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Enhancement of energy production efficiency from mixed biomass of *Chlorella pyrenoidosa* and cassava starch through combined hydrogen fermentation and methanogenesis

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HIGHLIGHTS

- Mixed biomass of *C. pyrenoidosa* and cassava starch was used for H_2 fermentation.
- Mixed biomass at C/N molar ratio of 25.3 gave the highest dark H_2 yield.
- Mixed biomass at C/N molar ratio of 15.6 gave the highest dark H_2 production rate.
- H_2 yield was improved to 664.2 mL/g TVS via combined dark and photo fermentation.
- Energy production efficiency was enhanced to 67.2% via cogeneration of H_2 and CH_4.

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ABSTRACT

To enhance energy production efficiency (EPE) from microalgae *Chlorella pyrenoidosa* (CP), cassava starch (CS) was mixed with CP to optimise the carbon/nitrogen (C/N) ratio and facilitate efficient dark hydrogen fermentation, followed by photo hydrogen fermentation and methanogenesis. Steam heating with dilute acid was a preferred pre-treatment method to hydrolyze mixed biomass. The maximum dark hydrogen yield of 276.2 mL/g total volatile solids (TVS) from the mixed biomass at C/N molar ratio of 25.3 showed 3.7-fold and 1.8-fold increases, respectively, compared with those from only CP and only CS. The maximum dark hydrogen production rate of 31.96 mL/g TVS/h from the mixed biomass at the C/N molar ratio of 15.6 showed 3.4-fold and 3.7-fold increases, respectively, compared with those from only CP and only CS. The dark and photo hydrogen yield of 664.2 mL/g TVS and the methane yield of 126.0 mL/g TVS corresponded to a total EPE of 67.2%.

1. Introduction

Energy and environmental problems caused by excess utilisation of non-renewable fossil fuels have been increasingly receiving worldwide attention [1–4]. Hydrogen is an ideal fuel because of its high energy density and clean combustion product [5–9]. Compared with conventional hydrogen-producing processes (e.g., steam reforming), fermentative hydrogen production from renewable biomass is more energy-saving and environment-friendly [10].

The choice of feedstock is the key factor for fermentative hydrogen production. Microalgae are considered excellent options because of their characteristics, such as rapid aquatic growth, high productivity, and wide distribution [11–15]. Some researchers have previously used microalgae as feedstock to produce hydrogen during dark fermentation [11,16–19]. However, the low hydrogen yield [⩽133.0 mL/g total volatile solids (TVS)] and energy production efficiency (⩽6.6%) of microalgae heavily constrain its use in large-scale applications [11,18]. Due to high protein content (15–70%) and relatively low carbohydrate content (10–70%), the carbon/nitrogen (C/N) ratio of microalgae is too low for hydrogen-producing bacteria (HPB) to efficiently produce fermentative hydrogen [11,12,20–22]. Therefore, biomass with high carbohydrate content and low protein content should be mixed with microalgae to adjust the C/N ratio for efficient hydrogen fermentation. Cassava, which can rapidly grow in barren and drought areas, is an important energy crop [23]. According to data from the Food and Agriculture Organization of the United Nations, the annual worldwide cassava production is approximately 257 million tonnes in 2012. The main

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organic component of cassava is starch, which has great potential as feedstock for hydrogen fermentation [23]. However, to date, no studies on hydrogen fermentation from mixed biomass of microalgae and cassava starch (CS) have been reported.

High-molecular weight compositions in biomass (e.g., starch and proteins) usually need to be hydrolyzed to low-molecular weight ones (e.g., reducing sugars and amino acids) for efficient utilisation by HPB prior to hydrogen fermentation. Previous studies have shown that proper pre-treatment methods (e.g., steam heating and steam heating with dilute acid) can enhance saccharification and subsequent hydrogen fermentation of biomass [11,24]. However, single-stage dark hydrogen fermentation has disadvantages of low hydrogen yield (theoretical hydrogen yield of glucose: 4 mol/mol glucose) and low energy production efficiency (EPE; theoretical EPE of glucose: 33.5%) because of energy waste in residues of fermentation [25]. The supernatants of dark fermentation, which mainly contain soluble metabolite products (SMPs) such as acetate and butyrate, can be reused by photosynthetic bacteria (PSB) to further produce hydrogen during photofermentation [10,25]. Furthermore, the solid residues of dark fermentation (mainly undegraded biomass) and residues of photofermentation (mainly PSB biomass and residual SMPs) can be reused by methane-producing bacteria (MPB) to cogenerate methane during methanogenesis [10].

In this study, the mixed biomass of microalgae *Chlorella pyrenoidosa* (CP) and CS was used as feedstock to produce hydrogen during dark fermentation. The effects of pre-treatments and C/N molar ratios on dark hydrogen fermentation were investigated. The hydrogen yield and EPE of the mixed biomass were remarkably enhanced through a three-stage process comprising dark fermentation, photofermentation, and methanogenesis.

