Fermentative Hydrogen Production from Molasses in an Activated Sludge Immobilized Bioreactor

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***Abstract*-This study evaluated the possibility of using granular activated carbon as support material for biohydrogen production in a continuous stirred tank reactor (CSTR). The CSTR was inoculated with aeration pre-treated sludge and operated at temperature of 36 °C and hydraulic retention time (HRT) of 6 h. It was found that both biogas and hydrogen yields increased with OLR at the range of 8-24 kg/m^3·d. The biogas was mainly composed of CO_2 and H_2 with the percentage of H_2 ranging from 38.4% to 41% in biogas. The maximum hydrogen production rate of 3.65 L was obtained in the reactor at OLR of 24 kg/m^{3}d. The granular activated carbon could make the hydrogen-producing system stable regarding hydrogen production, pH value and microbial by-products and could be used as support material for fermentative hydrogen production.**

***Keywords*-Hydrogen production; Continuous stirred tank reactor; Fermentation; Support material; Molasses**

I. INTRODUCTION

Since the oil crisis in the 1970s, biofuels produced from renewable biomass have been considered as a potentially important fuel generation technique [1]. Hydrogen is a clean and renewable energy that could be produced from waste/wastewater [2]. It has opened up the possibilities to reduce our reliance on fossil fuels and turn to a sustainable alternative. Direct and highly efficient conversion of H_2 into electricity by fuel cells makes the application of H_2 energy even more attractive [3]. Consequently, low-cost and sufficient supply of H_2 could soon become in urgent demand [4]. Currently, the primary source of H_2 is generated from fossil fuels, which are being depleted. Besides, energy-intensive thermal or chemical processes are often required for H_2 production using fossil fuels [5], making it expensive and polluting the environment [6]. In contrast, biological H_2 production using fermentative, photosynthetic bacteria, or algae is an environmentally friendly and energy saving process [7-8]. Therefore, it is a feasible alternative for global H_2 supply in the future [9].

Hydrogen production via dark fermentation has some advantages over other biological processes due to its capability of attaining high H_2 production rates [10]. Fermentative hydrogen production also has an additional merit of decomposing organic wastes into more valuable energy resources [11]. Therefore, using anaerobic cultures for H_2 production has caught the attention of researchers [12-13].

The continuous stirred tank reactor (CSTR) has been often used for fermentative hydrogen production. It allows good mass transfer efficiency by mixing, but it often encounters the problem of biomass washout at high dilution rate or high organic loading rate. In order to maintain higher biomass concentration, granular activated carbon was utilized to entrap acclimated sewage sludge for efficient and stable H_2 production in this study. Characteristics of H_2-producing fermentation with the immobilized mixed consortium were investigated using molasses as the sole carbon substrate. It was also conducted to evaluate the stability and durability of the immobilized cells. This work is the preliminary step towards the development of effective immobilized-cell systems for practical applications in continuous and mass production of biohydrogen from carbohydrate wastes.

II. MATERIAL AND METHODS

A. *Hydrogen-Producing Sludge and Cultivation*

The sludge obtained from a secondary settling tank in a local municipal wastewater treatment plant. It was first sieved through a mesh with a diameter of 0.5 mm in order to remove waste materials that could cause pump failure. Hydrogen productivity of the seed sludge was enhanced by aeration treatment for 30 d to inhibit the methanogenic activity prior to immobilization. The volatile suspended solid (VSS) was 17.74 g/L.

The molasses used in this study was obtained from a local beet sugar refinery (Harbin). Molasses was diluted by tap water to a COD of 10000 mg/L and the COD: nitrogen: phosphorus ratio was maintained at 1000:5:1 by the addition of synthetic fertilizer in the substrate to supply microorganisms with adequate nitrogen and phosphorus contents.

