Bioaugmentation of ammonia tolerant enriched methanogenic cultures: A microbiological process to efficiently digest ammonia-rich biomasses

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One of the most promising methods for treatment of ammonia-rich wastes is anaerobic digestion (AD). However, ammonia-rich substrates are well known to inhibit AD process and it is estimated that many full-scale biogas reactors are seriously affected by ammonia toxicity leaving up to 1/3 of their methane potential unutilised (Fotidis *et al.*, 2013). It is 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. 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 (Fotidis et al., 2013). Nevertheless, these acclimatized mixed cultures were never tested as bioaugmentation inocula in continuous anaerobic reactors. Thus, the aim of the current study was to use an 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. A hydrogenotrophic methanogenic enriched culture (MEC) used in the bioaugmentation process. Before introduced to the CSTR reactor, MEC culture was acclimatised stepwise up to 5 g NH_4^+-N L^{-1} in batch reactors. The feedstock used in the experiment was dairy slurry. 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 and ammonia levels were stepwise increased in the reactors to 5 g NH_4^+-N L^{-1} where an induced "inhibited steady-state" was established (P-I) for both reactors. The bioaugmentation of MEC in the R_{MEC} reactor took place twice on P-II.

After bioaugmentation (P-III), the R_{MEC} reactor demonstrated a significant improvement in methane production rate, which led to a new uninhibited steady-state (Figure 1). In this new steady-state, the R_{MEC} reactor was operating continuously with 40% higher methane production rate compared to the initial "inhibited steady-state" regaining 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. Bioaugmentation in R_{MEC} did not affect the reactor's operational parameters which were kept stable (e.g. HRT, OLR, temperature etc.). This technical approach is in contrast to conventional methods (dilution, temperature lowering, etc.), used today to alleviate ammonia toxicity in AD reactors. 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.

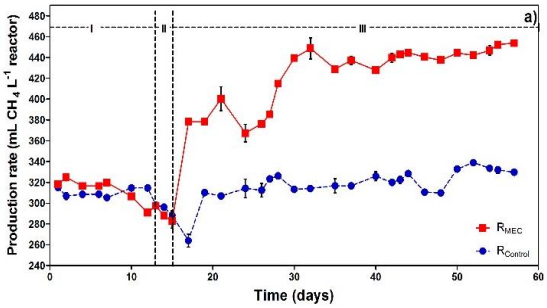

Figure 1. Methane production rate of the CSTR reactors

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.

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