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Different cultivation methods to acclimatise ammonia-tolerant methanogenic consortia

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Abstract

Bioaugmentation with ammonia tolerant-methanogenic consortia was proposed as a solution to overcome ammonia inhibition during anaerobic digestion process recently. However, appropriate technology to generate ammonia tolerant methanogenic consortia is still lacking. In this study, three basic reactors (i.e. batch, fed-batch and continuous stirred-tank reactors (CSTR)) operated at mesophilic (37°C) and thermophilic (55°C) conditions were assessed, based on methane production efficiency, incubation time, TAN/FAN (total ammonium nitrogen/free ammonia nitrogen) levels and maximum methanogenic activity. Overall, fed-batch cultivation was clearly the most efficient method compared to batch and CSTR.

Specifically, by saving incubation time up to 150%, fed-batch reactors were acclimatised to nearly 2-fold higher FAN levels with a 37%-153% methanogenic activity improvement, compared to batch method. Meanwhile, CSTR reactors were inhibited at lower ammonia levels. Finally, specific methanogenic activity test showed that hydrogenotrophic methanogens were more active than aceticlastic methanogens in all FAN levels above 540 mg NH_3-N L^{-1}.

Keywords

Batch reactor; Fed-batch reactor; CSTR; Specific methanogenic activity; Incubation time

1 Introduction

Anaerobic digestion (AD) is one of the most commonly used methods to treat a vast array of organic waste-slurries and wastewaters derived from different sources (e.g. agricultural waste, industrial waste, food waste, municipal sewage sludge etc.), which result in energy recovery (biogas; a mixture of CH_4 and CO_2) and in a nutrient-rich digestate used as biofertilizer (Bekkering et al., 2015). Additionally, AD reduces the greenhouse gas emissions and has lower energy requirements compared to other waste treatment methods (Westerholm et al., 2012). However, when ammonia-rich waste (e.g. animal manure, slaughterhouse wastewater etc.) are used as AD substrates, an instability or even complete process failure could occur from high total ammonia (TAN = NH_3 + NH_4^+) concentrations (Yenigün and Demirel, 2013). It has been reported that many commercial biogas plants lose up to 30% of their methane potential operating under an ammonia induced “inhibited steady state” (Fotidis et al., 2013a).

Among the microorganisms mediating the AD, methanogens are the most sensitive to ammonia and thus, methanogenesis becomes the rate-limiting step of the overall process (Singh and Olsen, 2011). There are two major methanogenic pathways using acetate as methanogenic substrate: aceticlastic methanogenesis (AM) and syntrophic acetate oxidation coupled by hydrogenotrophic methanogenesis (SAO-HM). AM pathway has been reported to be much more sensitive to ammonia compared to the SAO-HM pathway (Borja et al., 1996). Furthermore, many studies showed that free ammonia (FAN), which increases alongside with pH and temperature, is the most toxic form of TAN (Massé et al., 2014).

To solve the ammonia toxicity problem, many solutions have been proposed (e.g. reactor content dilution, addition of absorbents, air striping etc.) (Angelidaki and Ahring, 1992; Nielsen and Angelidaki, 2008; Zhang et al., 2012). However, these methods can alleviate ammonia inhibition to a certain extent, but they are either cost-expensive or some of them far from practical applicability. In the recent years, bioaugmentation of ammonia tolerant methanogenic consortia has been proposed as a promising method to attack this challenge. Bioaugmentation is the process of adding microorganisms with specific function or property into a biological system to improve the performance of the system (Stephenson and Stephenson, 1992). It has been successfully used in many areas, such as hazardous waste control, aerobic wastewater disposal (Ivanov et al., 2006; Schauer-Gimenez et al., 2010) and also in AD process to recover from organic overload and increase methane yield (Tale et al., 2015; Zhang et al., 2015).

Latterly, there have been different attempts to use bioaugmentation to solve the ammonia toxicity problem in AD reactors, with some encouraging results (Westerholm et al., 2012; Fotidis et al., 2014). These studies have identified that one of the major bottlenecks for a successful bioaugmentation process is the availability of ammonia-tolerant methanogenic consortia. Furthermore, it was suggested that bioaugmentation with mixed microbial consortia

is more attractive due to its robustness compared to pure cultures (Yang et al., 2016). However, to date, no study can be found assessing/proposing the most efficient method (in terms e.g. of incubation time, TAN and FAN levels achieved, methanogenic activity, etc.) to acclimatise ammonia tolerant methanogenic consortia.

Generally, there are three basic types of reactor configurations/processes that could practically be used to acclimatise ammonia tolerant methanogens, i.e. batch, fed-batch and continuous reactors. Batch cultivation is used to grow microorganisms where an initial supply of carbon source and nutrients is provided in the beginning and when these are consumed the culture cease growing (Minihane and Brown, 1986). Interestingly, in the existing bioaugmentation studies, batch reactors were used to acclimatise ammonia tolerant cultures, without assessing the efficiency of the process. However, considering the "one-time feeding", high cell density is not easy to get with batch process because, in specific cases, toxicity caused by the metabolic by-products can occur (Ding and Tan, 2006). Fed-batch is a cultivation process which starts with a batch culture, and then fed continuously or sequentially with substrate without fermentation broth removal until the reactor is filled up (Lee et al., 1999; Ding and Tan, 2006). Fed-batch reactors are widely used for biomass and specific metabolic product cultivation (Gordillo et al., 1998). However, different technical challenges could arise during fed-batch process, because some parameters, like the estimation or calculation of growth rate, sterility challenge due to pumping and other facilities and more attendance requirement needed compared to batch cultivations (Yoon et al., 1994; Wechselberger et al., 2013). Finally, a typical continuous AD reactor (e.g. continuous stirred tank reactor - CSTR), offers a more stable environment without too much toxicant accumulation due to daily input and output. However, washout effect of useful microorganism is the main drawback of this configuration (Fynn and Whitmore, 1984).

