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Optimization study on the hydrogen peroxide pretreatment and production of bioethanol from seaweed *Ulva prolifera* biomass

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HIGHLIGHTS

- *Ulva prolifera* residue was used as a feedstock for bioethanol production.
- H_2O_2 pretreatment is the first to use in marine biomass to obtain fermentable sugar.
- Reducing sugar yield was 0.42 g/g UPR higher than earlier report.

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ABSTRACT

The seaweed *Ulva prolifera*, distributed in inter-tidal zones worldwide, contains a large percentage of cellulosic materials. The technical feasibility of using *U. prolifera* residue (UPR) obtained after extraction of polysaccharides as a renewable energy resource was investigated. An environment-friendly and economical pretreatment process was conducted using hydrogen peroxide. The hydrogen peroxide pretreatment improved the efficiency of enzymatic hydrolysis. The resulting yield of reducing sugar reached a maximum of 0.42 g/g UPR under the optimal pretreatment condition (hydrogen peroxide 0.2%, 50 °C, pH 4.0, 12 h). The rate of conversion of reducing sugar in the concentrated hydrolysates to bioethanol reached 31.4% by *Saccharomyces cerevisiae* fermentation, which corresponds to 61.7% of the theoretical maximum yield. Compared with other reported traditional processes on *Ulva* biomass, the reducing sugar and bioethanol yield are substantially higher. Thus, hydrogen peroxide pretreatment is an effective enhancement of the process of bioethanol production from the seaweed *U. prolifera*.

1. Introduction

The increase in concern over issues of national security and environmental health has led to an overwhelming interest among researchers in developing economically viable processes for the production of alternative transportation fuels (Kumar et al., 2013). Bioethanol production from fermentable sugars has become an area of intense research (Kim et al., 2015). Among different feedstocks, seaweed is considered to be a preferable biomass because of its expansive area of productivity, lack of competition with conventional agriculture for land, recycling of carbon dioxide, and compatibility with processes for the integrated production of fuels (Trivedi et al., 2013). *Ulva prolifera*, a kind of thin flat green alga, has proliferated in coastal areas along the shore of Yellow Sea of China since 2007 (Zhuang et al., 2012). In 2015 alone, 10 million tons of algae bloomed in the Yellow Sea area of the Western Pacific Ocean. *U. prolifera* biomass is mainly used for the production of water-soluble polysaccharides (WSP), which show many bioactivities such as anticoagulant activity, antioxidant activity, antitumor activity, and immuno-modulatory activity (Li et al., 2013). The *U. prolifera* residue (UPR), as by-product of the WSP extraction process (Li et al., 2013), has caused severe environmental pollution due to its limited recycling capacity. From the aspect of bioethanol conversion, terrestrial biomass is hard to depolymerize because of the presence of complex lignin and hemicellulose over cellulose (Trivedi et al., 2013). Researches show *U. prolifera* is valuable as a bioethanol resource due to its high content of carbohydrate, fiber, and low lignin (Wal et al., 2013; Trivedi et al., 2013). Thus, UPR might be considered as a potential bioethanol biomass.

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However, obtaining adequate quantities of fermentable sugars might be the main challenge for this substitutable marine derived biomass. In general, lots of pretreatment methods including dilute acid, organic solvent, liquid hot water, and lime pretreatment have been developed for improving the efficiency of the hydrolysis of seaweed biomass (Kim et al., 2015, 2011; Jeong et al., 2015; Hyeon et al., 2011). Even though the above-mentioned pretreatments are effective and have facilitated high rates of conversion of cellulose from seaweed biomass, those pretreatment methods were usually performed under extreme conditions: high temperature (121–200 °C), high pressure (15 MPa), and high concentration of chemical solvents (1% H_2SO_4 or 4% liquid ammonia). Undoubtedly, those pretreatment methods also increased energy consumption, production cost, and environmental pollution (Ge et al., 2011). Moreover, especially under severe conditions, furan compounds (5-hydroxymethyl furfural, furfural), organic acids (levulinic acid, formic acid), and phenolic compounds, known inhibitors of cell growth and bioconversion, are generated as by-products (Jeong et al., 2015). Recently, hydrogen peroxide has been shown to be a good choice for the pretreatment of terrestrial plants, as it leads to high glucose yields and can be carried out in conditions of moderate temperature and pressure, without acids which leads to the formation of minor inhibitors (Rabelo et al., 2014). Finally, it is a typical environment-friendly agent that leaves no residues during the whole conversion process (Mou et al., 2013). However, no related study has been carried out to explore the pretreatment of hydrogen peroxide on seaweed biomass for bioethanol production.

This study was conducted to verify the effect and feasibility of the hydrogen peroxide pretreatment technique as a seaweed biomass pretreatment method for bioethanol production. The effects of hydrogen peroxide catalysis on delignification and reducing sugar yield from UPR were optimized. The efficacy of pretreatments was also evaluated through the conversion to bioethanol from reducing sugars achieved after the enzymatic hydrolysis of the pretreated solids.

