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Volatile fatty acids productions by mesophilic and thermophilic sludge fermentation: Biological responses to fermentation temperature

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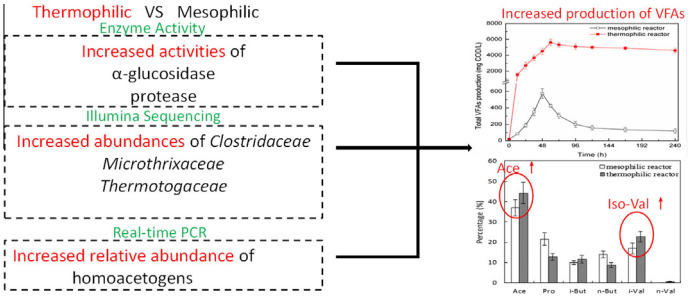
HIGHLIGHTS

- Thermophilic fermentation led to greater accumulation of VFAs.
- Carbohydrate and protein solubilized more at higher fermentation temperature.
- Extracellular hydrolases activities were much higher under thermophilic condition.
- The microbial communities were correlated well with reactor performance.
- Higher fermentation temperature raised the relative abundance of homoacetogens.

GRAPHICAL ABSTRACT



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ABSTRACT

The volatile fatty acids (VFAs) productions, as well as hydrolases activities, microbial communities, and homoacetogens, of mesophilic and thermophilic sludge anaerobic fermentation were investigated to reveal the microbial responses to different fermentation temperatures. Thermophilic fermentation led to 10-fold more accumulation of VFAs compared to mesophilic fermentation. α-glucosidase and protease had much higher activities in thermophilic reactor, especially protease. Illumina sequencing manifested that raising fermentation temperature increased the abundances of *Clostridiaceae*, *Microthrixaceae* and *Thermotogaceae*, which could facilitate either hydrolysis or acidification. Real-time PCR analysis demonstrated that under thermophilic condition the relative abundance of homoacetogens increased in batch tests and reached higher level at stable fermentation, whereas under mesophilic condition it only increased slightly in batch tests. Therefore, higher fermentation temperature increased the activities of key hydrolases, raised the proportions of bacteria involved in hydrolysis and acidification, and promoted the relative abundance of homoacetogens, which all resulted in higher VFAs production.

1. Introduction

Sludge anaerobic fermentation is regarded as a sustainable technology that can produce resources and solve environmental problems of excess sludge (Tchobanoglous and Burton, 1991). Up to now, large efforts have been made to convert the abundant organic matter in the sludge to methane or hydrogen (Cai et al., 2004; Palatsi et al., 2011). Recently, the production of volatile fatty acids (VFAs) is gaining increasingly attention since the produced VFAs can be provided as organic carbon source for nitrogen and phosphorus removal in wastewater treatment plant (Tong and Chen, 2007), or as raw material for bioplastic polyhydroxyalkanoates (PHAs) synthesis (Xiong et al., 2012).

Fermentation temperature, acting as a significant operational condition, can exert tremendous influences on fermentation

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products (Song et al., 2004; Xiong et al., 2012). Mesophilic condition (30–40 °C) has long been adopted for anaerobic digestion to recover biogas, showing good performances (Cai et al., 2004). However, VFAs accumulation is very low in this case. To improve VFAs production, increasing fermentation temperature is a succinct and effective approach (Liu et al., 2013; Xiong et al., 2012). Under thermophilic condition (50–60 °C), high concentration of VFAs can be acquired, as well as good stability and economic efficiency by proper control (Lu et al., 2007; Song et al., 2004). Although this process is very important, most studies have only focused on reactor performances, and there is still a lack of knowledge about the mechanism of VFAs production under thermophilic condition and the biological responses to different fermentation temperatures.

Three stages, hydrolysis, acidification and methanogenesis, are involved in fermentation process, and the initial hydrolysis of particulate organic matter to soluble substance is defined as the overall rate-limiting step (Yu et al., 2008). Accordingly, VFAs production largely depends on the catalyzing efficiency of extracellular hydrolases involved in hydrolysis reactions. The effect of heat-treatment on hydrolase activity of the sludge has been revealed (Yan et al., 2008). Besides, some researchers have compared the activities of four hydrolases of mesophilic and thermophilic dry anaerobic reactors digesting organic solid wastes (Lu et al., 2007). Although it was demonstrated that temperature could distinctly affect hydrolases activities, little attention has been paid to studying the temperature effects on fermentation in VFAs-producing anaerobic reactors during acid-forming phase.

