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Response of a biohydrogen-producing reactor to the substrate shift from sucrose to lactose

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ABSTRACT

The response of an upflow acidogenic granule-based reactor to the substrate shift from sucrose to lactose was investigated in this study. Experimental results show that it took 60 h for the reactor to completely degrade the new substrate. Hydrogen production performance, in terms of H_2 partial pressure, H_2 production rate and H_2 yield, was affected. Acetate, propionate, butyrate, valerate, caporate, ethanol and propanol were present in the reactor effluent, and their distribution changed significantly after the substrate shift. As the substrate was changed, the caproate- and ethanol-type fermentation was weakened, while the propionate-type fermentation was strengthened. Throughout the experiment, the butyrate-type fermentation played an important role. The H_2 yield had a close correlation with both propionate and B/A (butyrate/acetate) in this substrate shift process.

1. Introduction

Hydrogen is an efficient energy carrier with high energy content per unit mass. It is considered as the cleanest energy because its combustion product is only harmless water, but no greenhouse gases (Das and Veziroglu, 2001). Hydrogen is also a raw material for various industrial applications (Levin et al., 2003). To make H_2 become a more sustainable source of energy, it should be produced in cost-effective ways. Despite its green nature as a fuel, H_2 is still primarily produced from nonrenewable sources such as natural gas or petroleum hydrocarbons through steam reforming. Anaerobic fermentative production of H_2 decomposes organic substrates, and thus has an additional merit of converting organic wastes into more valuable energy sources (Levin et al., 2003; Yu et al., 2004). Thus, it has recently attracted considerable interests as an effective way of recovering H_2 from organic wastes (Levin et al., 2003; Oh et al., 2004).

Pervious studies have demonstrated that the fermentative H_2-producing process is influenced by many factors, such as substrate type, reactor configuration, hydraulic retention time (HRT), influent organic concentration, organic loading rate, pH, temperature, oxidation-reduction potential and nutritional requirements (Noike and Mizuno, 2000; Logan et al., 2002; Oh et al., 2004). Among these factors, substrate type is an important one. For example, proteins are proven to be inappropriate for H_2 production by anaerobic fermentation because of thermodynamic reasons (Yu et al., 2004).

Both sucrose and lactose are important disaccharides. Sucrose is obtained from sugarcane and sugar beet, while lactose exists in milk and cheese whey (Ferchichi et al., 2005). The hydrolyzation products of sucrose and lactose are different:

$$C_{12}H_{22}O_{11}\ (\text{sucrose}) + H_2O = C_6H_{12}O_6\ (\text{glucose}) + C_6H_{12}O_6\ (\text{fructose}) \quad (1)$$

$$C_{12}H_{22}O_{11}\ (\text{lactose}) + H_2O = C_6H_{12}O_6\ (\text{glucose}) + C_6H_{12}O_6\ (\text{galactose}) \quad (2)$$

A substantial number of studies have been performed to investigate H_2 production from sucrose in batch or continuous modes (Fang and Liu, 2002; Collet et al., 2004; Lin and Lay, 2004; Ferchichi et al., 2005). Because of the seasonal production of sucrose and lactose, the plants have to shift their main products. As a consequence, the substrate composition to the wastewater treatment plants has to be seasonally changed and the substrate of the bioreactors has also been shifted. On the other hand, anaerobic granules grown on sucrose-rich wastewater are sometimes used as the seed sludge to the reactors for the treatment of lactose-rich wastewater. In this case, substrate shift problem is also encountered. Since the H_2-producing bacteria are sensitive to environmental changes, an investigation into the response of the H_2-producing reactor to the substrate shift from sucrose to lactose might provide useful information for the operation of the reactors. However, little information is available in the literature concerning the shift of substrate in a continuous-flow H_2-producing reactor. Therefore, the main objective of this study was to explore the H_2 production performance of an upflow anaerobic sludge blanket reactor (UASB)

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with a shift of substrate from sucrose to lactose, and also to evaluate the influence of substrate shift on the distribution of the acidogenic products.

2. Methods

2.1. Reactors, seed sludge and substrates

The experiment was conducted in a bench-scale Plexiglas-made UASB reactor. The reactor consisted of a reaction portion of 2.0 l and a gas-solids separator portion of 2.6 l. The UASB reactor was seeded with the sludge taken from a full-scale anaerobic reactor treating citrate-producing wastewater. The pH and volatile suspended solids (VSS) of the seed sludge were 7.1 and 6.33 g/l, respectively. Prior to use, the seed sludge was washed with tap water five times, and was then sieved with a sieve of 60 Mesh to remove stone, sand and other coarse matters.

