Renewable Energy 127 (2018) 670–677

Contents lists available at ScienceDirect

Renewable Energy

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

Improvement of biohydrogen production by optimization of pretreatment method and substrate to inoculum ratio from microalgal biomass and digested sludge

Mishma S. Stanislaus, Nan Zhang, Yue Yuan, Hanying Zheng, Chenyu Zhao, Xiaohong Hu, Qi Zhu, Yingnan Yang*

Graduate School of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki, 305-8572, Japan

ARTICLE INFO

Article history:
Available online 4 May 2018

Keywords:
Biohydrogen
Chlorella vulgaris
energy balance
Pretreatment

ABSTRACT

Biohydrogen from microalgal biomass has shown particular advantage due to its high growth rate and high bioenergy production. As a representative of microalgae, *Chlorella vulgaris* was chosen as substrate along with digested sludge (DS) as inoculum in this research. In order to improve the hydrolysis of algal biomass and enhance biohydrogen production, pretreatment methods like acid and thermal pretreatment were employed. Thermal pretreatment showed better results than acid pretreatment of microalgal biomass. 100 °C for 60 min was identified as the optimum condition for the thermal pretreatment of *C. vulgaris* by response surface methodology (RSM) analysis. Experiments were also carried out to identify the optimum substrate to inoculum ratio (SIR) for the process. SIR of 8 generated the highest hydrogen yield of 190.90 mL H_2/g-VS. Moreover, the overall energy balance of the process was evaluated and the results showed a positive energy balance of 1790.13 kJ/kg. The results indicated that optimization of pretreatment methods and substrate to inoculum ratio was effective in enhancing biohydrogen production from microalgal biomass and digested sludge.

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1. Introduction

Hydrogen is widely acknowledged as an efficient and clean energy carrier among the various alternative forms of renewable energy since it has a high energy content of 122 kJ/g which is 2.75 times higher than fossil fuels [1]. By far, 40% of hydrogen is produced from natural gas or steam reforming of hydrocarbons, 30% from oil (mostly consumed within factories), 18% from coal, and the remaining 4% via water electrolysis across the globe [2]. However, they are energy intensive, expensive and eco-destructive processes. Owing to these issues, biological processes is an alternative method of biohydrogen production as it can be operated under ambient conditions, are less energy intensive and more eco-friendly [3]. Among the various biological hydrogen production methods, dark fermentative hydrogen production (DFHP) is widely recognized due to its high rate of cell growth, non-requirement of light energy and the potential for cost-effective hydrogen production [4–6].

The availability and cost of feedstock for fermentative hydrogen production is a major bottleneck. Recently, microalgal biomass has drawn worldwide attention due to its characteristics such as rapid aquatic growth, wide distribution, high bioenergy productivity, continuous supply and so on [7]. Yet only few studies have been conducted on the use of microalgal biomass as a feedstock for DFHP. Among the various microalgal biomass, *Chlorella* is a typical type of microalgal biomass composed of 10–70% carbohydrates and 15–70% proteins, indicating great potential to be used as feedstock [7]. However, some previous literature indicated that the intact and strong cell membranes of microalgae would result in a low biogas yield, limiting the efficient digestion in the DFHP process. To help disrupt the cell walls, pretreatment or disintegration of the microalgal biomass is needed [8,9]. Until now, some different pretreatment methods on microalgal biomass have been investigated. For instance, the ultrasonic pretreatment uses high shear forces resulting in extracting the intracellular organic and thereby increasing the biodegradability [10]. Although ultrasonic pretreatment is a good choice for microalgal biomass, it is energy-intensive for large scale applications and difficult to be used for practical application. Baccay and Hashimoto (1984) [11] investigated that

* Corresponding author.
E-mail address: yo.innan.fu@u.tsukuba.ac.jp (Y. Yang).

https://doi.org/10.1016/j.renene.2018.05.022
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acid pretreatment can bring about swelling of organic structure at low pH, thus making the substrate easier to be hydrolyzed. Furthermore, it has the characteristics such as low cost and simple operation. Thermal hydrolysis has also been accepted as the optimum pretreatment method especially for agricultural wastes as it is effective in increasing biogas production by thoroughly destroying the cell membrane [12]. Nevertheless, the optimum pretreatment method for microalgal biomass is still subject to much debate. Therefore, the acid and thermal pretreatment methods were further investigated in this study.

Digested sludge (DS) was chosen as a source of inoculum in this research due to its availability in abundance and demonstration of positive results from previous researches [13,14]. However, in a mixed culture system, under anaerobic condition some hydrogen consuming bacteria (HCB) existing in the DS, such as methanogens, homoacetogens and Archaea [15,16], often readily consume the hydrogen produced by HPB. Therefore, in order to harness hydrogen from a mixed culture system the HCB were restrained by thermal pretreatment as it was obtained as the optimum pretreatment method from previous research.

Consecutively, the substrate to inoculum ratio (SIR) was evaluated which is another key factor in DFHP. It was reported that as the substrate concentration increased the hydrogen production increased as well until a certain threshold. But beyond the threshold value it caused bioreactor upset leading to a decline in the hydrogen production [17]. Also, the higher concentration of inoculum could cause increased nutrient consumption and waste production which would inhibit the hydrogen production itself [18]. Therefore, an optimum SIR is important for enhancing the overall efficiency of DFHP. Pakarinen (2008) reported that the substrate to inoculum ratio of 2:1 increased H_2 production efficiently [19]. However, there is no report on the effect of SIRs higher than 2:1 on H_2 production from *Chlorella vulgaris* and digested sludge.

