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Short communication

Effects of bio-surfactants combined with alkaline conditions on volatile fatty acid production and microbial community in the anaerobic fermentation of waste activated sludge

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ABSTRACT

This study investigated the effects of three types of bio-surfactants (BS), namely, saponin (SP), surfactin (SF), and rhamnolipid (RL), with alkaline conditions on volatile fatty acid (VFA) production and microbial community in sludge fermentation. BS combined with a pH of 9 significantly enhanced hydrolase activities, promoted VFA production and inhibited methanation. SP & pH 9 was more efficient in converting organics to VFAs and thus achieved the highest yield of 425.2 mg COD/g VSS, which was 1.5 times that at pH 10. The lower negative effect of SP on metabolic potential and diversity than that of SF or RL was confirmed through a Biolog assay. Illumina-sequencing further proved that the relative abundances of acidogens in SP & pH 9 were higher than those in other tests, including *Proteiniclasticum*, *Fusibacter*, *Macellibacteroides* and *Petrimonas* et al. This study provided a strategy for plant-derived SP applications in sludge moderate alkaline fermentation to enhance VFA production.

1. Introduction

Large amounts of waste activated sludge (WAS) are produced in the process of wastewater treatment. Volatile fatty acids (VFAs) can be produced through the anaerobic fermentation of WAS, which contains large quantities of organic matter, such as proteins, polysaccharides, and humic acids (Feng et al., 2009). VFAs are considered cheap external carbon sources for wastewater treatment plants (Soares et al., 2010), they can also be used to biosynthesize high value-added products, such as polyhydroxyalkanoates (Morgan-Sagastume et al., 2010) and biodiesel (Liu et al., 2016). To increase VFA yields, alkaline conditions, especially an optimal pH of 10, has been generally applied to control the fermentation process in many studies (Chen et al., 2007; Huang et al., 2014; Jie et al., 2014). However, strong alkaline conditions could inhibit the metabolic activities of acid-producing bacteria, as a result, VFA yield was restricted (Jie et al., 2014). Moreover, high alkali concentrations compromises the anticorrosion capability of devices.

To solve these issues, adding accelerants, especially surfactants (Jiang et al., 2007), have become another acclaimed option to boost VFA production during WAS fermentation. Bio-surfactants (BS) produced from plants or microbes are desirable substitutes for traditional chemical surfactants because of their excellent surface/interface activity, biocompatibility, and environmental friendliness (Yi et al., 2013). Our previous study found that three types of BS can significantly enhance VFA concentrations (Huang et al., 2015). However, adding BS alone fails to release as many organics as alkaline conditions do and to effectively inhibit methane production. Consequently, it is particularly crucial to optimize the application strategy of BS to increase VFA yields.

Microorganisms play important roles in anaerobic sludge fermentation to metabolize soluble organics. Microbial studies on alkaline fermentation have revealed that *Actinobacteria*, *Clostridia*, *Proteobacteria* and *Firmicutes* were the major bacterial phyla in this process (Liu et al., 2014; Zheng et al., 2013). Considering surfactants, Zhou et al. (2015) reported that RL was more favorable than synthetic surfactants (sodium dodecyl sulfate and sodium dodecyl benzene sulfonate) to functional microbes for acidification. However, different BS possess different structures and properties. For example, saponin (SP) exhibited more biocompatibility but lower surface activity than surfactin (SF) and rhamnolipid (RL) do in WAS (Huang et al., 2015). Therefore, various biological effects of different

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BS on microbial community is required to be investigated. Illumina-MiSeq sequencing is a newly developed molecular biological technology with high coverage, high throughput, and short sequencing period. This technology can be utilized to reveal the biological effects of different fermentation conditions.

In this study, the effects of plant-derived SP and microbe-produced SF and RL, with the combination of alkaline conditions were compared in WAS fermentation. The organics solubilization, methane inhibition and key enzymatic activities were investigated. Biolog assay and Illumina-MiSeq sequencing were adopted to characterize the metabolic potential, functional diversity and microbial structures to reveal the microbial responses to the combination of BS and alkaline conditions.

2. Materials and methods

2.1. Batch experiments

The characteristics of WAS and BS (i.e. SP, SF, and RL), anaerobic fermentation processes and sampling frequencies were identical to those described in our previous study (Huang et al., 2015). The reactor with raw sludge was set as the blank reactor (BL group), and the pH value was approximately 7.0. Five groups were set (Table 1). One group was adjusted with sole alkaline adjustment, and the pH value was maintained as 9.0, 10.0 and 11.0, respectively. The second group of reactors was set as the BS group and used with dosages of 0.10, 0.05, and 0.05 g/g DS for SP, SF and RL, respectively. With the same BS and dosages, the three other groups with alkaline adjustment (pH of 9, 10, and 11) were defined as the experimental group (BS & pH group). The pH values of groups with alkaline fermentation conditions was adjusted with 6 M NaOH every 12 h.

