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Rapid establishment of thermophilic anaerobic microbial community during the one-step startup of thermophilic anaerobic digestion from a mesophilic digester

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ARTICLE INFO

Article history:
Received 4 August 2014
Received in revised form
25 October 2014
Accepted 2 November 2014
Available online 13 November 2014

Keywords:
One-step startup
Thermophilic anaerobic digestion
Thermophilic anaerobic microbial community
Establishment

ABSTRACT

The purpose of this study was to explore how fast the thermophilic anaerobic microbial community could be established during the one-step startup of thermophilic anaerobic digestion from a mesophilic digester. Stable thermophilic anaerobic digestion was achieved within 20 days from a mesophilic digester treating sewage sludge by adopting the one-step startup strategy. The succession of archaeal and bacterial populations over a period of 60 days after the temperature increment was followed by using 454-pyrosequencing and quantitative PCR. After the increase of temperature, thermophilic methanogenic community was established within 11 days, which was characterized by the fast colonization of *Methanosarcina thermophila* and two hydrogenotrophic methanogens (*Methanothermobacter* spp. and *Methanoculleus* spp.). At the same time, the bacterial community was dominated by *Fervidobacterium*, whose relative abundance rapidly increased from 0 to 28.52 % in 18 days, followed by other potential thermophilic genera, such as *Clostridium*, *Coprothermobacter*, *Anaerobaculum* and EM3. The above result demonstrated that the one-step startup strategy could allow the rapid establishment of the thermophilic anaerobic microbial community.

1. Introduction

Anaerobic digestion (AD) is a widely applied technology for sewage sludge disposal, which offers the advantage of rapid stabilization of organic matter, reduction in sludge volume, and generation of energy from the produced biogas (Maroun and El Fadel, 2007; Appels et al., 2008). The AD process can be applied at two temperature ranges, namely mesophilic (30–40 °C) and thermophilic (45–60 °C) (Cha and Noike, 1997), because of the presence of two groups of methanogens, and most of the full-scale AD digesters treating sewage sludge have been operated at the mesophilic condition because of the relatively low operation cost (Kardos et al., 2011). Though with a higher operation cost, the thermophilic digestion has also been focused because of the higher specific gas output

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http://dx.doi.org/10.1016/j.watres.2014.11.001
0043-1354/

(Mladenovska and Ahring, 2000; Zabranska et al., 2002). Another merit of the thermophilic digestion is the higher pathogen reduction rates (Watanabe et al., 1997), which is important for the use or disposal of sewage sludge (US. EPA, 1993). Recent studies have shown that thermophilic digestion is more efficient in reducing antibiotic resistance genes than mesophilic digestion (Ghosh et al., 2009; Diehl and LaPara, 2010; Ma et al., 2011). Due to the lack of bulk thermophilic inocula, however, it is necessary to transform mesophilic sludge to thermophilic for the startup of full-scale thermophilic digesters. The transformation of seed sludge has been considered a time-consuming process, usually taking two months, or even longer (Kugelman and Guida, 1989; de la Rubia et al., 2005).

Until now, two startup strategies have been adopted for the transformation of mesophilic sludge to thermophilic: one-step or step-wise temperature increase (Iranpour et al., 2002; Boušková et al., 2005). In general, anaerobic digestion involves numerous interactions among the four major groups of microbes, i.e., hydrolytic-fermentative bacteria, acetogenic bacteria, aceticlastic methanogens and hydrogenotrophic methanogens (Griffin et al., 1998). In a stable anaerobic digestion system, balanced carbohydrate fermentation proceeds by these microorganisms with methane and carbon dioxide as the main products (Sofer and Zaborsky, 1981). Among the four microbial groups, the methanogens are considered to be the most sensitive to temperature changes and the key to the stability of anaerobic digestion systems (Vanlier et al., 1993). It has been assumed that abrupt elevation of temperature may destroy the original metabolic community for methane production in mesophilic sludge, resulting in serious accumulation of hydrogen and volatile fatty acids (VFA) produced during fermentation and acetogenesis (Griffin et al., 1998), which might cause digester upset. Therefore, the step-wise approach has been frequently applied based on the assumption that gradual increase of temperature could be helpful in maintaining the stability of methanogenesis during the startup period (Garber, 1982; Rimkus et al., 1982; de la Rubia et al., 2005). However, Boušková et al. (2005) found that, in comparison with the step-wise temperature increase strategy, the one-step strategy could significantly reduce the transition time.

