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Ammonia tolerant enriched methanogenic cultures as bioaugmentation inocula to alleviate ammonia inhibition in continuous anaerobic reactors

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Abstract
Ammonia is the most common inhibitor of anaerobic digestion (AD) process, resulting in suboptimal exploitation of the biogas potential of the feedstocks, causing significant economic losses to the biogas plants. Ammonia is mainly inhibiting the aceticlastic methanogens, while the hydrogenotrophic methanogens are more robust to ammonia toxicity effect. It has been shown that bioaugmentation of a pure strain of a hydrogenotrophic methanogen (i.e. *Methanoculleus bourgensis*) in an ammonia inhibited continuous anaerobic reactor can improve methane production more than 30%. Nevertheless, cultivation of a pure culture, to be used as bioaugmentation inoculum, poses technical difficulties due to the required sterile conditions and the special growing media. On the contrary acclimatized enrichment methanogenic cultures have lower requirements to sterility. In the present study, we used an enriched ammonia tolerant methanogenic culture as potential bioaugmentation inoculum in a continuous stirred tank reactor (CSTR) operating under "inhibited steady-state", triggered by high ammonia levels (5 g NH_4^+-N L^{-1}). The results of the current study established for the first time that bioaugmentation of an enriched ammonia tolerant methanogen in a CSTR reactor could completely alleviate the ammonia inhibitory effect. Furthermore, it was found that bioaugmentation with the enriched culture resulted in 25% higher methane production compared to when the bioaugmentation was achieved with pure methanogenic strains. The bioaugmentation was performed without pausing the continuous operation of the CSTR reactor and without excluding the ammonia-rich substrate from the feedstock. Thus, bioaugmentation with mixed methanogenic cultures could potentially support the development of an efficient and cost-effective biomethanation process of ammonia-rich organic waste in full-scale continuous reactors.

Keywords
Ammonia inhibition; Biogas; Biomethanation; CSTR; Methane.

INTRODUCTION

The agricultural and the food industrial sectors produce vast amounts of ammonia-rich organic wastes. One of the most promising and effective methods for treatment of these wastes is anaerobic digestion (AD) as it provides energy (methane) and a digestate with high nutrient levels (bio-fertilizer). However, ammonia-rich substrates are well known to inhibit AD process and it is estimated that many full-scale biogas reactors worldwide are seriously affected by ammonia toxicity leaving up to 1/3 of their methane potential unutilised (Fotidis, et al., 2013a). It is widely accepted that ammonia is mainly inhibiting the aceticlastic methanogenic pathway, while the syntrophic acetate oxidation pathway followed by hydrogenotrophic methanogenesis is more robust to ammonia toxicity (Westerholm, et al., 2011). It is therefore, logical to assume that use of ammonia tolerant hydrogenotrophic methanogenic consortia could provide a new solution to alleviate ammonia inhibition in AD process. Bioaugmentation has been proposed as a potential method to deliver and establish these ammonia tolerant consortia (Costa, et al., 2012) in continuous anaerobic reactors operating under high ammonia levels. Fotidis, et al. (2014) has shown for the first time that bioaugmentation of an ammonia tolerant methanogen (Methanoculleus bourgensis) in lab-scale continuous reactors, was possible with more than 30% increase in methane production. However,

growing a pure culture to use it as bioaugmentation inoculum, poses technical difficulties due to the required sterile conditions and the special growing media. Previous studies have shown that is possible to acclimatize mixed methanogenic cultures (enriched cultures) to high ammonia levels (up to 7 g NH_4^+-N L^{-1}) in batch reactors (Fotidis, et al., 2013b). These complex communities could have a greater robustness and impact on the biomethanation process than a single strain. However, these acclimatized mixed cultures were never tested as bioaugmentation inocula in continuous anaerobic reactors. Thus, the aim of the current study was to use a mixed (enriched) ammonia tolerant methanogenic culture as potential bioaugmentation inocula in a continuous stirred tank reactor (CSTR) operating under "inhibited steady-state", caused by high ammonia levels.