2. Materials and methods

2.1. Feedstock and bacteria

The *U. prolifera* collected from the coasts of Qingdao, China was oven dried and powdered to 0.05–0.1 mm mesh size. It was homogenized to avoid compositional differences. The WSP was extracted by the hot water method. The milled seaweed (80 g) was dipped into 60 volumes of tap water, homogenized and refluxed at 100 °C for 1 h. The UPR was collected by centrifugation (4800*g*, 15 min) and dried at 60 °C for further analysis.

Saccharomyces cerevisiae (YSC2, type II) purchased from Angel Co., Ltd. (Yichang, China) was used for ethanol conversion. For the preparation of *S. cerevisiae* with inoculums, a loopful of cells was added to the culture medium in which the concentrations of nutrients in g/L were: glucose, 20; yeast extract, 0.5; urea, 1; Na_2HPO_4, 0.5; KH_2PO_4, 2.5; $MgSO_4$, 1; $(NH_4)_2SO_4$, 1; $FeSO_4$, 0.001. The flasks were incubated in a rotary shaker at 30 °C and 150 rpm for 24 h.

2.2. Chemical analysis of samples

The contents of cellulose, hemicelluloses, and acid insoluble lignin were determined by the method as described by Ge et al. (2011). Approximately 300 mg of sample was initially subjected to a primary 72% sulfuric acid hydrolysis at 30 °C for 60 min. The reaction mixture was diluted to 4% sulfuric acid and autoclaved at 121 °C for 1 h. The content of monosaccharide was quantified with gas chromatography (GC). The content of protein, lipid, and ash was determination by the methods of Lowry et al. (1951), Bligh and Dyer (1959), and Trivedi et al. (2013), respectively. The yields of glucose and bioethanol were analyzed by the SBA-40C biology analyzer (Biology Institute of Shandong Academy of Sciences, China). Reducing sugars were determined by the DNS (3,5-dinitrosalicylic acid) method with glucose as the standard (Fenice et al., 1997).

2.3. Scanning electron microscopy analysis

The morphology and the physical structure of UPR, and UPR pretreated with hydrogen peroxide were observed by using scanning electron microscopy (Field Emission Scanning Electron Microscope; S-3400N, Tokyo, Japan). Samples were dried at 60 °C to constant weight and coated with Au/Pd film. All images were taken at a magnification of 3000× and observed at a voltage of 10 kV.

2.4. Hydrogen peroxide pretreatment and enzymatic hydrolysis

The hydrogen peroxide pretreatment was conducted in a jacketed reaction vessel. It was optimized for different hydrogen peroxide dosages (0.1%, 0.2%, 0.5%, 1%, 2%), pH (4, 6, 8, 10), pretreatment duration (6, 12, 24 h), and temperatures (50, 70, and 90 °C), respectively.

Enzymatic hydrolysis of the pretreated UPR was performed in 40 mM citrate buffer with commercial cellulase (Jienuo Enzyme Co., China. 45 FPU/g) and cellobiase (Sigma Chemical Co., USA 250 U/mL). Cellulase and cellobiase were added at a loading corresponding to 7.5 FPU and 4.5 U/g pretreated UPR. The optimum conditions for enzymatic hydrolysis were pH 4.8, 50 °C, and 48 h. The same process using unpretreated UPR was performed as a control.

Samples were withdrawn after enzymatic hydrolysis for the analysis of glucose and reducing sugar. The chemical compositions of UPR and pretreated UPR were analyzed for the content of cellulose, hemicellulose, and acid insoluble lignin.

2.5. Bioethanol fermentation

The enzymatic hydrolyzate was concentrated by rotary evaporation and was subjected to fermentation using *S. cerevisiae*. Then, 100 mL of concentrated hydrolyzate was placed in a 250 mL Erlenmeyer flask and supplemented with optimum medium KH_2PO_4 (1 g/L), $(NH_4)_2SO_4$ (6 g/L), $MgSO_4{\cdot}7H_2O$ (0.4 g/L), and $FeSO_4{\cdot}7H_2O$ (0.4 g/L). The effects of fermentation conditions including fermentation time (24, 48, 72 h), hydrolysate concentration (0.5%, 1%, 2%, 5%, 10%), fermentation temperature (28, 30, 36, 40 °C), and inoculum size (2%, 5%, 10%, 15%) on the bioconversion of pretreated UPR were examined in this study. The corresponding fermentation efficiency was calculated as follows:

$$\text{Fermentation efficiency based on reducing sugar}(\%) = \frac{\text{Amount of fermented reducing sugar}}{\text{Amount of reducing sugar in the initial fermentated system}}$$

$$\text{Fermentation efficiency based on glucose}(\%) = \frac{\text{Amount of fermented glucose}}{\text{Amount of glucose in the initial fermentated system}}$$

Apart from that, bioethanol production was also scaled-up to a 2 L fermenter (Labfors, Infors AG, Switzerland) using the optimized conditions identified from shake flask experimentation.