Therefore, the main aim of the present study was to assess the efficiency of the three different cultivation methods (i.e. batch, fed-batch and CSTR) to acclimatise methanogenic consortia to high ammonia levels. Both mesophilic (37°C) and thermophilic (53°C) inocula were used to evaluate the effect of temperature in the different acclimation processes. The CH_4 production efficiency, incubation time, TAN/FAN levels achieved and methanogenic activity, were used as criteria to evaluate the three acclimation processes. On the other hand, different effects between stepwise and direct-exposure of methanogens to ammonia during batch cultivation was reported by a previous study (Fotidis et al., 2013b), thus both acclimation approaches were tested during the batch experimental assay. Finally, the specific methanogenic activity test was applied to the final consortia, derived from the acclimation methods, to evaluate the activity of methanogenic populations from each acclimatisation process.

2 Materials and methods

2.1 Inoculum and substrate

The inocula used in this study were obtained from two different Danish full-scale biogas plants; the mesophilic one (37±1°C) from Hashøj biogas plant, while the thermophilic one (53±1°C) from Snertinge biogas plant. Both plants are fed with 70-90% animal manure and 10-30% food industrial organic waste. The basic characteristics of these two inocula are presented in Table 1. The medium used in all the experiments was basal anaerobic medium (BA medium), which is a solution of basic nutrients for microbial growth (Angelidaki et al., 1990). Sodium acetate and ammonium chloride were used as carbon and ammonia sources, respectively.

2.2 Experimental setup

Three different experimental assays (batch, fed-batch and CSTR) were performed in this study to compare their efficiency on acclimating the two-different initial inocula to high ammonia levels. In all three assays, the inocula were incubated at their original TAN levels for lab-scale environment adaptation and determination of the baseline/uninhibited methane production. In all experimental assays, a low organic load (batch) or organic loading rate-OLR (fed-batch, CSTR) was chosen to avoid the ammonia-VFA synergistic inhibition effect (Lu et al., 2013) and thus, only assess the influence of ammonia. Overall, five different ammonia acclimation levels were tested for the mesophilic and four for the thermophilic inoculum, respectively (Table 2).

2.2.1 Batch experimental assay

Two subseries of experiments were performed during batch experimental assay. The first one was a direct-exposure of the baseline inoculum to different ammonia levels. The second was stepwise exposure of the baseline inoculum to increasing ammonia concentrations through successive batch cultivations, i.e. the inoculum used for the next ammonia level derived from the previous ammonia level batch cultivation. In this assay, glass serum bottles were used with 118 mL and 40 mL total and working volume, respectively. Inoculum (8 mL) and BA medium (32 mL) were added in each reactor, and then flushed with a mixture gas of N_2/CO_2 (80/20% v/v) to create anoxic conditions. Yeast extract (0.2 g L^{-1}) and vitamin solution (0.1 g L^{-1}) (Wolin et al., 1963) were introduced in the bottles and finally, $Na_2S•9H_2O$ (62.5 mg L^{-1}) was added as a reducing agent. All the reactors were closed with butyl rubber stoppers, sealed with aluminium caps, and incubated in their corresponding temperature. All experiments were performed in triplicates.

2.2.2 Fed-batch experimental assay

The fed-batch experiments were performed in two glass bottle reactors, one for the mesophilic and one for the thermophilic inoculum, with 2.3 L total volume. Starting with 45 mL inoculum, the later feeding was adopted by an exponential feeding strategy (i.e. the reactor content was converted at a specific rate to active inoculum). The feeding (as shown in Fig. S1, supplementary material) was performed every two days manually, with an increasing volume of feedstock. A constant OLR of 0.5 g HAc L^{-1} d^{-1} was used throughout the experimental period for both reactors. The ammonia levels (Table 2) were increased every 20 and 15 days, for mesophilic and thermophilic reactor, respectively. Before each new ammonia level, a small amount (15 mL) of sample was taken from the fed-batch reactor for analyses.

2.2.3 CSTR experimental assay

Two lab-scale CSTR reactors were used, one for the mesophilic and another for the thermophilic inoculum with 20 and 15 days HRT, respectively. Each reactor had a 2.3 and 1.8 L total and working volume, respectively, and an OLR of 0.5 g HAc L^{-1} d^{-1} was used throughout the experiment. Each reactor was consisted of an influent, an effluent bottle, a feeding peristaltic pump, an electrical heating jacket, a water-displacement gas meter and two magnetic stirrers for the homogenization of substrate and mixing of the reactor. To proceed to the next acclimation step (Table 2), the ammonia was spiked simultaneously into the reactors and the substrate.

2.3 Specific methanogenic activity test

A SMA test on specific substrates (formate, acetate and H_2/CO_2) was carried out for the final methanogenic consortia derived from the acclimation assays, to investigate the combined effect of high ammonia levels and each acclimation process on the methanogenic populations. Samples for SMA test were taken from the reactors at the end of each acclimation assay. The

test for fed-batch and CSTR was done in batch serum vials of 118 mL total volume and 40 mL working volume. The cultures derived for the batch experimental assay were incubated in vials with 58 mL and 20 mL total and working volume, respectively. Active biomass constituted the 25% of the working volume, and 75% was BA medium in all SMA tests. All the vials (liquid phase and headspace) were flushed with a mixture gas of N_2/CO_2 (80/20%, v/v) to create anaerobic conditions. Formate (80 mM), acetate (20 mM) and H_2/CO_2 (80/20%, v/v, under 1 atm) were used individually, as carbon sources (Luo et al., 2011). Finally, vials with only inoculum and BA medium were used as blanks and all the tests were made in triplicates.

