ENERGY REQUIREMENTS FOR THE CONTINUOUS BIOHYDROGEN PRODUCTION FROM SPIROGYRA BIOMASS IN A SEQUENTIAL BATCH REACTOR

Joana Ortigueira1*, Ana Ferreira2, Carla Silva2, Luísa Gouveia1 and Patrícia Moura1

1: Unidade de Bioenergia, Laboratório Nacional de Energia e Geologia, Estrada do Paço do Lumiar, 22; 1649-038 Lisboa, Portugal
* e-mail: joanaortigueira@gmail.com

2: IDMEC/LAETA, Instituto Superior Técnico, Universidade de Lisboa, Av. RoviscoPais, 1; 1049-001 Lisboa, Portugal

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Abstract
The current energy market requires urgent revision for the introduction of renewable, less-polluting and inexpensive energy sources. Biohydrogen (bioH₂) is considered to be one of the most appropriate options for this model shift, being easily produced through the anaerobic fermentation of carbohydrate-containing biomass. Ideally, the feedstock should be low-cost, widely available and convertible into a product of interest. Microalgae are considered to possess the referred properties, being also highly valued for their capability to assimilate CO₂ [1]. The microalga Spirogyra sp. is able to accumulate high concentrations of intracellular starch, a preferential carbon source for some bioH₂ producing bacteria such as Clostridium butyricum [2]. In the present work, Spirogyra biomass was submitted to acid hydrolysis to degrade polymeric components and increase the biomass fermentability. Initial tests of bioH₂ production in 120 mL reactors with C. butyricum yielded a maximum volumetric productivity of 141 mL H₂/L.h and a H₂ production yield of 3.78 mol H₂/mol consumed sugars. Subsequently, a sequential batch reactor (SBR) was used for the continuous H₂ production from Spirogyra hydrolysate. After 3 consecutive batches, the fermentation achieved a maximum volumetric productivity of 324 mL H₂/L.h, higher than most results obtained in similar production systems [3] and a potential H₂ production yield of 10.4 L H₂/L hydrolysate per day. The H₂ yield achieved in the SBR was 2.59 mol H₂/mol, a value that is comparable to those attained with several thermophilic microorganisms [3], [4].

In the present work, a detailed energy consumption of the microalgae value-chain is presented and compared with previous results from the literature. The specific energy requirements were determined and the functional unit considered was gH₂ and MJH₂. It was possible to identify the process stages responsible for the highest energy consumption during bioH₂ production from Spirogyra biomass for further optimisation.
1. INTRODUCTION
Biofuels are regarded as a viable alternative to fossil fuels for the production of renewable energy. Special attention has been given to biomass-derived fuels thanks to their renewable and largely non-polluting qualities. Biohydrogen (bioH₂) is one of such fuels, being easily convertible into energy through combustion, a process which yields solely water as sub-product [5]. BioH₂ production can be attained by anaerobic fermentation of carbohydrate-containing biomass and originates a highly rich biogas containing both H₂ and CO₂ [6]. A prime example of a feedstock adequate for bioH₂ production is microalgal biomass. Microalgae are photosynthetic organisms able to assimilate atmospheric CO₂ and store both lipids and carbohydrates in their intracellular space. They are also highly productive allowing for a near daily harvest and, unlike higher plant cultures, require no arable land or potable water [7]. BioH₂ production has already been successfully achieved by the authors, using Scenedesmus obliquus [8], [9], Chlorella vulgaris [10] and Spirogyra sp. biomass [2]. Spirogyra, in particular, is able to accumulate starch, a preferential substrate for anaerobic fermentation by certain bacterial strains, at very high concentrations [2]. In this work, the production of bioH₂ from Spirogyra biomass by Clostridium butyricum was evaluated in small-scale batch reactors and a bench-scale sequential batch reactor. Both processes were compared in terms of their H₂ yield, production rate and overall energy consumption.

2. MATERIALS AND METHODS
The Spirogyra biomass used in this work had the following average composition (% (w/w) dry weight basis): 45.1% total sugars, 22% crude protein, 3.6% fat, 25.9% ash and 3.4% others (by difference). The microalga was cultured and harvested as already described [2]. Biomass hydrolysis was performed with H₂SO₄ 1N (60 min, 121 ºC). Small scale fermentation was undertaken in 120 mL serum flasks containing 20 mL of MCM medium [11]. Bench-scale sequential batch fermentation was performed in a lab scale double jacketed reactor (1.65 L) with a total medium volume of 500 mL (10 g/L of total sugars, 37 ºC, 150 rpm). After the first batch assay, 250 mL of the medium were replaced with a 1:1 mixture of hydrolysate and concentrated MCM. The produced biogas was collected and stored in inverted serum flasks filled with water, and quantified by displacement of the liquid phase. Biomass dry weight was determined throughout the fermentation. Gas samples were analysed by GC and the fermentate samples by HPLC [9]. The final energy consumption inventory associated with the microalga culturing, harvesting, drying, hydrolysis and fermentation was assessed based on direct equipment energy measurements. The results are expressed in MJ/MJH₂.

