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Short-chain fatty acids production and microbial community in sludge alkaline fermentation: Long-term effect of temperature

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HIGHLIGHTS

- Mechanism of higher SCFAs production at mesotherm than microtherm was first studied.
- Acetic acid contributed to the higher SCFAs at mesotherm.
- Mesotherm increased bacterial diversity with Shannon index of 4.45.
- Sludge fermented at mesotherm saved more $6.7/year energy cost than at microtherm.

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ABSTRACT

Sludge alkaline fermentation has been reported to achieve efficient short-chain fatty acids (SCFAs) production. Temperature played important role in further improved SCFAs production. Long-term SCFAs production from sludge alkaline fermentation was compared between mesotherm (30 ± 2 °C) and microtherm (15 ± 2 °C). The study of 90 days showed that mesotherm led to 2.2-folds production of SCFAs as microtherm and enhanced the production of acetic acid as major component of SCFAs. Soluble protein and carbohydrate at mesotherm was 2.63-folds as that at microtherm due to higher activities of protease and α-glucosidase, guaranteeing efficient substrates to produce SCFAs. Illumina MiSeq sequencing revealed that microtherm increased the abundance of *Corynebacterium*, *Alkaliflexus*, *Pseudomonas* and *Guggenheimella*, capable of enhancing hydrolysis. Hydrolytic bacteria, i.e. *Alcaligenes*, *Anaerolinea* and *Ottowia*, were enriched at mesotherm. Meanwhile, acidogenic bacteria showed higher abundance at mesotherm than microtherm. Therefore, enrichment of functional bacteria and higher microbial activities resulted in the improved SCFAs at mesotherm.

1. Introduction

Biological nutrients removal (BNR) process has been widely used in wastewater treatment to simultaneously reduce nitrogen and phosphorus quantity discharging into receiving water, which protect natural water bodies from eutrophication. However, the shortage of carbon source in influent wastewater always restrains the performance of nitrogen and phosphorus removal. It has been reported that 6–9 mg of short-chain fatty acids (SCFAs) is required for removing 1 mg phosphorus in conventional BNR (Pitman et al., 1992) and 1.72–2.85 mg of SCOD for per mg of nitrogen. Actually, a significant amount of these COD flows into waste activated sludge (WAS) by cell synthesis, which needs to be further treated to achieve reduction, stabilization and unhazardous and thereby increasing the burden of wastewater treatment plants. However, it has been general realization that WAS is a valuable resource as it is mainly composed of organics. By fermentation, WAS (even primary sludge or a mixture of both) could produce SCFAs which have been demonstrated to be the preferred carbon source to support BNR. At the same time, sludge reduction could be accomplished. Moreover, the fermentative SCFAs previously have also been tested and confirmed its suitability as the carbon source in BNR (Elefsiniotis et al., 1996; Gao et al., 2011; Ucisik and Henze, 2008). And recovering SCFAs instead of exogenous carbon sources to enhance nutrients removal makes BNR more sustainable.

Generally, hydrolysis, acidification and methanogenesis are involved sequentially in a natural sludge digestion process. In respect of factors restricting SCFAs production, on one hand, initial solubilization of particulate organic matter to soluble substance is

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believed to be the rate-limiting step (Eastman and Ferguson, 1981). On the other hand, SCFAs produced during sludge anaerobic digestion can be consumed by methanogens. Therefore, if the solubilization, hydrolysis and acidification could be enhanced and the methanogenesis could be prevented or weaken, the production of SCFAs would undoubtedly increase. It has been reported SCFAs production was significantly improved under alkaline conditions (Chen et al., 2007). Since methanogens could be inhibited under alkaline conditions (Zheng et al., 2013; Yuan et al., 2015), high pH could guarantee the degradation of sufficient soluble organic substrates terminated with SCFAs.

Temperature was also considered as an important factor for sludge anaerobic fermentation system (Skalsky and Daigger, 1995; Yuan et al., 2011; Zhang et al., 2009). Maharaj and Elefsiniotis (2001) observed higher SCFAs concentrations were obtained at 25 °C than 16–8 °C, which was hypothetically attributed to the debilitating effects of the low temperatures on the acidogenic bacteria. Moreover, Cokgor et al. (2009) reported that temperature increase from 10 to 24 °C induced a 5-folds increase in SCFAs generation, from 610 mg/L at 10 °C to 2950 mg/L at 24 °C. Feng et al. (2009a) investigated that the hydrolysis and SCFAs production increased with increasing temperature from 10 to 35 °C at alkaline pH as well. However, all these studies only focused on the short-term performance of reactor, but the mechanism for the improved SCFAs production from WAS alkaline fermentation at mesotherm than at microtherm still remained vacuum. Since the production of SCFAs during WAS fermentation was a biological process (Feng et al., 2009b; Yuan et al., 2015), it is necessary to understand the mechanism for improved SCFAs production at mesotherm than at microtherm from the view point of microbial community involved in the anaerobic sludge fermentation.