2.4 Analyses

TS, VS, TAN and TKN were measured through the American Public Health Association-APHA's Standard Method (APHA, 2005). The pH fluctuation in the reactors was determined through PHM99 LAB pH meter. Total VFA concentration inside the reactors was determined by a gas-chromatograph (HP 5890 series II) equipped with flame ionization detector and a FFAP fused silica capillary column, (30 m × 0.53 mm i.d., film thickness 1.5 μm), and nitrogen was used as carrier gas. The biogas composition was measured by a gas-chromatograph (Trace 1310 GC-TCD, Thermo Fisher, Denmark) equipped with TracePLOT TG-BOND Q 26004-6030 column (30 m × 0.32 mm I.D., film thickness 10.0 μm) (Thermo Fisher). Helium was used as carrier gas.

2.5 Calculations and statistical analyses

2.5.1 Free ammonia calculation

The FAN concentration was calculated by the following equation:

$$FAN = \frac{TAN}{1+\frac{10^{-pH}}{K_a}} \quad \text{Eq. (1)}$$

Where Ka is the dissociation constant affected by temperature, and equals to 1.29×10^{-9} and 3.91×10^{-9} for mesophilic and thermophilic condition in the present study, respectively.

2.5.2 Maximum theoretical methane production

The maximum theoretical methane production used in the batch and fed-batch reactors' experiments was calculated based on the stoichiometry of biological CH_4 production from acetate (Angelidaki et al., 2011), where 1 g acetate can produce maximum 373.33 NmL CH_4. Furthermore, the real methane production of the batch reactors experiment, was expressed as percentage of the maximum theoretical methane production.

2.5.3 SMA test calculation

Considering the biofibers was part of the inoculum (e.g. lignin cellulose in the original inoculum), VS content cannot stand for the microbe cell quantity in this study. Therefore, the methanogenic activity was defined as the linear methane accumulation rate versus time, divided by the volume of the biomass tested.

2.5.4 Statistical analysis

All statistical analyses and the plotted data were made using the OriginLab program (OriginLab Corporation, Northampton, Massachusetts). Student's t-test was used for estimation of statistically significant difference ($p<0.05$) when compare SMA test result between the different acclimation methods. One-way ANOVA was used to evaluate the statistically significant differences ($p<0.05$) in methane production and VFA accumulation results derived from the fed-batch and CSTR reactors.

3 Results and discussion

3.1 Batch reactors performance

In general, the methane production in all batch reactors reached the theoretical value through different incubation times (Fig. 1). During direct-exposure acclimation method, the incubation time was prolonged alongside the ammonia levels, due to the longer lag phases (Fig. 1A and Fig. 1B). This was in accordance with a previous study, which reported a longer lag phase at high ammonia levels (6-7 NH_4^+-N L^{-1}) than low levels (1-3 NH_4^+-N L^{-1}) (Lu et al., 2013). An interesting finding was that a shorter incubation time was found at MP4 (TAN=6.56 g NH_4^+-N L^{-1}) compared to MP2 (TAN=4.56 g NH_4^+-N L^{-1}) and MP3 (TAN=5.56 g NH_4^+-N L^{-1}) (Fig. 1A). Considering that FAN is the most toxic form of TAN (Kroeker et al., 1979); this result could be explained by the relatively low FAN concentration (100 mg NH_3 L^{-1}) in MP4, compared to MP2 (180 mg NH_3 L^{-1}) and MP3 (200 mg NH_3 L^{-1}).

The stepwise acclimation method shortened the incubation time within each individual ammonia level both under mesophilic and thermophilic condition compared to direct-exposure (Fig. 1). For example, at the higher ammonia levels, 20 days were needed to fulfil the acclimation process during MP5 in stepwise-exposure, instead of 78 days in the same ammonia level in direct-exposure, which saved almost 300% of the incubation time.

However, even though the shorter incubation time within each individual acclimation step, the stepwise-exposure still took more time to acclimatise the consortia to high ammonia levels than the direct method. The incubation time for the highest ammonia levels was 78 and 91 days (Fig. 1A and Fig. 1B) under direct-exposure for mesophilic and thermophilic condition, respectively, while it was 125 and 158 days under stepwise-exposure (Fig. 1C and Fig. 1D). This was probably due to the accumulation of lag phases during every single step of the stepwise-exposure. On the other hand, it was reported that direct-exposure, results in higher

diversity of methanogens compared to stepwise acclimation (Fotidis et al., 2013b), which may provide a better chance for the methanogens to adapt to the high ammonia environment. Therefore, based on the results from the current study, the direct-exposure acclimation method seems to be preferable than the stepwise-exposure, with respect to methane production and especially the incubation time. However, it is still possible to have a process failure when using the direct-exposure acclimation method (Liu and Sung, 2002), since it depends on the initial microbiological composition of the inoculum (i.e. if ammonia tolerant methanogens are present even in low abundance).

