Available online at www.sciencedirect.com

SciVerse ScienceDirect

http://www.elsevier.com/locate/biombioe

Energy requirement and CO_2 emissions of bioH_2 production from microalgal biomass

A.F. Ferreira [a,*], J. Ortigueira [b], L. Alves [b], L. Gouveia [b], P. Moura [b,c], C.M. Silva [a]

[a] IDMEC – Instituto Superior Técnico, Tecnical University of Lisbon, Av. Rovisco Pais, 1, 1049-001 Lisbon, Portugal
[b] LNEG – National Laboratory of Energy and Geology, Bioenergy Unit, Estrada do Paço do Lumiar 22, 1649-038 Lisbon, Portugal
[c] Instituto Superior de Ciências da Saúde Egas Moniz, Quinta da Granja, Monte de Caparica, 2829-511 Caparica, Portugal

ARTICLE INFO

Article history:
Received 18 May 2012
Received in revised form
7 November 2012
Accepted 21 December 2012
Available online 29 January 2013

Keywords:
Scenedesmus obliquus
Dark fermentation
Clostridium butyricum
Biohydrogen
Life cycle inventory

ABSTRACT

This paper presents the life cycle inventory (LCI) of hydrogen production by *Clostridium butyricum* fermentation of *Scenedesmus obliquus* hydrolysate. The main purpose of this work was to evaluate the potential of H_2 production from microalgal biomass and the respective energy consumption and CO_2 emissions in the bioconversion process considering the microalga production, acid hydrolysis of *S. obliquus* biomass, preparation of the inoculum and culture media, and fermentation. The scale-up to industrial production was not envisaged.

The hydrogen yield obtained in this work was 2.9 ± 0.3 mol H_2/mol sugars in *S. obliquus* hydrolysate. Results show that this process of biological production of hydrogen can achieve 7270 MJ/MJ_{H_2} of energy consumption and 670 kg CO_2/MJ_{H_2}. The microalgal culture is the stage responsible for 98% of these total final values due to the use of artificial lighting. All stages and processes with the highest values of energy consumption and CO_2 emissions were identified for future energetic and environmental optimisation.

1. Introduction

The growing consumption of fossil fuels that consequently causes serious negative environmental impacts worldwide is at the center of global climate change policies discussion, aiming to implement new and alternative solutions to respond to these concerns. New energy sources like biofuels have been regarded as a potential commodity to reduce fossil fuel dependence [1]. The other potential solution is hydrogen that appears as an alternative fuel and "energy carrier". About 450 billion m^3 of hydrogen [2] are currently produced and consumed worldwide but mostly as raw material for the production of various chemicals rather than as a fuel itself. Hydrogen is mainly produced from natural gas (central steam reforming), oil, coal and water [2,3], although it can also be produced by biological processes such as dark and photo fermentation [4–8].

Microalgal biomass constitutes a potential source of renewable feedstock, as it can be used as substrate for the biological conversion into biofuels and biogas [9–11]. *Scenedesmus obliquus*, a robust microalga with good productivity rates, has been proved to be very versatile as a raw material for biofuels production (biodiesel, bioethanol and biohydrogen). This green microalga contains approximately 12–14% of oil and 10–17% of sugar [12] and is therefore a good source for biodiesel [11,13–15], bioethanol [16,17] and hydrogen production [18,19]. Starch is one of the major intracellular storage carbohydrate in microalgae and constitutes an important substrate for fermentation processes, e.g. bioethanol and biohydrogen production [15]. Zachleder et al. (1988) [20] found that starch

* *Corresponding author.*
E-mail address: filipa.ferreira@ist.utl.pt (A.F. Ferreira).
0961-9534/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biombioe.2012.12.033

accumulation occurred in *Scenedesmus* and that, in general, starch biosynthesis is increased under starvation. Moreover, Takeda (1996) [21] identified the major sugars in the rigid wall of *Scenedesmus* as being glucose, mannose and galactose, easily fermentable sugars after hydrolysis. In a study done by Miranda et al. (2012) [17], *S. obliquus* biomass accumulated a starch content of 24% g eq_{glu}/g dw (glucose equivalents) (glucose representing 70% of the total sugar content), corresponding to a total sugar production of 0.153 g eq_{glu}/L after 22 days of cultivation. Besides the fact that microalgae cultivation does not compete with arable land, the possibility of using waste water for growth and daily harvesting, reinforces the potential of the *S. obliquus* biomass as fermentation substrate [22].

Clostridium species are frequently found in hydrogen-producing bacterial consortia and are also very effective in producing H_2 from organic substrates, especially carbohydrates [23]. There are several studies about biohydrogen production by *Clostridium* sp. that have been published, reporting yields of 0.73–3.1 mol H_2/mol sugar [4,24–31].

Exhaustive work has been done by other authors on hydrogen production by biological methods with alternative feedstock [32]. Most recently, studies on biofuels production by microalgae, namely biodiesel, are being widely developed in terms of energy and CO_2 assessment, including their economic evaluation (e.g. [23,26]).

