Fermentative hydrogen production from microalgal biomass by a single strain of bacterium Enterobacter aerogenes — Effect of operational conditions and fermentation kinetics

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ABSTRACT

Biohydrogen production through dark fermentation is a promising technology for generating renewable energy, while using microalgal biomass as a third generation feedstock can further increase the sustainability of the process. In the present study, Scenedesmus obliquus was used as model microalga substrate for studying the impact of operational parameters in batch dark fermentation trials using a strain of Enterobacter aerogenes bacteria.

(i) The initial gas-liquid ratio in the bioreactor (from 1.3 to 8.2) was tested, resulting in higher bioH2 yields for ratios above 5.

(ii) Different bacterial growth, inoculation procedures and fermentation media were tested in combined experiments. The best conditions were chosen by maximising bioH2 yield and minimising production time and costs.

(iii) The autoclave sterilization effect on sugar extraction and bioH2 yield was tested for different microalga concentrations (2.5–50 g/L) with best results attained for 2.5 g/L (81.2% extraction yield, 40.9 mL H2/g alga).

For the best operational conditions, fermentation kinetics were monitored and adjusted to the Modified Gompertz model, with $t_{95}$ (time required for bioH2 production to attain 95% of the maximum yield) below 4.5 h. The maximum hydrogen production was higher when using wet algal biomass enabling the energy consuming biomass drying step to be skipped.

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1. Introduction

The production of hydrogen from renewable sources is a promising alternative for the future, considering the need for cleaner energy carriers and a reduction in carbon dioxide emissions. Hydrogen is a carbon-free fuel (upon oxidation produces only water) and the most energy-dense fuel per unit mass (142 kJ/g).

The European Strategic Energy Technology Plan [1] has identified hydrogen-based technologies among the technologies needed in Europe to achieve its target for 2020: a 20% reduction in greenhouse gas emissions; a 20% share of renewable energy sources in the energy mix and a 20% reduction in primary energy use.

Hydrogen can play an important role in the reduction of local air pollutants, as well as in the decarbonisation of Europe’s Transport system.

At present, however, hydrogen is mainly produced from fossil fuels (e.g. natural gas steam reforming, coal gasification) or water (e.g. electrolysis, photolysis) through energy intensive processes. It is therefore essential to develop and optimize more environmentally-friendly, energy-efficient and sustainable hydrogen production processes, namely through biological biomass fermentation.

The production of hydrogen through anaerobic fermentation is a relatively simple process and can use a wide spectrum of substrates, including waste products [2] and microalgae, such as Scenedesmus obliquus [3], Nannochloropsis sp. [4], Chlorella vulgaris [5], Dunaliella tertiolecta [5], Spirogyra sp [6]. Enterobacter aerogenes is known as an efficient biohydrogen producing bacterium, as it is one
of the most widely studied model strains [7–9]. Moreover, it is a facultative anaerobic bacterium, which is able to grow in the presence of oxygen, and therefore makes the manipulation in bioprocesses much easier compared with the use of strict anaerobes [10]. In this context, E. aerogenes has proven to be efficient in the bioconversion of the microalgae Anabaena sp. [11], Nannochloropsis sp. [4,12], Scenedesmus obliquus [3,13] and Chlorella vulgaris [13]. S. obliquus is a green microalga which is widely used for biofuel production purposes [14–16]. In fact, it can accumulate either oil or sugar and it is used for biodiesel [14,17] or bioethanol production [18,19], showing also its potential as a feedstock for biogas (bioH2 and bioCH4) through anaerobic production processes [3].

Dark fermentation is a complex system where environmental and operational factors such as temperature, pH and H2 partial pressure, regulate metabolic pathways of hydrogen producing microorganisms [20]; A lower partial pressure in the headspace of the reactors is one of the parameters which facilitate the mass transfer of hydrogen from the liquid to gas phase. Also, besides the type of substrate and its pretreatment, the inoculum sources and enrichments also greatly influence the biohydrogen production. In addition, the bacteria culture seed and inoculation procedure are also very important factors for the startup of the hydrogen production process. Thus, when dealing with complex substrates, such as microalgal biomass, it is crucial to study the effect of these parameters which influence the dark fermentation pathways and consequently the biohydrogen yields [20,21].