Granular activated carbon was used as support media for cell immobilization and retention. The particles were sieved

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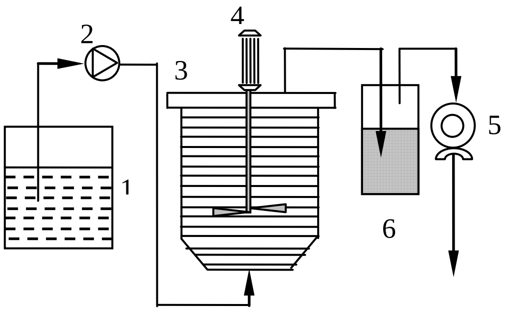
for uniformity of approximately 1.5–2 mm in diameter. The main physical characteristics of granular activated carbon were offered by supplier as follows: media real density = 1420 g/L; surface area = 1200–1350 m^2/g; bulk density = 450–500 g/L (Hainan Wen Chang Qiu Chi Activated Carbon. Co. Ltd.). H_2-producing sludge was mixed with granular activated carbon at a volume (ml) to weight (g) ratio of 1:10. It was observed that sludge predominantly covered the surface and interior portion of the immobilized disc.

B. Bioreactor Operation

The bioreactor used for H_2 fermentation in this study was a 12.5 L continuous stirred-tank reactor (CSTR) with an effective volume of 5.4 L (Fig.1). The reactor was constructed with transparent plexiglas with a gas-liquid-solid separating device, operated in continuous flow mode. The temperature was automatically maintained at 36 °C by electrothermal wire. The influent flow rate was controlled by a feed pump to regulate the HRT and OLR in the reactor.

The generated biogas was collected with water lock and measured by wet gas meter (Model LML-1, Changchun Filter Co., Ltd.) which were filled with acidized saturated combustion-supporting gas at 500 ml/min.



1. Waste water box 2. Feed pump 3. Reactor 4. Agitator 5. Biogas meter 6. Water lock

Fig. 1 Schematic diagram of the CSTR reactor for biohydrogen production from molasses wastewater

C. Analytical Methods

The biogas yield of the CSTR was measured daily at room temperature using a wet gas meter, and its constituents (H_2 and CO_2) were determined by gas chromatography (Model GC-122, Shanghai Anal. Inst.Co.). The gas chromatography system was equipped with a thermal conductivity detector and a stainless steel column (2m×5mm) filled with PorapakQ (80/100mesh, Agilent,USA). Nitrogen was used as the carrier gas at a flow rate of 40 ml/min.

Volatile fatty acids (VFAs) and ethanol in the fermentation solution were also analyzed by gas chromatography (Model GC-112, Shanghai Analytical Apparatus Corporation, China) with a hydrogen flame ionization detector and a stainless steel column (2 m×5 mm) packed with support (GDX103, 60/80 mesh, Shanghai Maikun Chemical Co., Ltd). The operation of the stainless steel column was amenable to temperature programming within 100–200°C. Nitrogen was used as the carrier gas at a flow rate of 50 ml/min. Hydrogen was the combustion gas at 50 ml/min, and oxygen was the combustion-supporting gas at 500 ml/min.

COD, pH and ORP were measured daily in the CSTR according to Standard Methods [22].

III. RESULTS AND DISCUSSION

A. The Performance of Hydrogen Production under Each OLR

Table 1 depicts steady-state results obtained at a varying OLR from 8 to 24 kg/ m^3·d. It was found that whenever there was a change in OLR, a climacteric variation would happen and a new steady-state was achieved. Both biogas and hydrogen production led the same trend and had a clear correlation with OLR in the reactor. Biogas and hydrogen production increased with OLR at the range of 8-24 kg/m^3·d, and reached the maximum yield of 9.2 L and 3.65 L, respectively, at the OLR of 24 kg/m^3·d. However, a great amount of VFAs (such as acetic acid and butyric acid) were produced by anaerobic microorganism, which caused pH decline to 3.56 at the OLR of 24 kg/m^3·d. One of the factors that affected the metabolic pathways and consequently the H_2-producing efficiency in fermentative H_2 production was pH value. The pH value had great effect on the biohydrogen production process, which would influence certain enzyme activity and fermentation pathway, and would further change nutrient supply and toxicity of harmful substances. Therefore, the COD removal decreased from 23% at OLR of 8 kg/m^3·d to 14.66% at OLR of 24 kg/m^3·d due to the activity of microorganism metabolity was inhibited with low pH value. However, it was found that the highest biogas yield of 9.2 L was obtained with a lower pH value when OLR was operated at 24 kg/m^3·d in the attached hydrogen-producing system. It could conclude that the attached hydrogen-producing system could endure low pH value. During the whole operation, the produced biogas was composed of hydrogen, carbon dioxide, and free of methane, indicating the absence of methanogens in the system might be as a consequence of the lower pH operation.

