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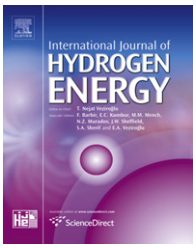

Phase holdups and microbial community in high-rate fermentative hydrogen bioreactors

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ABSTRACT

Phase holdups play an important role in high-rate hydrogen production in an anaerobic fermentative reactor, especially in understanding biomass content, biogas flow and distribution that significantly affect the flow regimes change in the reactor. In the present study three-phase hydrogen producing reactor with different configurations were tested to investigate the phase holdups phenomenon and microbial community. It was found that the major fatty acids produced from the reactors were acetate and butyrate (HBu), accounting for 74.4–93.5% of total soluble microbial products (SMP). When the HRT was shortened from 8 to 1 h, the HBu was the dominant acid product among the soluble metabolites and the ratio of Ethanol/SMP was lower than 15.1. Moreover, the gas holdup (ε_g) and solid holdup (ε_s) increased but liquid holdup (ε_l) decreased when the HRT was shortened. When the HRT was down to 1 h an increase in gas and solid holdups were noted. The gas holdups (ε_g) increased in the range of 0.30–0.34, and the solid holdups (ε_s) increased in the range of 0.32–0.34, which mean that the values of liquid holdups in high-rate fermentative hydrogen bioreactors could be decreased in the range of 0.38–0.32. Moreover, the empirical correlations of this study were satisfactory to predict the phase holdups in a dark-fermentation biohydrogen system. PCR-DGGE analysis revealed that bioreactor hydrodynamics under different HRTs significantly affects the occurrence of *Streptococcus* sp. and *Bacillus* sp. which mainly promotes granulation and retains high yielding hydrogen producing *Clostridium* sp. through their exopolysaccharides production. SEM results showed three dominant bacterial species namely *Clostridium pasteurianum*, *Streptococcus* sp. and *Propionibacterium* sp. in the bioreactors.

1. Introduction

The adverse effects of climate change caused by the depletion of fossil fuels have raised the demand for alternate fuels, and biohydrogen is one of them. Hydrogen produced in a biological way is energy-efficient and zero emission, providing a new alternative to environmental and energy issues as well as a platform for the hydrogen economy. Researchers have

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currently focused on the production of biohydrogen and the liquid fatty acids as byproducts through the process of dark fermentation from starch feedstock [1,2]. In addition, statistical model development has helped to better understand the behavior of biohydrogen production from dark fermentation [3]. In dark fermentation processes, lowering the hydraulic retention time (HRT) prior to hydrogen production causes the high speed substrate input to increase the risk of bacterial granules wash out and decreases the biomass content in the reactors.

Few case studies [4–7] indicate that the phase holdup in a three-phase bioreactor is of utmost importance for the good performance and productivity. Buffiere et al. [4] found that the overall gas holdup is higher when the gas is generated despite the fact that the average gas velocity in the whole bed is low. This is explained by the presence of entrapped bubbles without velocity all along the bed height. A modified correlation of the gas holdup was proposed as a function of gas velocity and particle size. They suggested that it would have required taking into account the bubble flow regime and the transition point between dispersed and coalesced bubble flow to predict details of the gas holdup. Wang et al. [5] reported based on the simulation results that the motion of the biogas bubbles is both upward and transverse. Sludge particle clusters were dragged transversely following the biogas bubbles. This behavior could lead to wash-out, and a lower biomass with a consequent reduction in biohydrogen yield.

Several studies have been carried out to investigate the function of phase holdups in three-phase reactors, for e.g. Tang and Fan [6] showed that the gas velocity is not affected by the solid holdup in the experimental reactors. They used dual-resistance and conductivity probes to detect the phase holdups and bubble sizes. Another study by Razzak et al. [7] showed that increasing the solid turn over rate and decreasing the liquid velocities, caused the solid holdup to increase, but the gas velocities did not change significantly. The authors used electrical resistance tomography, (ERT), to measure the velocity distribution and phase holdups. Buffiere et al. [4] claimed that the gas holdup in a gas producing bioreactor make a dramatic drop in the contact time between liquid and solid which directly affects the reactor performance. In one study the volume fraction (phase holdups) was one of the main parameter that was used to improve the reactor design and to optimize reaction conditions [5].