3.2 Fed-batch reactors performance

Throughout the whole experiment, methane accumulation for both fed-batch reactors followed the theoretical methane production with only small fluctuations and finally reached a high TAN concentration of 6.56 and 6.32 g NH_4^+-N L^{-1} for mesophilic and thermophilic conditions, respectively (Fig. 2A and Fig. 2B). The relatively higher methane accumulation (above the maximum theoretical) during baseline period (MP1, TP1) can be attributed to the background production from the initial inoculum. Even though a gas production delay was found at TP2 (4.32 g NH_4^+-N L^{-1}), the reactor recovered immediately during TP3. At the end of the experiment, the methane production was more than 83% of the theoretical expected production for both fed-batch reactors, which implied a stable AD environment without any serious ammonia inhibition. Notably, in the thermophilic reactor, the final FAN concentration was more than 1600 mg NH_3-N L^{-1}. To our knowledge, it has never been reported before an efficient biomethanation process acclimatised to these extremely high FAN levels at such a short time frame (64 days). This result suggests that the fed-batch cultivation could be appropriate as acclimatisation method.

Furthermore, pH ranged from 8.2 to 7.8 and VFA fluctuated from 0 to 2200 mg HAc L^{-1} for both reactors throughout the experiment (Fig. 2C and Fig. 2D), which were within the

appropriate levels reported by previous studies (Nissilä et al., 2012), indicating a stable digestion process. However, it should be mentioned that between the different ammonia levels there was significant difference ($p<0.05$) in the VFA concentrations, which means that ammonia increase applied some inhibitory pressure on methanogenesis, but the process adapted successfully.

To sum up, the initial inocula were successfully acclimatised to 6.56 and 6.32g NH_4^+-N L^{-1} ammonia concentrations under mesophilic and thermophilic condition, respectively. During the whole process (except TP2), no profound process instability due to high ammonia was observed. The uninhibited process could be explained by the relatively stable microorganism growth, which was controlled by exponential feeding strategy during fed-batch acclimation method (Ding and Tan, 2006). Another reason contributing to the process stability could be the lack of effluent (no washout effect), which ensured that all microorganisms remained in the reactor. The results derived from the fed-batch acclimation method further proved that adjusting the feeding to an exponential strategy played an important role for a better acclimation process (Liu, 2013).

3.3 CSTR reactors performance

Both CSTR reactors did not pass the second acclimation step (4.56 g NH_4^+-N L^{-1} for mesophilic and 4.32 g NH_4^+-N L^{-1} for thermophilic) due to strong ammonia inhibition. An ammonia induced "inhibited steady state" (Hansen et al., 1998) was established during MP2 and TP2, with a methane yield of around 30% of the theoretical, until the end of the experiment (Fig. 3A and Fig. 3B). The low methane yield indicated that methanogens were experiencing strong inhibition from the ammonia. In parallel, VFA concentration increased in both reactors from near zero, during the baseline period (MP1, TP1), to levels above 1500 mg HAc L^{-1} during the second acclimation step, which is an established threshold for non-healthy continuous AD process (Angelidaki et al., 2005). The pH (Fig. 3C and Fig. 3D), was within

the normal limits (6.5-8.5) for the AD process for both reactors throughout the experiment (Lay et al., 1998). However, the thermophilic reactor had a maximum pH of 8.44, which had a direct effect on the FAN levels (higher than 2000 mg NH_3-N L^{-1}) that consequently could have led to a serious inhibition.

The main reason for the failure of both CSTR reactors could be the washout of methanogenic communities (Fynn and Whitmore, 1984), which resulted in the loss of ammonia tolerant methanogens that were in low abundance in the initial inocula. Therefore, it can be concluded that CSTR reactors are not suitable for the acclimation of ammonia-tolerant methanogenic consortia in a realistic timeframe that will allow them to be used as bioaugmentation inocula. However, if time is not an issue, then it could be possible to use longer HRTs and slower exposure to higher ammonia levels to efficient acclimatise methanogenic inocula, as it has been shown before (Calli et al., 2005).

3.4 SMA test

Activity test (Fig. 4) indicated that hydrogenotrophic methanogens were significantly more active than aceticlastic methanogens among most of the acclimation methods at higher ammonia levels. That agreed with previous studies (Borja et al., 1996; Calli et al., 2003; Werner et al., 2014), which showed that aceticlastic methanogens were more sensitive to higher ammonia levels compared to hydrogenotrophic methanogens. Aceticlastic activity was higher only in mesophilic batch reactors, with no significant difference ($p>0.05$) between direct and stepwise acclimation approach. It is generally accepted that under optimum digestion conditions aceticlastic methanogens have higher growth rates compared to syntrophic acetate oxidizing bacteria, which are in an exclusive syntrophic collaboration with the hydrogenotrophic methanogens (Schnürer and Nordberg, 2008; Kato et al., 2014). This consequently means that aceticlastic have higher growth rates compared to the hydrogenotrophic methanogens, under the same optimum conditions. Thus, the higher

aceticlastic activity could be explained by the low FAN levels (210 mg NH_3-N L^{-1}) during the mesophilic batch acclimation that did not affect the growth rates of the aceticlastic methanogens. Overall, based on the SMA result and FAN levels in the present study, hydrogenotrophic methanogens had higher activity under high FAN levels (above 540 mg NH_3-N L^{-1}), while aceticlastic methanogens were more active at low FAN levels (below 210 mg NH_3-N L^{-1}).

Finally, in the samples with higher hydrogenotrophic activity, formate was consumed much faster than H_2/CO_2. Since it is known that most of the hydrogenotrophic methanogens can also utilize formate (Pan et al., 2016), the higher formate consumption rate could be attributed to the additional time needed for H_2 transfer from gas to liquid phase, compared to formate which was already available in the liquid phase (Boone et al., 1989; Pauss et al., 1990).

