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Differentiated stimulating effects of activated carbon on methanogenic degradation of acetate, propionate and butyrate

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ABSTRACT

Granular activated carbon (GAC) could promote methane production from organic wastes, but a wide range of dosages has been reported. In present study, different GAC dosages of 0, 0.5, 5 and 25 g/L were supplemented into anaerobic digesters and the methanogenic degradation kinetics of acetate, propionate and butyrate were characterized, respectively. At high organic load of 5 g/L, the degradation rates of propionate and butyrate increased by 1.5–4.7 and 2.5–7.0 times at varied GAC dosages. The methane production rates (R_{max}) from propionate and butyrate were significantly elevated when increasing GAC dosage up to 5 g/L. However, only a minor increment was found for acetate degradation either at 1 g/L or 5 g/L. The stimulatory mechanism of GAC for accelerated syntrophic degradation of propionate and butyrate can be primarily attributed to the triggering effect on acetogenesis, as evidenced by the enrichment of syntrophic bacteria e.g. *Thermovirga, Synergistaceae,* and *Syntrophomonas* etc.

1. Introduction

Biomethane production through anaerobic digestion is one of the most successful strategies utilizing bio-energy worldwide (Ferguson et al., 2016; Xiao et al., 2013). In general, anaerobic methanogenesis is carried out by several groups of microorganisms involved in the hydrolysis, acidogenesis, acetogenesis and methanogenesis processes. Fermentative bacteria and acetogens produce volatile fatty acids (VFAs) and other intermediates, such as lactate, ethanol and butanol and, etc., from the degradation of complex macromolecules (Karthikeyan and Visvanathan, 2013; Lee et al., 2016). Methanogens utilize simple organic substrates, such as acetate, CO_2/H_2, methanol, and formate to generate methane (Mcinerney et al., 1981; Pan et al. 2016; Stuckey and David, 1999).

As VFAs other than acetate can't be directly used by methanogens and therefore propionic and butyric acids are mostly found in the effluent from digester with high loads (Li et al., 2017; Viggi et al., 2014). In fact, the oxidation of propionate and butyrate are highly endergonic under standard conditions and occurs only if methanogens keep the concentrations of these intermediate products low (Müller et al., 2010). Propionate and butyrate are firstly converted to acetate and CO_2/H_2 by acetogens, and then they are utilized by aceticlastic- and hydrogenotrophic- methanogens. Syntrophic interspecies H_2 transfer is essential to make the reaction energetically favorable (Müller et al., 2010; Schink, 1997).

There are considerable studies aiming to strengthen the syntrophic metabolism within methanogenic conditions by supplementing conductive iron oxides, such as magnetite and Fe^0 (De Vrieze et al., 2016; Yamada et al., 2015) or conductive carbon materials, such as activated carbon (Liu et al., 2012; Xu et al., 2015), biochar (Luo et al., 2015), carbon cloth and graphite (Dang et al., 2017; Lee et al., 2016; Mumme et al., 2014; Zhao et al., 2015) etc. The stimulated methane production in reactors with conductive materials might be attributed to the promotion of direct interspecies electron transfer (DIET) (Li et al., 2017; Liu et al., 2012; Rotaru et al., 2014a, 2014b). One potential reason for this is that the availability of non-biological conductive materials may save cells energy because they do not need to produce as extensive extracellular biological electrical connections, such as electrically conductive pili and c-type cytochromes (Zhao et al., 2015).

Carbon materials could also provide high specific area for the effective immobilization of syntrophic microorganisms (Li et al., 2017; Luo et al., 2015; Kindzierski et al. (1992)). Zhao et al. (2016) found that the abundance of *Geobacter* species, such as *G. sulfurreducens* and *G. lovleyi* increased in the propionate- and butyrate-fed reactors, accounting for 20% of the community attached to biochar, meanwhile *Syntrophomonas* and *Smithella* species declined. Nevertheless, Dang et al. (2017) reported that granular activated carbon (GAC) seemed to significantly increase the

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abundance of syntrophic bacteria such as *Syntrophomonas, Symbiobacterium* and *Desulfotomaculum* species, whereas *Geobacter* were not enriched in any of the OFMSW reactors supplemented with GAC. The distinct results might either attributed to the different inoculums or different carbon sources, e.g. single/mixed VFAs, ethanol or complex organic matters (Kato et al., 2012; Wang et al., 2016). Thus further investigations are in demand to understand the syntrophic communities for propionate and butyrate in digester with carbon materials.

Furthermore, it is noted that only simple comparison between AC treated group and blank group has been reported in most studies, and the supplementing dosage of AC varied widely (e.g. from 0.005 to 50 g/L), as shown in Table S1. Nevertheless, Chen et al. (2014) reported that the metabolism rates of ethanol in methanogenic reactor increased when the amount of carbon cloth was doubled from 10 g/L to 20 g/L, but without further interpretation. Therefore, it is also necessary to clarify whether there is a dose-dependent effect and provide a quantitative basis for related practices.

Based on the above rationale, this study has investigated the degradation kinetics of acetate, propionate and butyrate, separately in methanogenic digesters supplemented with a series of GAC dosages (i.e. 0.5–25 g/L). Meanwhile, two different organic loads of substrate, i.e. 1 g/L and 5 g/L were compared. The rates of VFAs' degradation and methane generation were evaluated by using first-order kinetics and Modified Gompertz model. The high throughput technique was used for 16s rDNA sequencing to detect the microbial community structure, and the alternation of syntrophic VFAs degrading bacteria and methanogens due to GAC addition was discussed in this study.

