Algal Research 12 (2015) 295–299

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

# Fermentative hydrogen and polyhydroxybutyrate production from pretreated cyanobacterial blooms

Jinling Cai [a,c,*], Minglang Chen [b], Guangce Wang [d], Guanghua Pan [a], Peng Yu [b,c,**]

[a] College of Chemistry Engineering and Material Science, Tianjin University of Science & Technology, Tianjin Key Laboratory of Marine Resources and Chemistry, Tianjin 300457, China
[b] College of Biotechnology, Tianjin University of Science & Technology, Tianjin 300457, China
[c] Tianjin International Joint Academy of Biomedicine, Tianjin 300457, China
[d] Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China

ARTICLE INFO

*Article history:*
Received 8 April 2015
Received in revised form 14 September 2015
Accepted 23 September 2015
Available online xxxx

*Keywords:*
Hydrogen production
Polyhydroxybutyrate production
Algal biomass
Pretreatment
Fermentation

ABSTRACT

The cell wall and cytoplasmic membrane components of algal biomass (in cyanobacterial blooms) are resistant to biodegradation during anaerobic digestion. Various pretreatment methods including thermal, alkaline and acid pretreatments, were performed (each as a two-stage process) to increase the biodegradability of the algal biomass in terms of hydrogen and polyhydroxybutyrate (PHB) production. Among the pretreatment methods, thermal pretreatment achieved the highest hydrogen production (113 mL/g VS), followed by alkaline pretreatment (94 mL/g VS). Following hydrogen production, phototrophic bacteria were inoculated into the fermentative broth, for PHB production. The group that had undergone alkaline pretreatment produced the highest amount of PHB (about 1.69 g/L). Our studies indicate that pretreatment is a feasible option for transforming algal biomass into a bio-available material, for the purpose of microbial hydrogen production and PHB conversion.



## 1. Introduction

Due to the shortage of fossil fuels, as well as problems associated with environmental pollution, there is an urgent need to identify sources of clean, renewable, sustainable and carbon-neutral energy. Hydrogen is considered a favourable alternative energy source, because of its high energy density and clean-combustion products. Among the various hydrogen production technologies, fermentative hydrogen production, from biomass and organic wastes, is considered as one of the most promising processes for developing sustainable energy [1,2].

Cyanobacterial blooms frequently occur in eutrophic lakes, reservoirs, and polluted waters, worldwide. In recent years, cyanobacterial blooms have increased in frequency and intensity in several lakes in China [3]. The excessive growth of cyanobacteria in lakes can detrimentally affect ecosystem functioning in lakes. Moreover, many cyanobacterial strains contain microcystins, which pose a threat to human and animal health [4]. The treatment of algal blooms is a worldwide problem. Algal biomass in cyanobacterial blooms, which contain high levels of starch, polysaccharides, oligomers and hydrocarbons, are potentially suitable feedstocks for anaerobic fermentation [4–8]. The fermentative production of hydrogen from algal biomass is considered a low-cost, environment-friendly method for the treatment of algal blooms, and for energy production.

Cell walls and cytoplasmic membranes are components of the algal biomass that are resistant to biodegradation during anaerobic digestion. This complicates chemical processing, which means that biodegradation of these components becomes the limiting factor in the digestion of algal biomass [2,9]. Pretreatment destroys cell microstructure, resulting in a reduction in particle sizes within microalgae particles, facilitating the release of chemical contents and improvement to digestion efficiency [8,10,11]. Thermal, acid, and alkaline pretreatments are standard methods used for algal digestion [9,12]. Acid and alkaline pretreatment result in the disruption of the microalgal cell wall, rendering the inner complex organic matter more available to biodegradation [10–13]. Treatment with 2% (v/v) HCl results in higher cumulative hydrogen production (of 1230 mL/L), compared with that of the control, in which hydrogen is not produced [13]. Alkaline pretreatment at pH 13 has the capacity to produce about 3.8 times more hydrogen than that of the control [10]. Thermal pretreatment is effective in terms of solubilizing complex sugars in water, thus improving hydrogen production [13,14]. Thermal pretreatment (at 170 °C for 20 min) of *Laminaria japonica* resulted in a 62% higher production of hydrogen, compared with that of the control [14]. Comparisons of pretreatment methods have indicated that the suitability of the pretreatment method depends on the type of biomass and the nature of the biological conversion process. Algal cells have a simpler structure than agro-biomass, which allows them to be crushed under relatively mild conditions.

