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Fermentative hydrogen production in an up-flow anaerobic biofilm reactor inoculated with a co-culture of *Clostridium acetobutylicum* and *Desulfovibrio vulgaris*

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HIGHLIGHTS

- Dark fermentative H_2 production in continuous-flow anaerobic packed bed reactor.
- Artificial consortium of *Clostridium acetobutylicum* and *Desulfovibrio vulgaris*.
- Effect of main operating parameters on fermentation pathways and H_2 production.
- Stable H_2 production achieved after 3–4 days of continuous reactor operation.
- Stable global metabolism leading to H_2 production over a long period.

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ABSTRACT

Dark fermentation systems often show low H_2 yields and unstable H_2 production, as the result of the variability of microbial dynamics and metabolic pathways. Recent batch investigations have demonstrated that an artificial consortium of two anaerobic bacteria, *Clostridium acetobutylicum* and *Desulfovibrio vulgaris* Hildenborough, may redirect metabolic fluxes and improve H_2 yields. This study aimed at evaluating the scale-up from batch to continuous H_2 production in an up-flow anaerobic packed-bed reactor (APBR) continuously fed with a glucose-medium. The effects of various parameters, including void hydraulic retention time (HRTv), pH, and alkalinity, on H_2 production performances and metabolic pathways were investigated. The results demonstrated that a stable H_2 production was reached after 3–4 days of operation. H_2 production rates increased significantly with decreasing HRTv from 4 to 2 h. Instead, H_2 yields remained almost stable despite the change in HRTv, indicating that the decrease in HRTv did not affect the global metabolism.

1. Introduction

Nowadays, hydrogen gas (H_2) represents a promising alternative to fossil fuels, because it has a high energy content (120 MJ/kg), it can be stored under different physical and chemical forms (gas, liquid, and/or adsorbed in solid materials), and its combustion produces only energy and water. Therefore, research on the production and use of H_2 as an energy carrier has received a growing attention from politics and scientists during the last decades. Various processes are commonly used to produce H_2, including electrolysis of water, photolysis, photo fermentation, and dark fermentation. Compared to other processes, bacterial dark fermentation is considered one of the most attractive, because it employs the ability of strict or facultative anaerobes such as *Clostridium* or *Enterobacter* to produce bio-H_2 and volatile fatty acids from complex organic feedstocks such as organic waste and wastewater (Nath and Das, 2004; de Gioannis et al., 2013). This indicates a very interesting potential market of wastewater valorisation for the production of bio-H_2.

The technical feasibility of H_2 production from various types of synthetic and real wastewaters has been largely investigated in anaerobic biofilm reactors (Barca et al., 2015). Overall, biofilm

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reactors present several advantages compared to suspended biomass systems. Firstly, biomass concentration in biofilm reactors is higher than in suspended biomass ones. Indeed, biomass in biofilm reactors grows on the surface of the carrier material and it is accumulated into the reactor, whereas biomass in suspended systems is flushed out with the effluent (Jung et al., 2011). Secondly, the biofilm provides a good protection to microorganisms against sudden changes of operating parameters such as temperature, pH, organic load, O_2 concentration, etc., thus allowing more flexible operating conditions. Biofilm reactors can be divided into two main categories, according to the hydraulic behaviour of the bed: (i) anaerobic packed bed reactors (APBRs), where the carrier for biomass growth is fixed and the water flows through the void volume of the bed, and (ii) anaerobic fluidized bed reactors (AFBRs), where the water flow velocity is always equal or greater than the minimum velocity for which the upward drag force exerted by the fluid is equal to the apparent weight of the particles in the bed, thus leading to the suspension of the carrier material.

Several research groups have shown that the composition of microbial communities in the biofilm has a strong influence on H_2 production performances (Ren et al., 2008; Wong et al., 2014; Li and Fang, 2007; Koskinen et al., 2007). Most of the studies dealt with biofilm reactors inoculated with seed sludge from wastewater treatment plants and anaerobic digesters, thus resulting in a large variability of microbial communities (Ren et al., 2008; Wong et al., 2014). On the one hand, mixed bacterial communities may provide a large variety of metabolic pathways for the degradation of complex substrate (Li and Fang, 2007). On the other hand, the variability in the microbial composition may lead to unstable H_2 production (Koskinen et al., 2007). Therefore, the most important challenge for future research is to improve operation and design of the reactor in order to obtain a stable and efficient H_2 production. Metabolic control techniques might be a feasible way to redirect metabolic pathways and improve H_2 yields. Recent studies have shown that sub-dominant bacteria (such as *Escherichia coli*, *Ralstonia eutropha*, and *Desulfovibrio vulgaris*) can have a significant effect on H_2 production performances of dominant species (*Clostridia*) (Rafrafi et al., 2013; Benomar et al., 2015). Benomar et al. (2015) found that H_2 production of *Clostridium acetobutylicum* was improved by a factor 2.5 in the presence of *Desulfovibrio vulgaris* Hildenborough in sulphate-free solutions. Moreover, the results of Benomar et al. (2015) clearly indicated the presence of physical connections between the two species, which most probably promoted: (i) the exchange of nutrients and/or electrons, (ii) the formation of aggregates, and (iii) the adhesion properties of the cell clusters. However, most of these investigations were performed in batch, and further experiments are needed to evaluate the efficiency and long-term stability of H_2 production under continuous operating conditions.

