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Energetic and environmental evaluation of microalgae biomass fermentation for biohydrogen production

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Abstract

This paper presents an energetic and environmental evaluation of the fermentative hydrogen production from the sugars of *Scenedesmus obliquus* biomass hydrolysate by *Clostridium butyricum.* The main purpose of this work was to evaluate the potential of H_2 production and respective energy consumptions and CO_2 emissions in the global fermentation process: hydrolysis *of S. obliquus* biomass, preparation of the fermentation medium, degasification and incubation. The scale-up to industrial production was not envisaged.

Energy consumption and CO_2 emissions estimations were based on SimaPro 7.1 software for the preparation of the fermentation medium and the use of degasification gas, nitrogen. The functional unit of energy consumption and CO_2 emissions was defined as MJ and grams per 1 MJ of H_2 produced, respectively. The electricity consumed in all hydrogen processes was assumed to be generated from the Portuguese electricity production mix. 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 consumed 281-405 MJ/MJ_{H2} of energy and emitted 24-29 $kgCO_2/ MJ_{H2}$. The fermentation stages with the highest values of energy consumption and CO_2 emissions were identified for future energetic and environmental process optimisation.

Keywords: *biohydrogen; Scenedesmus obliquus; energy consumption; CO_2 emissions; Clostridium butyricum.*

1 Introduction

Presently, fossil fuels are at the center of global climate changes originating serious negative environmental impacts worldwide. In 2009, this energy source accounted for around 80% of the Portuguese primary energy consumption; oil (48.7%), coal (11.8%) and natural gas (17.5%) being the major fuel sources, whereas renewable energy sources accounted for the remaining 20% of energy consumption. The highest consumption sector was the road transportation, which represented approximately 38% of the total energy consumption in Portugal in 2009, and was responsible for about 31% of CO_2 emissions [1-2]. The final energy consumption in Portugal was 17499 ktoe in 2009,

showing a 3% decrease regarding 2008. Oil, electricity and natural gas have shown decreases of 2.8%, 0.9% and 8.4%, respectively, by replacement with renewable energy. Biofuels have been regarded worldwide as a potential commodity to reduce fossil fuel dependence. The 2003/30/EC European Directive aims to promote the use of biofuels and other renewable fuels instead of diesel or oil for transport purposes in each member state. In long term this is expected to contribute to the fulfillment of European climate change agreements [3].

Hydrogen appears as an alternative fuel and "energy carrier". As much as 450 billion m^3 of hydrogen are currently produced and consumed worldwide but mostly as raw material for the production of a variety of chemicals rather than as a fuel itself. Hydrogen is mainly produced from natural gas, oil, coal and water [4], though it can also be produced by biological processes, such as photo fermentation and dark fermentation [5-9]. Microalgae biomass constitutes a potential source of renewable feedstock, as it can be used as substrate for the biological conversion into biofuels and biogas [10]. *Scenedesmus obliquus* is a green microalgae that contains approximately 12–14% of oil and 10–17% of sugar [11] being promising for biodiesel and hydrogen production.

Table 1: Brief literature review of biohydrogen production by *Clostridium* sp.

Inoculums	Sugar	Fermentation type	H_2 Yield *	Ref.
C.acetobutylicum	Glucose	Batch	2.0	[5]
C.acetobutylicum	Glucose	Continuous	1.1	[5]
C. acetobutylicum	Xylose	Batch	0.7	[5]
C.pasteurianum	Sucrose	Batch	2.1	[5]
C.thermolaticum	Lactose	Continuous	3.0	[5]
C.butyricum	Glucose	Continuous	1.4-2.3	[12]
C.butyricum	Sucrose	Batch	2.78	[13]

*mol_{H2}/mol_{sugar}

Clostridium species are frequently found in hydrogen-producing bacterial consortia and are also very effective in producing H_2 from organic substrates, especially carbohydrates [14]. Several studies on biohydrogen production by *Clostridium* sp. have been published (Table 1), reporting yields of 1.1–2.8 mol H_2/mol sugar [5, 12-13].

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 [15, 16].

This paper presents experimental results of biohydrogen production from the sugars of *Scenedesmus obliquus* hydrolysate by *Clostridium butyricum* and evaluates the H_2 yield, respective energy consumptions and CO_2 emissions during the whole production process.

2 Methodology

Experimental methods

S. obliquus biomass was hydrolysed with 1N H_2SO_4 at 121ºC for 30 min followed by neutralisation with NaOH. The fermentation medium (BM1) was prepared according to Moura *et al.* (2007) [17] using *S. obliquus* hydrolysate as carbon and energy source. Fermentation was conducted during 144 h at 37ºC. Sugars and sugar degradation products in the hydrolysate (Table 2) were analysed by HPLC (Merck, L7100).

