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Mesophilic and thermophilic alkaline fermentation of waste activated sludge for hydrogen production: Focusing on homoacetogenesis

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ABSTRACT

The present study compared the mesophilic and thermophilic alkaline fermentation of waste activated sludge (WAS) for hydrogen production with focus on homoacetogenesis, which mediated the consumption of H_2 and CO_2 for acetate production. Batch experiments showed that hydrogen yield of WAS increased from 19.2 mL H_2/gVSS at 37 °C and pH 10–80.1 mL H_2/gVSS at 55 °C and pH 10. However, the production of volatile fatty acids (mainly acetate) was higher at 37 °C and pH 10 by comparison with 55 °C and pH 10. Hydrogen consumption due to homoacetogenesis was observed at 37 °C and pH 10 but not 55 °C and pH 10. Higher expression levels of genes relating with homoacetogenesis and lower expression levels of genes relating with hydrogen production were found at 37 °C and pH 10 compared to 55 °C and pH 10. The continuous experiment demonstrated the steady-state hydrogen yield of WAS was comparable to that obtained from batch experiments at 55 °C and pH 10, and homoacetogenesis was still inhibited. However, the steady-state hydrogen yield of WAS (6.5 mL H_2/gVSS) was much lower than that (19.2 mL H_2/gVSS) obtained from batch experiments at 37 °C and pH 10 due to the gradual enrichment of homoacetogens as demonstrated by qPCR analysis. The high-throughput sequencing analysis of 16S rRNA genes showed that the abundance of genus *Clostridium*, containing several homoacetogens, was 5 times higher at 37 °C and pH 10 than 55 °C and pH 10.

1. Introduction

In view of the shortage of fossil fuels and the pollution induced by the excessive utilization of that, we are facing serious environmental and energy challenges. Hydrogen is considered an attractive future fuel on account of its clean combustion product and high energy density by mass (Sarma et al., 2015). Fermentative hydrogen production from organic wastes has attracted much attention since it has advantages including stabilization of organic wastes and at the same time production of renewable energy (Arimi et al., 2015; Xia et al., 2015; Xiao et al., 2014).

Waste activated sludge (WAS) is a byproduct produced in huge quantities from municipal wastewater treatment plants. WAS has been considered as the substrate for fermentative hydrogen production (Xia et al., 2015). However, the hydrogen yields of WAS were generally low (Cai et al., 2004; Liu et al., 2013a; Massanet-Nicolau et al., 2008; Wang et al., 2015; Xiao and Liu, 2009; Yang et al., 2012; Zhao et al., 2010). Although WAS is rich in carbohydrate and protein, most of the organic content is enclosed inside the microbial cell membranes, which is difficult to be accessed by the hydrogen producing bacteria (Zheng et al., 2014). Therefore, different physical, chemical and biological methods have been employed to disrupt the cells of WAS to release the organics in order to increase the hydrogen yields (Guo et al., 2015; Liu et al., 2013a; Zheng et al., 2014). In addition, there are hydrogen-consuming microorganisms, which result in the production of methane (by methanogens) or acetate (by homoacetogens) from hydrogen and consequently reduce the hydrogen yields of WAS (Cai et al., 2004; Saady, 2013; Zhao et al., 2010). The inhibition of methanogens could be achieved by pretreating the sludge with heat, acid, alkali and so on, since they are not able to form spores under extreme conditions (Luo et al., 2010a). However, the inhibition of homoacetogens is very challenging, since some of them are able to form spores (e.g. *Clostridium ljungdahili*, *Clostridium autotrophicum*, *Clostridium aceticum*, et al.) and therefore still survive after the pretreatments (Luo et al., 2011; Saady, 2013).

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A previous study reported that fermentation at 37 °C and constant pH 10 inhibited hydrogen consumption by homoacetogenesis and improved the solubilisation and acidification of heat-pretreated WAS, and thereby achieved the highest hydrogen yield (26.9 mL H_2/gVSS) by comparison of a range of fermentation pH from 4 to 11 (Zhao et al., 2010). However, only batch experiments were carried out and therefore the long-term hydrogen production performance could not be revealed, since it would be possible that spore-forming homoacetogens could be revived in subsequent fermentation. Our previous study (Luo et al., 2011) found that the effects of heat pretreatment on the inhibition of homoacetogenesis disappeared after long-term cultivation due to the reactivation of spore-forming homoacetogens. Therefore, it is necessary to investigate the presence of homoacetogenesis and the performance of hydrogen production during the fermentation of WAS at 37 °C and constant pH 10 without heat pretreatment, and also the long-term performances of hydrogen production. Futhermore, temperature was shown to be a key factor affecting the hydrogen yields of various organic wastes by fermentation (Luo et al., 2010b; Wong et al., 2014; Zheng et al., 2014). The hydrogen yield of WAS was found to be higher at 55 °C (19.9 mL H_2/gVSS) compared to 37 °C (14.2 mL H_2/gVSS) with the fermentation pH around 6.5 (Zheng et al., 2014), which was still lower than that (26.9 mL H_2/gVSS) obtained at 37 °C and pH 10 (Zhao et al., 2010). Until now, alkaline fermentation at pH 10 for hydrogen production was only studied under mesophilic condition (Cai et al., 2004; Wang et al., 2015; Zhao et al., 2010), and the hydrogen yield of WAS by thermophilic alkaline fermentation remained to be investigated. In fact, fermentation of WAS at pH 10 has been reported to be beneficial for VFAs production in previous studies, which was mainly due to the enhancement of the solubilisation of WAS and the inhibition of methanogens (Chen et al., 2007; Su et al., 2013). Fermentation of WAS at pH 10 to produce VFAs has been carried out in pilot scale (Li et al., 2011). The present study mainly focused on the H_2 production from WAS at pH 10, which would provide new insights to the alkaline fermentation of WAS.

