Hydrogen production from wastewater by acidogenic granular sludge

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Abstract Sludge was granulated in a hydrogen-producing acidogenic reactor when operated at 26°C, pH 5.5 treating a sucrose-rich wastewater. The influence of hydraulic retention time (HRT) and sucrose concentration on hydrogen production by the acidogenic granular sludge was investigated at a constant loading rate of 25 g-sucrose/(l·day). Results show that the gas composition was not greatly influenced by HRT or sucrose concentration. The hydrogen accounted for 57% to 68% of the biogas at HRT ranging 4.6–28.6 h and sucrose concentration ranging 4,800–29,800 mg/l. However, the hydrogen yield was more dependent on HRT and sucrose concentration. It ranged from 0.19 to 0.27 l/g-sucrose with the maximum yield occurring at HRT 13.7 h and sucrose concentration 14,300 mg/l in the wastewater. The acidified effluent was composed of volatile fatty acids and alcohols. The predominant products were butyrate (59–68%) and acetate (10–25%), plus smaller amounts of i-butyrate, valerate, i-valerate, caproate, methanol, ethanol, propanol, and butanol. The sludge yield averaged 0.2 g-VSS/g-sucrose. The carbon balance was 98–107% throughout the study.

Keywords Acidification; anaerobic; granule; hydrogen; sucrose; wastewater

Water Science and Technology **Vol 47 No 1 pp 153–158** © IWA Publishing 2002

Introduction

In a conventional anaerobic reactor, organic pollutants in wastewater are converted to methane through interactions of various anaerobes. Full-scale applications of anaerobic wastewater treatment technology have become widely accepted in the past decades (Fang and Liu, 2000). This is largely due to the successful development of several high-rate reactors. Among them, the upflow anaerobic sludge blanket (UASB) reactor (Lettinga *et al.*, 1980) has become most popular for the treatment of a variety of industrial effluents. In a UASB reactor, sludge agglutinates into granules, resulting in the increase of biomass concentration and the reduction of sludge washout. Furthermore, the formation of biogranules also facilitates the syntrophic interactions among microbes involved in the methanogenic degradation of organic pollutants (Fang, 2000).

However, many industrial wastewaters containing complex pollutants prefer the two-stage anaerobic process (Fang and Yu, 2000), in which hydrolysis and acidification are conducted in the first reactor at pH, temperature, and hydraulic retention time (HRT) favored by the fermentative and acidogenic bacteria. The acidic effluent is subsequently treated in a separate methanogenic reactor. In the acidogenic reactor, hydrogen is often a by-product under proper conditions. Since hydrogen is a valuable raw material for many industrial applications as well as a clean energy source, harvesting hydrogen from the two-stage anaerobic wastewater treatment process has attracted much research attention recently (Mizuno *et al.*, 2000; Nakamura *et al.*, 1993). In most previous studies, hydrogen was produced by microbes grown in suspension. However, it was found in a recent study that the hydrogen-producing sludge could also be developed into granules with high bioactivity (Fang *et al.*, 2001).

Microbial hydrogen production is greatly influenced by the operational parameters, such as pH (Tanisho *et al.*, 1989) and HRT (Nakamura *et al.*, 1993). However, the reported optimal HRT values for hydrogen production are conflicting, varying from 6 h (Lin and Chang, 1999) to 17 h (Lay, 2000). This study was conducted to examine the influence of HRT, and hence substrate concentration, on continuous hydrogen production by the granular acidogenic sludge at a constant loading rate. The physical and microscopic characteristics of such granules were also investigated.

Material and methods

Experimental conditions

The hydrogen-production experiment was conducted in a 3 litre fermentor (Biostat B, B. Braun Biotech, Germany) treating a sucrose-containing wastewater at pH 5.5, 26°C. The acidogenic granular sludge from a stirred reactor treating a similar wastewater was used to seed the acidogenic reactor at an initial concentration of 10 g-VSS/l. The HRT was kept at 4.6 h and sucrose concentration 4,800 mg/l in startup. Startup was completed after 30 days as the hydrogen production and effluent composition become steady. The HRT was then increased from 4.6 to 28.8 hours, corresponding to the increase of sucrose concentrations from 4,800 to 29,800 mg/l to keep the loading rate at the constant level of 25 g-sucrose/(l·day). The reactor was operated at each HRT/concentration level for 14–21 days to ensure the reactor reaches a pseudo-steady state operation before increasing to the next level. Trace metals and balanced nutrients were added to the wastewater following the formula reported in a previous study (Fang *et al.*, 1996). The pH was automatically controlled by two peristaltic pumps feeding NaOH (6 mol l^{-1}) and HCl (4 mol l^{-1}) solutions, respectively. The fermentor was mixed continuously. A level probe and a harvest pump were used to keep the mixed liquor volume at a constant 1.7 litres.

