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Enzymatic characterization of acid tolerance response (ATR) during the enhanced biohydrogen production process from Taihu cyanobacteria via anaerobic digestion

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ABSTRACT

Enhancement of biohydrogen production via anaerobic digestion from Taihu cyanobacteria (blue algae) after acid stress on anaerobic sludge, and the enzymatic characterization of the acid tolerance response (ATR) during the enhanced biohydrogen production process were investigated in this study. Comparing to those of the control, biohydrogen accumulation and hydrogen content increased by 1.9 and 1.7 times, when 12.5 and 7.5 g/L of acid stress on anaerobic sludge were performed respectively. Other than that, activities of hydrolytic enzymes, such as β-glucosidase, BAA-proteolytic enzyme and phosphatase were all improved during the enhanced biohydrogen process after appropriate acid stress. Significantly, activity of glutamate decarboxylase (GAD), the main microbial ATR stimulated by excessive acids, was increased consistently with the biohydrogen accumulation. Therefore, acid stress might be a practical approach to improving the biochemical traits of the anaerobic sludge. In turn, improved hydrolysis of organic substances would help the anaerobic sludge better survive excessive organic acids, and then enhance biohydrogen production from Taihu cyanobacteria.

1. Introduction

As a result of the conflict between fast economic development and regional environment pollution, the annual blooming of cyanobacteria (commonly referred to as blue algae) of Taihu Lake located in Yangtze River Delta, China, always poses a major threat to water supply of the lakeside Wuxi city, and finally led to the water crisis during the summer of 2007 [1]. To withdraw as more pollutants as possible from the seriously eutrophicated Taihu Lake, refloatation of cyanobacteria after their blooming was considered as one of the most efficient methods. Meanwhile, as it was now considered as a competitive option to make bioenergy including both hydrogen and methane, and other valuable chemicals including organic acids, solvents and even biopolymers from organic solid wastes [2], the authors proposed a coupled production of hydrogen and PHA from cyanobacteria via anaerobic digestion (Fig. 1), in order to better the potential of reclaiming Taihu cyanobacteria refloated annually [3]. On the other hand, as the peptidoglycan layers of cyanobacteria were

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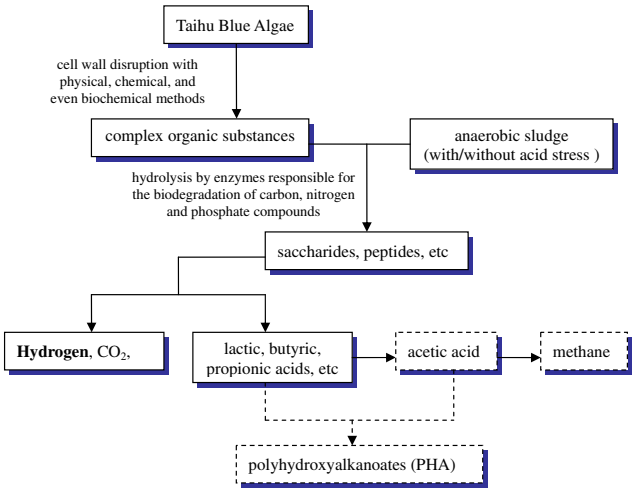

Fig. 1 – Schematic diagram of the reclaimation of Taihu cyanobacteria. ——— biohydrogen pathway described in this study - - - - - other reclaimation pathways of cyanobacteria.

about tens of times thicker than those of other gram negative bacteria [4], disruption of the algal cell wall, release of intracellular contents, together with the subsequent anaerobic hydrolysis would be crucial for the anaerobic digestion of the Taihu cyanobacteria [5]. Hence, hydrolytic enzymes responsible for the decomposition of organic contents facilitated by hydrolytic microbes should be enhanced uppermost taking into account any viable option to promote the biohydrogen efficiency.

