Bioresource Technology 221 (2016) 324–330

Contents lists available at ScienceDirect

Bioresource Technology

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

Biohydrogen and methane production via a two-step process using an acid pretreated native microalgae consortium

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HIGHLIGHTS

- A native microalgae consortium was pretreated using thermal-acidic hydrolysis.
- Hydrogen and methane were produced sequentially with the acidic hydrolysates.
- The lower acid concentration gave the highest H_2 and CH_4 production.
- H_2 and CH_4 yields were up to 45.4 and 432 mL g VS^{-1}, respectively.

ARTICLE INFO

Article history:
Received 21 July 2016
Received in revised form 7 September 2016
Accepted 11 September 2016
Available online 13 September 2016

Keywords:
Dark fermentation
Hydrogen
Methane
Acidic hydrolysis
Microalgae
Consortium

ABSTRACT

A native microalgae consortium treated under thermal-acidic hydrolysis was used to produce hydrogen and methane in a two-step sequential process. Different acid concentrations were tested, generating hydrogen and methane yields of up to 45 mL H_2 g VS^{-1} and 432 mL CH_4 g VS^{-1}, respectively. The hydrogen production step solubilized the particulate COD (chemical oxygen demand) up to 30%, creating considerable amounts of volatile fatty acids (up to 10 g COD L^{-1}). It was observed that lower acid concentration presented higher hydrogen and methane production potential. The results revealed that thermal acid hydrolysis of a native microalgae consortium is a simple but effective strategy for producing hydrogen and methane in the sequential process. In addition to COD removal (50–70%), this method resulted in an energy recovery of up to 15.9 kJ per g of volatile solids of microalgae biomass, one of the highest reported.

1. Introduction

The energy crisis has driven a search for renewable fuels that can be produced using substrates such as photosynthetic biomass or wastes. The term microalga often generalizes all photosynthetic unicellular or simple multi-cellular prokaryotic and eukaryotic microorganisms, such as cyanobacteria, green and red algae, and diatoms. Microalgae are a potential biomass for biofuel production because of their fast growth rate, their lipid and carbohydrate content, and their cultivation in wastewater, which is coupled to their effective role in nutrient removal. When compared to terrestrial crops, microalgae cultures consume less water, reaching higher productivities per culture area, and do not compromise the production of food (Brennan and Owende, 2010). In this sense, a sustainable biofuel production based on microalgae is only possible under a biorefinery approach, producing gaseous biofuels and other value-added products from microalgae biomass (Sarkar et al., 2015).

Microalgae cultivation in wastewater will promote the development of a consortium, contrasting with the mono-algal cultures evaluated in most studies of fuel production. The importance of evaluating a native microalgae consortium lies in the wide diversity of cell wall composition among microalgae species (Domozych et al., 2012), implying different grades of resistance between species. The high carbohydrate content in microalgae makes them a suitable substrate for fermentative fuel production, producing fuels such as biohydrogen, bioethanol, and methane. However, carbohydrates are difficult to extract from microalgae because they are part of the microfibrillar polysaccharides embedded in matrix of polysaccharides and proteoglycans, making necessary a pretreatment step to liberate them (Domozych et al., 2012; Günerken et al., 2015). Different pretreatment technologies have been suggested to break down complex biopolymers in microalgae cells, among them biological, mechanical or chemical.

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http://dx.doi.org/10.1016/j.biortech.2016.09.050
0960-8524/

Biological pretreatments involve the cell degradation by purified enzymes or by microorganisms with enzymatic activity capable of hydrolyzing the microalgae cell wall (Carrillo-Reyes et al., 2016). In this sense, significant differences were observed in the fermentative step when microalgae biomass (*Chlorella vulgaris*) was used directly or received an enzymatic pretreatment. Specifically, the former resulted in a yield of 11.3 mL H_2 g(volatile solids) VS^{-1} (Lakaniemi et al., 2011), whereas enzymatic pretreatment produced 135 mL H_2 g VS^{-1} (Wieczorek et al., 2014).

Methane production from microalgal biomass has been improved by applying pretreatments to solubilize the microalgae and digest their organic content. For instance, applying a thermal pretreatment there was an increase of 50% in the methane production from *Chlorella vulgaris* (Mendez et al., 2014).

Chemical pretreatment has an economic advantage over enzymatic pretreatment or thermal pretreatments; however, its application to native microalgae biomass for hydrogen and methane production is still limited (Passos et al., 2014). To the best of our knowledge, the scarce works applying chemical pretreatments are combined with harsh physical disruption strategies, such as ultrasonic or high pressure (Cheng et al., 2014; Liu et al., 2012; Yun et al., 2013). Among chemical pretreatments for microalgae, acidic hydrolysis has been successful in carbohydrate recovery for bioethanol production. For instance, thermal-acidic hydrolysis, under optimized conditions, achieved 95.6% sugar extraction from *Scenedesmus obliquus* (Miranda et al., 2012), and 97% from *Chlorella vulgaris* (Ho et al., 2013). Regarding biohydrogen production, acidic hydrolysis recovered almost 100% of the carbohydrate concentrations as reducing sugars; however, this procedure has only been optimized for pure microalgae strains (Liu et al., 2012), which is different from the microalgae consortium that could be recovered from wastewater treatment. Moreover, the acidic hydrolysate concentration is a key parameter to evaluate for increasing the specific hydrogen-producing potential from microalgae biomass, since it has been observed the generation of inhibitors such as furans and 5-hydroxymethyl furfural (HMF) (Yun et al., 2013).

