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The performance efficiency of bioaugmentation to prevent anaerobic digestion failure from ammonia and propionate inhibition

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HIGHLIGHTS

- Bioaugmentation could prevent unstable digestion system against further deteriorate.
- Bioaugmentation was able to effectively recover the failing digester.
- The increasing populations of *Methanosaetaceae* enhanced methane production.

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ABSTRACT

This study aims to investigate the effect of bioaugmentation with enriched methanogenic propionate degrading microbial consortia on propionate fermentation under ammonia stress from total ammonia nitrogen concentration (TAN) of 3.0 g N L^{-1}. Results demonstrated that bioaugmentation could prevent unstable digestion against further deterioration. After 45 days of 1 dosage (0.3 g dry cell weight L^{-1} d^{-1}, DCW L^{-1} d^{-1}) of bioaugmentation, the average volumetric methane production (VMP), methane recovery rate and propionic acid (HPr) degradation rate was enhanced by 70 mL L^{-1} d^{-1}, 21% and 51%, respectively. In contrast, the non-bioaugmentation reactor almost failed. Routine addition of a double dosage (0.6 g DCW L^{-1} d^{-1}) of bioaugmentation culture was able to effectively recover the failing digester. The results of FISH suggested that the populations of *Methanosaetaceae* increased significantly, which could be a main contributor for the positive effect on methane production.

1. Introduction

Anaerobic digestion (AD) is a proven technology that offers many environmental benefits, such as efficient treatment of organic material and the generation of renewable energy. Despite this, research is still required to improve the operational stability and efficiency of AD (Banks et al., 2011; Fisgativa et al., 2016). Bioaugmentation is the practice of adding specific microorganisms to a system to enhance a desired activity and could provide a means to improve the efficiency of AD (Deflaun and Steffan, 2002; Maier et al., 2000; Rittmann and Whiteman, 1994).

Over the past decade, bioaugmentation has successfully reduced the start-up period (Lins et al., 2014), shortened hydraulic retention time (Baek et al., 2016; Neumann and Scherer, 2011) and decreased the recovery time of anaerobic digesters stressed by oxygen (Schauer-Gimenez et al., 2010) or organic overloading (Acharya et al., 2015; Tale et al., 2011, 2015). Furthermore, bioaugmentation also has been studied to improve the performance of AD, including increase in methane production from cellulosic waste (Cater et al., 2015; Lu et al., 2013; Martin-Ryals et al., 2015; Nielsen et al., 2007; Nkemka et al., 2015; Peng et al., 2014; Weiss et al., 2016, 2010; Yu et al., 2016; Zhang et al., 2015), digested sludge (mainly proteins and polysaccharides) (Lu et al., 2014), lipid-rich wastes (Cirne et al., 2006), ammonia-rich substrate (Fotidis et al., 2014), and long-chain fatty acids (LCFA) (Cavaleiro et al., 2010).

Compared to enrichment of individual cultures to enhance AD process for each specific substrate, a more practical and time-saving approach may be to target key, ubiquitous intermediates to improve digestion performance (Tale et al., 2015). Propionate and acetate as bioaugmentation targets are of great interest, which at high concentration may cause the deterioration of digester performance. Several studies found that adding propionate-utilizing cultures (Schauer-Gimenez et al., 2010; Tale et al., 2015) or VFA-degrading culture (Acharya et al., 2015) could

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reduce propionate accumulation and improve digestion. In addition, bioaugmentation has proven an effective way to counteract ammonia inhibition, with the introduction of hydrogenotrophic methanogens showing increased methane production at high ammonia levels (Fotidis et al., 2013, 2014). However, not all bioaugmentation cases result in a positive impact on digestion performance. The addition of syntrophic acetate-oxidizing cultures did not affect digestion performance or stability against ammonia inhibition (Fotidis et al., 2013; Westerholm et al., 2012). This might be due to methanogens playing a more important role than syntrophic acetate-oxidizing culture in anaerobic digestion under high ammonia levels (Fotidis et al., 2013).

Most of the successful cases of bioaugmentation have occurred in batch AD processes. For continuous reactors the major challenge for bioaugmentation is to ensure that the introduced microorganisms are able to thrive and are not washed out of the reactor (Fotidis et al., 2014; Mohan et al., 2005). In order to favor survival and prolonged activity of the exogenous microbial population, routine bioaugmentation for the continuous reactor might a more effective strategy (Martin-Ryals et al., 2015).

As described above, previous studies have shown that bioaugmentation is effective in enhancing poor digestion performance from either ammonia or propionate inhibition. Implementation of bioaugmentation under synergetic stress of ammonia and propionate has been less well addressed. Further studies are needed in this area since the accumulation of propionate, together with high acetate concentration is considered to be a major problem in digesters with high ammonia concentrations (Westerholm et al., 2015). It is also important to consider the nutrient concentrations, in particular trace elements, as well as the dosage of the bioaugmentation culture, both of which may have a significant effect on the microbial diversity and abundance within the digester.

With consideration of previous work, this study will investigate the routine bioaugmentation with solid methanogenic cultures enriched for propionate degradation to prevent deterioration of digester performance and recovery of the digester from the double stress of ammonia and propionic acid (HPr) accumulation. This work looks at the effect of different culture dosages of bioaugmentation and the functional microbial groups as a result of this.