3.5 Batch vs fed-batch vs CSTR

An overall evaluation (Table 3) of the three different reactor configurations, based on the assessment indexes that were set before (i.e. methane production efficiency, incubation time, TAN/FAN levels achieved and methanogenic activity), clearly showed that fed-batch was the best acclimation method compared to batch and CSTR methods, under both mesophilic and thermophilic conditions. With respect to the production efficiency, only 30% of the theoretical production was detected during CSTR acclimation method, while more than 83% in batch and fed-batch reactors was achieved. Furthermore, CSTR reactors didn't operate stably (during the chosen acclimation approach) even with only 1 g NH_4^+-N L^{-1} increase. Even though high activity results can be found among CSTR reactors, it was explained by the combined outcome of high microbial density due to high initial inoculation ratio in the CSTR, ideal growth conditions during the SMA test (Angelidaki and Ahring, 1993; Boe et al., 2009) and the fact that ammonia inhibition is reversible when the ammonia levels decrease (Parkin

et al., 1983; Wu et al., 2009), instead of the result of acclimation. Thus, CSTR method is not suitable to efficiently acclimatise ammonia tolerant methanogenic consortia in a realistic timeframe, compared to the alternative methods.

Among the successful acclimation methods, the shortest incubation period was achieved together with the highest FAN levels and the highest methanogenic activity, with fed-batch reactors for both mesophilic and thermophilic conditions. Even though mesophilic batch direct-exposure method had a few days shorter incubation time than fed-batch, the FAN levels and the maximum methanogenic activity of the mesophilic fed-batch was 164% and 138% higher than batch direct-exposure method, respectively. The thermophilic Fed-batch acclimation method not only had the highest FAN levels and activity, but also had more than 40% shorter incubation time compared to batch acclimation methods. Thus, based on the comprehensive comparison of production efficiency, incubation time, TAN/FAN level and methanogenic activity between the different acclimation methods, it can be concluded that fed-batch could be the acclimation method that will potentially supply the necessary ammonia-tolerant bioaugmentation inocula in the near future.

4 Conclusions

The present study assessed different methods to acclimatise ammonia-tolerant methanogenic consortia and found that fed-batch is the best acclimation method based on the production efficiency, incubation time, TAN/FAN levels achieved and methanogenic activity. Fed-batch reactor worked efficiently at FAN levels as high as 1633 mg NH_3 L^{-1} with a higher methanogenic activity compared to others. SMA test indicated that hydrogenotrophic activity was significantly higher than aceticlastic activity, during fed-batch acclimation process. Thus, this study offers an efficient method to create ammonia-tolerant methanogenic consortia,

which is necessary for a successful bioaugmentation process to alleviate ammonia toxicity problem in biogas reactors.

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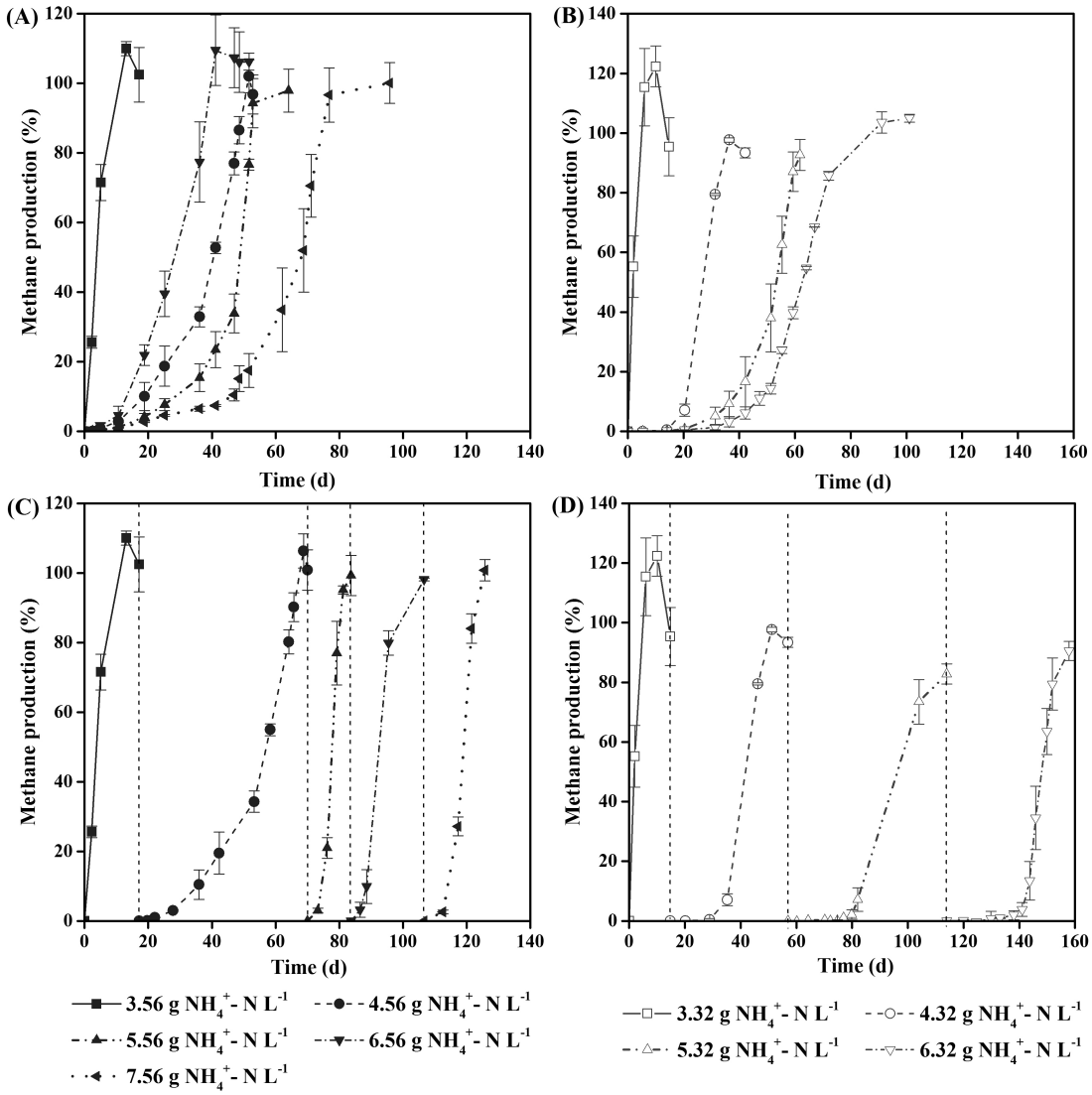
Figure Legends