With comparison to an independent methane process, it would be more efficient to conduct the sequential hydrogen and methane process, as the biohydrogen production process first may provide more organic acids for the successive biomethanation, and then improve the exploitation efficiency of organic wastes [6]. However, if not being removed or utilized instantly, accumulated organic acids during the acidogenic stage would in turn inhibit the biohydrogen production, and even lead to the failure of any anaerobic treatment process [7]. On the other hand, inducible acid tolerance response (ATR) could be observed when microorganisms grow under acid stress, in order to maintain an appropriate cytoplasmic pH environment [8]. As composed of numerous types of microbes, it was obviously that anaerobic sludge would also develop similar ATR mechanisms to survive the surplus organic acids during the anaerobic biohydrogen process. Considering the importance of various microbial communities in the performance of any anaerobic digestion [9], it seemed to be substantial to enhance the biohydrogen efficiency through improving the acid tolerance capacity of the anaerobic sludge. However, few publications could be tracked on this field till now.

In this study, enhancement of biohydrogen production from Taihu cyanobacteria via acid stress on anaerobic sludge was investigated. Moreover, hydrolytic behavior of enzymes associated with the degradation of organic carbon, nitrogen and phosphate, and the ATR of glutamate decarboxylase (GAD) were analyzed in order to reveal the mechanism for the enhancement of biohydrogen after acid stress on anaerobic sludge.

2. Material and methods

2.1. Experimental apparatus and operating procedures

Alkaline pretreatments of Taihu cyanobacteria, source of anaerobic granular sludge and the size of anaerobic digester used in this study were the same as previously described in Ref. [3]. Firstly, the digester was flushed with nitrogen to maintain an anaerobic environment, then the pretreated cyanobacteria was inoculated with anaerobic sludge with/without acid stress to conduct the biohydrogen production process.

2.2. Acid stress of anaerobic sludge

Acid stress was conducted in a 1 L tightly sealed reaction bottle for 48 h with appropriate shaking. The stress medium contains (g/L): anaerobic sludge 200, glucose 20, $(NH_4)_2SO_4$ 5, 10 mL mineral solution (g/L in 1 mol/L HCl): $FeSO_4 \cdot 7H_2O$ 10, $ZnSO_4 \cdot 7H_2O$ 2.25, $CuSO_4 \cdot 5H_2O$ 2.25, $MnSO_4 \cdot 5H_2O$ 0.5, $CaCl_2 \cdot 2H_2O$ 2, H_3BO_4 0.3, $(NH_4)_6Mo_7O_{24}$ 0.1), and acetate at 0, 5, 7.5, 10, 12.5 or 15 g/L, respectively. The pH was adjusted to 5.5 to maintain a protonated environment. After acid stress, the sludge was washed twice with tap water to remove residual acids, and was then kept for biohydrogen production.

Prior to the assay of GAD activity, the anaerobic sludge collected after acid stress was re-cultured with 5 g/L of glutamate, acetate and glucose for 8 h respectively, to distinguish the inducibility by a specific substrate. Afterwards, the procedure was the same as Section 2.3.2.

2.3. Analytical procedures

2.3.1. TS, VSS, hydrogen and organic acids

TS and VSS were determined with the gravimetric method according to the standard protocol of State Environmental Protection Administration of China (SEPA) [10]. Hydrogen and organic acids were detected using GC and HPLC, respectively [3].

2.3.2. Sample preparation for the enzymatic activity assay

The anaerobic sludge was collected after centrifugation at 8000 rpm for 5 min, then was grinded with normal saline solution (9 g NaCl in 1 L water) at 30 Hz for 5 min (Tissuelyzer II, Qiagen, USA). After being kept overnight at 4 °C, the sludge was centrifuged again at 10,000 rpm for 10 min, and the supernatant was then collected for the assay of enzyme activities.

2.3.3. GAD, β-glucosidase, BAA-proteolytic enzyme and phosphatase

GAD was detected as the amounts of γ-Aminobutyric acid (GABA) obtained from glutamate (Sigma, USA) using HPLC (Ultimate 3000, Dionex, USA), equipped with a Dionex Acclaim C18 column (250 × 4.6, USA) and a UV detector at 254 nm after derivatization. Comprised of MeOH and H_2O (62:38), the mobile phase is kept at a flow rate of 1.5 mL/min. The injection volume was 10 μL. For derivatization, 2 mL of the sample was mixed with 2 mL of a buffer (0.2 M $NaHCO_3$) and 2 mL of a derivatization