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Production and storage of biohydrogen during sequential batch fermentation of *Spirogyra* hydrolyzate by *Clostridium butyricum*

Joana Ortigueira, Tiago Pinto, Luísa Gouveia, Patrícia Moura*

Unidade de Bioenergia, Laboratório Nacional de Energia e Geologia, Estrada do Paço do Lumiar 22, 1649-038 Lisboa, Portugal

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ABSTRACT

The biological hydrogen production from *Spirogyra* sp. biomass was studied in a SBR (sequential batch reactor) equipped with a biogas collecting and storage system. Two acid hydrolysis pre-treatments (1N and 2N H_2SO_4) were applied to the *Spirogyra* biomass and the subsequent fermentation by *Clostridium butyricum* DSM 10702 was compared. The 1N and 2N hydrolyzates contained 37.2 and 40.8 g/L of total sugars, respectively, and small amounts of furfural and HMF (hydroxymethylfurfural). These compounds did not inhibit the hydrogen production from crude *Spirogyra* hydrolyzates. The fermentation was scaled up to a batch operated bioreactor coupled with a collecting system that enabled the subsequent characterization and storage of the biogas produced. The cumulative hydrogen production was similar for both 1N and 2N hydrolyzate, but the hydrogen production rates were 438 and 288 mL/L.h, respectively, suggesting that the 1N hydrolyzate was more suitable for sequential batch fermentation. The SBR with 1N hydrolyzate was operated continuously for 13.5 h in three consecutive batches and the overall hydrogen production rate and yield reached 324 mL/L.h and 2.59 mol/mol, respectively. This corresponds to a potential daily production of 10.4 L H_2/L *Spirogyra* hydrolyzate, demonstrating the excellent capability of *C. butyricum* to produce hydrogen from microalgal biomass.

1. Introduction

The demand for alternative energy sources has been growing in the last few decades. H_2 (hydrogen) is an interesting alternative to the increasingly scarce and polluting fossil fuels as it is easily and efficiently converted into energy. Furthermore, it has a high heating value (142–120 MJ/kg) and produces only water as a by-product [1]. Hydrogen can be produced biologically ($bioH_2$) through anaerobic fermentation of organic substrates, particularly those rich in carbohydrates [2], a process known as DF (dark fermentation). The production of $bioH_2$ is also accompanied by the production of organic acids with industrial use [3].

Microalgae have been recently regarded as a prime substrate for third generation bioconversion processes [4,5]. They are photosynthetic CO_2 (carbon dioxide) consuming organisms, easy to grow in fresh, salt or wastewater streams and capable of storing carbohydrates and lipid compounds in the intracellular space and as part of the cell walls [6,7]. Microalgae also display high photosynthetic efficiencies, can be cultivated all year round and harvested daily, and grow faster when compared to higher plants, without the need for arable land [5,7]. Furthermore, the use of microalgal biomass as a feedstock for DF is recommended, as the CO_2 produced in the fermentation can be reused for the autotrophic culture of microalgae [4,8]. Several studies have already shown that this type of feedstock is adequate and easily convertible in a $bioH_2$ production setting. *Scenedesmus obliquus*, a microalga with the capacity to store glucose-based carbohydrates, was efficiently converted into hydrogen by *Clostridium butyricum* and *Enterobacter aerogenes* [9,10]. The production process required no biomass pre-treatment, relied solely on wet or dry microalgal biomass as a carbon and energy source and the hydrogen production yield by *C. butyricum* and *E. aerogenes* was 69% and 34% of the maximum theoretical yield from glucose (4 mol/mol), respectively. In a similar way, the thermophilic *Thermotoga neapolitana* and *C. butyricum* CGS5 were able to produce hydrogen from pre-treated *Chlamydomonas reinhardtii* and *Chlorella vulgaris* biomass, respectively [11,12]. Comparatively, the high-potential for sugar accumulation that the microalga *Spirogyra* presents, makes it extremely adequate as a fermentative substrate. In a recently proposed model of a microalgae biorefinery for hydrogen and pigments production, 156 mL H_2/g of total sugars

* Corresponding author. Tel.: +351 210924600; fax: +351 217163636.
E-mail address: patricia.moura@lneg.pt (P. Moura).

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