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Pretreatment of macroalgal *Laminaria japonica* by combined microwave-acid method for biohydrogen production

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ABSTRACT

Suitable pretreatment can effectively enhance the fermentative hydrogen production from algae biomass. In this study, combined microwave-acid pretreatment was applied to disintegrate the biomass of macroalgae *L. japonica*, and dark fermentation in batch mode was conducted for hydrogen production. The results showed that combining microwave pretreatment at 140 °C and 2450 MHz with 1% H_2SO_4 for 15 min could effectively disrupt macroalgal cells and release the organic matters, and soluble chemical oxygen demand (SCOD) concentration increased by 1.92-fold and achieved 5.12 g/L. During the fermentation process, both polysaccharides and proteins were consumed. Hydrogen production process was dominated by acetate-type fermentation, and the dominance of genus *Clostridium* contributed to more efficient hydrogen production. After the pretreatment, hydrogen yield increased from 15 mL/g TS_{added} to 28 mL/g TS_{added}, and energy conversion efficiency increased from 9.5% to 23.8%. Combined microwave-acid pretreatment is potential in enhancing hydrogen production from the biomass of *L. japonica*.

1. Introduction

Energy crisis and the demand for carbon emission reduction makes it urgent to explore low-carbon renewable energy. Hydrogen is an ideal candidate for it is the only fuel with no carbon, owns high energy content, totally clean with water as sole product, easy transportation and utilization with the ready-made facilities for natural gas, especially with the development of hydrogen fuel cell, hydrogen can be easily converted to electricity efficiently. Traditional hydrogen production process still relies on fossil fuel, in which extreme conditions are usually required, leading to the environment deterioration. Hydrogen production through biological ways owns more environmental benefits with mild reaction conditions and potential for wastes management. Dark fermentation possesses more prospective in commercial application for its high hydrogen production rate, easy control. Moreover, it can use a wide range of organic wastes as feedstock for hydrogen production and resource recovery (Rajhi et al., 2016; Dennehy et al., 2017; Ding et al., 2017; Wang and Yin, 2017), which simultaneously achieves waste treatment and renewable energy generation.

As dark fermentative hydrogen production is a complex microbial metabolism process, high hydrogen production efficiency and stability are required to accomplish a practical application of dark fermentative hydrogen production. Among the various influencing factors, substrate plays an important role: source of substrate determines the cost, structure of substrate defines the pretreatment required, and composition of substrate affects the fermentation type thus hydrogen production efficiency (Wang and Yin, 2018a,b). Substrate used for hydrogen production include the first-generation land-based crops, which may cause the competition for food resources; the second-generation non-edible lignocellulosic biomass, which has been widely studied as substrate for its wide distribution and large amount, but the high requirement for pretreatment and low degradation of lignin restricted its application; and the third-generation algae-based feedstock (Doshi et al., 2016). Especially for the marine algae, they can grow in a variety of marine environments, without requiring fresh water or cultivable land; they are lack of hemicellulose or lignin, thus the depolymerization can be easier; furthermore, they are high efficient in fixing carbon dioxide to biomass, shows great potential for carbon dioxide remediation (Wei et al., 2013). Marine algae has been widely used in recovering value-added products like hydrocarbons, syngas, and polyhalomethanes (Doshi et al., 2016). Up to now, various marine algae species have been studied for biohydrogen production, like *Codium fragile*, *Gelidium amansii*, *Fucus vesiculosus*, *Laminaria japonica*, and *Gracilaria verrucosa* etc. (Jung et al., 2011b; Barbot et al., 2015). *L. japonica* was proved to be more preferable with high carbohydrate content, readily soluble and easily hydrolyzable sugars. Furthermore, it is presenting as a kind of

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waste in some European countries. Thus, *L. japonica* is considered as an ideal feedstock for hydrogen production.

However, intact macro-algae cell membranes and walls restrict the disintegration and prolong the hydrogen production process. Thus, pretreatment is a fundamental and essential step for a high efficient hydrogen production (Wang and Yin, 2018). Widely studied treatment methods include heat-shock, ultrasonication, acid/base and electric current. Park et al. (2009) explored heat-shock at different conditions in enhancing hydrogen production from *L. japonica*, results showed that highest hydrogen production was obtained at 65 °C for 20 min treatment. Liu and Wang (2014) compared hydrogen yield from heat-shock, ultrasonication, acid and base treated *L. japonica*, found that heat-shock treatment at 121 °C for 20 min group achieved highest hydrogen yield, followed by acid treatment with 1 mol/L HCl group. Jung et al. (2011a) combined heat-shock and acid in disintegrating *L. japonica*, obtained hydrogen yield was 1.5 times higher than sole heat-shock treated group. Xia et al. (2016) also obtained significant increase in hydrogen yield with combined heat-shock and acid pretreatment. It can be seen that heat-shock has been proved to be efficient in disrupting *L. japonica*, and enhanced effect can be obtained by combined heat-shock and acid treatment. Besides heat pretreatment, microwave pretreatment comprises both thermal and non-thermal effect, which has been used in disrupting microbial cells in waste activated sludge for hydrogen production, and the results demonstrated that microwave pretreatment was more efficient in solubilizing waste activated sludge and enhancing hydrogen production than heat-shock (Kuglarz et al., 2013). Combined microwave-acid treatment could achieve the synergistic effect effective in sludge disintegration (Liu et al., 2016). However, there is little information about the effect of microwave treatment on macroalgae disintegration, neither the effect of microwave on biohydrogen production from *L. japonica*.

