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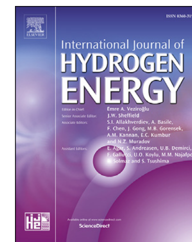

Effects of various dilute acid pretreatments on the biochemical hydrogen production potential of marine macroalgal biomass

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ABSTRACT

This research investigated the effects of various dilute acid (HCl, H_2SO_4, HNO_3, H_3PO_4) pretreatment on the solubilization of marine macroalgal biomass *Gelidium amansii* and subsequent hydrogen fermentation in a batch vials. The dry grounded biomass was hydrolyzed at temperature of 121 °C, solid/liquid (S/L) ratio of 5% (w/v), dilute acid concentration (1%) of various reagents, and reaction time of 30 min. The hydrolyzates obtained at these conditions were then fed to batch hydrogen fermentation. Results revealed that only H_2SO_4 pretreatment method had a significant effect on improvement of biohydrogen production from *Gelidium amansii*, whereas the other pretreatment conditions were even worse than the control experiment. Dilute sulfuric acid (1%) provided a maximum hydrogen production of 52 mL-H_2/g-dry biomass, whereas control experiment provided a hydrogen efficiency of 27 mL-H_2/g-dry biomass. The results showed that selection of appropriate pretreatment method is essential for enhanced hydrogen production from macroalgal biomass.

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Introduction

In modern world, energy is considered as an essential supply for many sectors. Day by day the need for energy is increasing due to the rise in population and the development of higher energy consumption [1]. Therefore, due to the environmental problems and to meet out the upcoming energy demand, researchers are now finding alternative fuel resources to replace the fossil fuels [2,3]. H_2 is considered to be an ideal clean energy carrier in near future because of its high energy storage capacity, no GHG emission when combusting, and can be even generated from water without CO_2 emissions [4]. Additionally, the renewable energy produced from solar and wind power can be transformed to H_2 via electrolysis of water

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for efficient storage and utilization of the renewable energy source for further purposes. Biohydrogen production via dark fermentation is a striking method towards waste minimization along with generation as win–win situation. Several studies have been carried out to measure the potency of bioH_2 production with different kinds of biomass which are varied from cellulosic [5], lignocellulose [6], wastewater [7] and recently algal biomass [8,9].

Macroalgae biomass based H_2 fermentation is recently explored and gaining heed towards its bio refinery along with energy generation. The preference of macroalgal biomass for hydrogen production was due to the lignin free structure, low lipid and high carbohydrates compositions as polysaccharides units [10]. The major sugar present in the red algal biomass is galactose, which is extensively studied by particular group of researchers from South Korea and gradually spreading in other countries [11–14]. Unlike the glucose to hydrogen production, the conversion of galactose to hydrogen needed additional energy requirement for breakdown of galactose to glucose-1-phosphate via Leloir pathway [15] to produce hydrogen and other organic acids [13].

In general, the production of biohydrogen from macroalgal biomass is based on the diverse nature of the microbial source which digests the complex polymers under anaerobic conditions and the hydrogen yield (HY) depends mainly on the available carbon and nitrogen source [10]. The direct conversion of macroalgal biomass to biohydrogen is often limited due to the poor hydrolysis rate. Mild/dilute acid (HCl) hydrolysis towards sugar recovery from various lignocellulose biomasses has been extensively researched, due to its cost-effectiveness and easier operation in large scale [16–19]. Likewise, several studies have been investigated the application of dilute acid pretreatment method to assess the potential of hydrogen production from macroalgal biomass [10], however limited studies have focused on investigation of different pretreatment agents. Thus considering all these factors mentioned above, hydrogen fermentation process for macroalgae based acid hydrolyzate and the process performance and parameters were evaluated in this study.

Materials and methods

Algal biomass and seed source

The algal biomass *Gelidium amansii* was collected from littoral zone in Morocco and washed with distilled water to remove the impurities, then dried in an oven at 40 °C for overnight, further grounded with hammer mill to make the particle size of 300 μm and used for the experiment. The detailed composition of the *G. amansii* was mentioned elsewhere with a total carbohydrate content of 67.3% and protein of 15.6% [20]. The seed source for the hydrogen producers were obtained from the granular digester sludge operated in our laboratory. The heat pretreatment at 90 °C for 30 min was adopted to suppress the hydrogen consumers and enriching the spore forming hydrogen producers. The seed sludge had initial characteristics of pH 7.4, total solids (TS) 42.9 g/L. volatile solids (VS) 32.5 g/L, with a TS/VS ratio of 75.8%.

Biomass pretreatment

Separate hydrolysis pretreatment method was performed using 5% dried biomass with four kinds of 1% dilute acid as H_2SO_4, HCl, HNO_3, H_3PO_4. The pretreatment reactions for breakdown of the complex sugars to simpler monomers were performed in an autoclave at 121 °C for 30 min. After the post hydrolysis step, the pretreated slurry was cooled down to room temperature in 3 h, followed by pH neutralization to 6.5–7.0 with 6 N NaOH solution. Control experiment was performed with addition of distilled water as a catalyst and the reaction conditions were followed as described above.

Batch hydrogen fermentation

Hydrogen fermentation was performed in a 150 mL batch vials with total working volume of 65 mL. In each vial, 50 mL of pretreated slurry and 10 mL of heat treated seed sludge and 5 mL of the Endo nutrient medium was added. The Endo medium consists of the following essential macro and micronutrients as follows (g/L): 5.24, NH_4CO_3; 6.72, $NaHCO_3$; 0.125, K_2HPO_4; 0.1, $MgCl_2 \cdot 6H_2O$; 0.015, $MnSO_4 \cdot 6H_2O$; 0.025, $FeSO_4 \cdot 7H_2O$; 0.005, $CuSO_4 \cdot 5H_2O$; 0.00012, $CoCl_2 \cdot 5H_2O$. After the batch vials were added with the aforementioned components, the gas sparing with pure N_2 (99.9%) for 3 min to ensure the suitable anaerobic condition to the microbes present in the seed sludge. There forward, the reactors were kept in a shaker incubator with a fixed rpm of 150 and a temperature of 35 ± 0.1 °C. The initial pH of the reactor was maintained as 7.0. The experimental sets were run as duplicates and the average mean and median values were reported.

Analytical procedure for gas and liquid products

The compositions of biogas (CH_4, H_2, CO_2, and N_2) were analyzed by a gas chromatograph (SHIMADZU GC-8A) equipped with a thermal conductivity detector (TCD, 80 mA) and a 2 m stainless steel column packed with Shincarbon ST (SHIMADZU GLC). Helium was employed as carrier gas at a flow rate of 30 mL/min. The column temperature was 70 °C, and the injector and detector temperatures were both 100 °C. The volume of H_2 was measured using glass syringes as per the expected production and then calculated as the values at standard temperature and pressure conditions (STP, $T = 273.15$ K, $p = 100$ bar). Other analytical procedures such as COD, TS, and VS were measured by following the standard methods of APHA [21]. DNS method was used to analyze the total reducing sugar (RS) concentration, by using glucose as a reference standard [22]. Total sugar recovery was calculated based on the ratio of the RS concentration of the pretreated hydrolyzate to its total carbohydrate content of the untreated biomass [16]. Sugar utilization rate was measured based on the residual RS after the fermentation reactions.

Modified Gompertz equation

Kinetic analysis was performed based on the cumulative H_2 production for each experiment from the experimental data. Each graph represents the duplicate values of H_2 production

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