Sucrose- or lactose-rich synthetic wastewater was separately used as the substrate, supplemented with buffering chemicals and a sufficient amount of inorganic nutrients as follows (in mg/l): NH_4HCO_3, 405; $K_2HPO_4 \cdot 3H_2O$, 155; $CaCl_2$, 50; $MgCl_2 \cdot 6H_2O$, 100; $FeCl_2$, 25; NaCl, 10; $CoCl_2 \cdot 6H_2O$, 5; $MnCl_2 \cdot 4H_2O$, 5; $AlCl_3$, 2.5; $(NH_4)_6Mo_7O_{24}$, 15; H_3BO_3, 5; $NiCl_2 \cdot 6H_2O$, 5; $CuCl_2 \cdot 5H_2O$, 5; and $ZnCl_2$, 5.

2.2. Experimental

The UASB reactor was operated for 5 months to enrich the H_2-producing granules and establish a stable operation. Then, the reactor was operated at a fixed loading rate of 10 g-COD/l and an HRT of 12 h for 2 months.

After the UASB reactor reached a pseudo-steady state, experiments were conducted to evaluate the influence of substrate shift. The reactor was extensively sampled for 40 h when sucrose was used as the substrate, after that the substrate was changed to 10 g-COD/l lactose with the same alkalinity. Aqueous effluent, biogas amount and composition were analyzed. The reactor was operated at an HRT of 12 h and 35 ± 1 °C throughout the entire operation.

2.3. Analyses

The volume of biogas produced was recorded daily using a gas meter with water displacement method. The contents of H_2 and CH_4 in biogas were analyzed with a gas chromatograph (Lunan, Model SP-6800A) equipped with a thermal conductivity detector and a 1.5 m stainless-steel column packed with 5 Å molecular sieve. The temperatures of injector, detector and column were kept at 100 °C, 105 °C and 60 °C, respectively. Argon was used as the carrier gas at a flow rate of 30 ml/min. The concentrations of ethanol and volatile fatty acids (VFA), including acetate, propionate, butyrate, *i*-butyrate, valerate and caproate, in the effluent were determined with another gas chromatograph (Agilent, Model 6890NT) equipped with a flame ionization detector and a 30 m × 0.25 mm × 0.25 μm fused-silica capillary column (DB-FFAP). The liquor samples were first centrifuged at 12000 rpm for 5 min, and were then acidified by formic acid and filtrated through 0.2 μm membrane and finally measured for free acids. The temperatures of the injector and detector were 250 °C and 300 °C, respectively. The initial temperature of the oven was 70 °C for 3 min followed with a ramp of 20 °C/min for 5.5 min and to final temperature of 180 °C for 3 min. Nitrogen was used as carrier gas with a flow rate of 2.6 ml/min. The concentrations of sucrose and lactose were determined by using anthrone-sulfuric acid method (Dubois et al., 1956), while the VSS was measured according to the Standard Methods (APHA, 1995). The concentration of total organic carbon (TOC) was measured with a TOC analyzer (Shimadzu Co., TOC-V_{CPN}).

3. Results and discussion

3.1. Variation of substrate degradation and effluent pH

As shown in Fig. 1, the degradation efficiency of sugar exceeded 99.5% before substrate was shifted at hour 40. After the shift from sucrose to lactose at hour 40, the degradation efficiency decreased sharply to a minimum of 53.5% at hour 67. This could result from the incomplete lactose degradation in the reactor after the substrate was changed. This phenomenon could be explained by the fact which different enzymes are needed to hydrolyze sucrose and lactose. From hour 67 to hour 98, the lactose degradation efficiency increased gradually, indicating that lactose was started to be hydrolyzed and degraded. After hour 98, the substrate degradation efficiency was resumed to around 99% again.

The reactor effluent pH was around 3.90 before the substrate shift (Fig. 1). After the shift, the effluent pH increased rapidly to 4.70 at hour 70, resulting from the poor lactose degradation and the relatively decreasing VFA production (Fig. 1). After hour 70, the pH decreased to pH 4.50 and kept unchanged later.

3.2. Evolvement of H_2 production

The H_2 partial pressure declined from 0.41 atm at hour 40 to 0.34 atm at hour 60 (Fig. 2). It slightly increased at hour 67, but decreased to 0.07 atm at hour 200. The biogas was free of methane, due to the suppression of methanogenic activity at a very lower pH.