Table 2 – Composition in sugars and sugar degradation products of *S. obliquus* hydrolysate (g/L)

	S. obliquus hydrolysate (g/l)
Glucose	5.7
Arabinose	1.0
Galactose	0.7
Mannose	1.9
Xylose	0.9
Formic acid	n.d.
Furfural	n.d.
HMF	n.d.

HMF – hydroxymethylfurfural
n.d. – not detected

Biogas produced by *C. butyricum* fermentation was analysed through GC (Varian CP 3800) equipped with a TCD. Figure 1 shows the scheme of all the experimental stages.

Energy Consumption and CO_2 emissions

During the stages of hydrogen production there are energy demands, mainly of electricity, and associated CO_2 emissions. Figure 2 shows the scheme of the whole fermentation process and corresponding *inputs*. The main stages considered were the preparation of the fermentation medium, which included BM1 preparation and hydrolysis of microalgae biomass, degasification and incubation. Table 3 shows the respective energy consumptions and CO_2 emissions.

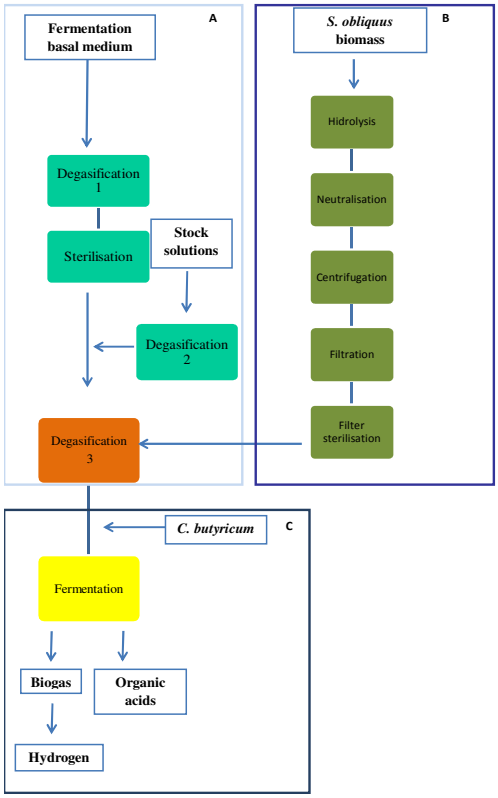

Figure 1: Scheme of the experimental stages of the whole fermentation process: (A) BM1 medium preparation, (B) Biomass hydrolysis and (C) Fermentation.

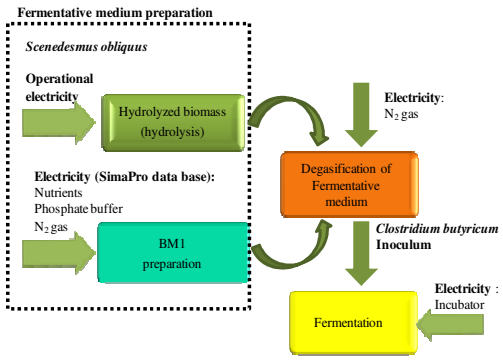

Figure 2: Scheme of the fermentation process and corresponding *inputs*.

The consumed electricity is assumed to be obtained from the Portuguese electricity production mix which is composed by 65 % of non renewable energy and 35 % of renewable energy (2009 data), with 8 % of energy losses in distribution [18-19].
The resulting energy consumption and CO_2 emissions per 1 MJ of electricity produced are 1.27 (0.98-1.41) MJ and 95.13 (84.62-101.88) g respectively, representing an electricity mix efficiency of 44%. The uncertainty of the Portuguese electricity generation mix considered minimum and maximum deviation values for each energy source based on the Concawe study [20]. Only operational processes were accounted i.e. equipment production, storage, production of microalgae biomass and pre-inoculum preparation were not included. The remaining energy inputs, from the equipment used, were derived by device specifications and working hours.

Hydrolysys
The energy requirements for weighing was estimated by the decimal balance power, 11W, multiplied by the working time, 15 minutes, and electricity conversion factor:

$$E_{weighing} = P_{decimal\ balance} * \Delta t * 1.27\ (MJ) \qquad (Eq.\ 1)$$

The acid hydrolysis of *S. obliquus* biomass was performed in a 6000W autoclave for 30 minutes:

$$E_{hidrolysis} = P_{autoclave} * \Delta t * 5.08 * 10^{-4}\ (MJ) \qquad (Eq.\ 2)$$

The capacity factor of $5.08*10^{-4}$ was obtained by dividing the volume used (30 ml) by the total autoclave capacity (75 l) and multiplied by the electricity conversion factor.
An agitation with 600W was used during the neutralisation process, for 10 minutes.

$$E_{agitation} = P_{shaker\ equipment} * \Delta t * 0.003\ (MJ) \qquad (Eq.\ 3)$$