* Correspondence to: Jinling Cai, College of Chemistry Engineering and Material Science, Tianjin University of Science and Technology, Tianjin 300457, P. R. China.
** Correspondence to: Peng Yu, College of Biotechnology, Tianjin University of Science & Technology, Tianjin 300457, China.
*E-mail addresses:* jinlingcai@tust.edu.cn (J. Cai), yupeng@tust.edu.cn (P. Yu).

http://dx.doi.org/10.1016/j.algal.2015.09.014
2211-9264/

Cyanobacterial biomass-based carbohydrates, mainly comprised of glycogen, cellulose and exopolysaccharides (with the absence of lignin), are thus more easily converted to monosaccharides than is the case for lignocellulosic materials [15].

The production of valuable by-products during processing can greatly increase the value and applicability of various hydrogen production methods. Polyhydroxybutyrate (PHB), a completely biodegradable and biocompatible plastic is a common intracellular storage compound found in many microorganisms, such as photosynthetic bacteria [16]. With increasing environmental pollution, the replacement of synthetic plastic with biodegradable plastic becomes an important issue. Nevertheless, the use of PHB is currently limited by its high production cost. Substrate costs represent a significant proportion of the total cost of PHB production. The broth that forms after fermentative hydrogen production is rich with organic acids, which maybe used as a substrate by photosynthetic bacteria for PHB production. This would greatly increase its commercial application potential.

Relatively little data is currently available for the purpose of an intensive investigation into the maximum potential hydrogen and PHB production from cyanobacterial blooms. In the present study, to demonstrate the potential of hydrogen and PHB production from a cyanobacterial bloom, we investigated the influence of pretreatment methods on hydrogen production.

## 2. Materials and methods

### 2.1. Inoculum

Anaerobic sludge, for the purpose of hydrogen production, was obtained from an anaerobic municipal wastewater treatment plant in Tianjin, China. The inoculum was stored at 4 °C until the test. Prior its use in the hydrogen production experiment, the sludge was thermally pretreated (at 90 °C for 30 min) to inhibit non-hydrogen-producing bacteria.

The phototrophic bacterial consort for use in of PHB production was collected from the lake water of the Meiliang Bay, Taihu Lake (120°30′N, 31°27′E). The phototrophic bacterial consort was enriched by a culture medium, according to the methods described in Cai et al. [17] with some modifications. Butyrate (concentration: 20 mmol/L) and ammonium chloride (concentration: 10 mmol/L) were used as carbon and nitrogen sources, respectively. A 20 mL aliquot of lake water was inoculated into 80 mL of culture medium in a 130 mL glass vial at 35 °C and pH 7.5 ± 0.2. Light intensity was maintained at 80 μmol/$m^2$·s and argon gas was purged into the vial, to create anaerobic conditions. Under the experimental conditions, phototrophic bacterial growth would result in a purple-colored broth, after which a 20 mL aliquot of the purple culture was injected, as an inoculum, into 80 mL of culture medium. This process was repeated five times, to harvest the purple non-sulfur photosynthetic bacteria. The phototrophic bacterial consort was used for PHB production.