The key to successfully transform mesophilic digestion to thermophilic operation is the establishment of a thermophilic metabolic community for methane production. Although in low abundance, thermophiles, which are responsible for methane production in thermophilic conditions, were found to be present in mesophilic sludge (Chen, 1983). So, the one-step increase of temperature to the optimum conditions for thermophiles will provide an advantage for their rapid colonization of a reactor, which was assumed to be the main reason for the reduction in transition time (Boušková et al., 2005). However, direct evidence for the above hypothesis is limited and it is still not clear how fast a stable thermophilic metabolic community could be established in an anaerobic digestion system using the one-step strategy.

Therefore, the aim of this study was to investigate the adaptation processes of the bacterial and archaeal populations during the transition of anaerobic digestion from mesophilic (35 °C) to thermophilic conditions (55 °C) using the one-step strategy. The performance of a completely stirred tank reactor (CSTR) was continuously monitored over a period of 80 days after the hike of temperature to 55 °C, and the changes of bacterial and archaeal populations were followed by 454-pyrosequencing and quantitative PCR (qPCR). The result of this study will be helpful in achieving quick startup of thermophilic AD systems.

2. Material and methods

2.1. Reactor system, startup and operation of anaerobic digester

The experiment was carried out in one CSTR reactor of 6 L working volume. The reactor system consists of a feed tank (4 °C), feed pump, reactor, effluent pump, effluent bottle and wet gas meter. The temperature was controlled by circulating hot water in the water jacket of the reactor. The reactor was initially inoculated with mesophilic digested sludge (35 °C) from Gaobeidian WWTP, from which sewage sludge, a mixture of primary sludge and secondary sludge fed to the reactor, was also collected. The characteristics of the substrate are listed in Table S1.

The CSTR was operated at constant feed rate of 300 mL/day and solid retention time (SRT) of 20 days, which is often used for anaerobic mesophilic digestion studies (Griffin et al., 1998; Bolzonella et al., 2005; Boušková et al., 2005). Substrate was fed into the reactor once a day. The reactor was maintained at mesophilic condition (35 °C) for more than 100 days and was at steady state before the experiment started. Characteristics obtained from the steady state mesophilic operation were used as initial values. On day 1, the temperature of the reactor was directly increased to 55 °C, but each day after that, 300 mL of digester content was still removed from the reactor and 300 mL of the substrate was still added to keep a constant SRT. Meanwhile, $NaHCO_3$ was added to the feed during the first SRT (from day 1 to day 20) after temperature elevation to prevent any pH drop that might lead to digester failure. Biogas production, VFA content and pH of the digested sludge were chosen as the main parameters to monitor the process stability.

2.2. Chemical analyses

Biogas production and pH were measured daily. The individual VFA concentrations, dissolved chemical oxygen demand (dCOD), NH_4^+–N and alkalinity were measured daily for the first week and 2–3 times per week thereafter. Solids were measured 1–2 times per week. A portion of the 300-mL digested sludge sample was centrifuged at 10,000 rpm for 10 min and the resulting supernatant passed through a 0.22 μm filter before dCOD, NH_4^+–N and alkalinity analyses. dCOD and NH_4^+–N were measured by a spectrophotometric method (Shimadzu UV-160) (Wei, 2002). Bicarbonate and total alkalinity were determined by titrating to pH 5.8 and 4.3, respectively (Greenberg et al., 1992). For VFA, the samples were acidified with 6M HCl to lower the pH below 3, and centrifuged at 10,000 rpm for 10 min. The supernatant was filtered with a 0.22 μm membrane and 1-butanol was added as