



Third generation biohydrogen production by *Clostridium butyricum* and adapted mixed cultures from *Scenedesmus obliquus* microalga biomass



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HIGHLIGHTS

- A mixed culture and *C. butyricum* were used to ferment dried and ground *S. obliquus* biomass.
- The best adapted mixed culture LE37 produces 1.52 mol H₂/mol from 50 g/L of dried and ground *S. obliquus*.
- *C. butyricum* produces 116.3 mL H₂/g alga vs, attaining a H₂ yield of 2.74 mol/mol.
- The biogas produced by *C. butyricum* has the highest purity, H₂/CO₂ (v/v) of 1.7.

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ABSTRACT

Scenedesmus obliquus biomass was used as a feedstock for comparing the biological production of hydrogen by two different types of anaerobic cultures: a heat-treated mixed culture from a wastewater treatment plant and *Clostridium butyricum* DSM 10702. The influence of the incubation temperature and the carbon source composition were evaluated in order to select the best production profile according to the characteristics of the microalgal biomass. *C. butyricum* showed a clear preference for monomeric sugars and starch, the latter being the major storage compound in microalgae. The highest H₂ production reached by this strain from starch was 468 mL/g, whereas the mixed culture incubated at 37 °C (LE37) produced 241 mL/g. When the mixed culture was incubated at 58 °C (LE58), a significant increase in the H₂ production occurred when xylose and xylan were used as carbon and energy source. The highest H₂ yield reached by the LE37 culture or in co-culture with *C. butyricum* was 1.52 and 2.01 mol/mol of glucose equivalents, respectively. However, the ratio H₂/CO₂ (v/v) of the biogas produced in both cases was always lower than the one produced by the pure strain. In kinetic assays, *C. butyricum* attained 153.9 mL H₂/L h from *S. obliquus* biomass within the first 24 h of incubation, with a H₂ yield of 2.74 mol/mol of glucose equivalents. H₂ production was accompanied mainly by acetate and butyrate as co-products. In summary, *C. butyricum* demonstrated a clear supremacy for third generation bioH₂ production from *S. obliquus* biomass.

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1. Introduction

Traditionally, large-scale hydrogen (H₂) is produced through steam reforming of natural gas [1]. This process involves the conversion of methane and water into carbon dioxide (CO₂) and molecular hydrogen at high temperature. H₂ can also be obtained through electrolysis of water and thermocatalytic reformation of other H₂ containing organic compounds [2]. Both are polluting and expensive processes [3]. Biological hydrogen (bioH₂) can be produced mainly by two routes: photobiologically and by fermentative processes

(photofermentation and dark fermentation) [4]. The first one is based on the uptake of CO₂ or other organic substrates and water by photosynthetic organisms. Its major drawbacks are the requirement of a constant light source supply and the low yields of the overall process [5]. Dark fermentation consists in the conversion of sugars into H₂, CO₂ and organic acids by microorganisms, through the acidogenic pathway. Theoretically, any sugar-containing biomass can be used as a feedstock [6] and if the chosen biomass is readily available and low-cost, the appeal of the process could be increased.

Microalgae could meet these requirements as they have the capability to absorb solar energy and CO₂ and convert it into chemicals; being able to store them as either natural oil, primary subject for biodiesel production, or other carbon storage

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