In the light of the above research background, this study aimed at optimization of the overall DFHP process for biohydrogen production from *Chlorella vulgaris.* Acid and thermal pretreatment of *Chlorella* was carried out in order to investigate the optimum pretreatment method. Furthermore, a response surface methodology (RSM) with a central composite design (CCD) was used to find the optimum thermal pretreatment conditions and analyze the data statistically. Also, the SIR of *Chlorella* and DS were investigated for the optimization of the overall DFHP process. Along with the optimization of the process, the overall energy balance of the process was evaluated for practical application and future prospects.

2. Materials and methods

2.1. Inoculum and substrate preparation

The digested sludge was obtained from a wastewater treatment plant in Ibaraki prefecture, Japan. After sub packaging in plastic bottles, the digested sludge was stored in the refrigerator at 4 °C before using. The pH, total solid (TS), volatile solid (VS) and DOC (dissolved organic carbon) of the DS were 6.8, 11.40 g/L, 7.80 g/L, and 808 mg/L respectively. DS was acclimatized by incubating them at 35 °C in 500 mL serum bottles containing trace mineral solution (200 mL/L). The composition of trace mineral solution is as follows: $FeSO_4{\cdot}7H_2O$ (1000 mg/L), $CaCl_2{\cdot}2H_2O$ (125 mg/L), $MgCl_2{\cdot}6H_2O$ (125 mg/L), $CoCl_2{\cdot}6H_2O$ (25 mg/L), $MnSO_4$ (25 mg/L), $ZnSO_4{\cdot}7H_2O$ (25 mg/L), $NiCl_2{\cdot}6H_2O$ (25 mg/L), $CuSO_4{\cdot}5H_2O$ (25 mg/L), $Na_2MoO_4{\cdot}2H_2O$ (25 mg/L) and H_3BO_3 (25.0 mg/L) [20]. In addition, 0.5 g glucose was added every alternate day to enable the acclimatization of DS.

The acclimatized DS was then thermally pretreated by using a hot air oven (WFO-600PD, EYELA) at 90 °C for 60 min to inhibit the hydrogen consuming bacteria (HCB). Thermal pretreatment at 90 °C for 60 min was obtained as the optimum pretreatment condition of inoculum from our previous researches.

Chlorella vulgaris biomass used in this study was bought from the company (CHLORELLA INDUSTRY CO., LTD, Japan). Centrifugation was chosen as a method to harvest microalgae as its the most widely used method [7]. The centrifugation was carried out at 100 × 100 rpm for 5 min using a centrifugal machine (6800, KUBOTA) and the residue was used in the further experiments.

2.2. Pretreatment of Chlorella vulgaris

Different pretreatment methods were employed on *C. vulgaris* biomass. For acid pretreatment, the pH of *Chlorella vulgaris* biomass was adjusted to 3 using 3% HCl, and then stored in refrigerator at 4 °C for 24 h. After 24 h, the pretreated microalgal biomass was adjusted to room temperature. Finally, the pH was set to 5.5 using 3% NaOH for hydrogen fermentation. In case of thermal pretreatment, the *C. vulgaris* was subjected to heating using a hot air oven (WFO-600PD, EYELA) at the corresponding temperature and residence time. Initially to identify the best pretreatment method among acid and thermal pretreatment, 100 °C for 60 min was used as thermal pretreatment condition. Later the *C. vulgaris* was pretreated thermally according to the temperature and corresponding residence time as designed by RSM software.

2.3. Experimental design using RSM

RSM, including two factors and a central composite design (CCD) was used in this research to study the effect of independent variables on dependent variables. The maximum hydrogen concentration, cumulative hydrogen production and hydrogen yield (HY) were chosen as the response or dependent variables, while temperature (X_1: 100°C-140 °C) and pretreatment time (X_2: 20–60 min) were chosen as independent variables. Design expert version 6.0.6 was used as the software. The response was fitted using a polynomial quadratic equation to correlate it to the independent variables. The general form of the predictive polynomial quadratic equation used to code variables is as shown in Eq. (1) [21].

$$Y = b_0 + \sum_{i=1}^{k} b_i x_i + \sum_{i=1}^{k} b_{ii} x_i^2 + \sum_{i=1}^{k} \sum_{i<j=2}^{k} b_{ij} x_i x_j \quad (1)$$

where X_i are the input variables, which influence the response variable Y, b_0 the offset term, b_i the *i*th linear coefficient, b_{ii} the quadratic coefficient and b_{ij} is the ij^{th} interaction coefficient.

2.4. Batch fermentation

During the initial experiments to identify the optimum pretreatment condition 2.5 g of *C. vulgaris* and 25 mL of the heat treated DS was used. For the experiments to determine the optimum SIR, appropriate amounts of substrate and inoculum were added. SIR was defined to be the initial ratio of volatile solids (VS) contained in the substrate to the VS contained in the inoculum in each reactor. The inoculum volume in each reactor contributed to 0.24-g VS. Consecutively, different amounts of harvested and pretreated *C. vulgaris* were added to the reactors to get the desired SIRs of 2, 3, 5, 8, 11 and 14 corresponding to 0.48-g VS, 0.72-g VS, 1.20-g VS, 1.92-g VS, 2.64-g VS and 3.36-g VS respectively.

All batch experiments were carried out in 50 mL serum bottles. The pH of each bioreactor was adjusted to 5.5 using 2 M HCl or 2 M

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