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Improvement of the energy conversion efficiency of *Chlorella pyrenoidosa* biomass by a three-stage process comprising dark fermentation, photofermentation, and methanogenesis

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HIGHLIGHTS

- Saccharification of *C. pyrenoidosa* is promoted by steam heating with diluted acid.
- Dark H_2 production is remarkably enhanced by using hydrolyzed biomass as feedstock.
- H_2 yield is dramatically increased by the combined dark and photo fermentation.
- Energy conversion efficiency is markedly improved via co-generation of H_2 and CH_4.

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ABSTRACT

The effects of pre-treatment methods on saccharification and hydrogen fermentation of *Chlorella pyrenoidosa* biomass were investigated. When raw biomass and biomass pre-treated by steam heating, by microwave heating, and by ultrasonication were used as feedstock, the hydrogen yields were only 8.8–12.7 ml/g total volatile solids (TVS) during dark fermentation. When biomass was pre-treated by steam heating with diluted acid and by microwave heating with diluted acid, the dark hydrogen yields significantly increased to 75.6 ml/g TVS and 83.3 ml/g TVS, respectively. Steam heating with diluted acid is the preferred pre-treatment method of *C. pyrenoidosa* biomass to improve hydrogen yield during dark fermentation and photofermentation, which is followed by methanogenesis to increase energy conversion efficiency (ECE). A total hydrogen yield of 198.3 ml/g TVS and a methane yield of 186.2 ml/g TVS corresponding to an overall ECE of 34.0% were obtained through the three-stage process (dark fermentation, photofermentation, and methanogenesis).

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1. Introduction

The global use of fossil fuels has resulted in serious energy and environmental problems. Hydrogen has attracted attention because of its high energy density and clean combustion product (Kim et al., 2006; Sun et al., 2011; Xia et al., 2013). In contrast to traditional hydrogen-producing processes (e.g., water electrolysis), fermentative hydrogen production from renewable biomass is considered as an energy-saving and carbon–neutral process (Cheng et al., 2012; Su et al., 2010).

Microalgae are a promising biomass feedstock for biofuel production because of several reasons. First, biomass doubling times of microalgae in exponential growth are usually as short as 3.5 h (Chisti, 2007). Second, biofuel yields from microalgae in the same cultivation area are 10–100 times higher than those from land-based crops (Demirbas, 2010; Dismukes et al., 2008; Williams, 2007). Third, microalgae can provide a continuous biomass supply because they can be cultivated and harvested continuously almost throughout the year (Schenk et al., 2008). Fourth, microalgae can be cultivated in seawater or wastewater, thereby significantly reducing freshwater consumption (Phukan et al., 2011; Schenk et al., 2008). Fifth, biomass compositions (e.g., carbohydrates and lipids) can be easily adjusted by altering the growth conditions (Dismukes et al., 2008). *Chlorella* is one of the typical microalgae used for commercial large-scale cultivation and has been widely cultivated since the 1980s (Spolaore et al., 2006). This organism can be cultivated in wastewater and flue gas in an industrial scale and has great potential to be used as biomass feedstock to produce biofuels (Cheng et al., 2013). The compositions of *Chlorella* biomass (10–70% carbohydrates and 15–70% proteins) are suitable for hydrogen fermentation (Biller and Ross, 2011; Brown and Jeffrey, 1992; Lv et al., 2010; Ogbonna and Tanaka, 1996; Phukan et al., 2011; Spolaore et al., 2006; Tokusoglu and Unal, 2003; Xia and Gao, 2005; Yeh et al., 2010). Previous studies (Cheng et al., 2011a,b; Elbeshbishy et al., 2012; Guo et al., 2008; Su et al.,

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2010, 2009b) have shown that proper pre-treatment methods (e.g., steam heating, microwave heating, enzymatic hydrolysis, ultrasonication, steam heating with diluted acid/alkali, and microwave heating with diluted acid/alkali) can significantly improve saccharification and subsequent hydrogen fermentation of biomass such as cassava starch, rice straw, microalgae, water hyacinth, food waste, and waste sludge. Therefore, the effects of pre-treatment methods on hydrolysis and hydrogen fermentation of *Chlorella* biomass should be investigated.

Researchers have previously investigated hydrogen production from *Chlorella* biomass through dark fermentation (Ho et al., 2012; Liu et al., 2012; Sun et al., 2011; Yun et al., 2012). However, experimental hydrogen yields [$\leqslant$85.3 ml/g total volatile solids (TVS)] and energy conversion efficiencies (ECE $\leqslant$ 3.6%) from *Chlorella* biomass are extremely low for industrial applications because of the low theoretical hydrogen yield (4 mol H_2/mol glucose) in dark fermentation (Ho et al., 2012; Liu et al., 2012; Su et al., 2009a; Sun et al., 2011; Yun et al., 2012). Residues produced by dark fermentation can be reused to improve hydrogen yield and ECE from *Chlorella* biomass. The supernatant of dark fermentation, which mainly contains acetate, butyrate, and ammonium, can be first treated with zeolite to extract ammonium and then be reused by photosynthetic bacteria (PSB) to produce hydrogen during photofermentation. The theoretical hydrogen yield is markedly improved to 12 mol/mol glucose in the combined dark fermentation and photofermentation (Su et al., 2009a). The solid residues of dark fermentation and the solution residues of photofermentation can be reused by methane-producing bacteria (MPB) to produce methane during methanogenesis.