MATERIAL AND METHODS

Methanogenic enriched culture

The hydrogenotrophic methanogenic enriched culture (MEC) used in the bioaugmentation process derived from a mesophilic manure based full-scale reactor (Hashøj, Denmark). Before introduced to the CSTR reactor, MEC culture was acclimatised in stepwise increasing ammonia concentrations (up to 5 g NH_4^+-N L^{-1}) in fed-batch reactors with bicarbonate buffered basal media-BM (Zehnder, et al., 1980)(data not shown). Furthermore, the methanogenic pathway of the MEC culture at 5 g NH_4^+-N L^{-1} was determined using [2-^{14}C] acetate as demonstrated before (Fotidis, et al., 2013b).

Inoculum and feedstock

The inoculum used in the CSTR reactors was obtained from a full-scale mesophilic anaerobic reactor (Hashøj Biogas, Denmark). The feedstock used in the experiment was dairy slurry derived from Hashøj municipality (Denmark). The dairy slurry was sieved to remove coarse materials and kept at 4ºC before it was introduced to the reactors (Table 1).

Table 1. Characteristics of the inoculum and the dairy manure used as feedstock.

Parameter	Inoculum Value ± SD	Feedstock Value ± SD
TS ($g \cdot L^{-1}$)	29.9±0.2	56.1±0.0
VS ($g \cdot L^{-1}$)	18.5±0.1	41.7±0.0
Total Kjeldahl nitrogen (g N L^{-1})	4.0±0.2	2.7±0.1
Ammonia (g NH_4^+-N$\cdot L^{-1}$)	3.2±0.2	1.7±0.1
pH	7.88	7.2
Total VFA (g L^{-1})	2.2±0.3	11.0±0.9

Experimental setup

The bioaugmentation experiment was carried out in two identical CSTR reactors (R_{MEC}: MEC culture bioaugmentation and $R_{Control}$: abiotic augmentation) with 2.3 L and 1.8 L total and working volume, respectively. Both reactors had organic loading rate (OLR) and hydraulic retention time (HRT) of 1.74 g VS L^{-1} d^{-1} and 24 days, respectively. Ammonium chloride was used as additional ammonia source. Each reactor's setup had a feedstock tank, a feeding peristaltic pump, an effluent tank, two magnetic stirrers, a water-displacement gas meter and an electrical heating jacketed system. Both CSTR reactors started-up with an ammonia level of 1.65 g NH_4^+-N L^{-1} and methane production rate, at steady-state, of 457±12 mL CH_4 $L^{-1} \cdot d^{-1}$. Ammonia levels were stepwise increased in the reactors to 5 g NH_4^+-N L^{-1} (data not shown). The complete bioaugmentation experiment was divided to three distinct experimental periods. One HRT after ammonia concentration in the reactors reached 5 g NH_4^+-N L^{-1}, an induced "inhibited steady-state" was established (Period-I, days 1-12) for both reactors. The bioaugmentation of MEC in the R_{MEC} reactor took place twice on days 13 and 15 (Period-II) with a total of 200 mL of the MEC culture (OD_{600}=0.21-0.23 and μ_{max}=0.024 h^{-1}, under exponential growth phase) introduced into the reactor. At the same time, 200 mL of sterile BM medium with 5 g NH_4^+-N L^{-1}, were also introduced in

reactor $R_{Control}$, in order to replicate the same hydraulic effect (abiotic augmentation) that the volume of the MEC inoculum had on the R_{MEC} reactor. Period-III (days 16-57) of the experiment was defined as the period after the bioaugmentation and abiotic augmentation processes took place. Throughout the duration of the experiment, both reactors were operated continuously and the ammonia concentration, the HRT and the OLR were kept stable.

