Renewable Energy 75 (2015) 1–7

Contents lists available at ScienceDirect

Renewable Energy

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

Effect of inoculum concentration, pH, light intensity and lighting regime on hydrogen production by phototrophic microbial consortium

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ARTICLE INFO

Article history:
Received 22 October 2013
Accepted 18 September 2014
Available online

Keywords:
Phototrophic bacterial consortium
Culture conditions
Organic acids
COD removal efficiency

ABSTRACT

Photobiological hydrogen production using a bacterial consortium has advantages over pure cultures regarding application to wastewater treatment. Photo-H_2 production from organic acids, which were produced by dark fermentation, must be studied in detail to make a hydrogen production process in two stages efficient. In this scenario, our study aimed to determine the optimized culture conditions for hydrogen production by a phototrophic bacterial consortium. The inoculum concentration, pH, light intensity and illumination protocols were the parameters that we evaluated. The optimal conditions for hydrogen production were inoculum concentration of 0.2 g VSS L^{-1}, pH 7.0, light intensity of 5 klux, with a constant illumination regime. The highest hydrogen production potential (P) and substrate conversion efficiency (SCE) were 41.5 mmol H_2 L^{-1} culture and 25.4%, respectively, with a COD removal of 95%.

1. Introduction

Considering the environmental damage caused by the combustion of fossil fuels, researchers have been looking for a new and sustainable energy system [1]. H_2 is considered a clean nonpolluting fuel that can be produced biologically through photosynthetic and fermentative pathways [2]. Purple non-sulfur bacteria (PNS) (*Rhodobacter*, *Rhodopseudomonas*, *Rhodospirillum* and *Rubrivivax*) can produce hydrogen through photoheterotrophic metabolism by organic acid consumption (acetate, butyrate and propionate). This process is an attractive alternative because dark fermentation effluents containing such acids can be used as substrate for PNS bacteria to produce H_2 simultaneously to chemical oxygen demand removal (COD) [3].

The type and amount of organic acids produced during dark fermentation depends mainly on the metabolic pathway carried out by different bacterial species [4]. Acetate and butyrate has been identified as the main fermentation products; lactate, propionate and ethanol have been reported in fewer amounts [3]. Specialized literature indicates that *Rhodopseudomonas palustris* WP3-5 was able to produce hydrogen from acetate, butyrate and lactate individually [5] and also from a mixture of acetate and butyrate [6]. Other PNS bacteria, *Rhodobacter sphaeoides* O.U.001, grew and produced hydrogen from a mixture of acetate, butyrate and propionate [3]. *Rubrivivax gelatinosus* were able to grow on acetate and butyrate; however, the hydrogen production occurred in the presence of glucose and lactate as the sole carbon sources [7]. The feasibility of hydrogen production was also assessed by phototrophic sludge from a mixture of acetate, butyrate and ethanol [8].

The main challenge of the process is to enhance the hydrogen yield (HY) and hydrogen production rate (HPR) making it economically viable. Thus, is necessary to study and evaluate the effects of as many culture conditions as possible [9]. The concentration of the inoculum is an important parameter because an excess of biomass can cause self-shading by reducing light penetration [10,11]. pH is another parameter that influences the hydrogen production (HP) by altering the enzymatic activity and the biochemical reactions [12]. Furthermore, HP in large-scale outdoor under natural sunlight is desirable. Thus, to carry out experiments under light intensity and lighting regime as close as possible to outdoor conditions is therefore recommended [9]. The effect of various parameters has been studied, but there are discrepancies among the results obtained, even for different strains of the same bacterial species, and further variation among species will probably be encountered [10].

The previous studies primarily used pure cultures to investigate the effects of those parameters on the performance of HP [3,6,9,11,13–19], however for scale-up it is not practical. Although a few studies evaluated HP using mixed cultures [20–23], the use of a microbial consortium could be advantageous over pure cultures in wastewater biotechnology processes [24]. The present study

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http://dx.doi.org/10.1016/j.renene.2014.09.034
0960-1481/

therefore aimed to optimize culture conditions (concentration of inoculum, pH, light intensity and lighting regime) to enhance photobiological HP by PHPBC using a mixture of acetate and butyrate in batch reactors.

2. Material and methods

2.1. Inoculum, culture medium and culture conditions

The inoculum was PHPBC composed by microorganisms related to the genera *Rhodobacter*, *Rhodospirillum*, *Rhodopseudomonas* and *Sulfurospirillum*, that was provided by Lazaro et al. [21]. Prior to the HP experiments the bacterial consortium was submitted to an adaptation phase, in which the cells grew on a mixture of carbon sources described hereafter. The culture medium was prepared using the following compounds (g L^{-1}): 0.6 KH_2PO_4; 0.9 K_2HPO_4; 0.2 $MgSO_4$; 0.4 NaCl; 0.05 $CaCl_2$; 0.2 yeast extract; 5 mL L^{-1} of iron citrate solution (0.1 g 100 mL^{-1}); 1 mL L^{-1} of trace metal solution described by Lee et al. [25]; 0.8 mL L^{-1} of HCl (30%); 0.1 (mg L^{-1}) vitamin B_{12}. Carbon sources were 1.93 g L^{-1} of sodium acetate trihydrate and 1.4 g L^{-1} of sodium butyrate.