In this study, microwave was applied to pretreat *L. japonica*, and different dosages of H_2SO_4 were added to explore the effect of combined microwave-acid pretreatment on the saccharification of *L. japonica*. Hydrogen production from differently pretreated *L. japonica* was conducted. Hydrogen production, metabolites formation, and energy conversion during the fermentation process were comprehensively examined. Furthermore, microbial communities in different test groups were analyzed, revealing the mechanism of pretreatment in enhancing the hydrogen yield from the perspective of microbiology.

2. Materials and methods

2.1. Inoculum and algal biomass pretreatment

Anaerobic sludge collected from a mesophilic anaerobic digester in a local sewage treatment plant was used as inoculum. Prior to inoculation, anaerobic sludge was boiled at 100 °C for 15 min to inhibit the hydrogen consumers (Wang and Wan, 2008), and then pre-cultured to enrich the hydrogen producers. During the pre-culture process, 150 mL flask was filled with 10 mL boiled anaerobic sludge, 80 mL deionized water, and 10 mL nutrient solution. 5 g glucose, 1 g peptone and 0.05 g yeast extract was added to supply the organic nutrients for microbial growth. Composition of nutrient solution was the same as described in reference (Yin et al., 2018). After that, the mixture was adjusted to pH 7.0, flushed with N_2 for 3 min, and then cultivated at 36 °C for 24 h. Then, the mixtures after the cultivations were centrifuged at 5000 rpm for 5 min to separate the microbial biomass from the medium, and the obtained microbes were washed by 0.9% NaCl solution for 3 times before being used for biohydrogen production.

L. japonica was washed by fresh water for 5 times, dried at 80 °C for 24 h, then comminuted. The macroalgal particles were sieved by an 18-mesh screen to obtain the powders with diameter less than 0.88 mm. Combined microwave-acid pretreatment was applied to enhance the saccharification of *L. japonica* and hydrogen production. During the pretreatment process, 12.5 g-TS /L of *L. japonica* was used in each sample. Microwave oven (APL-MD6H, Chengdu, China) with maximum power of 1000 W was used for the microwave treatment. Microwave frequency of 2450 MHz was adopted, and temperature was controlled at 140 for 15 min. Different amounts of 0%, 0.5%, 1% and 2% (v/v) H_2SO_4 were added, making the concentration of H_2SO_4 to be 0, 93.4, 186.7 and 373.5 mmol/L, respectively. The added acid worked together with microwave pretreatment to enhance the algae saccharification and hydrogen production.

2.2. Experimental set-up for biohydrogen production

Batch tests were designed to evaluate the influence of pretreatment on biohydrogen production from *L. japonica*. Dark fermentation was conducted in 150 mL Erlenmeyer flasks with 100 mL working volume. For the control group, 1 g comminuted and sifted *L. japonica* was used as substrate. For the experimental groups, 80 mL pretreated *L. japonica* mixture (equivalent to 1 g TS added during pretreatment process) was used as substrate. Then, 10 mL inoculum and 10 mL nutrient solution were added in each flask to make the working volume of 100 mL. Composition of nutrient solution was the same as given in (Yin et al., 2014). The initial pH of all the reactors was adjusted to 7.0 with 5 mol/L NaOH and 5 mol/L HCl. Before the fermentation process, N_2 gas was passed through the samples for 3 min to establish an anaerobic environment. Thereafter, all flasks were put in a reciprocal shaker with an agitation speed of 120 rpm at 36 °C. All batches were conducted in duplicate.

2.3. DNA extraction and PCR amplification

For the microbial community analysis, inoculum after heat treatment, sediment of all the test groups after the termination of fermentation were collected and kept at -80 °C until DNA extraction. E.Z.N.A. soil DNA extraction kit (Omega Bio-tek, Norcross, GA, U.S.) was used for the DNA extraction. PCR amplication was performed from the obtained DNA. Two primers: 515F (5′-barcode- GTGCCAGCMGCCG CGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′), and V4-V5 region of 16S rRNA gene was used for PCR amplification. For the PCR reactions, 10 ng of template DNA, 0.8 μL of each primer (5 μM), 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, and 0.4 μL of FastPfu Polymerase were involved in a total volume of 20 uL. The conditions used for PCR reactions were 95 °C for 2 min, 95 °C for 30 s (repeat 25 cycles), 55 °C for 30 s, 72 °C for 30 s and then a final extension at 72 °C for 5 min. PCR products were purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.), and detected by electrophoresis in a 2% agarose gel. A further quantification of PCR products was conducted using QuantiFluor™ -ST (Promega, U.S.) according to the results obtained from electrophoresis.

2.4. MiSeq sequencing and data analysis

Amplicon sequencing was performed by Shanghai Majorbio Biopharm Biotechnology (Shanghai, China) using Illumina MiSeq PE250. QIIME (version 1.17) was used to demultiplex and quality-filter the raw fastq files. Sequences were clustered according to their similarity, and operational taxonomic units (OTUs) were defined based on the similarity threshold of 97% using UPARSE (version 7.1). UCHIME was used to identify and remove the chimeric sequences.

2.5. Analytical methods

For the characteristics of *L. japonica*, VS, TS, total oxygen demand (TCOD), soluble chemical oxygen demand (SCOD) and volatile suspended solids (VSS) were measured according to the standard methods. The contents of hydrogen, carbon, nitrogen and oxygen were determined by an elemental analyzer (Exeter Analytical CE 440, UK). Protein content was calculated by the nitrogen content multiplied by a