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Different cultivation methods to acclimatise ammonia-tolerant methanogenic consortia

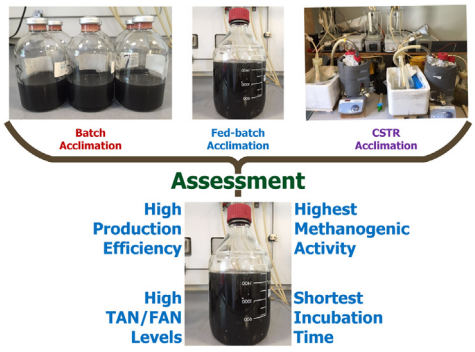
Hailin Tian, Ioannis A. Fotidis *, Enrico Mancini, Irini Angelidaki

Department of Environmental Engineering, Technical University of Denmark, Bygningstorvet Bygning 115, DK-2800 Kgs. Lyngby, Denmark

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.

GRAPHICAL ABSTRACT



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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}.

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

* Corresponding author.
E-mail address: ioanf@env.dtu.dk (I.A. Fotidis).

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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 synergis-

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).