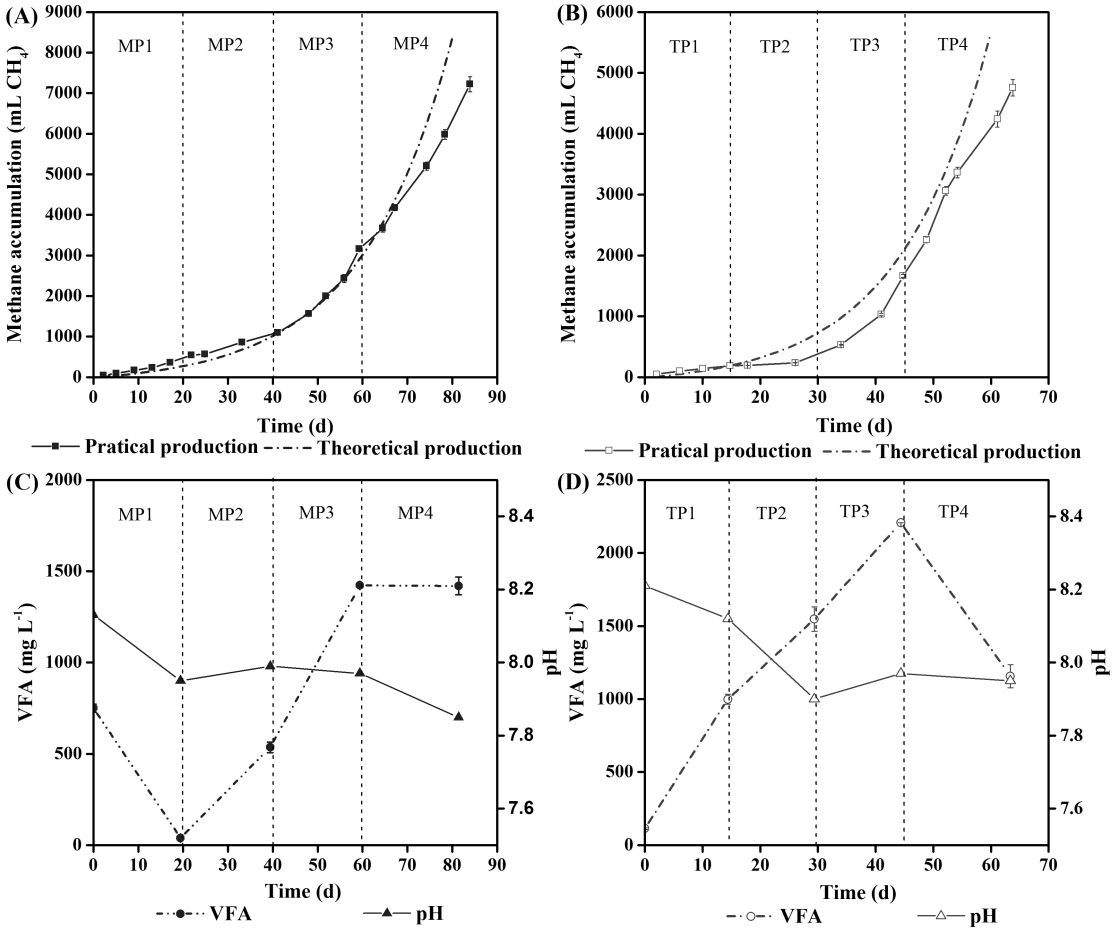
Fig. 1. Methane production of batch acclimation method, (A) direct-exposure at mesophilic condition, (B) direct-exposure at thermophilic condition, (C) stepwise-exposure at mesophilic condition, (D) stepwise-exposure at thermophilic condition.

