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Bioaugmentation for overloaded anaerobic digestion recovery with acid-tolerant methanogenic enrichment

Ying Li [a,1], Gaixiu Yang [a,1], Lianhua Li [a], Yongming Sun [a,b,c,*]

[a] Laboratory of Biomass Bio-chemical Conversion, Guangzhou Institute of Energy Conversion, Chinese Academy of Sciences, Guangzhou 510640, PR China
[b] Key Laboratory of Renewable Energy, Chinese Academy of Sciences, Guangzhou 510640, PR China
[c] Guangdong Provincial Key Laboratory of New and Renewable Energy Research and Development, Guangzhou 510640, PR China

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ABSTRACT

This study aims to investigate the performance of bioaugmentation with an acid-tolerant methanogenic culture to recover deteriorated anaerobic digestion caused by organic overloading. The function of bioaugmentation was evaluated in terms of substance metabolism, microbial community structure, and gene function. Our findings demonstrate that routine bioaugmentation effectively recovered the failing digester by degrading accumulated volatile fatty acids and increasing pH. In contrast, a non-bioaugmentation reactor (control) did not recover by itself, while abiotic augmentation restored the digestion performance temporarily but the digester failed again at an organic loading rate of 1.5 g L^{-1} d^{-1}. Using whole genome pyrosequencing analysis, we found that after bioaugmentation, the populations of *Methanothrix* (acetoclastic methanogens) and *Methanolinea* (hydrogenotrophic methanogens) increased significantly, which may be the main contributors for the positive effect on methane production. On the genic level, bioaugmentation may enhance the function of genes involved in cell motility, signal transduction mechanisms for methanogens, and energy production and conversion for bacteria.

1. Introduction

Conversion of organic waste by anaerobic digestion (AD) for biogas generation is one of the corner stones of sustainable waste management and renewable energy production (Koch et al., 2017; Nghiem et al., 2017). The AD process involves a series of reactions such as hydrolysis, acidogenesis, acetogenesis, and methanogenesis during degradation of organics and recovery of biogas (Khalid et al., 2011). A delicate balance exists in AD between the phases of acidogenesis, acetogenesis, and methanogenesis that ensures the optimum conversion of organic substrate to methane (Cohen et al., 1982). Therefore, any imbalances between these phases may affect the stability of an anaerobic system and even lead to system failure (Ketheesan & Stuckey, 2015). Organic overloading is one of the most typical process imbalance in anaerobic digesters, resulting in the accumulation of volatile fatty acids (VFA), pH drop, and a reduction and cessation of methanogenesis (Regueiro et al., 2015). To reach a compromise between stable process performance and desired fermentation efficiency, it would be ideal to operate an anaerobic process as close as possible to the maximum organic loading rate (OLR). Thus, organic overloading might be often encountered in the pursuit of a higher OLR for AD.

Various strategies have been examined to control or recover from the adverse responses of overloading in anaerobic systems. For instance, regulating the supply of trace metals, such as Fe, Ni, Co, and Se, to enhance the degradation rate of VFA (Banks et al., 2012; Moestedt et al., 2016; Zhang et al., 2015), and adjusting the bioreactor pH to improve digestion performance (Ghasimi et al., 2009b; Yang et al., 2015). Nevertheless, it was found that using large amounts of sodium hydroxide to adjust pH inhibited metabolic activity of methanogenic bacteria that are involved in methane production (Ghasimi et al., 2009a). The method of pH adjustment or supply of trace metals could be an indirect solution for recovering overloaded anaerobic systems because the environment for microorganism growth is improved first, which then leads to enhancement of microbial density in the AD system.

Bioaugmentation, a practice of introducing specific microorganisms to a system to enhance a desired activity (Maier et al., 2000; Rittmann and Whiteman, 1994;Schauer-Gimenez et al., 2010), has also gained considerable attention in controlling VFA accumulation during overloading conditions. As previous studies reported, adding acetate-utilizing cultures and formate/H_2 utilizers improved butyrate and propionate degradation (Voolapalli & Stuckey, 1999), while using an acetate-catabolizing methanogenic

* Corresponding author at: No. 2 Nengyuan Rd, Tianhe District, Guangzhou 510640, PR China.
E-mail address: sunym@ms.giec.ac.cn (Y. Sun).
[1] These authors contributed equally to this paper.

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consortium induced methane production (Town & Dumonceaux, 2016), Similarly, introducing a propionate-utilizing culture improved propionate degradation and system recovery (Li et al., 2017b; Ma et al., 2009; Schauer-Gimenez et al., 2010; Tale et al., 2015), whereas inoculating with a VFA-degrading enrichment reduced VFA concentration and shortened system recovery time (Acharya et al., 2015; Amani et al., 2011). Since VFA accumulation is often accompanied with a drop in pH, an acidotolerant methanogenic community may overcome the failure of anaerobic digesters (Steinberg & Regan, 2011).

Compared to pH adjustment or the supply of trace metals, bioaugmentation may be more efficient in the recovery of the overloaded anaerobic systems because it shortens the response time of the microbial community to environmental changes, thereby directly increasing the population of beneficial microbes. Therefore, an acid-tolerant methanogenic propionate degradation culture, acclimated in our previous work (Li et al., 2018), was used as a bioaugmentation culture to reduce recovery time following organic overload.