Analyses and calculations

Methane content in CSTR reactors were measured with GC-TCD (MGC 82-12, Mikrolab a/s, Denmark). Volatile fatty acids (VFA) were determined using gas-chromatograph (HP 5890 series II) as described previously (Fotidis, et al., 2014). The pH was measured with PHM99 LAB pH meter. The maximum growth rate (μ_{max}) of the MC culture was calculated as has been described before (Fotidis, et al., 2013a). The optical density at 600 nm (OD_{600}) was determined with a Spectronic 20D+ Spectrophotometer (Thermoscientific, Soeborg, Denmark). All the analyses were made in triplicate (n=3) and the averages with the standard deviations (± SD) are presented.

RESULTS AND DISCUSSION

During the initial "inhibited steady-state" (Period-I), both reactors were producing approximately 30% less methane, compared to the start-up period (Fig. 1a). Immediately after bioaugmentation (Period-III), the R_{MEC} reactor demonstrated a significant improvement in methane production rate, which led to a new uninhibited steady-state (days 33-57). In this new steady-state, the R_{MEC} reactor was operating continuously for more than one HRT with approximately 40% higher methane production rate compared to the initial "inhibited steady-state". Surprisingly, the R_{MEC} reactor regained the same methane production rate (~450 mL CH_4 $L^{-1}\cdot d^{-1}$) it had before the introduction of the additional ammonia to the feedstock alleviating completely the ammonia inhibitory effect. Additionally, a previous study, performed under the same experimental conditions using a pure methanogenic strain (*Methanoculleus bourgensis* $MS2^T$) as bioaugmentation inoculum, demonstrated a 30% improvement of methane production (Fotidis, *et al.*, 2014). The comparison between the two studies indicates that the enriched culture (MEC) alleviated ammonia toxicity more efficiently (by ~25%) than the pure culture (*M. bourgensis*). This finding strengthens the hypothesis that ammonia tolerant enriched cultures could potentially be the appropriate bioaugmentation inocula to alleviate ammonia toxicity in full-scale biogas reactors. Finally, bioaugmentation in R_{MEC} did not affect the methane production rate of the reactor since the operational parameters were kept stable (e.g. HRT, OLR, temperature etc.). Thus, ammonia-rich feedstock was fed continuously in the reactor retaining the same high ammonia levels (5 g NH_4^+-N L^{-1}). This technical approach is in contrast to conventional methods (dilution, temperature lowering, etc.), used today to alleviate ammonia toxicity in AD reactors (Nielsen & Angelidaki, 2008).

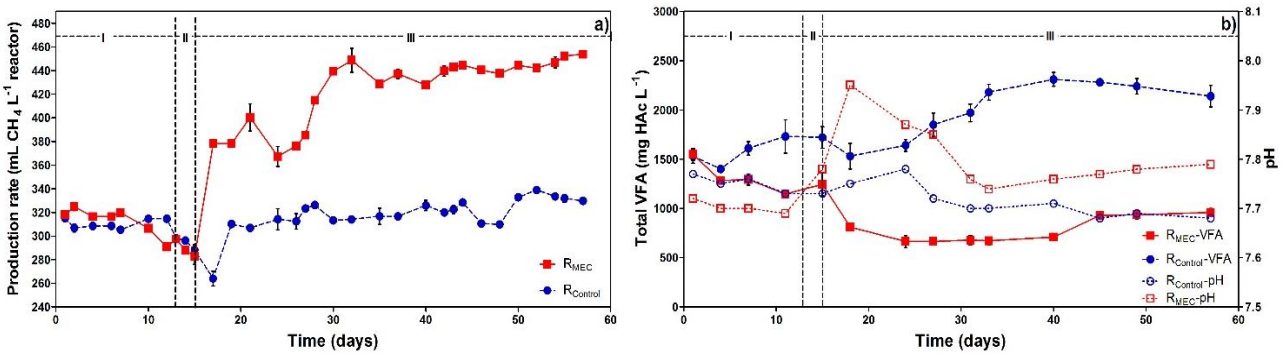

Figure 1. Methane production rate (a) and VFA accumulation and pH fluctuation (b) of the CSTR reactors.

