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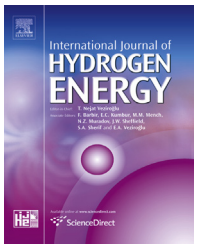

Mesophilic biohydrogen production by *Clostridium butyricum* CWBI1009 in trickling biofilter reactor

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ABSTRACT

This study investigates the mesophilic biohydrogen production from glucose using a strictly anaerobic strain, *Clostridium butyricum* CWBI1009, immobilized in a trickling bed sequenced batch reactor (TBSBR) packed with a Lantec HD Q-PAC® packing material (132 ft^2/ft^3 specific surface). The reactor was operated for 62 days. The main parameters measured here were hydrogen composition, hydrogen production rate and soluble metabolic products. pH, temperature, recirculation flow rate and inlet glucose concentration at 10 g/L were the controlled parameters. The maximum specific hydrogen production rate and the hydrogen yield found from this study were 146 mmol H_2/L.d and 1.67 mol H_2/mol glucose. The maximum hydrogen composition was 83%. Following a thermal treatment, the culture was active without adding fresh inoculum in the subsequent feeding and both the hydrogen yield and the hydrogen production rate were improved. For all sequences, the soluble metabolites were dominated by the presence of butyric and acetic acids compared to other volatile fatty acids. The results from the standard biohydrogen production (BHP) test which was conducted using samples from TBSBR as inoculum confirmed that the culture generated more biogas and hydrogen compared to the pure strain of *C. butyricum* CWBI1009. The effect of biofilm activity was studied by completely removing (100%) the mixed liquid and by adding fresh medium with glucose. For three subsequent sequences, similar results were recorded as in the previous sequences with 40% removal of spent medium. The TBSBR biofilm density varied from top to bottom in the packing bed and the highest biofilm density was found at the bottom plates. Moreover, no clogging was evidenced in this packing material, which is characterized by a relatively high specific surface area. Following a PCA test, contaminants of the *Bacillus* genus were isolated and a standard BHP test was conducted, resulting in no hydrogen production.

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Introduction

Biohydrogen production by microorganisms has attracted increasing global attention, owing to its potential to be used as an inexhaustible, low-cost and renewable source of clean energy [1]. Among the biological processes, the anaerobic hydrogen fermentation called dark fermentation seems to be more favorable, since hydrogen is yielded at a high rate and various organic wastes or wastewaters enriched with carbohydrates could be used as substrates, thus reducing production costs [2]. The dark fermentation can be conducted in either suspended or immobilized systems. Previous studies on immobilization were conducted using pure cultures, mixed cultures, different modes of operation, different packing materials and different operating conditions. Biohydrogen production in sequenced batch reactors with microbial biofilm has been studied by Bhaskar et al. [3] and Venkata Mohan et al. [4]. The immobilization of *Clostridium* species, *i.e. Clostridium tyrobutyricum* ATCC 25755 [5] and *C. tyrobutyricum* JM1 [6], was studied to optimize continuous biohydrogen production under various hydraulic retention times and inlet glucose concentrations. Different immobilization techniques [7–12] were investigated in order to improve the biofilm formation, the biohydrogen production rate and the hydrogen yield and composition.

The effect of the hydraulic retention time (HRT) and glucose concentration on hydrogen production in a mesophilic anaerobic fluidized bed reactor (AFBR) was studied by Zhang et al. [13]. They achieved a maximum yield of 1.7 mol H_2/mol glucose at HRT of 0.25 h, pH 5.5 and a glucose concentration of 10 g/L. They used a Continuous Stirred Tank Reactor (CSTR) and an AFBR to study the effect of different inocula on biohydrogen production. A 20-fold increase of the biohydrogen production rate was recorded in the AFBR compared to the CSTR that used a suspended culture for reactor operation. One of the problems associated with AFBR is the washout of biomass from the reactor. An anaerobic fixed bed sequenced batch reactor [14] was operated for 1435 days using synthetic wastewater and vegetable wastewater under different time periods. The reactor produced hydrogen without inhibition and microbial community analysis confirmed the presence of four species among which *Bacillus* sp. and *Clostridium* sp. were dominant in the biofilm. Among the biofilm reactors, the Trickle Bed Reactor (TBR) offers advantages such as high mass transfer rate between the gas–liquid interface, an easy control of pH in the circulating liquid phase and low liquid hold up [15]. The first continuous thermophilic TBR study was conducted using glucose as substrate and a mixed culture grown on a fibrous support matrix [15]. The optimal pH, temperature and hydrogen yield were 5.5, 60 °C and 1.11 mol H_2/mol glucose respectively. The same TBR was further studied for continuous biohydrogen production and a microbial analysis confirmed the presence of *Clostridia* and *Bacillus* as dominant species [16]. More importantly, it was found that the biomass concentration in the TBR gradually decreased as the reactor bed height increased.