This study aimed at evaluating the H_2 production performances of a laboratory-scale up-flow APBR inoculated with a co-culture of *Clostridium acetobutylicum* and *Desulfovibrio vulgaris* Hildenborough. The novelty and the importance of this study consist in the interdisciplinary approach that involves the integrated skills of chemical engineering and microbiology applied to the scale-up from batch to continuous flow systems. Also, this study demonstrates for the first time a transition from a molecular-based approach in batch experiments to a systemic study in a continuous flow APBR: the procedures for inoculum and sterile medium preparation, metabolic and molecular analyses, as well as temperature conditions were adapted from Benomar et al. (2015) in order to better assess the feasibility of the scale-up. Firstly, the effects of various operating parameters, including void hydraulic retention time (HRTv), pH, and inlet water alkalinity, on H_2 production stability and performances were investigated in order to determine the best operating conditions for the reactor. Secondly, molecular biology and metabolic analyses were performed to evaluate the effects of the main operating parameters on microbial dynamics and metabolic pathways. Finally, some perspectives and technical solutions to improve reactor performances were proposed.

2. Materials and methods

2.1. Reactor design

An up-flow APBR with a cylindrical jacketed glass design was set up for the experiments. The inner diameter and the height of the reactor were 10 and 40 cm, respectively (Fig. 1). The reactor was packed with 4 mm-diameter glass beads as biofilm carriers. The porosity of the packed bed was 0.38 and its height was 24 cm, thus leading to a void volume of the packed bed of around 0.72 L. The ratio of diameter of the reactor to height of the bed was lower than 0.5 in order to favour dissipation of initial turbulence, while the ratio of diameter of the reactor to diameter of the beads was higher than 10 in order to limit wall effects and improve hydraulic performances (Zeiser et al., 2001).

2.2. Feeding solution and inoculum

The main purpose of this study was to evaluate the feasibility of the scale-up from batch to continuous flow reactors when using an artificial bacterial consortium. Therefore, a sterile feeding solution was used in order to avoid external microbial contamination. This solution was prepared with deionised water and glucose (1 g/L) as the main organic carbon source, with the addition of nutrients (100 mg N/L and 20 mg P/L) and micronutrients (1 mL/L of Starkey solution according to Bauchop and Elsden, 1960). The molar ratio of C/N/P was 100/21.4/1.9, which is in the range of typical C/N/P molar ratios of municipal wastewater (Tchobanoglous et al., 2003). Yeast extracts (0.5 g/L) were added to the feeding solution in order to promote biomass growth. The total alkalinity (TA) of the feeding solution was stabilized around a value of 500 mg $CaCO_3$/L by the addition of a buffer agent ($NaHCO_3$). This was done to limit the decrease in pH due to the production of organic acids during the fermentation process (Jung et al., 2011). The feeding solution was sterilized at 120 °C for 20 min, and then cooled by a

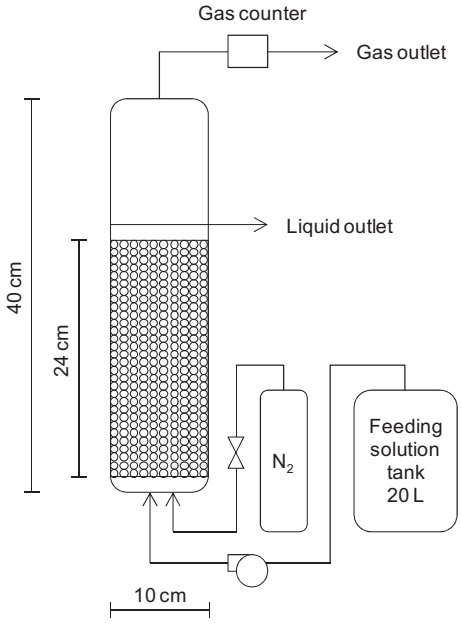

Fig. 1. Design of the reactor.