Two sets of anaerobic sludge fermentation reactors at mesotherm (30 ± 2 °C) and microtherm (15 ± 2 °C) were respectively operated under alkaline condition over 90 days. This study aimed to investigate the long-term effect of temperature on SCFAs production and microbial community distribution in sludge alkaline fermentation. Additionally, the activities of key enzymes during hydrolysis and acidification phases were also measured to reveal the microbial activity.

2. Materials and methods

2.1. Source of WAS

The WAS used in present study was obtained from a pilot-scale sequencing batch reactor (SBR) treating municipal wastewater in Beijing, China (Yuan et al., 2015). The sludge was concentrated by settling at 4 °C for 24 h before anaerobic fermentation and the composition of the WAS after settlement are listed in Table 1.

Table 1
Characteristics of the concentrated wasted activated sludge (WAS) used in the experiments.

Parameters	Units	Value[a]
pH	–	7.4 ± 0.2
Total suspended solids (TSS)	mg/L	7345.8 ± 372.1
Volatile suspended solids (VSS)	mg/L	6603.8 ± 333.2
Total chemical oxygen demand (TCOD)	mg/L	9732.6 ± 283.5
Soluble chemical oxygen demand (SCOD)	mg/L	27.6 ± 12.7
Soluble protein (SP)	mg/L	3.5 ± 0.6
Soluble carbohydrate (SC)	mg/L	1.4 ± 0.3
Short-chain fatty acids (SCFAs)	mg COD/L	21.3 ± 4.7

All data were made from samples obtained over the 90 days.
[a] Stands for average ± standard deviation.

2.2. Semi-continuous fermentation reactors and operation modes

To determine the optimal alkaline pH value and sludge retention time (SRT) applied in WAS fermentation at microtherm (15 ± 2 °C) and mesotherm (30 ± 2 °C), a series of pH and SRT was selected and primarily evaluated in the batch tests. Ten batch reactors (with working volume of 1.5 L each) were divided equally into two groups, one group maintained at 15 ± 2 °C (microtherm) and the others at 30 ± 2 °C (mesotherm), respectively. The pH conditions of five reactors of each group were set at 8, 9, 10, 11 and uncontrolled (at pH of 6.8–7.6), respectively, by adding 2 M sodium hydroxide (NaOH). 1.5 L WAS was fed to each reactor with preset pH condition. Then all the reactors were maintained at corresponding temperature and 10 mL fermented sludge was withdrawn from each reactor to analyze SCFAs every day. The highest amount of SCFAs (751.3 mg COD/L) at microthermal condition appeared at pH 10 on 8th day (Supplementary material, Fig. S1). In mesophilic fermentation, the SCFAs production at pH 10 with SRT of 6 d reached the highest (1674.4 mg COD/L).

Then two semi-continuous fermentation reactors (working volume: 5 L) were built and anaerobically operated at 30 ± 2 °C (Reactor 1) and 15 ± 2 °C (Reactor 2), respectively. After the addition of sludge, these reactors were sealed and mechanically stirred at a speed of 90 rpm. According to the predetermined results in batch tests, the fermentation pH in two reactors was maintained at pH 10.0 ± 0.2 during the entire fermentation period by adding 2 M NaOH. 833 mL of fermented sludge was withdrawn from Reactor 1 and an equal amount of WAS was injected while the Reactor 2 had 625 mL WAS withdrawn and 625 mL WAS injected every day. The SCFAs concentrations in these reactors were measured every three days during the fermentation period of 90 days.

2.3. DNA extraction, PCR amplification and Illumina MiSeq sequencing

For the analysis of bacteria in fermentation reactors, sludge samples were collected before feeding fresh sludge on the 90th day. The samples were freeze-dried by LABCONCO (Model 195, England). DNA extraction, PCR amplification and the analysis of Illumina MiSeq sequencing data were proceed according to the method described by Yuan et al. (2015). The raw sequences can be achieved by the accession number SRA304040.

2.4. Analytical methods

The analyses of COD, VSS, and TSS were conducted in accordance with standard methods (APHA, 1998). To measure the concentration of the produced SCFAs, soluble proteins (SP) and carbohydrates (SC), the fermentation mixture was withdrawn from each reactor and immediately filtered through a Whatman GF/C glass fiber filter. The measurements of SCFAs, SP and SC as well as the conversion factors to COD were the same as described in our previous publication (Yuan et al., 2015). Alkaline/Acid phosphatase, Protease and α-Glucosidase activities, the key hydrolytic enzymes, were determined according to the method proposed by Goel et al. (1998). The key acetic acid-forming enzyme phosphotransacetylase (PTA) was measured according to the method reported in Feng et al. (2009c), and the other acetic acid-forming enzyme, acetate kinase (AK), was tested using Fast ACK kit according to the manufacture's instruction (Suzhou, China).

2.5. Statistical analysis

All measurements were performed in triplicate. An analysis of variance was applied to evaluate the significance of results, and $p < 0.05$ was considered to be statistically significant.