The aim of the present work was to explore the operational conditions which were best suited for biohydrogen production using S. obliquus microalga as a model substrate. The experiments were conducted in batch mode and the influence of the following parameters on the bioH2 production was tested: (i) Initial gas-liquid volume ratio in the bioreactor; (ii) Bacteria growth and inoculation procedure; (iii) Sterilization. Finally, the fermentation kinetics parameters were also monitored.

2. Material and methods

2.1. Microalga production

Scenedesmus obliquus ACOI 204/07 from Coimbra University Algotec (Portugal) was used in this study. The culture was grown in a Bristol culture medium at pH 7 [22]. Initially, 1 L glass bubble column photobioreactors, with filtered bubbling air, at a constant temperature of 25 ± 1 °C, under low light (150 μE/m²/s) were used. Afterwards, S. obliquus biomass was produced in two outdoor open raceway ponds (300 L capacity, 2 m² each), agitated by paddle-wheels at approximately 5 m/min, with natural light (light/dark cycles). For the recovery of the microalgal biomass from the raceway ponds the agitation was stopped, the biomass settled down, most of the liquid phase was poured out and the biomass collected was centrifuged (10000 rpm; 10 min) (Avanti J25, Beckman). The biomass was dried in an oven at 80 °C overnight (16 h), and had a proximate composition of: 30.7% total sugars, 17.1% crude fat, 20.4% crude protein and 20.2% total minerals (all % w/w in dry weight) [3]. The algal biomass was used as carbon and energy source in the fermentations for H2 production. For biohydrogen production kinetics assays (see Section 3.4), wet microalgal biomass, collected after centrifugation, was also tested.

2.2. Fermentative bacterium

Enterobacter aerogenes ATCC 13048 Sputum (American Type Culture Collection, Manassas, USA), was used for the fermentation experiments, being harvested from exponentially grown cultures (Section 2.3.2). The original culture was kept at 4 °C in solid CASO Agar (MERCK: 15 g/L peptone from casein, 5 g/L peptone from soymeal, 5 g/L sodium chloride and 15 g/L agar–agar).

2.3. Biohydrogen production experiments

Batch fermentation assays were performed in 159 mL serum bottles closed with butyl rubber stoppers and crimped with aluminium seals.

Initially, the bioreactors containing both fermentation medium and microalgal biomass (substrate) were sterilized in an autoclave (121 °C, 15 min and 1.4 bar). Afterwards, the sterilized bioreactors were aseptically purged with bubbling N2 (2 min) to replace O2 and were inoculated with different initial concentrations of exponentially grown E. aerogenes, according to the objectives.

The fermentation was carried out under orbital shaking (220 rpm), for 24 h at 30 °C. Control fermentation tests, without microalgal biomass, were also prepared for all assays. Peptone water (PW) and Complex Fermentation Medium (CFM) (see composition in Section 2.3.2), were used as pre-inoculum and fermentation medium, respectively [except in the cases indicated in Table 1 for the assays on bacteria growth and inoculation procedure (Section 2.3.2)].

2.3.1. Initial gas-liquid volume ratio

The serum bottles (159 mL) were filled with determined volumes of liquid medium (containing CFM and 10 g/L microalga) in

| Medium | Pre-Inoculum | Inoculation | Fermentation |
|--------|--------------|-------------|--------------|
|        | Procedure    | Concentration | Fermentation medium | Specific yield | Biogas purity |
| PW     | Aerobic      | 1% (v/v)    | PW            | 11.3          | 1.1          |
| PW     | Anaerobic    | 1% (v/v)    | PW            | 9.6           | 1.1          |
| PW     | Anaerobic    | 1% (v/v)    | CFM           | 19.9          | 1.5          |
| PW     | Anaerobic    | 0.003       | CFM           | 20.9          | 1.1          |
| PW     | Anaerobic    | 1% (v/v)    | CFM           | 23.5          | 1.4          |
| PW     | Anaerobic    | 0.03        | BM1           | 14.2          | 1.3          |
| PW     | Anaerobic    | 0.01        | BM1           | 11.8          | 1.3          |
| PW     | Anaerobic    | 0.03        | BM1           | 7.5           | 1.2          |

* PW: Peptone Water; CFM: Complex Fermentation Medium; RCM: Reinforced Clostridia Medium; BM1: Basal Medium.
