Innovative bioaugmentation strategies to alleviate ammonia inhibition in anaerobic digestion process - DTU Orbit (11/10/2019)

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Anaerobic digestion (AD) is a widely used biotechnology to recover energy (in the form of biomethane, CH4) from various biowastes and biomasses. In recent years, nitrogen-rich substrates, such as chicken manure, slaughterhouse waste, microalgae, etc. are becoming attractive AD substrates due to their high methane potential. However, high ammonia levels caused by the degradation of these nitrogen-rich substrates usually inhibit the AD process, resulting in low methane production and volatile fatty acids (VFAs) accumulation. In this Ph.D. project, an efficient bioremediation method, i.e. bioaugmentation, was developed to overcome the ammonia inhibition in the AD process and thereby improving the methane production.

First of all, the prerequisite of a successful bioaugmentation is to acquire the ammonia-tolerant methanogenic consortia and use them as bioaugmentation inocula. Thus the possibility of acclimatizing microbial community to high ammonia levels was assessed in this study. The results showed that microbial inocula taken from two mesophilic and three thermophilic full-scale biogas plants were successfully acclimatized to high ammonia levels with a batch cultivation method. In addition, another acclimatization performed in a continuously stirred tank reactor (CSTR) was also achieved. The microbial community composition changed significantly in both studies, and Methanosarcina spp. were found to be dominant in the final ammonia-tolerant methanogenic community, indicating their important role in overcoming the ammonia toxicity.

Moreover, focusing on the future full-scale application of bioaugmentation, a fast and efficient method to enrich the ammonia-tolerant consortia was developed based on the comparison of different acclimatization methods. The results demonstrated that the fed-batch was the most efficient method to acclimatize the ammonia-tolerant consortia. Specifically, compared to the batch acclimatization method, fed-batch saved up to 150% incubation time, achieved two times higher free ammonia (FAN) levels and improved 37%-153% methanogenic activity.

Thereafter, two bioaugmentation studies were performed to investigate the bioaugmentation efficiency and unravel the working mechanism. The results demonstrated a positive effect of bioaugmentation on improving the performance of the ammonia inhibited reactors. Firstly, after the bioaugmentation of pure hydrogenotrophic Methanoculleus bourgensis, 28% methane production improvement was observed in a mesophilic CSTR reactor. Secondly, two bioaugmentation inocula were used under thermophilic conditions: an enriched ammonia-tolerant methanogenic consortium, and a mixed inoculum of a pure hydrogenotrophic methanogen and the enriched consortium. Compared to the control, 11-13% higher methane production was detected in the bioaugmented reactors with both inocula. In addition, compared to the bioaugmentation only with the enriched consortium, a faster methane recovery rate was observed by using the mixed inoculum. Based on the results, it was proposed that the instant hydrogen partial pressure reduction by the bioaugmented hydrogenotrophic methanogens played a significant role in alleviating ammonia inhibition in the AD process. Moreover, the "microbiological domino effect" was identified as the key mechanism of a successful bioaugmentation. In other words, even though the bioaugmented microorganisms were not the most abundant members in the microbiome, they were able to trigger an overall microbial community change towards a more efficient AD microbiota.

Additionally, the impacts of other physicochemical factors, i.e. long chain fatty acid (LCFA) and different ammonia sources, on ammonia inhibition in the AD process were also assessed in this Ph.D. project. An ammonia-LCFA synergistic co-inhibition effect was identified in both batch and CSTR reactors. The results suggested that β-oxidation of LCFA was inhibited by high ammonia levels, thus resulted in the excess LCFA levels, which triggered the synergistic co-inhibition between ammonia and LCFA. Moreover, urea was found to be a stronger inhibitor compared to NH4Cl. The higher toxicity of urea was attributed to the momentary higher FAN and pH levels during urea hydrolysis. Meanwhile, the results also demonstrated that the hydrogenotrophic methanogens were more robust than aceticlastic methanogens regardless of ammonia sources.

Overall, this study developed an efficient method to acquire bioaugmentation inocula and proved the effectiveness of bioaugmentation. Deep insight into the microbial community dynamics unravelled the bioaugmentation working mechanism and expanded the understanding of the ammonia-tolerant microbiota. The obtained findings suggested a practical solution for the future efficient utilization of nitrogen-rich substrates in the full-scale anaerobic digesters.