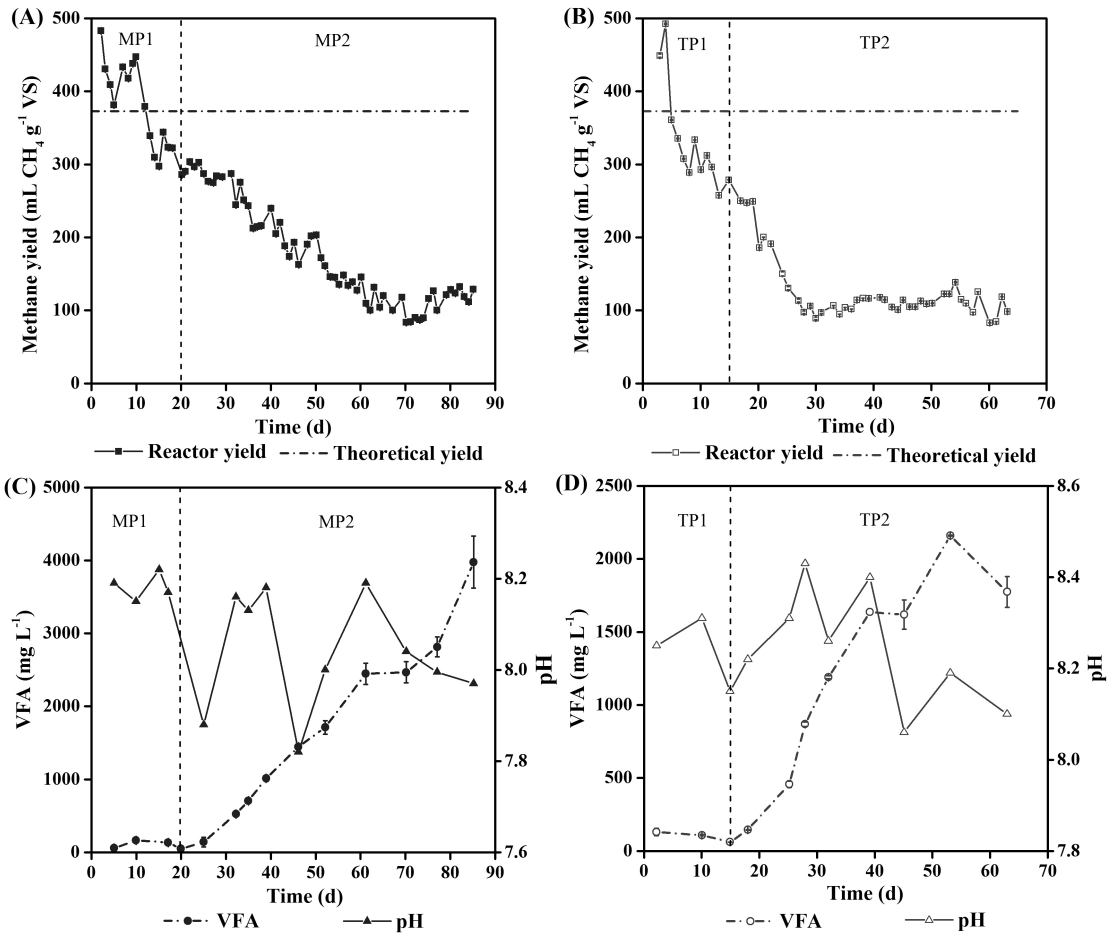
Fig. 2. Methane accumulation at (A) mesophilic and (B) thermophilic reactor; pH fluctuation and total VFA accumulation at (C) mesophilic and (D) thermophilic condition during fed-batch acclimation method

Fig. 3. Methane yield at (A) mesophilic and (B) thermophilic reactor, pH fluctuation and total VFA accumulation at (C) mesophilic and (D) thermophilic condition during CSTR acclimation method

Fig. 4. SMA test results of mesophilic batch direct-exposure (MBD), mesophilic batch stepwise-exposure (MBS), mesophilic fed-batch (MFB), mesophilic CSTR (MCS), thermophilic batch direct-exposure (TBD), thermophilic batch stepwise-exposure (TBS), thermophilic fed-batch (TFB) and thermophilic CSTR (TCS).