Moreover, most bioaugmentation inocula from previous studies were added in the form of liquid culture (Fotidis et al., 2013; Tale et al., 2015; Town & Dumonceaux, 2016). Some nutrients, in particular trace elements present in the culture media may remain in the liquid bioaugmented culture, and that these "spillover" nutrients may have a significant effect on endogenous microbial activity and digestion performance. Thus, to distinguish contributions between the microorganisms and nutrients in the liquid culture, the recovery performances of active and sterile liquid enrichment cultures were compared in this study.

Due to technological limitations of microbial community analysis in the past, some in-depth studies related to the effect of bioaugmentation on a microbial community have rarely been well investigated. In the present study, our evaluation of bioaugmentation function was characterized in terms of microbial community structure, functional microbial density, and gene function. Further, our study provides a deeper understanding of the correlative relationship between the added culture and endogenous microbes in the digester for substance metabolism in the recovery process.

2. Materials and methods

2.1. Experimental set-up

Inoculum was taken from an anaerobic digester treating food waste at the Guangzhou Institute of Energy Conversion at the Chinese Academy of Sciences (China). Before use it was sieved through a 1-mm mesh to remove grit and other solids. The experiment was carried out in mesophilic continuously stirred tank reactors with working volumes of 1 or 2 L that were initially inoculated with sieved digestate, and the headspace was flushed with a N_2:CO_2 gas mixture (80:20 ratio v/v). An identical hydraulic retention time (HRT) of 20 days was maintained by removing the appropriate volume of reactor content and replacing it with the same volume of feed daily.

The substrate was artificial food waste consisting of rice flour, whole egg powder, and milk powder with a dry weight ratio of 3:1:1, respectively. The basic characteristics of artificial feedstock were as follows: total solid (TS): 94.68%, volatile solid (VS): 93.52%, C%: 42.85, N%: 1.12, H%: 6.9, S%: 0.04, and C/N: 38.26. The volume of the fresh feed was made up by distilled water.

2.2. Experimental procedure

The entire experiment lasted for 200 days with three different experimental phases: phase I (days 0–96), phase II (days 96–160), and phase III (days 160–200). The main strategic operational conditions of each experimental reactor are shown in Fig. 1. During the first phase (days 0–96), the experiment was carried out in a laboratory-scale semi-continuous reactor (Reactor 0, R0) with a working volume of 2 L. OLR was step-wise increased from 0.5 g VS L^{-1} d^{-1} to 2.0 g VS L^{-1} d^{-1} by adding the appropriate amount of artificial food waste. To ensure that the reactors for bioaugmentation comparison operated from the same starting point, the digestate in R0 was divided into three homogeneous parts on day 88 and maintained in three 1-L conical flasks termed Reactor 1 (R1), Reactor 2 (R2), and Reactor 3 (R3). The initial volume of digestate in each reactor was 600 mL. To bring the working volume to 1 L, the three reactors were running under an OLR of 2.0 g VS L^{-1} d^{-1} with daily feed but without discharge from day ~88 to 96. The reactors were restarted to operate in daily fill-and-draw mode once the working volume was supplemented to 1 L. During phase II (days 96–160), R1 was bioaugmented by adding an acid-tolerant enrichment (20 mL d^{-1}), R2 was amended with a sterile acid-tolerant culture (20 mL d^{-1}), which was autoclaved for 20 min at 121 °C to rule out an impact by microbial organisms, and R3 was the control digester without bioaugmentation. During this phase, OLR was decreased to 0.5 g VS L^{-1} d^{-1} and held in this rate from day 97 to 117, and was then elevated to 1.5 g VS L^{-1} d^{-1} on day 118. For phase III (days 160–200), augmentation of both reactors ceased. OLR was maintained at 1.5 g L^{-1} d^{-1} during this period.

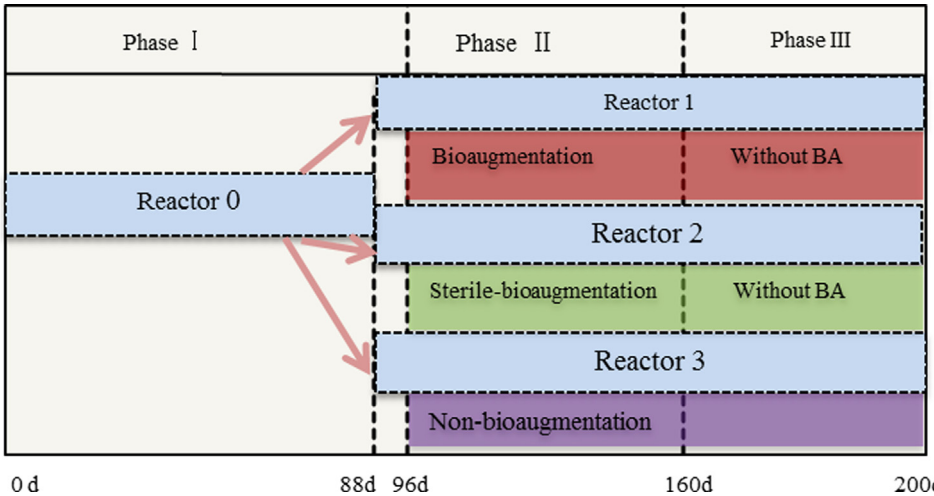

Fig. 1. The main strategic operation for the experiment. Phase I (0–96 d), Phase II (96–160 d), Phase III (160–200 d). (BA: bioaugmentation).