As illustrated in Fig. 2, the substrate shift also induced a fluctuation of the H_2 production rate. It decreased from 93 ml/l/h at hour 40 to 39 ml/l/h at hour 50. It increased to 128 ml/l/h at hour 64 and decreased to 11 ml/l/h at hour 230. The H_2 yield had a similar changing trend to that of the H_2 production rate. It decreased from 1.55 mol-H_2/mol-sugar at hour 40 to 0.18 mol-H_2/mol-sugar at hour 135, and kept stable thereafter.

During the shift, the H_2 partial pressure, H_2 production rate and H_2 yield all decreased with different degrees. This might be attributed to the poor microbial adaptability and the change of fermentation type in the reactor.

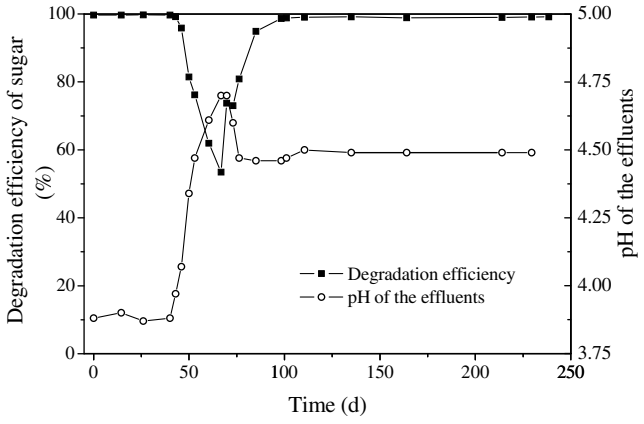

Fig. 1. Variation of substrate degradation efficiency and effluent pH in the substrate shift.

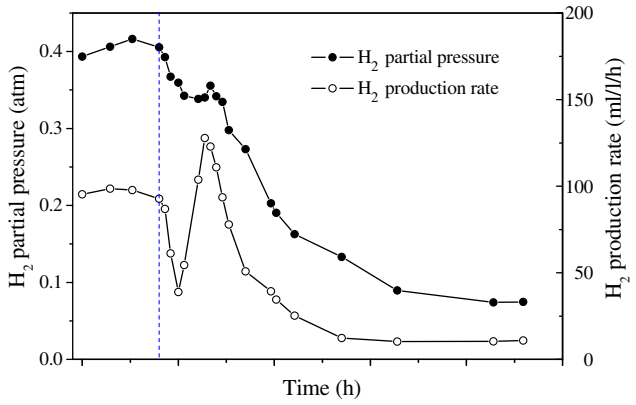

Fig. 2. Variation of H2 partial pressure and H2 production rate in the substrate shift.

3.3. Formation of aqueous products

In this study, caproate, butyrate and ethanol were found to be dominant aqueous products before the substrate shift (Fig. 3). The concentrations of caproate, butyrate and ethanol were 1500, 1110, and 770 mg/l, respectively, whereas the concentrations of acetate, valerate, propionate and propanol were 380, 160, 100 and 30 mg/l, respectively. Such an aqueous product distribution indicates that a mixed-type fermentation existed in this H_2-producing UASB reactor before the substrate shift.

As shown in Fig. 3, after the substrate shift, the caproate concentration decreased. The butyrate concentration decreased quickly from 1110 mg/l at hour 40 to 750 mg/l at hour 60, but then increased to 1455 mg/l at hour 110. After that, it decreased gradually to around 1060 mg/l. Acetate and propionate had similar changing patterns. It should be noticed that all the acetate, propionate and butyrate concentrations had a maximum value at hour 110. The level of ethanol decreased from 750 mg/l at the beginning to 500 mg/l in the end. In the shift process, the concentration of valerate increased from 147 mg/l to 450 mg/l and propanol kept at a low level of less than 40 mg/l.

After the substrate shift, acetate became the major product and a significant production of propionate was also observed. This suggests that the fermentation type was converted from the caproate- and ethanol-type to the propionate-type. However, it should be noticed that the butyrate-type fermentation was present through this experiment.

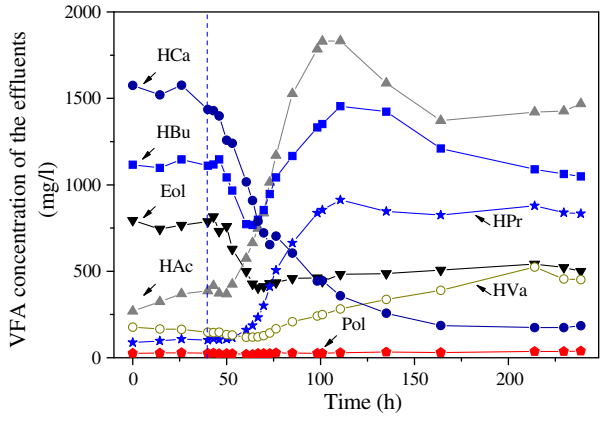

Fig. 3. Variation of aqueous products in the substrate shift (HAc = acetate; HPr = -propionate; HBu = butyrate; HVa = valerate; HCa = caproate; Eol = ethanol; and Pol = propanol).