The experiments were carried out in glass bottles with a total volume of 0.6 L (0.2 L working volume) that were kept inside an incubator with a controlled temperature (30 °C) and constant illumination by tungsten lamps (60 W) or different (when mentioned in the text). The light intensity was measure at the surface of the bottles, see Table 1. N_2 gas was flushed for 10 min to create anaerobic conditions in the headspace prior to incubation. The pH adjustment was made with NaOH solution (2 M) or HCl (30%). Optical density (OD) equal to 1 (OD at 660 nm) correspond to 0.4 g VSS L^{-1}. Each set of experiments was performed in triplicate. A summary of the experimental conditions is presented in Table 1.

Table 1
Experimental conditions.

Runs	Parameters evaluated			
	Inoculum concentration (g VSS L^{-1})	pH	Light intensity (klux)	Lighting regime
Run 1	0.03	6.5	5	24 h light
Run 2	0.2			
Run 3	0.4			
Run 4	0.6			
Run 5	0.2	5.0	5	24 h light
Run 6		6.0		
Run 7		7.0		
Run 8		8.0		
Run 9	0.2	7.0	3	24 h light
Run 10			5	
Run 11			8	
Run 12	0.2	7.0	5	12 h light − 12 h dark
Run 13				18 h light − 6 h dark
Run 14				24 h light

2.2. Chemical and chromatographic analysis

The H_2 content in the biogas was determined by gas chromatograph [26]. Acetic and butyric acid was measured by high-performance liquid chromatograph [26]. COD and VSS (volatile suspended solids) were measured according to Standard Methods [27]. The OD was measured in a spectrophotometer (Hach DR/2010) at 660 nm. The light intensity was measured using a digital luximeter (Testo 545).

2.3. Experimental data fitting

The experimental data were fit to the mean values obtained from the triplicates using Statistica® software (version 8). The maximum HPR was obtained by nonlinear sigmoidal adjustment of the modified Gompertz function [28].

3. Results and discussion

HP varies according to the carbon source. Considering acetate and butyrate, the sources applied in the present study, the theoretical hydrogen yield is 4 mol of H_2 for each mol of acetate (equations (1) and (10) moles of H_2 for each mol of butyrate (equation (2)). Thus, in case of a mixture using both substrates, the theoretical yield will be 14 mol of H_2.

$$C_2H_4O_2 + 2H_2O \rightarrow 4H_2 + 2CO_2 \quad (1)$$

$$C_4H_8O_2 + 6H_2O \rightarrow 10H_2 + 4CO_2 \quad (2)$$

Besides the theoretical HY, it is possible to calculate the SCE (equation (3)), to evaluate the microbial HP based on the stoichiometric conversion of each substrate.

$$SCE(\%) = \frac{\text{moles of } H_2 \text{ that have actually been produced}}{\text{moles of } H_2 \text{ expected through stoichiometric equation}}(100) \quad (3)$$

To produce H_2, PNS bacteria are influenced by several culture conditions and the present study evaluated the effect of inoculum concentration, initial pH, light intensity and lighting regime to determine an optimal condition for biological HP by the PHPBC. The influence of each condition will be discussed in detail hereafter.

3.1. Effect of initial biomass concentration

Regarding to inoculum concentration effect, the first known point is that excessive cell density leads to a decrease in the substrate/microorganism (S/M) ratio that causes a shortage of substrate to support microbial metabolism. In addition, the excess of cells can favor the formation of cell aggregates, which can hamper the penetration of light due to the self-shading effect or can even limit the diffusion of substrate into bacterial flocs [10].

It was observed that hydrogen production potential (HPP) and SCE increased slightly from 25 mmol H_2 L^{-1} culture and 13.7 % to 25.9 mmol H_2 L^{-1} culture and 14.6% with an increase in the cell concentration from 0.03 to 0.2 g VSS L^{-1}. However, a further increase in biomass concentration (0.4 and 0.6 g VSS L^{-1}) caused a decrease either in the HPP and SCE that reached the lowest value (12.7 mmol H_2 L^{-1} culture and 10.3%) at a biomass concentration of 0.6 g VSS L^{-1} (Table 2).

According to Argun et al. [29] the highest HP (19.85 mmol H_2 L^{-1} culture) was obtained at 1.1 g L^{-1} cell concentration. Ren et al. [30] achieved the highest SCE (51.5%) at an inoculant volume of 10% (v/v). Kim et al. [10] observed that HP was positively proportional to