A novel bioaugmentation using long-term stored specialised inocula in ammonia inhibited biomethanation process

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Bioaugmentation with ammonia-tolerant methanogens into inhibited anaerobic digestion (AD) systems was proposed as a successful in-situ remediation strategy to alleviate ammonia toxicity (Fotidis et al., 2014). Nevertheless, methanogens are slow growing and vulnerable to fluctuating environment (Thauer et al., 2008). The strict preservation and transfer of stock cultures can be extremely limiting for commercial application (Yan et al., 2020). Thus, 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. The current study aims to assess the shelf life of lyophilized ammonia-tolerant consortia and its bioaugmentation performance to alleviate ammonia inhibition in AD reactors.

Inoculum from full-scale biogas plant was acclimatised in batch AD reactors through stepwise ammonia increases. The acclimatisation process included four steps (30 days each), the concentration of ammonia nitrogen was increased to 3.0, 4.0, 5.0 and 6.0 g NH_4^+-N L^{-1}, respectively for each step. After centrifugation at 1500 rpm, the inoculum solution was lyophilized at -80°C for 15 hours. Three groups of batch mesophilic AD reactors were set up with digestate (degassed for 10 days before use) and 1.2 g L^{-1} Avicel (carbon source), each group had triplicate reactors with 640 mL working volume. Group A was used as the control group with 1.39 g L^{-1} ammonia concentration. Group B and C were operated under 5.0 g NH_4^+-NL^{-1} L^{-1}. After lyophilization, the consortia were stored at room temperature for 180 days and then were added into Group C' reactors to boost the AD process. Meanwhile, another reactor DG (containing only basal anaerobic medium (Fotidis et. al., 2013) and Avicel) was used for the lyophilized consortia reactivation experiments.

Results showed that at low ammonia concentration (1.39 NH_4^+-N L^{-1}), *Methanosaeta* sp. was the dominant strain without ammonia inhibition (Fig. 1). After 120 days of acclimatization, *Methanoculleus bourgensis* became the dominant methanogens with a relative abundance of 63.2% in the consortia, which was used to the lyophilization process.

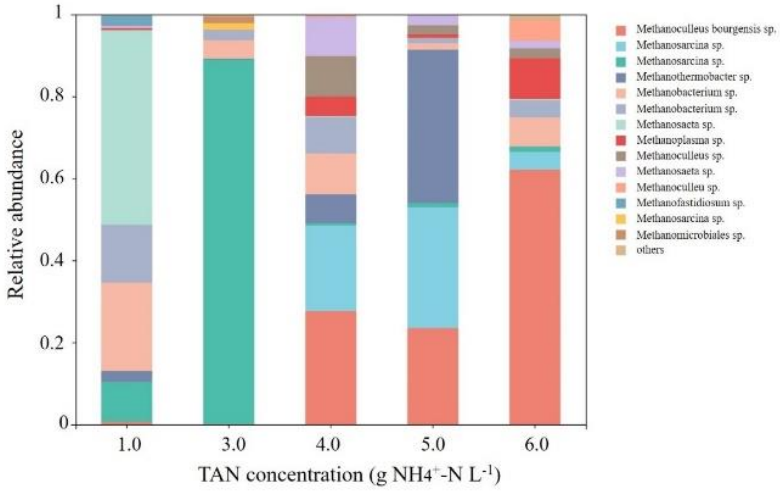

Figure. 1 Relative abundance of methanogens in ammonia tolerance acclimatized cultures

The average methane production of A, B and C was 436.5±48.0, 274.8± 27.5 mL and 392.0±45.135 mL, respectively (Fig. 2). Bioaugmentation with lyophilized inoculum recovered 28.9% of the uninhibited methane yield. In addition, the maximum daily methane production rate of group A, B and C was 104.1, 49.0 and 77.4 mL CH_4 L^{-1} d^{-1}, respectively. Moreover, group C had experienced significantly shorter lag-phase during methanogenesis. The results clearly showed that bioaugmentation with lyophilized inoculum improved methanogenic activity under ammonia stress.

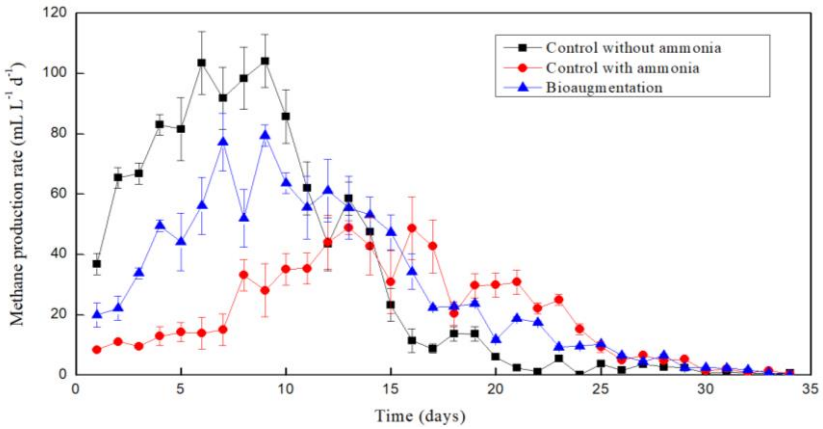

Figure 2. Effect of lyophilized inoculum (stored for 180 days) on methane production performance

The microbial community composition in the four groups, at species level, are visualized with 5 archaea OTUs and 21 bacteria OTUs. In control group, the relative abundance of *Methanosaeta sp*. and *Methanoculleus Bourgensis* were 39.2% and 42.6%, respectively. Microbial community structure of the three reactors in the ammonia inhibited group B was very distinct among each other.

Methanosarcina spp., a polytrophic methanogen with an average relative abundance of 89.4%, was the most abundant methanogenic bacterium in Group C reactors. In reactor DG, all methanogens had poor recovery performance, only *Methanosarcina* sp. *Methanoculleus bourgensis* recovered slightly with a total relative abundance of less than 0.01%. *Castellaniella* sp. and *Sporanaerobacter* sp., showed prominent reactivation performance. Interestingly, the average relative abundance of *Geobacillus sp*. in group C reactors was 11.3%, which is the direct interspecific electron transfer partner of *Methanosarcina* sp. (Li et al., 2022). The enhancement of direct interspecific electron transfer was the potential reason for successful bioaugmentation. Bioaugmentation with lyophilized inoculum promoted the methanogenic efficiency under ammonia stressed. However, the long-term maintenance of methanogenic activity and the reactivation method need further study.

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