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Process Biochemistry 42 (2007) 1165–1171

Process Biochemistry

www.elsevier.com/locate/procbio

Short communication

Simultaneous production of biohydrogen and bioethanol with fluidized-bed and packed-bed bioreactors containing immobilized anaerobic sludge

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Received 23 January 2007; received in revised form 2 May 2007

Abstract

Hydrogen and ethanol are promising biofuels and of great potential to become alternatives to fossil fuels. In this work, two bioreactor systems, namely fluidized-bed (FBR) and packed-bed (PBR), were developed to produce H_2 and ethanol simultaneously from dark fermentation of carbohydrate substrates using polyethylene–octane elastomer immobilized anaerobic sludge as the biocatalyst. The H_2 and ethanol production in FBR essentially increased with increasing upflow velocity (v_{up}), as sucrose and fructose was better substrate for the yield of H_2 and ethanol, respectively. With FBR operated at $v_{up} = 0.91\,cm\,s^{-1}$, sucrose gave the highest H_2 production rate (59 mmol $h^{-1}\,l^{-1}$) among the three sugar substrates (sucrose, glucose, and fructose) tested, but the best H_2 yield (1.04 mol mol hexose^{-1}) was obtained with glucose at $v_{up} = 0.55\,cm\,s^{-1}$. For ethanol production in FBR, fructose was the favorable substrate, resulting in maximum ethanol production rate and yield of 378 mmol $h^{-1}\,l^{-1}$ and 0.65 mol mol hexose^{-1}, respectively, when operating at $v_{up} = 0.91\,cm\,s^{-1}$. At a hydraulic retention time of 4 h, the PBR system produced H_2 and ethanol at a slower rate of 16 and 6 mmol $h^{-1}\,l^{-1}$, respectively, by using glucose. However, the yields of H_2 and ethanol were comparable to those for FBR. The soluble metabolites were dominated by ethanol, accounting for 43–65% of total soluble microbial products. The production of acetate and butyrate was less significant when compared to cultures optimized for H_2 production. Comparison of the yield of H_2 and ethanol shows that production of H_2 and ethanol was reversely correlated. The total energy generation based on the heat values of H_2 and ethanol was calculated to assess the overall efficiency of energy production. In FBR, the energy generation rate was higher when a faster upflow velocity was used. The best energy generation rate and yield was 526 kJ $h^{-1}\,l^{-1}$ and 1048 kJ mol hexose^{-1}, respectively, both occurred with fructose-feeding FBR operated at $v_{up} = 0.91\,cm\,s^{-1}$. The PBR system displayed a lower energy generation rate, whereas the energy yield was comparable or even higher than those for FBR.

Keywords: Biohydrogen; Bioethanol; Dark fermentation; Cell immobilization; Polyethylene–octene elastomer; Fluidized-bed; Packed-bed

1. Introduction

Biomass energy has emerged as one of the most attractive and promising alternative energy carriers to fossil fuels. Ethanol can be supplemented to gasoline as a fuel for transportation and could replace MTBE (methyl *tert*-butyl ether) as a more environment-compatible gasoline additive in the future [1,2]. Ethanol can also be used as a substrate for biodiesel production [3]. Hence, converting biomass feedstock to bioethanol and/or biodiesel is heavily focused bioenergy technology at this moment [4]. Nevertheless, as a clean, recyclable, and efficient energy carrier, hydrogen is still considered to play a pivotal role in future energy supply [5]. Producing H_2 via fermentative routes is more environmentally friendly and less energy intensive, thereby being competitive to conventional H_2-producing methods (e.g., thermo-chemical means) [5,6]. In particular, dark H_2 fermentation carried out with heterotrophic anaerobic microorganisms (e.g., *Clostridium* sp.) [7–14] is considered the most commercially viable bioH_2 process, because it has a higher H_2 production rate and can be easily incorporated into existing waste treatment systems for simultaneous waste reduction and clean energy generation [15]. In addition to H_2 production, anaerobic fermentation also produces a significant amount of alcohols (such as ethanol) [16]. Although formation of alcohols that would consume free electrons from NADH is usually unfavorable for H_2 production, it might be feasible to allow

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doi:10.1016/j.procbio.2007.05.012

a balanced production of both gaseous and liquid biofuels (i.e., H_2 and ethanol) through proper operation of dark fermentation processes toward an optimal total energy gain.

Entrapped-cell systems were shown to be suitable for batch and continuous H_2 fermentation with mixed cultures [17–19]. In particular, the immobilized cells could be integrated with a variety of bioreactor design, such as fluidized-bed [20,21] or fixed bed [22] for efficient bioH_2 production. In this study, acclimated H_2-producing sludge were immobilized by polymeric matrix polyethylene–octene elastomer (POE), which is a commercially available elastomer widely used in various industrial applications due to its excellent balance of mechanical properties along with favorable processability [23–25]. In the present work, the POE-entrapped cells were used for H_2 production in a fluidized-bed reactor (FBR) possessing favorable characteristics for the production of gaseous products [20] as well as in a packed-bed reactor (PBR) that is cost effective with simple setup and easy operation [26]. Using three types of sugar (namely, sucrose, glucose, and fructose) as the sole carbon substrate, the performance of continuous H_2 and ethanol production was investigated at different upflow velocity for FBR and at a fixed hydraulic retention time for PBR. The objective of this work was to develop innovative fermentation technology for dual production of two most critical biomass energy products—H_2 and ethanol.

