**Mitigation of Ammonia Inhibition Through Bioaugmentation in Anaerobic Digestion: Selection of Strains and Reactor Performance Evaluation.**

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**Highlights**

* Bioaugmentation could achieve 71% increased methane production and 85% biodegradability.
* Bioaugmentation with *Syntrophaceticu smithii* + *Methanobrevibacter* spp. was optimum.
* Enhancement of both aceticlastic and hydrogenotrophic methanogenesis was important.
* *Methanobrevibacter* and *Methanosarcina* spp. were non-dominant but important archaea.

**1. Introduction**

Anaerobic digestion (AD) has gained extreme attention since producing renewable energy [1]. Ammonia is considered as a key parameter affecting AD efficiency [2]. Bioaugmentation could be a potential method to improve AD efficiency [3]. Researchers have attempted to increase methane production (MP) by strengthening the hydrogenotrophic methanogenesis bioaugmentated with certain strains [4]. However, other studies didn’t indicate significant improvements [5]. Knowledge of mitigating ammonia inhibition effect with hydrogenotrophic methanogens is lacking. On the other hand, *Methanosaeta* and *Methanosarcina* were found to be dominant archaea [6]. Whether bioaugmentation with aceticlastic methanogens could achieve efficient MP remains unknown. The objectives of the present research are as follows: (1) to compare the bioaugmentation effects of different strains and (2) to develop an ammonia-tolerant environment for MP.

**2. Methods**

Seven pure strainswere selected (Table 1). The total and working volumes were 500 and 350 mL. Glucose and NH4Cl were applied as carbon and ammonium nitrogen sources. Organic loading rate was 1 g glucose/L/d. Hydraulic retention time was 10 days. The reactors were carried out in a 37 °C continuous stirred shaker. The ammonia level was 4 NH4+-N/L. Biogas production, NH3-N, were determined as publications [7]. Specific methanogenic activity (SMA), stable isotopic analysis (based on αc values), and 16S rRNA gene sequencing analysis were described in publications [7,8].

**Table 1.** Abbreviation of different strains used in this study

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| Pure strain | Abbreviation | Pure strain | Abbreviation |
| *Methanosaeta harundinacea* | MSH | *Methanosarcina barkeri* | MSB |
| *Methanobacterium bryantii* | MBB | *Syntrophaceticus schinkii* | SS |
| *Methanoculleus bourgensis* | MCB | *Tepidanaerobacter acetatoxydans* | TA |
| *Methanobrevibacter smithii* | MBS |  |  |

**3. Results and discussion**

Bioaugmentation with SS + MBS was the optimal choice (Figure 1; MP was 71.1% higher than that in Blank), the activity of hydrogenotrophic methanogenesis was greatly heightened according to SMA (Figure 2). And bioaugmentation with MSB alone was also proven efficient (MP was 59.7% higher than that in Blank), both aceticlastic and hydrogenotrophic methanogenesis were enhanced. Further evaluation with carbon isotope fractionations analysis (Figure 3) indicated that balancing the activities of the aceticlastic and hydrogenotrophic methanogenic pathways is of great importance. 16s rRNA gene sequencing results (Figure 4) showed that showed that *Methanobacterium* spp. and *Methanosaeta* spp. were the dominant archaea in all 14 reactors. Nevertheless, bioaugmentation with *Methanosaeta* spp. did not result in a positive effect on MP. On the other hand, *Methanobrevibacter* spp. and *Methanosarcina* spp. were non-dominant archaea (even after bioaugmentation with MBS or MSB, the relative abundances were still poor (< 2%)), but displayed pivotal roles in determining the overall microbial consortium and, in turn, improved the overall performance.

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| **Figure 1.** MP and biodegradability of different reactors | **Figure 2.** SMA and average MP in different reactors  |
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| **Figure 3.**  αc values and pathway in different reactors | **Figure 4.** Relative abundance of different methanogens |

**4. Conclusions**

Bioaugmentation could be an efficient way with over 59% increased on MP. aceticlastic and hydrogenotrophic methanogenic pathway should be both taken into account during bioaugmentation and the balance could result in a better reactor performance. Non-dominant archaea might display pivotal roles in improving AD performance.

**References**

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