3.4. Relationship between H_2 yield and propionate and B/A ratio

The propionate concentration increased sharply from hour 64 to hour 98 and the corresponding H_2 yield decreased quickly. Fig. 4a shows a negative relationship between the propionate concentration and H_2 yield. This may be because the propionate-acetate type fermentation contributed little to H_2 production, although lactose was increasingly consumed. Similar phenomenon was observed in a fluidized-bed bioreactor for H_2 production from sucrose (Koskinen et al., 2007).

The distribution of aqueous products in anaerobic H_2 fermentation is often a crucial signal in assessing the efficiency of the H_2-producing cultures (Cha and Noike, 1997). Usually, a high H_2 yield is often achieved accompanied with a high butyrate to acetate (B/A) ratio (White, 1995; Annous et al., 1996; Kim et al., 2006). This was confirmed by our experimental results. The B/A ratio dropped from 2.88 at hour 40 to 0.74 at hour 230. At the same time, the H_2 yield decreased from 1.55 mol-H_2/mol-glucose at hour 40 to 0.18 mol-H_2/mol-glucose at hour 230. As shown in Fig. 4b, there was a close correlation between the H_2 yield and the B/A ratio, suggesting that more electrons went to acetate, rather than for H_2 production.

In theory, acetate production can result in a higher H_2 yield than butyrate production (4 mol-H_2/mol-glucose vs. 2 mol-H_2/mol-glucose) (Nandi and Sengupta, 1998). However, the acetate production may also be related to the H_2 consumption according to the following reaction (Kim et al., 2006):

$$4H_2 + 2HCO_3^- + H^+ = CH_3COO^- + H^+ + 4H_2O \quad (3)$$

Therefore, the low H_2 production after substrate shift might be attributed to the H_2 consumption for acetate production.

Butyrate and ethanol are usually found as the dominant aqueous products in fermentative H_2-producing reactors (Lin and Jo, 2003; Chang and Lin, 2004; Collet et al., 2004). However, in a UASB reactor at pH 3.10–4.00, a high level of caproate was detected and a thermodynamic analysis was performed to justify the caproate formation pathway (Wang et al., 2007). In the present study, the

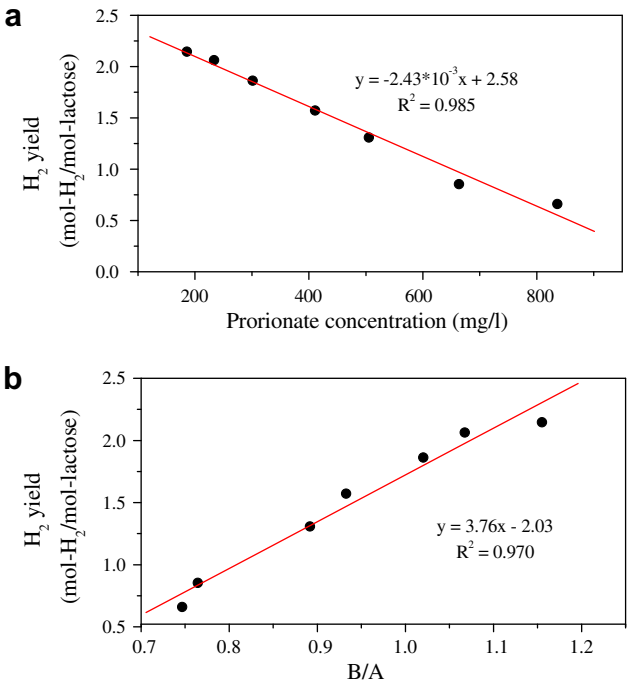

Fig. 4. Relationship between the H2 yield and: (a) propionate concentration; and (b) B/A.

caproate-type fermentation was also found to be dominant before the substrate was changed. However, the complex VFA distribution may result from different microbial community structure and different fermentation types in reactors (Koskinen et al., 2007). In this study, the substrate shift from sucrose to lactose might activate the propionate-producing bacteria and inhibit the caproate-producing bacteria. However, it warrants a further investigation into the change of the microbial community.

