effect" (Tian et al., 2019), which enabled the adaptation of methanogenic communities to high lignocellulose ammonia levels. This emphasizes the resilience of the lyophilised hydrolytic and methanogenic consortia in catabolising unwanted lignocellulose-and ammonia-rich biomasses.

Interestingly, ongoing preliminary lab-scale continuous reactor experiments, bioaugmented with the same lyophilized hydrolytic and methanogenic inocula and operated under high ammonia (5 g NH_4^+-N L^{-1}) and lignocellulose (4 g VS L^{-1}) levels, increased overall biomethanation production by at least 25% compared to the abiotic augmentation control reactor (data not shown). Thus, it is expected that the new bioaugmentation strategy developed in the MicroPower project, it is comparably effective both in batch and continuous AD reactors. These findings underscore the potential of bioaugmentation with lyophilized hydrolytic and methanogenic consortia as a cost-effective and scalable approach to optimize AD of unwanted biomasses and future research should be focused on the upscaling of this novel process.

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