### 2.2. Feedstocks and pretreatments

Mixtures of algae and lake water, collected from Meiliang Bay, Taihu Lake were used as substrates in the experiment. Mixtures were stored at 4 °C until further use. The cyanobacterial biomass was rich in organic compounds (volatile solid: VS) (Table 1). Thermal, acid, and alkaline, pretreatments were performed, for the purpose of comparison of the hydrogen yields. In the acid pretreatment experiment the algal biomass samples were adjusted to pH 3 by addition of 6 mol/L HCl, for 30 min at room temperature, after which neutral pH was maintained by the addition of NaOH. In the alkaline pretreatment experiment, the algal biomass samples were adjusted to pH 13 by the addition of 6 mol/L NaOH for 30 min at room temperature, after which the neutral pH was maintained by the addition of HCl. In the thermal pretreatment experiment, the algal biomass was heated to 170 °C and maintained at this temperature for 20 min, after which it was cooled to room temperature.

**Table 1**
Characteristics of the cyanobacterial bloom.

| Constituent | Cyanobacterial bloom | inoculum |
|---|---|---|
| Total solids (TS, %) | 6.47 ± 0.26 | 6.53 ± 0.46 |
| Volatile solids (% of TS) | 74.29 ± 2.99 | 61.63 ± 2.61 |
| Reducing sugar (% of TS) | 5.57 ± 0.28 | NA |

NA—no analysis.
Data are the means of three measurements, and numbers in parentheses are the standard deviations.

### 2.3. Experimental procedure

Hydrogen production tests were carried out in 500 mL glass bottles, with a working volume of 200 mL. Approximately 150 mL of pretreated biomass solution and 50 mL of inoculum were added to each bottle. Prior to purging with $N_2$ gas, to create anaerobic conditions, the initial pH was adjusted to 7.5 ± 0.2. Cultures were placed in a shaker-incubator at 35 °C and 150 rpm. A control, containing raw cyanobacteria and lake water, was also set up.

PHB production tests were carried out in 130 mL glass vials with a 100 mL working volume. After the cessation of hydrogen production, a 20 mL aliquot of precultured phototrophic bacteria was harvested and inoculated into 100 mL of the fermentative broth. The pH was regulated to 7.5 ± 0.2. Batch fermentation was carried out in dark aerobic conditions in a shaker–incubator maintained at 35 °C and 150 rpm. The PHB accumulation was analyzed after 3 d cultivation. All experiments were carried out in triplicate and results were expressed as means.

### 2.4. Analytical methods

Total solid (TS) and volatile solid (VS) were measured according to standard methods [18]. The pH value was measured with a digital pH meter (delta-320, Mettler, USA). Total reducing sugars were evaluated by the phenol–sulfuric acid method as described by Dubios et al. [19]. PHB content was measured using the FT-IR methods according to Cai et al. [16]. The water replacement method was used to measure gas. A gas chromatograph (Agilent, Model 6820, China) equipped with a thermal conductivity detector (TCD) and a 2-m-long column packed with 5 Å molecular sieves (80/100 mesh), was used to determine hydrogen content. Nitrogen was used as a carrier gas, at a flow rate of 30 mL/min. The temperatures of the injector, detector, and column oven were 200 °C, 200 °C, and 40 °C, respectively.

The concentrations of organic acids, including acetic acid, propanoic acid and butyric acid, were analyzed by gas chromatography (Agilent, Model 6890 N, USA), using a flame ionization detector (FID) and a 30 m × 0.25 mm × 0.25 μm fused-silica capillary column (DB-FFAP). The supernatants were centrifuged (12,000 rpm for 5 min), acidified (by formic acid) and filtered (through a 0.2 μm membrane). The temperatures of the injection port and the detector were 250 °C and 300 °C, respectively. The initial temperature of the oven was 70 °C, for 3 min, followed with a ramp of 20 °C/min for 5.5 min, to reach a final temperature of 180 °C for 3 min. Nitrogen was used as the carrier gas at a flow rate of 2.6 mL/min.

## 3. Results and discussion

### 3.1. Effect of pretreatment methods on algal biomass

During fermentative processes, an intact algal cell wall can protect the cell from biodegradation [11,20]. Pretreatment can effectively disrupt the cell microstructure and subsequently enhance hydrogen production [8,11,13,20]. In this study, three pretreatment methods (thermal, acid, and alkaline) were used to treat algal biomass.