Therefore, the present aim was to study the phase holdups in three-phase biohydrogen producing continuously stirred anaerobic bioreactors (CSABR) with different aspect ratio and fed with sucrose as the substrate. The effect of different HRTs on the gas-solid-liquid phase holdups has been investigated in this study. The relationship of microbial community structure and soluble microbial products with the hydrodynamics behavior is also simultaneously evaluated.

2. Materials and methods

2.1. Experimental setup and analytical methods

The experimental setup of the CSABR is shown in Fig. 1. Three configurations of reactors were employed with working volumes of 10 L (bioreactor A: H 50 cm, ID 16 cm, H/D 3.1), 1.2 L (bioreactor B: H 25 cm, ID 7.5 cm, H/D 3.2) and 1.2 L (bioreactor C: H 50 cm, ID 5.5 cm, H/D 9.1). The substrate and nutrients were fed from the bottom and the effluent was collected at the top of the bioreactor. The pH, volatile suspended solids (VSS) and total solids (TS) concentration of the sludge were 6.81, 33.30 g/L and 65.10 g/L, respectively. The hydrogen productivity of the seed sludge was enhanced by thermal pretreatment followed by the acid pretreatment and finally neutralized by alkali. The thermal and acid treated sludge mostly dominated by *Clostridium* species was further acclimated in continuous culture operated at HRT of 12–8 h with an influent sucrose concentration of 20 g COD/L for 4–5 h. The effluent sludge was then collected for fermentative H_2 production in a CSABR. The medium used for H_2 fermentation contained 20 g COD/L (17.8 g sucrose/L) as the sole carbon source along with a sufficient amount of inorganic supplements.

The hydrogen content of the bioreactors was determined with a gas chromatography (GC-14A, Shimadzu, Tokyo, Japan) equipped with a thermal conductivity detector. The carrier gas was argon and the column was packed with Porapak Q (80/100 mesh, Waters Corp., USA). The sucrose concentration in the effluent was also determined according to the phenol-sulfuric acid method [8]. The volatile fatty acid (VFA) and ethanol concentrations were also detected by gas chromatography (Shimadzu GC-14A) using a flame ionization detector. The carrier gas was N_2 and the packing material was FON (Shimadzu, Japan). Volatile suspended solids (VSS, representing the biomass concentration) were measured according to the procedures described in Standard Methods [9].

2.2. Phase holdups measurements

The gas-liquid-solids holdups were obtained by measuring the biogas production rate and overflow rate of liquid-solids at steady state with uniform biomass concentration in the bioreactors. The granular density means bioparticle density, ($\bar{\rho}_s$). The bioparticle density is very difficult to measure, due to the particle having low density, porous inside and various shapes. The terminal velocity and U_s methods [10–12] are used to measure the bioparticle density in this study. The experimental column was consisted of acrylate with height of 20 cm and inner diameter of 5 cm, also with two marks, up and down having 10 cm distance. An introduce pipe was set at the top of column with height of 5 cm and inner diameter of 1 cm. The granular bioparticle was introduced from this pipe through the column at room temperature (25 °C). The water was filled in the column at first, and then a granular bioparticle was introduced from the pipe through the column. The time was recorded while the bioparticle moves from the up and down marks. By repeating the steps as mentioned above, the average terminal velocities were obtained. Using the Eq. (1) [10–12] the average granular bioparticle densities were obtained.

$$U_s = \left[\frac{8gf}{\pi}\left(\frac{1}{\rho_w} - \frac{1}{\rho_s}\right)\frac{W_d}{C_D d_a^2}\right]^{\frac{1}{2}} \quad (1)$$

where U_s stands for terminal velocity of granules, g for gravity forces, f for the fractional dry and wet weight of bacteria, W_d