TABLE 1 HYDROGEN PRODUCTION PERFORMANCE OF GRANULAR ACTIVATED CARBON SLUDGE UNDER DIFFERENT OLR

OLR (kg/m^3·d)	Biogas yield (L)	H_2 content (%)	COD removal (%)	Effluent pH
8	5.89	38.4	23	4.25
16	7.7	41	16.8	3.63
24	9.2	39.7	14.66	3.56

It was observed that the production of VFAs was higher at high OLR than at low OLR. The pH value decreased with a great amount of VFAs (such as acetic acid, butyric acid) were produced, which lead to the activity of microorganism (VSS/SS) declined. The collection between OLR and VSS/SS can be observed from Fig. 2, showing that, the VSS/SS was in general correlated irrespective of the different OLR. Linear regression results show that the correlation between VSS/SS (y) and OLR (x) can be expressed as $y = -0.6x + 78$ ($r^2 = 0.4948$).

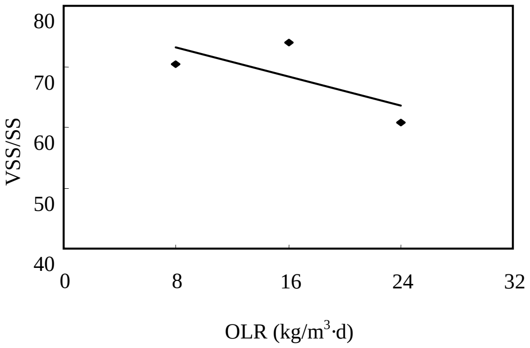


Fig. 2 The collection between OLR and VSS/SS

B. *Variation in Soluble Metabolites*

Fermentative H_2 production is always associated with substrate metabolic conversion of organic fraction to acid intermediates (secondary metabolites) in the anaerobic microenvironment. Production of acidic intermediates (VFA) reflects the changes in the metabolic process involved and provides information to improve the conditions favorable for H_2 production. Visible difference in VFA productions, including typical soluble metabolite ethanol (EtOH), acetic acid (HAc), propionic acid (HPr), and butyric acid (HBu), were observed between feed compositions.

TABLE 2 THE VARIATION IN LIQUID FERMENTATION PRODUCTS

OLR (kg/m^3·d)	VFAs (mmol/L)	EtOH (mmol/L)	HAc (mmol/L)	HPr (mmol/L)	HBu (mmol/L)
8	41.8	16.9	14.7	0.8	8.9
16	56.5	24.2	23.5	0.72	7.2
24	90	44	36.4	1.24	7.53

HAc: acetic acid; HPr: propionic acid; HBu: butyric acid; EtOH: ethanol; VFAs = EtOH + HAc + HPr + Hbu

Table 2 reveals the distribution of soluble fermentation products during the attached bioreactor operation. The production of VFAs and their characteristics were closely correlated with OLR. Both VFAs and ethanol kept increasing with OLR. The total concentration of liquid fermentation products ranged from 41.8 to 56.5 mmol/L when OLR increased from 8 to 16 kg/m^3·d, and further increased to 90 mmol/L at OLR of 24 kg/m^3·d. The dominant metabolic products were ethanol and acetic acid with the yield of 16.9-44 mmol/L and 14.7-36.4 mmol/L, respectively.

IV. CONCLUSIONS

This work demonstrates a feasible cell immobilization method for entrapment of activated sludge for anaerobic hydrogen production from molasses. Acclimation of the immobilized sludge via continuous operation was required in upgrading the performance of H_2 production. Organic loading rate (OLR) was shown to significantly affect the effectiveness of H_2 fermentation. The maximum hydrogen production rate of 3.65 L was obtained in the reactor at OLR of 24 kg/m^3d. The immobilized H_2-producing biocatalyst developed here may have potential for practical applications for conversion of carbohydrate-bearing wastewater into clean energy (H_2).

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