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Short Communication

Thermophilic hydrogen production from sludge pretreated by thermophilic bacteria: Analysis of the advantages of microbial community and metabolism

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HIGHLIGHTS

- Pretreated sludge had lower metabolism inhibition and higher buffering capability.
- Better DOM utilization and higher H_2 yield were achieved at thermophilic condition.
- Bacteria community was quantitatively characterized by high-throughput sequencing.
- Thermophilic hydrogen-producing bacteria community had extremely high specificity.

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ABSTRACT

In this study, the effects of thermophilic bacteria pretreatment and elevated fermentation temperature on hydrogen production from sludge were examined. The highest hydrogen yield of 19.9 ml H_2 g^{-1} VSS was achieved at 55 °C by using pretreated sludge, which was 48.6% higher than raw sludge without pretreatment, and 28.39% higher than when fermented at 35 °C. To explore the internal factors of this superior hydrogen production performance, the microbial community and the metabolism analysis were performed by using high-throughput sequencing and excitation–emission matrix. The pretreated sludge showed better utilization of dissolved organic matter and less inhibition of metabolism, especially at thermophilic condition. The 454 sequencing data indicated that microbial abundance was distinctly reduced and extremely high proportion of hydrogen-producing bacteria was found in the thermophilic community (*Thermoanaerobacterium* accounted for 93.75%). Thus, the pretreated sludge and thermophilic condition showed significant advantages in the hydrogen production using waste sludge as substrate.

1. Introduction

Developing new sustainable energy sources for substituting fossil fuels is key to averting and reducing an impending energy and environmental crisis. In particular, hydrogen has a high heating value and provides a good source for clean energy conversion. Bio-hydrogen production from renewable resources or organic waste has especially become an environmentally friendly and promising alternative research direction for clean energy conversion (Hallenbeck and Ghosh, 2009; Lee et al., 2011). Using sewage sludge as a substrate for hydrogen production through dark fermentation can also be especially effectively at solving severe pollution and mitigating potential environmental risks (Astals et al., 2013).

The optimization of sludge pretreatment and fermentation conditions are the two valuable and critical research aspects. Though rich polysaccharide and protein content are contained in sludge, most of these organic matter are enclosed inside the microbial cell membranes and extracellular polymeric substances (EPS), which can lead to a relatively low hydrogen yield and long retention time (Coelho et al., 2011). Thus, various pretreatment methods which can accelerate the hydrolysis rate were studied to release sufficient cellular organic materials into liquid to make them more accessible to hydrogen-producing bacteria (Yang et al., 2012). Sludge solubilization by thermophilic bacteria is an economical and promising pretreatment method having the advantages of efficient

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inactivation of hydrogen consumption methanogens, rapid removal of volatile solids, and simple control requirements and better prospect of application (Guo et al., 2012). The method is to add thermophilic bacteria into sludge at thermophilic conditions ranging from 40 °C to 80 °C to hydrolyze the complex structure of sludge through lysis enzyme secreted from thermophilic bacteria (Guo et al., 2012). The purpose of this procedure was conducted to enhance the biodegradability of sludge.

The previous study has revealed that hydrogen production at high temperature was more efficient and practical than at mesophilic conditions, especially when it was applied to substrates with complex structures, although it is not easily utilized under normal temperature (Kargi et al., 2012). Since complexly structured waste sludge is largely produced in China, taking advantage of the large volumes of waste sludge produced there meets both environmental and reclamation needs. A considerable amount of excess heat was contained in the pretreated products after thermophilic bacteria pretreatment. The primary objective of this study was to investigate whether thermophilic hydrogen production had a higher yield compared to hydrogen production at mesophilic conditions. Thus, better utilization of excess heat also could be achieved. So far, most studies concentrated on hydrogen production from sludge have already been conducted. At most, there has only been preliminary research done on fermentation pathways and microbial community (Nitipan et al., 2014). The advantages of hydrogen production have not been addressed from the metabolic perspective, especially the quantitative analysis of microbial communities. Therefore, another main objective was to analyze the internal mechanisms of the promotion effects induced by pretreatment and fermentation control on the metabolic performance with the microbial community.