2. Materials and methods

2.1. Inoculum and bioaugmentation seed

The inoculum was taken from an anaerobic digester treating municipal wastewater biosolids (Millbrook Wastewater Treatment works, Southampton, UK). Before use the digestate was sieved through a 1 mm mesh to remove grit and other solids.

The bioaugmentation culture was taken from a propionate-degrading enrichment digester. To avoid the culture medium impact on the digester, the enriched culture suspensions was centrifuged at 7000*g* for 5 min at room temperature and resuspended with ddH_2O, then centrifuged again under the same conditions to collect the microbial precipitate for bioaugmentation. The 454 whole genome pyrosequencing data of the bioaugmentation seed was deposited in NCBI Sequence Read Archive database with bioproject accession number PRJNA359412. According to the 454 whole genome pyrosequencing, the bacteria belong to *Proteobacteria*, *Firmicutes*, *Chloroflexi*, *Bacteroidetes*, *Synergistetes*, *Actinobacteria* and 24 other phyla, and the dominant archaeal groups are *Methanosaetaceae* (above 90%), *Methanospirillum* (below 5%) and *Methanosphaerula* (below 5%).

2.2. Experimental set-up and procedure

The whole experiment lasted for 125 days with four different experimental phases: phase I (0–50 day), phase II (50–75 day), phase III (75–95 day), and phase IV (95–125 day). The main strategic operational conditions of each experimental reactor are shown in Fig. 1.

During the first phase, the experiment was carried out in a laboratory-scale semi-continuously stirred tank reactor (Reactor 0, R0) with a working volume of 1.5 L. From day 51 the digestate in R0 was divided into two parts homogeneously and maintained in two 1 L conical flasks with 0.75 L of working volume (Reactor1, R1 and Reactor 2, R2). Each flask was connected to a gas sampling bag (Tedlar, SKC Ltd., UK) and connected to the flask by a stainless steel tube inserted through a butyl rubber bung. The flasks were maintained at 36 ± 1 °C in an orbital shaking incubator operating at 100 rpm continuously. They were operated in daily fill-and-draw mode with identical hydraulic retention time (HRT) of 15 days by removing the appropriate volume of reactor content and replacing it with the same volume of feed once per day.

The feed comprised a certain amount of sodium propionate and the volume was made up with nutrient medium. The nutrient medium contained the following [mg/L]: NH_4Cl [400]; $MgSO_4 \cdot 6H_2O$ [250]; KCl [400]; $CaCl_2 \cdot 2H_2O$ [120]; $(NH_4)_2HPO_4$ [80]; $FeCl_3 \cdot 6H_2O$ [55]; $CoCl_2 \cdot 6H_2O$ [0.5]; $NiCl_2 \cdot 6H_2O$ [0.5] the trace metal salts $MnCl_2 \cdot 4H_2O$, $CuCl_2 \cdot 2H_2O$, $AlCl_3 \cdot 6H_2O$, $Na_2WO_4 \cdot 2H_2O$, H_3BO_3, Na_2SeO_3 and $ZnCl_2$ [each at 0.5]; $NaHCO_3$ [5000] (Tale et al., 2011).

The organic loading rate (OLR) started at 0.5 g VS L^{-1} d^{-1} in HRT1 and was then increased to 0.625 g VS L^{-1} d^{-1} by adding the

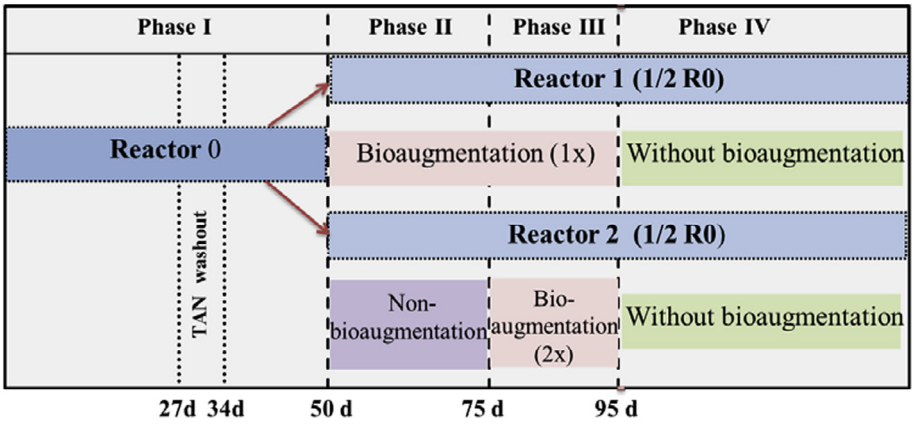

Fig. 1. Main strategic operational conditions of experimental reactors. Phase I (0–50 d), Phase II (50–75 d), Phase III (75–95 d), Phase IV (95–125 d)). 1× presented 1 dosage of bioaugmentation seed (0.3 g DCW L^{-1} d^{-1}), 2× presented 2 dosage of bioaugmentation seed (0.6 g DCW L^{-1} d^{-1}).