Based on the above considerations, the hydrogen yields of WAS at pH 10 under both mesophilic and thermophilic conditions were compared, and the long-term hydrogen production performance and stable microbial community composition were also analyzed. Anaerobic fermentation at 55 °C and pH 10 was found to be an efficient method for hydrogen production from WAS, and the mechanism for the high hydrogen yield (80.1 mL H_2/gVSS) was also elucidated, providing new understanding towards the inhibition of homoacetogenesis.

2. Materials and methods

2.1. Waste activated sludge

WAS was collected from a secondary sedimentation tank in a municipal wastewater treatment plant in Shanghai, China. The main characteristics of WAS were as follows: total suspend solids (TSS) 10.6 ± 1.1 g/L, volatile suspend solids (VSS) 6.9 ± 1.3 g/L, pH 6.9 ± 0.1, total carbohydrate 1261 ± 51 mg/L, total protein 3784 ± 37 mg/L, total chemical oxygen demand (TCOD) 9800 ± 150 mg/L, and soluble chemical oxygen demand (SCOD) 120 ± 21 mg/L.

2.2. Fermentative hydrogen production from WAS at pH 10 under different temperatures

Four 320 mL serum bottles were used, and 100 mL WAS was added to each bottle. The pH of WAS in the bottles was adjusted to 10.0 by adding 4 M sodium hydroxide (NaOH). All the bottles were then flushed with pure nitrogen to maintain anaerobic condition, capped with rubber stoppers, and then sealed. The bottles were divided into two groups (two bottles in each group as duplicate), and placed in the shakers at either 37 °C or 55 °C. The pH in all the bottles was controlled at 10.0 by adding 4 M NaOH with an automatic titrator in the whole fermentation process. The gas composition in the headspace of the bottles was measured periodically, and the volatile fatty acids (VFAs) were analyzed at the end of the fermentation (the fermentation was lasted for 8 days).

The hydrogen production from WAS at 55 °C at different alkaline pH (8, 9 and 10) was also compared, and the experimental procedure was the same as described above. pH above 10 was not investigated since it had serious toxic effect on the microorganisms as demonstrated before (Zhao et al., 2010).

2.3. Acidification during mesophilic and thermophilic fermentation at pH 10

Hydrogen is mainly produced in the acidification step during the fermentation of WAS (Luo et al., 2011). In order to understand the effects of mesophilic and thermophilic alkaline fermentation on acidification, the hydrolysate of WAS and monomers including glucose (representative monomer of carbohydrate) and glutamate (representative monomer of protein based on Table S1) were used for fermentation. The hydrolysate was obtained by heat-pretreatment (120 °C, 1 h) of WAS, and the characteristics of the hydrolysate were as follows: soluble chemical oxygen demand (SCOD) 2387 ± 70 mg/L, total carbohydrate 295 ± 21 mg/L, total protein 910 ± 25 mg/L 20 mL WAS (as inoculum) and 80 mL hydrolysate were added to each bottle for fermentative hydrogen production. For monomers, 100 mL mixtures containing 20 mL WAS (as inoculum) and 500 mg/L of either glucose or glutamate solutions were added to each bottle. The pH of each bottle was adjusted to 10.0. The bottles were then flushed with nitrogen, capped with rubber stoppers, sealed, and placed in shakers at 55 °C or 37 °C. pH was controlled at 10.0 during the fermentation process. Gas composition and the concentration of VFAs in each bottle were measured.

2.4. Hydrogen consumption tests

The fermentation experiment was repeated as described in chapter 2.2 with the following modifications in order to determine whether the sludge in the batch experiments could consume the produced hydrogen. Hydrogen production ceased after 8 days' fermentation, and then the sludge in the duplicate bottles at 37 °C and pH 10 was mixed together and then distributed equally into four bottles. Two of the bottles were flushed with a gas mixture (25% hydrogen, 15% carbon dioxide, 60% nitrogen) to test the possible hydrogen consumption by the sludge, and the left two bottles were used as controls and were flushed with pure nitrogen. The pH of the four bottles was controlled at 10. All the bottles were incubated in the shaker at 37 °C. The same procedure was also adopted to the sludge at 55 °C and pH 10, except that the bottles were incubated in the shaker at 55 °C. During the fermentation, gas composition was measured periodically, and VFAs were analyzed at the end of the experiment. The sludge from the continuous reactor as described in the following part was also used for the hydrogen consumption tests.

2.5. Reverse transcription and quantitative PCR of the key genes involved in the hydrogen production

The hydrogenanse gene hydA has been identified as a gene encoding hydrogenase to catalyze the reduction of protons as terminal electron acceptors to produce hydrogen (Xu et al., 2009), and