In this paper, the performance of hydrogen production using thermophilic bacteria pretreated sludge under thermophilic and mesophilic conditions was first compared. Aside from measuring the composition of the volatile fatty acid (VFA), the dissolved organic matter (DOM) species and content of the fermentation liquid was also examined using excitation–emission matrix (EEM) for microbial metabolism analysis. Above all, the detection of nicotinamide adenine dinucleotide (NADH) would deeply explain the enhanced effect of hydrogen production under optimal conditions from the perspective of internal metabolic regulation pathways during fermentation. High-throughput sequencing technology was also applied to quantitatively characterize the bacterial community responsible for enhanced hydrogen production performance.

2. Methods

2.1. Sludge pretreatment and hydrogen-producing inocula enrichment

The characteristics of the sludge used as substrate for hydrogen production were shown in Table S1. The sludge was first pretreated by thermophilic bacteria to increase the DOM content suitable for hydrogen production. The information on the thermophilic bacteria *Geobacillus stearothermophilus* TP-12 that was used for pretreatment in this study and the sludge pretreatment steps are shown in Supplementary Information (SI).

The mesophilic and thermophilic hydrogen-producing mixed seed cultures were enriched from the sludge which was stored at strictly anaerobic conditions for 2 months. A combination of heat-shock and repeated batch cultivation was then used to enrich hydrogen-producing bacteria. The detailed steps in this operation were shown in SI.

2.2. Fermentation procedure

All the sludge which was used as substrate was first heated at 90 °C for ten minutes in a water bath to inhibit the activity of hydrogen-consuming anaerobes. The bio-hydrogen production experiments were conducted in a series of 100-ml serum bottles. Each serum bottle had a mixture of 50 ml substrate and 5 ml seed sludge, continuously sparged with N_2 for five minutes to drive air out. After, the batch reactors were placed on a magnetic stirring apparatus and stirred at 140 rpm at 55 ± 1 °C for thermophilic fermentation and at 35 ± 1 °C for mesophilic fermentation. The biogas contents and volumes were detected at intervals of 4 h. All the tests were performed in triplicate to determine the reproducibility of the experiments.

2.3. Analytical methods

The analytical methods of all the parameters are given in SI, which also includes the steps describing high-throughput sequencing and EEM fluorescence spectroscopy.

3. Results and discussion

3.1. Comparison studies of biohydrogen production under thermophilic and mesophilic conditions

Hydrogen and carbon dioxide were the main biogas components. No methane was detected in all batch tests. As shown in Fig. 1a, the maximum hydrogen yield from pretreated sludge was 19.9 ml H_2 g^{-1} VSS, 48.6% higher than that from raw sludge. The logarithmic phase of fermentation from raw sludge appeared after 30 h. In contrast, the lag time of pretreated sludge was too short to be recorded, indicating that hydrogen-producing bacteria had fast adapted to this micro-environment and that the resulting metabolism was accelerated. The higher hydrogen yield from pretreated tests also demonstrated that the released soluble substrate concentration of raw sludge was increased by thermophilic bacteria pretreatment, and hydrogen production capability was subsequently enhanced due to an increase in available utilizable substances. The hydrogen yield from pretreated sludge at 55 °C was 28.39%

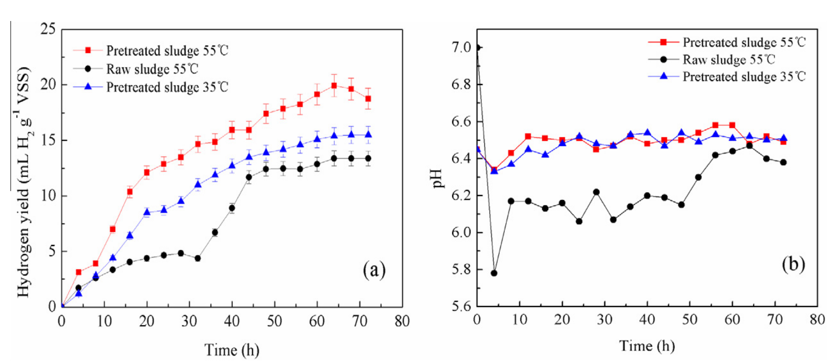

Fig. 1. Hydrogen yield (a) and pH change (b) at thermophilic and mesophilic condition.