The capacity factor of 0.003 was obtained by dividing the volume used (20 ml) by the total shaker capacity (10 l) and multiplied by the electricity factor.
The energy requirements for centrifugation were estimated by the centrifugue power (155.6 W, 8500 rpm) multiplied by the working time (10 min) and capacity factor:

$$E_{centrifugation} = P_{centrifuge@8500rpm} * \Delta t * 0.173\ (MJ) \qquad (Eq.\ 4)$$

The capacity factor of 0.173 was obtained by dividing the volume of the centrifuge used (30 ml) by the maximum operational volume (220 ml) and multiplied by the electricity conversion factor.
For filtration, the pump potential was considered, 180W multiplied by the working time, 1 minute, and multiplied by the electricity conversion factor.

$$E_{filtration} = P_{filtration\ pump} * \Delta t * 1.27\ (MJ) \qquad (Eq.\ 5)$$

Preparation of BM1medium
The energy consumption regarding the nutrients used for the preparation of the fermentation medium and N_2 gas for degasification was determined by the respective energy required for their production, which was based in the SimaPro 7.1 software [21].

$$E_{nutrient,\ N2gas} = E_{Simapro} * 1.27\ (MJ) \qquad (Eq.\ 6)$$

BM1 medium was sterilised in a 6000W autoclave for 15 minutes:

$$E_{sterilisation} = P_{autoclave} * \Delta t * 1.69 * 10^{-4} \text{ (MJ)} \quad \text{(Eq. 7)}$$

The capacity factor of $1.69*10^{-4}$ was obtained by dividing the volume used (10 ml) by the total autoclave capacity (75 l) and multiplied by the electricity conversion factor.

Fermentation

After inoculation with *C. butyricum*, fermentation was conducted in an incubator under 150 rpm (145.3W) for 144 hours.

$$E_{incubation} = P_{incubator@150rpm} * \Delta t * 8.3 * 10^{-5} \text{ (MJ)} \quad \text{(Eq. 8)}$$

The capacity factor of $8.3*10^{-5}$ was obtained by dividing the volume used (10 ml) by the total incubator capacity (154.8 l) and multiplied by the electricity conversion factor.

All energy consumptions estimated for the nutrients of the fermentation medium, N_2 gas, and operational equipments were afected by the Portuguese electricity and CO_2 emission factors, which possess a resulting associated uncertainty, as mentioned above. Table 3 shows the energy results and respective CO_2 emissions.

Table 3: Energy and CO_2 emissions of fermentation

	Energy (MJ)			CO_2 (g)		
	Value	**Min**	**Max**	**Value**	**Min**	**Max**
Hidrolysis:						
Weighing	0.013	0.010	0.014	0.94	0.84	1.01
Acid hydrolysis	0.005	0.004	0.006	0.41	0.37	0.44
Agitation	0.001	0.001	0.001	0.07	0.06	0.07
Centrifugation	0.016	0.013	0.018	1.21	1.08	1.30
Filtration	0.014	0.011	0.015	1.03	0.91	1.10
BM1 Medium:						
Nutrients	0.0005	0.0002	0.0010	0.04	0.02	0.07
Phosp. buffer	0.001	0.001	0.001	0.06	0.06	0.07
N_2 gas(1)	0.019	0.014	0.021	1.40	1.24	1.50
N_2 gas (2)	0.034	0.027	0.038	2.56	2.28	2.74
Sterilisation	0.0009	0.0007	0.0010	0.07	0.06	0.07
N_2 gas(3)	0.031	0.024	0.035	2.33	2.07	2.49
Fermentation:						
Incubation	0.006	0.005	0.007	0.46	0.41	0.50

(1) for degasification of fermentation basal medium
(2) for additional degasification of YNB and cysteine HCl solutions
(3) for degasification of BM1 medium after addition of *S. obliquus* hydrolysate

Rough energy requirements may be summarized by equations 9 and 10:

$$\frac{MJ_{expended}}{MJ_{H2}} = \frac{\sum_i \left[\left(\frac{MJ}{kg_{hydrogen}} \right)_i \right]}{LHV} \quad \text{(Eq. 9)}$$

or

$$\frac{MJ_{expended}}{MJ_{H2}} = \frac{\sum_i \left[\left(\frac{MJ}{kg_{biomass}} \right)_i \right]}{\eta . LHV} \quad \text{(Eq. 10)}$$

LHV stands for the hydrogen low heating value of 120 MJ/kg [22]. Hydrogen density is assumed to be 0.084 kg/m^3 [23] and CO_2 density is assumed to be 1.848 kg/m^3 [24]. In the Equation 10 [16], η represents the hydrogen yield (kg H_2/kg biomass).
In this study we used all the energy requirements which were obtained from the experimental data.

3 Results and Discussion

Table 4 shows the values of H_2 production and maximum yield.