3. RESULTS AND DISCUSSION
H₂ production from Spirogyra sp. hydrolysate was first attempted in a small set-up consisting of individual flasks with the purpose of evaluating whether C. butyricum was able to successfully convert the sugars made available by the acid hydrolysis. The fermentation results are displayed in figure 1.
As seen in figure 1 (A), H$_2$ production occurred rapidly between 0 and 12 hours of incubation with no visible lag phase. The maximum H$_2$ production was achieved at 42 hours of fermentation (2.1 L H$_2$/L). The maximum H$_2$ percentage in the biogas produced was 28% (v/v). The highest H$_2$ production rate (141 mL/L.h) was detected from up to 12 hours and corresponded to a H$_2$ yield of 3.78 mol H$_2$/ mol glucose equivalents. These results show that not only H$_2$ production from Spirogyra hydrolysate was viable as it was comparable or higher to already published results [8], [9].

With the objective of scaling-up the bioH$_2$ production, a sequential batch reactor (SBR) was set-up. The use of a sequential batch system maintains the concentration of biomass inside the reactor in a quasi-exponential status, virtually eliminating the lag-phase between consecutive batches and lessening the operation time of each batch. This enables to increase the number of batches per day and the overall H$_2$ production rate. Figure 1 (B) depicts the results of bioH$_2$ production from Spirogyra hydrolysate in SBR during three consecutive batches. In comparison to the small-scale batch reactor, the H$_2$ production rate increased almost two-fold (324 mL/L.h) and the biogas produced was richer in H$_2$ (>50% (v/v)). The bioH$_2$ production did not change significantly during the consecutive batches, displaying a steady, uninterrupted production profile up to 4.4 L H$_2$/L.

Table 1 summarises the inventory of both production processes and the energy consumption associated to each stage of H$_2$ production. The results are presented in MJ per g of H$_2$ produced.
Table 1. Inventory results of bioH\textsubscript{2} production from *Spirogyra* biomass (MJ/gH\textsubscript{2})

<table>
<thead>
<tr>
<th>Production stage</th>
<th>BioH\textsubscript{2} production in small-scale batch reactor</th>
<th>BioH\textsubscript{2} production in SBR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microalga culture</td>
<td>0.11</td>
<td>0.03</td>
</tr>
<tr>
<td>Biomass harvesting and drying</td>
<td>1.87</td>
<td>0.55</td>
</tr>
<tr>
<td>Biomass hydrolysis</td>
<td>1.71</td>
<td>0.07</td>
</tr>
<tr>
<td>Fermentation</td>
<td>1.96</td>
<td>3.09</td>
</tr>
</tbody>
</table>

In order to show the results in MJ per MJ of hydrogen produced, a lower heating value of 120 MJ/kg was used [1], [2]. A total energy consumption of 47 and 31 MJ/MJ\textsubscript{H\textsubscript{2}} was obtained in the small-scale batch reactor and the SBR, respectively. Previous studies on the fermentation of dried and ground microalgal biomass achieved total energy consumption values of 88 MJ/MJ\textsubscript{H\textsubscript{2}} with *Scenedesmus obliquus* as feedstock and 207 MJ/MJ H\textsubscript{2} with *Spirogyra* sp., values which are visible higher than those attained in this study [1], [2]. The use of less energy consuming harvesting and drying procedures (electrocoagulation and solar dewatering) and the use of a simpler culture medium contributed for this energy consumption decrease. In the small-scale process, the production and processing of the microalgal biomass (harvesting, drying, hydrolysis) was responsible for a considerable energy consumption (30 MJ/MJ\textsubscript{H\textsubscript{2}}), in accordance to what was already reported by other authors [12]. In contrast, the fermentation was clearly the stage which consumed more energy in the SBR. This result is directly related to the high energy consumption of the heating bath used for controlling the reactor temperature. Together, the biomass hydrolysis and the fermentation stages accounted for 85% (26 MJ/MJ\textsubscript{H\textsubscript{2}}) of the total energy consumption in the SBR. The comparison between small-scale and bench-scale allowed us to assess that the SBR improved the cumulative H\textsubscript{2} production, the H\textsubscript{2} production rate and the global energy consumption. Although it is still necessary to reduce the ratio of energy input per energy output, the increase in the scale of bioH\textsubscript{2} production allowed for a reduction of 34% of the energy requirements. The comparison of the results obtained in this work with others already published [2] shows a clear improvement in the process performance, likely due to the refinement of the microalga harvesting process, the culture medium optimisation and the bioconversion efficiency.

4. CONCLUSIONS

The purpose of the current study was to evaluate the effect of scaling-up the bioH\textsubscript{2} production from microalgal biomass in the energy requirements and production yield of the process. The fermentation results show that *Spirogyra* biomass is an adequate feedstock for the fermentation by *C. butyricum*, achieving H\textsubscript{2} production yields close to the maximum theoretical value. The SBR system improved significantly the H\textsubscript{2} production yield (from 2.1 to 4.4 L H\textsubscript{2}/L) and H\textsubscript{2} production rate (from 141 to 324 mL/L.h), while supporting at the same time the operation in a continuous mode. The energy inventory analysis revealed that the process scale-up decreased the energy consumption in 34%. It is
possibly that pursuing with the optimisation of the fermentation stage, the energy requirements may decrease to values which make the biological H\(_2\) production more sustainable.

REFERENCES