2. Materials and methods

2.1. Hydrogen-producing sludge and fermentation medium

The seed sludge was collected from the final sedimentation tank of a municipal wastewater treatment plant located in central Taiwan. The sludge was pretreated with HCl at pH 3.0 for 24 h to eliminate the methanogenic activity [22]. The acid pretreated sludge was acclimated in continuous culture operated at a HRT of 12 h to maintain stable H_2-producing activity. The medium used for cell growth and H_2 production contained 20 g COD l^{-1} of sugar (sucrose, glucose, or fructose) as the sole carbon source along with a sufficient amount of inorganic salts, including (mg l^{-1}) NH_4HCO_3, 5240; $NaHCO_3$, 6720; K_2HPO_4, 125; $MgCl_2{\cdot}6H_2O$, 100; $MnSO_4{\cdot}5H_2O$, 15; $FeSO_4{\cdot}7H_2O$, 25; $CuSO_4{\cdot}5H_2O$, 5; and $CoCl_4{\cdot}5H_2O$, 0.125.

2.2. Cell immobilization

Fifty milliliters of acclimated H_2-producing sludge (ca. 0.15 g VSS) was well mixed with 90 g polyethylene–octene elastomer (POE). The resulting mixture was introduced to an extruder at a temperature of 70 °C and was extruded to form cell-entrapped colloid beads (1.5 mm in diameter). After being rinsed with deionized water, the colloid bead was immersed into a solution containing 0.5 g sodium alginate, 0.6 g zirconium oxide and 5 g of the acclimated sludge and was then transferred to 0.1 M $CaCl_2$ for solidification. The resulting immobilized-cell beads had an average density of 1.1 g cm^{-3}.

2.3. Setup and operation of the fluidized-bed reactor

Schematic description of the fluidized-bed reactor (FBR) used in this study is shown in Fig. 1a. Main body of the FBR reactor was a glass column with a diameter of 2.7 cm and a height of 120 cm. In the beginning of FBR operation, 100 g of the immobilized cells and 1.4 l of the aforementioned medium were placed in the reactor. The static bed height of the immobilized-cell particles was 4.5 cm. Argon gas (device no. 1) was used to sparge the reaction liquid thoroughly to create an anaerobic condition. The medium (device no. 13) was fed from the bottom into the immobilized-cell-loaded FBR reactor (device no. 2). The effluent of the reactor was introduced to a gas–liquid separator (device no. 9), where the gaseous and soluble products were collected separately. The operation temperature and pH in the bed was controlled at 35 °C and 5.8–6.8 throughout the operation. The medium was fed into the reactor with the adjustment of liquid flow rate ($v_{up} = 0.55-0.91$ cm s^{-1}) to fluidize the bed materials. When a steady state was reached, the hydraulic retention time was controlled at a designated value with the adjustment of recycle stream (device no. 3). The steady-state operation was defined as a nearly constant average H_2 and ethanol production rate (within 10% variation) for 3–4 days. The biogas (consisting of CO_2 and H_2) production was monitored by a gas flow meter (Type TG1; Ritter Inc., Germany). The gas volumes were calibrated to 25 °C and 760 mmHg. The compositions of gaseous and soluble products were also analyzed by gas chromatography at designated time intervals.

2.4. Setup and operation of the packed-bed bioreactor

The pack bed reactor (PBR) was composed of a glass column 3.2 cm in diameter and 120 cm in height (Fig. 1b). Before PBR operation, 100 g of immobilized-cell beads was packed into the reactor with a bed height of 5 cm, a porosity of 65%, and a working volume of 3.86 l. The sugar-containing medium was then fed from bottom into the immobilized-cell-loaded column for continuous H_2 production. The reactor was operated at HRT of 4 h. The definition of steady-state operation was similar to that for FBR. Operation temperature of the reactor was controlled at 35 °C and initial pH of the medium was controlled at 6 ± 0.1. The composition of gas products and soluble metabolites produced was determined at designated time intervals. A gas meter was used to record the quantity of gas products generated.

2.5. Analytical methods

The gas products were analyzed by gas chromatography (GC) using a thermal conductivity detector (TCD). The volatile fatty acids and ethanol were also detected by GC using a flame ionization detector (FID). The conditions and columns used for GC analysis were identical to those reported previously [26–28]. Standard Methods [29] were used to determine biomass concentration (in terms of volatile suspended solid; VSS). The carbohydrate concentration in the effluent was also measured using Standard Methods (via phenol-sulfuric acid method) [29].

3. Results and discussion

3.1. H_2 and ethanol production with fluidized-bed bioreactor

The performance of H_2 and ethanol (EtOH) production with the fluidized-bed bioreactor is indicated in Table 1. Regardless of the type of sugar substrate, the H_2 production rate essentially increased with an increase in upflow velocity (v_{up}) as the highest v_{up} tested (0.91 cm s^{-1}) gave the highest H_2 production rate of 59, 53, and 33 mmol h^{-1} l^{-1}, for sucrose, glucose, and fructose, respectively (Table 1). The highest ethanol production rate also reached 287, 196, and 378 mmol h^{-1} l^{-1}, for sucrose, glucose, and fructose, respectively, when the fluidized-bed was operated at a v_{up} of 0.91 cm s^{-1}. The positive effect of upflow velocity on the production rate of H_2 and ethanol is most likely due to better mass transfer efficiency arising from higher upflow velocity. Among the three sugar substrates used (all at a concentration of 20 g COD l^{-1}), sucrose appeared to be the preferable substrate stimulating the rate of H_2 production, while fructose led to better ethanol production rate (Table 1). On the other hand, Table 1 shows that glucose seemed to exhibit higher