2.2. Biolog assay

The samples were pretreated to remove substrates and solid particles contained in sludge according to Smalla et al. (1998) with some modifications. The average well color development (AWCD) for six types of carbon sources in the microplate was used to evaluate microbial utilization potential. Shannon (H'), McIntosh (U) and Simpson ($1/D$) indexes, as well as principal component analysis (PCA), were calculated to reflect metabolic characteristics. H' is closely related to the substrate metabolic diversity, U reflects the community evenness, and $1/D$ represents the species diversity (Magurran, 1988). Detailed pretreatment methods, AWCD calculation and diversity indexes can be referred in the Supplementary Material.

Table 1
Batch experimental group settings.

Group	Category	BS type	BS dosage	pH value
/	BL	/	/	/
Alkaline	pH 9	/	/	9
	pH 10	/	/	10
	pH 11	/	/	11
BS	SP	saponin	0.10 g/g DS	/
	SF	surfactin	0.05 g/g DS	/
	RL	rhamnolipid	0.05 g/g DS	/
BS & pH 9	SP & pH 9	saponin	0.10 g/g DS	9
	SF & pH 9	surfactin	0.05 g/g DS	9
	RL & pH 9	rhamnolipid	0.05 g/g DS	9
BS & pH 10	SP & pH 10	saponin	0.10 g/g DS	10
	SF & pH 10	surfactin	0.05 g/g DS	10
	RL & pH 10	rhamnolipid	0.05 g/g DS	10
BS & pH 11	SP & pH 11	saponin	0.10 g/g DS	11
	SF & pH 11	surfactin	0.05 g/g DS	11
	RL & pH 11	rhamnolipid	0.05 g/g DS	11

2.3. DNA extraction and PCR amplification

Two biological replicates were conducted per fermentation condition for the sludge on day 6 to analyze the bacteria and archaea. Microbial DNA was extracted with E.Z.N.A.® (Omega Biotek, 182 Norcross, GA, USA.). The V4 + V5 region of the bacteria 16S ribosomal RNA gene was amplified by PCR, with initial denaturation at 95 °C for 2 min, followed by 30 cycles of denaturing at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 45 s using primers 338F-806R for bacteria and Arch334F-915R for archaea. PCR reactions were performed in triplicate with a 20 μL mixture containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, and 10 ng of template DNA.

2.4. Illumina-MiSeq sequencing and processing of sequencing data

Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 250) on an Illumina-MiSeq platform according to standard protocols. Raw fastq files were demultiplexed and quality-filtered by QIIME (version 1.17) with the following criteria. First, the 300 bp reads were truncated at any site receiving an average quality score of <20 over a 50 bp sliding window; truncated reads that were shorter than 50 bp were discarded. Second one is the exact barcode matching. Two nucleotide mismatch in the primer matching, and reads containing ambiguous characters were removed. Third, only sequences that overlap longer than 10 bp were assembled according to their overlap sequence. Reads that could not be assembled were discarded. Operational taxonomic units (OTUs) were clustered with a 97% similarity cutoff by UPARSE (version 7.1 http://drive5.com/uparse/), and chimeric sequences were identified and removed using UCHIME (Amato et al., 2013).

2.5. Analytical methods

For batch experiments, the treatment methods for fermentation liquid samples, the measurement methods of basic parameters (including VFAs, SCOD, and soluble organics), and key enzyme activities were in accordance with those in our previous study (Huang et al., 2015). All experiments were conducted in triplicate and mean values are presented. One way analysis of variance was applied to test the significance of the results; $P < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Effects of BS combined with alkaline condition on fermentation products

The VFA yields, VFA compositions and soluble organics under sole alkaline adjustment (pH of 9, 10, and 11), sole BS addition (SP, SF, and RL) and the combination of BS and alkaline adjustment are illustrated in Fig. 1. Maximal VFA yields of approximately 420 mg COD/g VSS were obtained with BS & pH 9, which facilitated VFA accumulations best among all conditions. For the alkaline group, pH 10 (a stronger alkaline condition than BS & pH 9) achieved the highest VFA yield (293 mg COD/g VSS), which was only 70% of those in BS & pH 9. The pH value of 10 was consistent with that in the results of alkaline sludge fermentation by Jie et al. (2014), which indicated that a pH value that higher than 10 is unfavorable for VFA production.

Fig. 1B shows that the soluble organics (polysaccharides, proteins and humic acids) concentrations in BS & pH 9 were lower than those at pH 10. This result indicated that the addition of BS could produce higher yields of VFAs from fewer substrates under