Two-step processes have been proposed to improve the energetic gain from microalgal biomass (Yang et al., 2011; Lü et al., 2013; Wieczorek et al., 2014). In such processes, the carbohydrates are first fermented producing hydrogen and volatile fatty acids (VFA). Then, in a second step, the VFA are easily digested under methanogenic conditions to generate methane. This two-step strategy has been applied in lipid-extracted microalgal biomass residues increasing the methane yield by 22% (Yang et al., 2011), and up to 67% compared to methanogenesis using a single step (Wieczorek et al., 2014). Lü et al. (2013) found a 9.4% increase in the energy yield in a two-step process when compared with the one-step process, using bacterial bioaugmentation. Despite the advances on microalgal pretreatments, most of the previously cited works used mono-algal cultures as feedstock, leaving unresolved the potential barriers of hydrolyzing mixed cultures, such as the developed in wastewater.

Therefore, the aim of the present work was to evaluate the energy recovery through the hydrogen and methane production using a two-step process. A thermal acidic pretreated native microalgae consortium was evaluated under different hydrolysate conditions.

2. Materials and methods

2.1. Microalgae biomass

A native microalgae consortium enriched from a local lake in Queretaro, Mexico (20°42′07.0″N 100°27′36.7″W) was used as the biomass source. The microalgae culture was enriched in Bold's medium in tubular plastic bags (8 L) as reactors illuminated by 12 h light-dark cycles supplied via a 54 W daylight neon lamp with an intensity of 100 μmol m^{-2} s^{-1} (LT 300, Extech Instruments, Nashua, NH, USA) and aeration flow rate of 1 L min^{-1} (Cea-Barcia et al., 2014). The culture composition was determined by optical microscopy (Leica DM500, Japan), and direct counting was performed with a 0.1 mm Neubauer chamber (Wehr and Sheath, 2003). The main genera identified was *Scenedesmus* (79%), with the remaining microalgae belonging to *Keratococcus* (19%), *Oscillatoria* and undetermined species (<2%). The microalgal culture was concentrated by centrifugation (4500 rpm, 10 min). Biochemical fractioning of the microalgae consortium revealed that biomass was composed of 20% of carbohydrates, 19% of lipids and 50% of proteins.

2.2. Thermal-acidic pretreatment

The microalgae biomass was hydrolyzed in 2% HCl solution (v/w) at 40 g TS (total solids) L^{-1}, heated at 90 °C for 2 h with constant mixing at 300 rpm in a total volume of 300 mL. After the pretreatment, the hydrolysate was neutralized with 10 N NaOH. The pretreatment protocol was carried out in triplicate to evaluate its reproducibility. After evaluating different dilutions of 2% HCl hydrolysates, microalgae biomass was hydrolyzed with 1% HCl using 20 and 10 g TS L^{-1}, at the same temperature and mixing conditions. Hydrolysis conditions were based on a previous work that optimized the saccharification using dilute acid hydrolysis for microalgae biomass (Castro et al., 2015).

2.3. Hydrogen- and methane-producing inoculum

The inoculum for hydrogen and methane tests was a granular anaerobic sludge from a digester treating wastewater from the brewery industry; the sludge had a solids content of 27 g TS L^{-1} and 19 g volatile solids (VS) L^{-1}. Prior to its utilization for hydrogen tests, a thermal pretreatment was applied to the inoculum (105 °C, 24 h) to select those hydrogen-producing bacteria capable of sporulating. Then, the dried sludge was ground with a mortar to homogenize, and the resulting powder was used as inoculum (Buitrón and Carvajal, 2010). For methane tests, the granular anaerobic sludge was kept under endogenous conditions for 2 weeks to reduce the remaining substrate and the exogenous biogas generation. No further treatment was applied to that sludge.

2.4. Hydrogen production batch tests

Hydrogen production batch tests were performed in triplicate in sealed 120 mL serum bottles in a 60 mL working volume and 6.7 g VS L^{-1} of inoculum. The specific hydrogen production and rate were evaluated using two different acid concentrations for the hydrolysate. In a first round, three dilutions of 2% HCl hydrolysates were tested and named according to the final solid concentrations obtained as 40, 20 and 10 g TS L^{-1}. Then, a second set of experiments using 1% HCl hydrolysates was evaluated. Here, the solids concentrations of 20 and 10 g TS L^{-1} were selected. The mineral medium composition was described previously (Mizuno et al., 2000). The initial pH was adjusted to 6.5 with 5 N HCl or 5 N NaOH. The head space was purged with N_2 for 1 min to ensure anaerobic conditions, incubating the bottles at 36 °C with 150 rpm of horizontal shaking (WiseCube, Daihan Scientific Co., Korea) until the gas production stopped. Blank tests containing only inoculum and mineral medium were carried out to determine the endogenous hydrogen production from the inoculum. Gas production was measured daily by the liquid displacement method (an acidic