Some culture-independent analyses, such as clone library and fluorescent in situ hybridization (FISH), have been used to reveal the microbial diversity of methanogenic reactors with different temperatures (Sekiguchi et al., 1999). Nevertheless, there has been no related research comparing the microbial communities of acidification reactors operated at different temperatures. In addition, the culture-independent methods not only costs a long time, but also may underestimate the microbial diversity. Next-generation sequencing technologies can overcome these drawbacks and make it more credible to reveal the microbial community of environmental samples (Ju et al., 2014; Wang et al., 2012; Zheng et al., 2013). Among these technologies, Illumina high-throughput sequencing is a relatively cost-effective method with high coverage. For example, some researchers found that the microorganisms of the activated sludge from a full-scale wastewater treatment plant appeared regular seasonal variations over 4 years by Illumina sequencing platform (Ju et al., 2014). To understand the mesophilic and thermophilic VFAs-producing process more deeply, next-generation sequencing can be applied to comprehensively reveal the microorganisms of the reactors, and figure out the major bacteria groups involved in VFAs production.

In most cases, the largest proportion of the fermented VFAs is acetate (Jia et al., 2013; Liu et al., 2013; Wang et al., 2013), and one important group of acetate-producing bacteria is the homoacetogen. Homoacetogens have the capability of autotrophic growth on H_2/CO_2 and heterotrophic growth on a wide range of simple or complex compounds (Drake et al., 2006). Homoacetogens are among the most phylogenetically diverse bacterial groups, but they have a shared functionality due to their ability to convert CO_2 to acetate via the acetyl-CoA pathway. A key enzyme in this CO_2 fixation pathway, formyltetrahydrofolate synthase (FTHFS), is conservative in structure and function (Xu et al., 2009). So the gene encoding FTHFS combined with clone library or real-time PCR assay can be used to identify and enumerate homoacetogens (Ryan et al., 2008; Xu et al., 2009). Though it was found that homoacetogens favored low temperature (Kotsyurbenko et al., 2001), FTHFS clone libraries revealed that they could have high diversity under mesophilic and thermophilic conditions (Ryan et al., 2008; Siriwongrungson et al., 2007). However, up to now little has been known about homoacetogens abundances under mesophilic and thermophilic conditions, and their relationships with the fermented VFAs are also needed to be investigated.

The main objective of this study was to compare the VFAs productions by mesophilic and thermophilic sludge fermentation and provide thorough elucidations to clarify the biological responses to different fermentation temperatures. The activities of key hydrolases during hydrolysis and acidification phases were measured to reveal the microbial biomass and activity. Additionally, microbial communities and homoacetogens, one of the main functional groups, were investigated by Illumina sequencing and real-time PCR, respectively. It should be noted that this is the first time that enzyme activities, microbial communities and homoacetogens have been comprehensively concerned to evaluate the effects of fermentation temperature on VFAs production.

2. Methods

2.1. Dewatered sludge and experimental sludge

To facilitate storage of the sludge and control of the solids concentration, dewatered sludge was collected from a municipal wastewater treatment plant in Beijing, China, and stored at 4 °C before use. TS (total solids) and VS (volatile solids) of the dewatered sludge were 14.1 ± 0.2% (w/w) and 8.9 ± 0.7% (w/w), respectively. The sludge was diluted to a certain ratio by adding deionized water to 300 g dewatered sludge to the final volume of 1 L, with continuous mixing and homogenizing. The above diluted sludge was divided into the same aliquots and used for all batch and semi-continuous tests in this study, and no extra inoculum was needed. The characteristics of the diluted sludge are listed in Table 1.

Compared with other studies which used primary or secondary sludge, or synthetic materials as substrates for fermentation (Jia et al., 2013; Wang et al., 2013; Zheng et al., 2013), the sludge used in this study had higher solids concentration, as it was reported previously that higher solids concentration could improve VFAs production (Xiong et al., 2012).

2.2. Operation of batch and semi-continuous fermentation reactors

500 mL glass bottles were used as fermentation reactors, with working volume of 300 mL. For 10-day batch tests, after adding 300 mL diluted sludge, the reactors were purged with nitrogen gas for 3 min to displace oxygen, and inserted with rubber plugs to keep anaerobic environment. Then the reactors were operated in two rotary shakers at 165 rpm, 35 ± 1 °C for MR and 55 ± 1 °C for TR. The pH of the reactors was monitored but not controlled, and the value fluctuated from 6.6 to 7.1. During fermentation process, samples were withdrawn from the reactors at 12 h or 24 h intervals for the measurement of different parameters, and nitrogen gas was filled for 3 min after every sampling.

Table 1
Characteristics of the experimental sludge.[a]

Parameter[b]	Sludge sample
TSS (total suspended solids)	36,829 ± 1014
VSS (volatile suspended solids)	23,378 ± 731
TCOD (total chemical oxygen demand)	28,383 ± 286
SCOD (soluble chemical oxygen demand)	508 ± 29
Total carbohydrate (as COD)	4139 ± 194
Total protein (as COD)	9352 ± 533
Lipid and oil (as COD)	180 ± 7

[a] Results are the averages and their deviations of triplicate measurements.
[b] All values are expressed in mg/L.