2. Materials and methods

2.1. Feedstock and bacteria

HPB, PSB, and MPB were isolated from anaerobic digestion sludge obtained from a methane plant located in Zhejiang Province, China. The isolation and enrichment processes of HPB, PSB, and MPB have been previously described in detail [19,26]. The enriched HPB, whose dominant strain was *Clostridium butyricum*, were used as the inocula for dark fermentation [16]. The enriched PSB, whose dominant strain was *Rhodopseudomonas palustris*, were used as the inocula for photofermentation [27]. The enriched MPB, whose primary bacterial species were *Methanothrix* and *Methanosarcina*, were used as the inocula for methanogenesis [28].

CS was purchased from Shanghai Heyu Trade Co., Ltd., China. CP was cultivated in raceway ponds supplied with flue gas from a power plant located in Shandong Province, China. The biomass of CP was harvested by centrifugation and then spray dried for storage and use in succeeding fermentation experiments.

2.2. Pre-treatment, fermentation, and methanogenesis

For pre-treatment of steam heating, 5.0 g mixed biomass (2.5 g CS and 2.5 g CP) at CS/CP ratio of 1, which corresponds to C/N molar ratio of 10.8, and 100 mL deionised water were added to a conical flask. For pre-treatment of steam heating with dilute acid, 5.0 g mixed biomass (5.0–1.67 g CS and 0–3.33 g CP) at different CS/CP ratios [∞ (only CS), 8, 4, 2, 1, and 0.5, corresponding to C/N molar ratios of ∞, 44.5, 25.3, 15.6, 10.8, and 8.4, respectively] and 100 mL dilute H_2SO_4 solution (1% v/v) were added to each conical flask. The conical flasks were placed in an autoclave (Sanyo MLS-3780, Japan), and then heated by steam at 135 °C for 15 min.

Dark fermentation, photofermentation, and methanogenesis were performed in 300 mL-scale glass fermenters. Approximately 100 mL pre-treated biomass solution (5.0 g mixed biomass equivalent) and 130 mL deionised water were added to each dark fermenter. The initial pH was adjusted to 6.0 ± 0.1 using 6 M HCl solution and 6 M NaOH solution. The dark fermenters were subsequently inoculated with 20 mL activated HPB, sealed with rubber stoppers, purged with nitrogen for 5 min, and then maintained at 35.0 ± 1.0 °C for dark fermentation. Supernatants and solid residues of dark fermentation were separated by centrifugation. The supernatants were transferred to a photo fermenter, diluted to approximately 15 mM SMPs, and inoculated with 25 mL activated PSB mixed with autoclaved medium [29]. The total liquid volume of photofermentation was 250 mL. The initial pH was adjusted to 7.0 ± 0.1 using 6 M HCl solution and 6 M NaOH solution. The photo fermenters were subsequently sealed with rubber stoppers, purged with nitrogen for 5 min, subjected to an illumination intensity of approximately 6000 lux (incandescent lamp), and then maintained at 30.0 ± 1.0 °C for photofermentation. The solid residues of dark fermentation mixed with 200 mL deionised water were transferred to a methane fermenter, and the residues of photofermentation(250 mL) were transferred to another methane fermenter. The initial pH was adjusted to 8.0 ± 0.1 using 6 M HCl solution and 6 M NaOH solution [30]. The methane fermenters were subsequently inoculated with 15 mL activated MPB, sealed with rubber stoppers, purged with nitrogen for 5 min, and then maintained at 35.0 ± 1.0 °C for methanogenesis. The material flow of the overall process is as shown in Fig. 1. Gases (mainly hydrogen, methane, and carbon dioxide) produced during dark fermentation, photofermentation, and methanogenesis were released from the headspace of fermenters and collected in graduated containers [10]. All experiments were conducted in duplicate, and results were expressed as the average (±standard deviation).

2.3. Instrumental analysis

The carbon, hydrogen, and nitrogen contents in the biomass were determined using an elemental analyser (Changsha Kaiyuan 5E-CHN2000, China). The sulfur content in biomass was determined using an infrared sulfur analyser (Changsha Kaiyuan 5E-IRS-II, China). The oxygen content was assumed to be the remaining content of biomass [31]. The heating value of biomass was determined using a fast calorimeter (Changsha Kaiyuan5E-KC5410, China) [31]. The TVS content in the biomass was determined as described in a previous study [10]. The concentration of reducing sugars was determined through 3,5-dinitrosalicylic acid method (glucose was used as the standard) [19]. Hydrogen, methane, and carbon dioxide contents in the gas phase were determined using a gas chromatography (GC; Agilent 7820A, USA) equipped with a thermal conductivity detector, as described in a previous study [21]. The contents of SMPs in the liquid phase were determined using another GC (Thermo Finnigan TRACE 2000, USA) equipped with a flame ionization detector, as described in a previous study [21].

The cumulative volumes of hydrogen, methane, and carbon dioxide were calculated as described in a previous study [10]. The cumulative yields of hydrogen, methane, and carbon dioxide were defined as the ratios of the cumulative volumes of hydrogen (mL), methane (mL), and carbon dioxide (mL) to the original TVS weight of mixed biomass (g TVS), respectively. The cumulative yields of hydrogen and methane were fitted using a modified Gompertz equation, and the kinetic parameters [H_m, hydrogen or methane production potential (mL/g TVS); R_m, hydrogen or methane production peak rate (mL/g TVS/h); λ, lag phase time (h); and T_m, peak time (h)] were calculated using Origin 8.0 [21]. The carbon production efficiency [CPE (%)] of dark fermentation was defined