2. Methods

2.1. Preparation of sludge inoculum and experimental design

Inoculum sludge taken from Quyang Sewage Treatment Plant (Shanghai, China) was pre-cultured in a laboratory scale anaerobic digester. And then the sludge was transferred to three reactors fed with different VFAs, i.e. acetate, propionate and butyrate, respectively, to enrich the specific fatty acids degradation bacteria. After several sequential batches of cultivation, 1 g/L of each VFA species could almost be degraded after 5–7 days. The cultivation temperature was maintained at 35 ± 2 °C.

During experiments, the determined volume of enriched sludge was put into 500 mL serum bottle with 400 mL of digestate liquid to make a final concentration of total volatile suspended solid (TVSS) at 1 g/L. Different dosages of GAC was supplemented to serum bottles i.e. 0, 0.5, 5 and 25 g/L, respectively, which were recorded as GAC0, GAC0.5, GAC5 and GAC25. GAC was purchased from Sinopharm Chemical Reagent CO. LTd. 20–40 mesh GAC was obtained by shive, which apparent density and specific surface area was 430 ± 30 g/L and 875–1185 m^2/g, respectively. Sequentially, the conversion rates of acetate, propionate and butyrate into methane with specific enriched cultures were evaluated in batch studies at the concentration of 1 g/L and 5 g/L. Data was collected after two batches of pre-culture, and each test was carried out in triplicate. The temperature of reactors was maintained at 35 ± 2 °C with an incubator shaker (DKY-II, Shanghai Duke Auto Co., China).

The substrate formula: specific carbon source (e.g. acetate, propionate and butyrate), the corresponding qualities of NH_4Cl and KH_2PO_4 were added to the reactors according to the C: N: P = 100:5:1. Additionally, 2 mL/L of the trace element solution was added (El-Mamouni et al., 1995) and the pH was adjusted to 7.2 with HCl and NaOH solutions. Finally, all reactors were flushed with nitrogen gas for more than 10 min before startup.

2.2. Physiochemical analyses

The volume of methane generation was automatically measured by AMPTS II (Bioprocess, Sweden) equipped with a gas flowmeter. The liquid of each reactor was sampled and analyzed to monitor the variations of total organic carbon (TOC) and VFAs. After filtrated by 0.45 μm filter membrane, the concentration of VFAs was analyzed by high performance liquid chromatography (Waters 2695/2489, USA) equipped with refractive index detector. The TOC was analyzed by Total Carbon/Total Nitrogen analyzer (Multi N/C 3100, Jena Co., Germany).

2.3. Microbial community analyses

The sludge samples were collected from GAC0 and GAC5 reactors at the end of experiment. The total DNAs of all samples were extracted using the Power Soil™ DNA isolation kit (Mo-Bio Laboratories Inc., CA). Labels of "HAc0", "HPr0", "HBu0" stand for the sludge samples taken from GAC0 with respective substrate, and "HAc1", "HPr1", "HBu1" stand for the samples taken from GAC5. The microbial community of samples was analyzed by using high-throughput pyrosequencing on an Illumina platform (Illumina Miseq PE300). Amplicon libraries were constructed for pyrosequencing using bacterial primers 515F (50-GTG CCA GCM GCC GCG GTA A-30) and 806R (50-GGA CTA CHVGGG TWT CTA AT-30) for the V4–V5 region of the microbial 16SrRNA gene (Xu et al., 2015). Sequencing data has been deposited into public database NCBI, and the accession number is SRP134710 (https://www.ncbi.nlm.nih.gov/sra/SRP134710).

2.4. Data analysis

Modified Gompertz model (Eq. (1)) was fitted to the experimentally observed curve of cumulative CH_4 production (Lü et al., 2013). The variations of VFAs were fitted with first-order kinetics (Eq. (2)).

$$M_{CH_4}(t) = P_{CH_4} \times \exp\left\{-\exp\left[\frac{R_{CH_4} \times e}{P_{CH_4}} \times (\lambda_{CH_4} - t) + 1\right]\right\} \quad (1)$$

$$\ln\frac{C_0}{C(t)} = kt \quad (2)$$

Where, in Eq. (1), M(t), P, R and λ is cumulative production (mmol-C/mmol-C_{added}) at time t, ultimate methane yield (mmol-C/mmol-C_{added}) at the end of the incubation, maximum production rate (mmol-C/mmol-C_{added}/d) and lag phase (d), respectively for CH_4 and CO_2 production; e is 2.71828. In Eq. (2), C_0 and $C(t)$ is the initial concentration of particular substrate and the concentration at time t; k is the first order degradation constant.

3. Results and discussion

3.1. Profile of VFAs degradation and methane generation at low strength

Syntrophic interaction is essential to overcome the thermodynamic barriers in the anaerobic oxidation of fermentation intermediates especially propionate and butyrate (Hattori, 2008). In present study, we examined the methanogenic degradation of HAc, HPr and HBu, respectively with the supplementation of GAC at different organic loads, i.e. 1 g/L and 5 g/L, which profiles of cumulative methane production and VFAs declination are presented in Figs. 1 and 2.

With the initial concentration of 1 g/L, three species of VFAs i.e. HAc, HPr and HBu were rapidly degraded, which almost vanished after 5 days. Similarly, the lag phase of methane generation could

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