In this study, the effects of pre-treatment methods of *Chlorella pyrenoidosa* biomass on saccharification and fermentative hydrogen production were investigated. The hydrogen yield and ECE of *C. pyrenoidosa* biomass were significantly improved by a three-stage process comprising dark fermentation, photofermentation, and methanogenesis.

2. Methods

2.1. Inocula and feedstock

Hydrogen-producing bacteria (HPB), PSB, and MPB were isolated from the anaerobic digestion sludge collected from a methane plant located in Zhejiang Province, China. Bacteria isolation and enrichment of HPB, PSB, and MPB were described in detail in previous studies (Cheng et al., 2012; Xia et al., 2013). HPB, PSB, and MPB were used as the inocula in dark fermentation, photofermentation, and methanogenesis, respectively.

C. pyrenoidosa was cultivated in raceway ponds supplied with flue gas (12.5–14.0% CO_2, 45–75 ppm SO_2, and 80–120 ppm NO_X) from a power plant located in Shangdong Province, China. *C. pyrenoidosa* biomass was harvested by centrifugation, spray dried, and stored for use in the succeeding experiments.

2.2. Experimental processes

C. pyrenoidosa biomass was pre-treated using the following methods: (1) steam heating; (2) microwave heating; (3) ultrasonication; (4) steam heating with diluted acid; and (5) microwave heating with diluted acid. In method (1), 5.0 g of *C. pyrenoidosa* biomass was placed in a conical flask and deionized water was added until the mixture reached a final volume of 100 ml. The conical flasks were then placed in an autoclave (Sanyo MLS-3780, Japan) and heated by steam at 135 °C for 15 min. In method (2), 1.25 g of *C. pyrenoidosa* biomass was placed in a polytetrafluoroethylene (PTFE) reactor and deionized water was added until the mixture reached a final volume of 25 ml. The PTFE reactors were placed in a microwave digestion system (Shanghai Yiyao WX-4000, China) and heated by microwave at 140 °C for 15 min. In method (3), 5.0 g of *C. pyrenoidosa* biomass was placed in a beaker and deionized water was added until the mixture reached a final volume of 100 ml. The beakers were then placed in an ultrasonic cell disruptor (Autoscience UH-500A, China) and pre-treated by ultrasonic wave for 15 min. In method (4), 5.0 g of *C. pyrenoidosa* biomass was placed in another conical flask and diluted acid (1% v/v) was added until the mixture reached a final volume of 100 ml. The conical flasks were placed in an autoclave and heated by steam at 135 °C for 15 min. In method (5), 1.25 g of *C. pyrenoidosa* biomass was placed in another PTFE reactor and diluted acid (1% v/v) was added until the mixture reached a final volume of 25 ml. The PTFE reactors were placed in a microwave digestion system and heated by microwave at 140 °C for 15 min.

The three-stage process comprising dark fermentation, photofermentation, and methanogenesis is shown in Fig. 1. Fermentation and methanogenesis experiments were performed in 300 ml scale fermenters. Approximately 100 ml of pre-treated solution (5.0 g of *C. pyrenoidosa* biomass equivalent) and 125 ml of deionized water were added to each fermenter. The initial pH was adjusted to 6.0 ± 0.1 by using 6 M HCl solution and 6 M NaOH solution. The fermenters were subsequently inoculated with 25 ml of HPB, sealed with rubber stoppers, purged with nitrogen gas for 10 min, and maintained at 35 ± 1.0 °C for dark fermentation. The supernatant and the solid residues of dark fermentation were separated by centrifugation at 6000×*g* for 10 min. The supernatant was treated with modified zeolite to extract ammonium prior to photofermentation as described in a previous study (Cheng et al., 2012). The treated supernatant was transferred to a fermenter, diluted to 15 mM soluble metabolite products (SMPs), and added to 25 ml of PSB mixed with autoclaved medium (Su et al., 2009a). The liquid volume of photofermentation was 300 ml. The initial pH was adjusted to 7.0 ± 0.1 by using 6 M HCl solution and 6 M NaOH solution. The fermenters were subsequently sealed with rubber stoppers, placed in an incubator (Shanghai Yiheng LRH-250F, China) at 30.0 ± 1.0 °C, and subjected to an illumination intensity of approximately 6000 lux (incandescent lamp). The solid residues (undegraded biomass and HPB cells) of dark fermentation were mixed with 225 ml of deionized water and the solution residues (residual SMPs and PSB cells) of photofermentation (250 ml) were transferred to the fermenters. The initial pH was adjusted to 8.0 ± 0.1 by using 6 M HCl solution and 6 M NaOH solution. The fermenters were subsequently inoculated with MPB (10 ml of MPB for the solid residues of dark fermentation and 25 ml of MPB for the solution residues of photofermentation) sealed with rubber stoppers, purged with nitrogen gas for 10 min, and maintained at 35 ± 1.0 °C for methanogenesis. The gas produced during fermentation (rich in hydrogen and carbon dioxide) and the gas produced during methanogenesis (rich in methane and dioxide) were released from the fermenter headspace and collected in graduated containers, respectively.

2.3. Analytical methods

Carbohydrates, proteins, lipids, ash, moisture, and TVS in *C. pyrenoidosa* biomass were determined as described in previous studies (Cheng et al., 2012; Su et al., 2010). The carbon content of *C. pyrenoidosa* biomass was determined using an elemental analyzer (5E-CHN2000, China). The concentration of total reducing sugars was determined based on 3,5-dinitrosalicylic acid method (Su et al., 2010). The concentration of ammonium was determined using an ion meter (Mettler Toledo SG8-B, Switzerland) with a DX218-NH_4^+ ion electrode (Cheng et al., 2012).

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