However, none of the previous studies covers both biohydrogen production from microalga and energy and CO_2 emission analysis. The most similar study to this approach was recently done using cyanobacteria for the photoautotrophic production of biohydrogen [33] through a photoautotrophic process and additional dark fermentation.

Given the expected market penetration of hydrogen technologies and the fact that the relative environmental impacts of biological hydrogen production systems have not been scientifically established to date, there is still a need to produce reliable impact studies on the issue [33,34].

The methodology used in this work follows the international standards for Life Cycle Assessment (LCA) ISO 14040–14041, as closely as possible. The findings clearly show the relative environmental and energetic magnitude of each life cycle phase, providing the feedback required to focus future critical processes.

This paper presents experimental results of biohydrogen production from the sugars contained in *S. obliquus* hydrolysate by means of dark fermentation using *Clostridium butyricum*. It was also evaluated the H_2 yield, the energy consumptions and CO_2 emissions during the whole bioconversion process, using the LCI methodology.

2. Methodology

2.1. *Hydrogen production by* C. butyricum *from the biomass of* S. obliquus

2.1.1. *Production of microalgal biomass*

The growth of *S. obliquus* was performed in Bristol culture medium, in a 5 L closed photobioreactor agitated by compressed filtered air, for 18 days [16]. The alga was grown with continuous irradiance, with 72 W (4 lamp × 18 W/BRL) of lamp intensity and the temperature was kept constant at 25 °C. Bristol medium contains (g/5 L) $NaNO_3$ (2.5 g), K_2HPO_4 (0.75 g), $CaCl_2.H_2O$ (0.33 g), $MgSO_4.7H_2O$ (0.75 g), NaCl (0.25 g), Fe-EDTA (0.3 g), KH_2PO_4 (1.75 g), H_3BO_3 (2.86 g), $MnSO_4.4H_2O$ (2.03 g), $ZnSO_4.7H_20$ (0.22 g), $Na_2MoO_4.2H_2O$ (0.06 g), $CuSO_4$ (0.05 g) and $CoCl_2.7H_2O$ (0.09 g). The microalgal biomass was collected and recovered by decantation and centrifugation, and dried (<10% (w/w) H_2O) before hydrolysis.

2.1.2. *Acid hydrolysis of* S. obliquus *biomass and biohydrogen production*

Dried microalgal biomass was introduced into an acid-resistant flask and suspended in 1 N sulphuric acid at a 1:10 mass/volume ratio. This mixture was submitted to a high temperature treatment of 121 °C during 30 min. The resulting suspension was allowed to cool to room temperature before the pH value was adjusted to 7.0 with 2 M sodium hydroxide solution [16]. After this step, the suspension was centrifuged at 8500 rpm for 10 min for the separation of algal residues. The supernatant was sterilised by filtration and diluted 1:2 with concentrated Basal Medium (BM1) to serve as carbon and energy source in the fermentation experiments. BM1 was prepared according to Moura et al. (2007) [35]. An overnight grown culture of *C. butyricum* DSM 10702 (DSMZ, Germany) was inoculated at 1% (v/v) in BM1 medium supplemented with *S. obliquus* hydrolysate and the fermentation was conducted for 144 h at 37 °C.

Table 1 shows the inputs of all the experimental stages comprising microalgae production, preparation of pre-inoculum, acid hydrolysis of *S. obliquus* biomass, preparation of the fermentation medium and stock solutions, and fermentation by *C. butyricum*.

2.1.3. *Analytical methods*

Sugars and sugar degradation products in the hydrolysate were quantified by HPLC (LaChrom L7100, Merck) equipped with a refraction index (RI) detector (LaChrom L7490, Merck). Sugars were quantified in an Aminex HPX-87P column at 90 °C, elution was performed with purified water, at a flow rate 0.6 mL min^{-1}. Sugar degradation products and organic acids were quantified in an Aminex HPX-87H column at 50 °C, with the use of 5 mM H_2SO_4 as mobile phase, at a flow rate of 0.4 mL min^{-1}. Device calibration was made individually with the use of external standard concentration solutions.

The biogas produced by *C. butyricum* was characterised in a gas chromatography device (Varian CP-3800) equipped with a thermal conductivity detector (TCD). H_2 and CO_2 analysis was performed with the use of a stainless steel column (1/8″ × 3), packed with Porapak S and N_2 as carrier gas (flow rate of 4 psi). GC column was kept at 30–60 °C, the injector at 60 °C and the TCD at 150 °C.

The calculation of hydrogen yield was based on the ratio of mol of H_2 produced by mol of glucose, arabinose, galactose, mannose and xylose in *S. obliquus* hydrolysate.

2.2. *Energy consumption and* CO_2 *emissions*

During the stages of hydrogen production there are energy demands, mainly of electricity, and associated CO_2 emissions. Fig. 1 shows the microalgal biomass production and whole fermentation process and corresponding *inputs*.