Table 4: Values of H_2 production and respective yield

Inoculum	Production		H_2 yield (mol H_2/mol sugars*)
	Hydrolysate (ml)	BioH2 (mmol)	
C. butyricum	10	1.7 ± 0.2	2.9 ± 0.3

* in Table 2

Hydrogen production reached 1.7 ± 0.2 mmol from 10 ml of microalgae hydrolysate, and the H_2 yield was 2.9 ± 0.3 mol H_2 per mol of glucose, arabinose, galactose, mannose, and xylose quantified in *S. obliquus* hydrolysate. Although the H_2 yield may be slightly over evaluated due to the possible presence of carbon sources which were not quantified by HPLC, it can still be considered competitive when compared with results from the literature (Table 1).
These results will determine the estimation of energy consumptions and CO_2 emissions. Considering the hydrogen production in kg there is a range of $3.24*10^{-06}$ - $3.70*10^{-06}$ kg of hydrogen produced. Considering equation 8, the functional units of energy consumption and CO_2 emissions were defined as MJ and grams per 1 MJ of H_2 produced, respectively. Figures 3 and 4 show the energy consumptions (MJ/MJ_{H2}) and CO_2 emissions (g/MJ_{H2}) of the whole fermentation process. A total energy consumption of 364.3 (281.2-404.9) MJ/MJ_{H2} and 27198 (24149-29218) gCO_2/MJ_{H2} of CO_2 emissions was obtained.

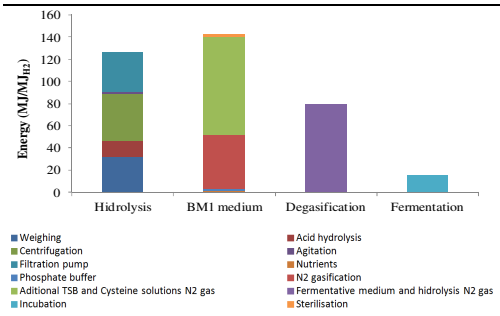

Figure 3: Energy consumption (MJ/MJ_{H2}) of each step of the fermentation process.

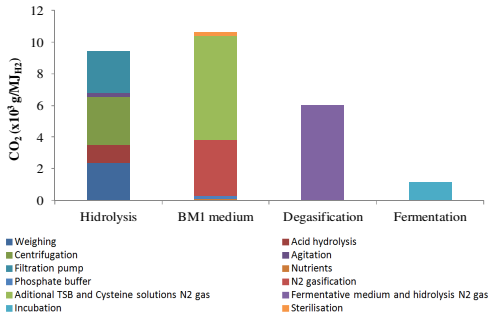

Figure 4: CO_2 emissions (g/MJ_{H2}) of each step of the fermentation process

The preparation of BM1 medium was the stage that consumed more energy and emitted more CO_2, with 39% of contribution to overall energy consumption. Namely, the degasification of stock solutions and of the fermentation medium were the processes which generated the highest values of energy consumption and CO_2 emissions with 80-88 MJ/MJ_{H2} and 5985-6584 gCO_2/MJ_{H2} respectively, which corresponds to 46.2% of the total consumptions and emissions. According to the obtained results and taking into account all the possibilities of process optimisation, the substitution of "degasification 1" (Figure 1) by an unique step of degasification of BM1 medium would be feasible, rendering a 13.2% of electricity savings. Moreover, the use of the whole acid-treated *S. obliquus* biomass as carbon substrate would avoid the steps of centrifugation and filtration for solid-liquid separation, resulting in a further decrease of 21.2% of electricity consumption.
The results obtained in this study are in the same order of magnitude when compared with a recent laboratorial study of the energy and CO_2 balance applied to both photoautotrophic and fermentative H_2 production processes [16]. Nevertheless, there is still a long way to go to reach comparable values to other industrial scale H_2 production pathways, *e.g.* natural steam reforming (NG – 0.83-0.92 MJ/MJ_{H2} and 104.1-108.8 gCO_2/MJ_{H2} [25]) or electrolysis (3.43-3.81 MJ/MJ_{H2} and 200.4-217.5 gCO_2/MJ_{H2}[25]).

4 Conclusions

Biological hydrogen production from the fermentation of the sugars of *Scenedesmus obliquus* biomass hydrolysate by *Clostridium butyricum* produced a hydrogen yield of 2.9 ± 0.3 mol H_2/mol sugars. This H_2 yield was obtained at the expense of 281.2 - 404.9 MJ/MJ_{H2} of energy consumption and 24.0 - 29.0 kg CO_2/MJ_{H2} of CO_2 emissions. The biological process of hydrogen production is still not comparable to the industrial scale H_2 production processes, e.g. natural steam reforming, but innumerous possibilities of process optimisation can be identified for future implementation.

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