On contrary to R_{MEC}, $R_{Control}$ reactor remained in an ammonia induced "inhibited steady-state" in Period-III, increasing its methane production rate only by 5% compared to Period-I (Fig. 1a). This

statistically significant ($p<0.05$), but small improvement in methane production efficiency, could be attributed to a slow acclimation of the methanogenic populations to the high ammonia levels (Chen, et al., 2008). A direct comparison between R_{MEC} and $R_{Control}$ during the final steady-state (days 33-57), R_{MEC} had an average of more than 36% higher methane production rate.

Finally, VFA accumulation and pH fluctuation after bioaugmentation process (Fig. 1b) of R_{MEC} reactor, were kept stable and within the normal limits for AD of dairy slurry in CSTR reactors (Fang, et al., 2011). On contrary, VFA accumulation (Fig. 1b) of $R_{Control}$ reactor verified the ammonia "inhibited steady-state" period (days 33-57) at 5 g NH_4^+-N L^{-1}, with the VFA levels higher than the identify threshold (1500 mg HAc L^{-1}) for a healthy AD process in lab-scale CSTR reactors (Boe, *et al.*, 2010). Despite the VFA accumulation, pH of the $R_{Control}$ remained inside acceptable pH range for the AD process due to high buffer capacity of the dairy slurry (Liu, et al., 2008).

CONCLUSIONS

In the current study was established that an enriched ammonia tolerant methanogenic culture was successfully bioaugmented in an ammonia inhibited CSTR reactor and could completely alleviate ammonia toxicity. This new method, is very promising, for development of an efficient and cost-effective biomethanation process of ammonia-rich organic waste in full-scale reactors.

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REFERENCES

Boe, K., Batstone, D. J., Steyer, J.-P. & Angelidaki, I. (2010) State indicators for monitoring the anaerobic digestion process. *Water Research* **44**(20), 5973-5980.

Chen, Y., Cheng, J. J. & Creamer, K. S. (2008) Inhibition of anaerobic digestion process: A review. *Bioresource Technology* **99**(10), 4044-4064.

Costa, J. C., Barbosa, S. G., Alves, M. M. & Sousa, D. Z. (2012) Thermochemical pre- and biological co-treatments to improve hydrolysis and methane production from poultry litter. *Bioresour. Technol.* **111**(0), 141-147.

Fang, C., Boe, K. & Angelidaki, I. (2011) Anaerobic co-digestion of by-products from sugar production with cow manure. *Water research* **45**(11), 3473-3480.

Fotidis, I. A., Karakashev, D. & Angelidaki, I. (2013a) The dominant acetate degradation pathway/methanogenic composition in full-scale anaerobic digesters operating under different ammonia levels. *International Journal of Environmental Science and Technology* **11**(7), 2087-2094.

Fotidis, I. A., Karakashev, D., Kotsopoulos, T. A., Martzopoulos, G. G. & Angelidaki, I. (2013b) Effect of ammonium and acetate on methanogenic pathway and methanogenic community composition. *FEMS Microbiol Ecol* **83**(1), 38-48.

Fotidis, I. A., Wang, H., Fiedel, N. R., Luo, G., Karakashev, D. B. & Angelidaki, I. (2014) Bioaugmentation as a solution to increase methane production from an ammonia-rich substrate. *Environ Sci Technol* **48**(13), 7669-7676.

Liu, C.-f., Yuan, X.-z., Zeng, G.-m., Li, W.-w. & Li, J. (2008) Prediction of methane yield at optimum pH for anaerobic digestion of organic fraction of municipal solid waste. *Bioresource Technology* **99**(4), 882-888.

Westerholm, M., Dolfing, J., Sherry, A., Gray, N. D., Head, I. M. & Schnürer, A. (2011) Quantification of syntrophic acetate-oxidizing microbial communities in biogas processes. *Environmental Microbiology Reports* **3**(4), 500-505.

Zehnder, A. J. B., Huser, B. A., Brock, T. D. & Wuhrmann, K. (1980) Characterization of an acetate-decarboxylating, non-hydrogen-oxidizing methane bacterium. *Archives of Microbiology* **124**(1), 1-11.