Glucose fermentation was conducted using a pure culture of *Clostridium acetobutylicum* ATCC 824 grown on glass beads in TBR [17]. The reactor was tested for various glucose concentrations and the head-space average hydrogen composition was 74% (v/v). The major drawback of this study was the clogging of beads due to biomass formation after 72 h. Two bioreactor systems, *i.e.* trickle bed reactor and fluidized bed reactor, were compared [18] for thermophilic biohydrogen production and the TBR showed yield of 3 mol H_2/mol glucose. However, to achieve this yield, nitrogen gas had to be stripped throughout the experiment. A TBR was packed with perlite and fed with oat straw hydrolyzate [19]. By varying HRT and inlet OLR, Arriaga et al. [19] obtained a maximum specific hydrogen production rate of 3.3 mmol $H_2/L_{reactor}$.h and a hydrogen yield of 2.9 mol H_2/mol hexose. The maximum hydrogen composition was 45% (v/v), the rest being CO_2. Globally the major drawback of many of these studies was the clogging of the trickling filter bed with biomass [17,19].

It is usually not recommended to use pure cultures in non-sterile conditions due to contamination risks, which can generate deterioration of reactor performances. However thermophilic biohydrogen production was conducted in a 400 L non-sterile trickling bed reactor starting with a pure culture of *Caldicellulosiruptor saccharolyticus* using sucrose as major substrate [20]. It was found that contaminants were outcompeted by the pure culture and a hydrogen yield of 2.8 mol H_2/mol hexose could be achieved.

At CWBI, extensive research studies had been conducted using *Clostridium butyricum* CWBI1009 to improve biohydrogen production in batch, sequenced batch and continuous mode under various operating conditions and using different substrates. Fermentative hydrogen production was conducted using a co-culture of pure *C. butyricum* and *Citrobacter freundii* with five different carbon sources [21]. To investigate the optimal culture conditions for production of hydrogen using *C. butyricum*, batch and sequenced batch experiments were conducted using glucose and starch as substrates [22]. For glucose degradation, it was found that the maximum hydrogen yield could be obtained when pH was controlled at 5.2. In order to characterize the biohydrogen potential of different strains and sludge inocula growing on glucose, a series of experiments using serum bottles was conducted [23], showing that the pure *C. butyricum* strains achieved the highest hydrogen yield. To further improve the performances of *C. butyricum*, experiments were conducted using horizontal tubular fixed bed and biodisc-like anaerobic reactors [24]. The major objective was to improve biofilm formation by simultaneously enhancing liquid to gas mass transfer. For the anaerobic biodisc-like reactor, when the reactor bulk volume was reduced from 500 mL to 300 mL, both hydrogen production rate and yields were improved significantly. Experiments conducted in a 20 L fixed bed SBR [25] using polyurethane as a support material and an artificial co-culture, composed initially of *C. butyricum* CWBI1009 and *Clostridium pasteurianum* DSM525, achieved maximum hydrogen yields when a mixed substrate was used in this reactor. Drawbacks found in this reactor set up were the poor hydrodynamics and susceptibility for clogging due to biomass build up.

The purpose of the current study was to further investigate the biohydrogen production by developing a new reactor configuration such as TBR for improving biofilm formation and high L/G transfer. In this study, a 20 L fermenter was converted into Trickle Bed Sequenced Batch Reactor (TBSBR) to produce hydrogen using Lantec HD Q-PAC® as packing material with