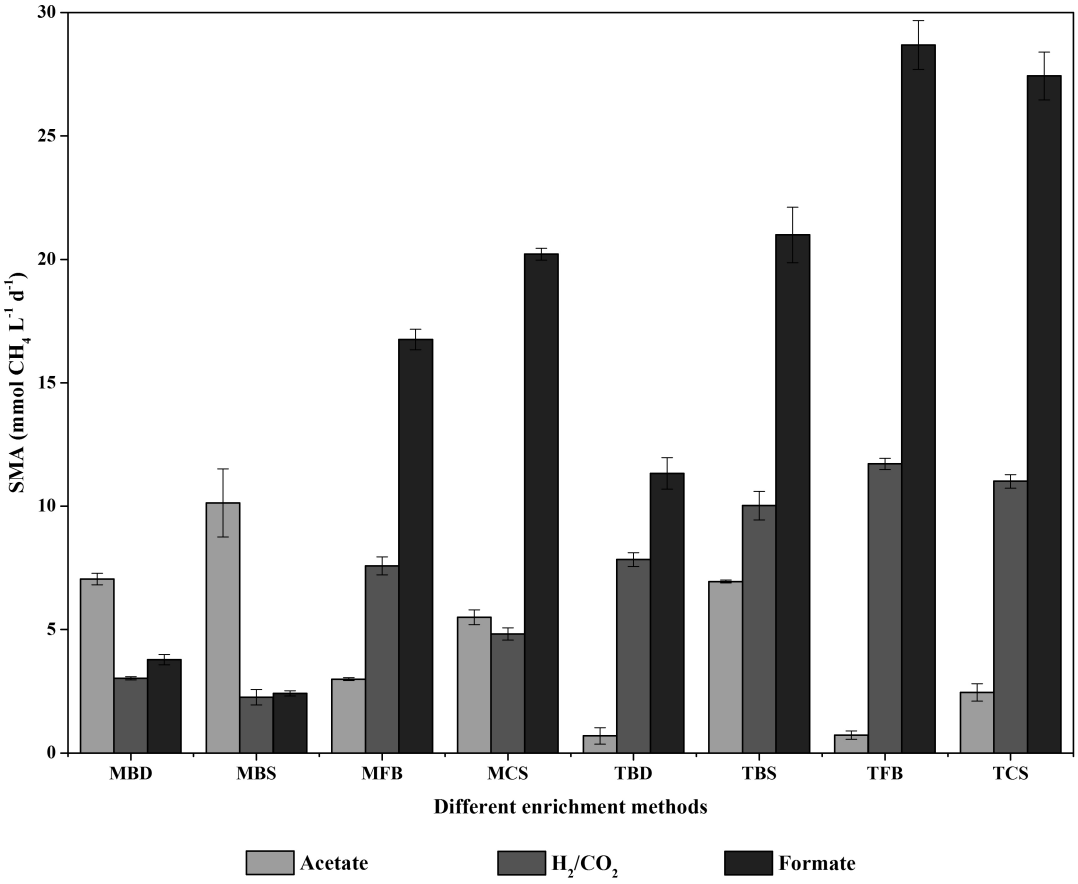


Table 1. Characteristics of the inocula

Parameter	Mesophilic value ± SD [a]	Thermophilic value ± SD [a]
Total solids, TS (g L^{-1})	39.68 ± 0.98	30.12 ± 0.02
Volatile solids, VS (g L^{-1})	27.82 ± 0.01	19.22 ± 0.00
Total Kjeldahl nitrogen, TKN (g N L^{-1})	5.15 ± 0.50	5.39 ± 0.04
Total ammonia nitrogen, TAN (g NH_4^+-N·L^{-1})	3.56 ± 0.09	3.32 ± 0.04
Free ammonia [b], FAN (g NH_3-N·L^{-1})	0.53 ± 0.01	1.29 ± 0.02
pH	8.13	8.21
Total volatile fatty acids, VFA (g L^{-1})	0.750 ± 0.028	0.113 ± 0.08

[a] Standard deviation

[b] Calculated according to Eq. (1)

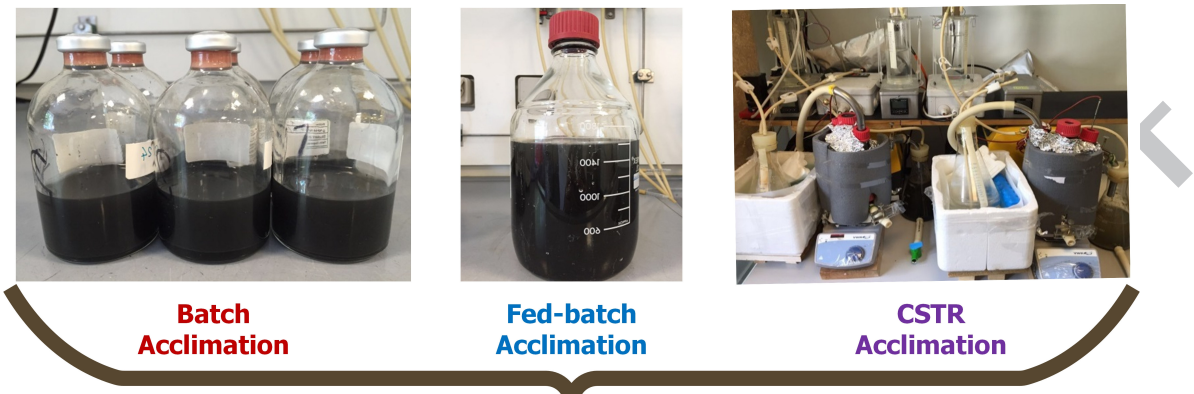
Table 2. Ammonia levels during the different acclimation steps for the three experimental assays

Mesophilic inoculum				**Thermophilic inoculum**			
Acclimation step	**Batch**	**Fed-batch**	**CSTR**	**Acclimation step**	**Batch**	**Fed-batch**	**CSTR**
	(g NH_4^+-N L^{-1})				**(g NH_4^+-N L^{-1})**		
MP1 (baseline)	3.56	3.56	3.56	**TP1 (baseline)**	3.32	3.32	3.32
MP2	4.56	4.56	4.56	**TP2**	4.32	4.32	4.32
MP3	5.56	5.56	5.56	**TP3**	5.32	5.32	5.32
MP4	6.56	6.56	6.56	**TP4**	6.32	6.32	6.32
MP5	7.56	-	-	-	-	-	-

Table 3. Comprehensive comparison between the three different acclimation methods

	Mesophilic inoculum				Thermophilic inoculum			
	Batch direct	**Batch stepwise**	**Fed-batch**	**CSTR**	**Batch direct**	**Batch stepwise**	**Fed-batch**	**CSTR**
Highest TAN (g NH_4^+-N L^{-1})	7.56	7.56	6.56	4.56	6.32	6.32	6.32	4.32
Highest FAN (mg NH_3 L^{-1})	208	181	549	490	614	542	1633	1425
Incubation time (d)	78	125	84	-	91	158	64	-
Methanogenic activity (mmol CH_4 L^{-1} d^{-1}) [a]	7.04	10.13	16.75	20.21	11.33	20.99	28.68	27.43
Production efficiency (%)	100	100	86.5	32	100	100	83.9	30

[a]The maximum methanogenic activity from SMA results.



Highlights

- Fed-batch was the most efficient method to acclimatise ammonia tolerant consortia
- Fast acclimation of methanogens at extremely high FAN levels (1633 mg NH_3-N L^{-1})
- Hydrogenotrophic methanogens were dominant at FAN levels above 540 mg NH_3-N L^{-1}
- CSTR acclimation failed at low TAN level (< 4.6 g NH_4^+-N L^{-1}) due to washout effect
- Fed-batch is a promising acclimation method to be coupled with bioaugmentation