4. Conclusions

The experimental results show that the substrate shift from sucrose to lactose caused a disturbance in the performance of the H_2-producing UASB reactor, and it took 60 h for the reactor to completely degrade the new substrate after such a shift. In the substrate shift process, the H_2 partial pressure and H_2 yield decreased from 0.41 to 0.07 atm and 1.55 to 0.18 mol-H_2/mol-sucrose, respectively. The aqueous product distribution was affected by the substrate shift, and the caproate- and ethanol-type fermentation was shifted to the propionate-type fermentation. The decrease in hydrogen production was related to the low substrate degradation efficiency and the change of fermentation type. The H_2 yield had a good relativity with propionate and B/A (butyrate/acetate) in this substrate shift process.

Acknowledgements

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References

Annous, B.A., Shieh, J.S., Shen, G.J., Jain, M.K., Zeikus, J.G., 1996. Regulation of hydrogen metabolism in Butyribacterium-Methylotrophicum by substrate and pH. Appl. Microbiol. Biotechnol. 45, 804–810.

APHA, AWWA, WEF, 1995. Standard Methods for the Examination of Water and Wastewater, 19th ed. American Public Health Association, Washington, DC.

Cha, G.C., Noike, T., 1997. Effect of rapid temperature change and HRT on anaerobic acidogenesis. Water Sci. Technol. 36, 247–253.

Chang, F.Y., Lin, C.Y., 2004. Biohydrogen production using an up-flow anaerobic sludge blanket reactor. Int. J. Hydrogen Energy 29, 33–39.

Collet, C., Adler, N., Schwitzguebel, J.P., Peringer, P., 2004. Hydrogen production by *Clostridium thermolacticum* during continuous fermentation of lactose. Int. J. Hydrogen Energy 29 (14), 1479–1485.

Das, D., Veziroglu, T.N., 2001. Hydrogen production by biological processes: a survey of literature. Int. J. Hydrogen Energy 26, 13–28.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination sugars and related substance. Anal. Chem. 28, 350–356.

Fang, H.H.P., Liu, H., 2002. Effect of pH on hydrogen production from glucose by a mixed culture. Bioresource Technol. 82, 87–93.

Ferchichi, M., Crabbe, E., Gil, G.H., Hintz, W., Almadidy, A., 2005. Influence of initial pH on hydrogen production from cheese whey. J. Biotechnol. 120, 402–409.

Kim, S.H., Han, S.K., Shin, H.S., 2006. Effect of substrate concentration on hydrogen production and 16S rDNA-based analysis of the microbial community in a continuous fermenter. Process Biochem. 41, 199–207.

Koskinen, P.E.P., Kaksonen, A.H., Puhakka, J.A., 2007. The relationship between instability of H2 production and compositions of bacterial communities within a dark fermentation fluidised-bed bioreactor. Biotechnol. Bioeng. 97 (4), 742–758.

Levin, D.B., Pitt, L., Love, M., 2003. Biohydrogen production: prospects and limitations to practical application. Int. J. Hydrogen Energy 29, 173–185.

Lin, C.Y., Jo, C.H., 2003. Hydrogen production from sucrose using an anaerobic sequencing batch reactor process. J. Chem. Technol. Biotechnol. 78 (6), 678–684.

Lin, C.Y., Lay, C.H., 2004. Effects of carbonate and phosphate concentrations on hydrogen production using anaerobic sewage sludge microflora. Int. J. Hydrogen Energy 29 (3), 275–281.

Logan, B.E., Oh, S.E., Kim, I.S., van Ginkel, S., 2002. Microbial production of hydrogen: an overview. Crit. Rev. Microbiol. 36, 2530–2535.

Nandi, R., Sengupta, S., 1998. Biological hydrogen production measured in batch anaerobic respirometers. Environ. Sci. Technol. 24, 61–84.

Noike, T., Mizuno, O., 2000. Hydrogen fermentation of organic municipal wastes. Water Sci. Technol. 42 (12), 155–162.

Oh, Y.K., Kim, S.H., Kim, M.S., Park, S., 2004. Thermophilic biohydrogen production from glucose with trickling biofilter. Biotechnol. Bioeng. 60, 690–698.

Wang, Y., Mu, Y., Yu, H.Q., 2007. Comparative performance of two upflow anaerobic biohydrogen-producing reactors seeded with different sludges. Int. J. Hydrogen Energy 32 (8), 1086–1094.

White, D., 1995. The Physiology and Biochemistry of Prokaryotes. Oxford University Press, New York.

Yu, H.Q., Mu, Y., Fang, H.H.P., 2004. analysis on formation of alcohols and high-molecular-weight VFA in acidogenesis of lactose. Biotechnol. Bioeng. 87, 813–822.