Biogas and effluent analyses

The amount of biogas produced was recorded daily using the water displacement method. The content of hydrogen, carbon dioxide, methane and nitrogen were analyzed by a gas chromatograph (GC) (Model 5890II, Hewlett Packard, USA) equipped with a thermal conductivity detector and a 2 m × 2 mm (inside diameter) stainless-steel column packed with Porapak N (80–100 mesh). Injector, detector and column temperatures were kept at 57°C, 180°C and 50°C, respectively. The concentrations of volatile fatty acids (VFA) and alcohols in the effluent were determined by a second GC of same model, which was equipped with a flame ionization detector and a 10 m × 0.53 mm HP-FFAP fused-silica capillary column, following the procedures described previously (Yu and Fang, 2000). Contents of volatile suspended solids (VSS) and ashes in the granular sludge were determined according to the *Standard Methods* (1992). The concentration of sucrose was measured using the anthrone method (Gaudy, 1962).

Physical and microscopic characteristics of granule

The average granule diameter was analyzed from the digital photographic images. The settling velocity was measured in a plexiglas column (i.d. 200 mm). The microstructure of the granules was examined using scanning electron microscopy (SEM, Stereoscan 360, Cambridge, USA). The sample preparation procedures were as reported previously (Fang, 2000).

Results and discussion

Production of hydrogen

Figure 1 illustrates the effects of HRT and sucrose concentration in wastewater on (a) sucrose conversion, (b) biogas content, and (c) hydrogen yield. Figure 1a illustrates that

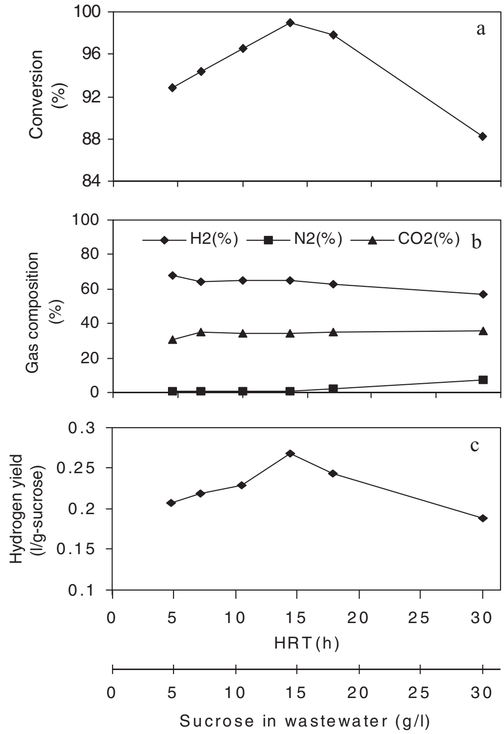


Figure 1 Effects of HRT and sucrose concentration on (a) sucrose conversion, (b) biogas composition, and (c) hydrogen yield

sucrose conversion increased from 92.9% at HRT 4.6 h (sucrose 4,800 mg/l) to 98.9% at HRT 13.7 h (sucrose 14,300 mg/l), and then decreased to 88.2% when HRT increased to 28.8 h (sucrose 29,800 mg/l). The low conversion rate at high HRT and sucrose concentration was probably due to the inhibition of high products concentration. Van den Heuvel *et al.* (1988) reported that free butyrate inhibited the acidogenic degradation of glucose. Figure 1b illustrates that the biogas comprised mostly hydrogen and carbon dioxide, plus a small amount of nitrogen. The gas composition was not greatly influenced by HRT and sucrose concentration. The hydrogen accounted for 57% to 68% of the biogas with carbon dioxide ranging from 31 to 36% and nitrogen from 1 to 7%. The biogas was free of methane, due to the suppression of methanogenic activity under acidic conditions (pH 5.5). The hydrogen yield ranged from 0.19 to 0.27 l/g-sucrose with the maximum yield occurring at HRT 13.7 h and 14,300 mg/l of sucrose, as illustrated in Figure 1c.

Degrading each gram of sucrose produced 0.27 litre of hydrogen at HRT 13.7 h (sucrose 14,300 mg/l). Such a yield is higher than 0.22 l/g (Lin and Chang, 1999) for the degradation of glucose by mixed culture at pH 5.7 and HRT 12 h, and comparable to the yields 0.29 l/g (Kataoka *et al.*, 1997) for the degradation of glucose by *Clostridium butyricum* at pH 6.7 and HRT of 8 h. Converting sucrose into acetate and butyrate with hydrogen as a by-product may be expressed as follows:

$$C_{12}H_{22}O_{11} + 5H_2O \rightarrow 4CH_3COOH + 8H_2 + 4CO_2 \quad (1)$$

$$C_{12}H_{22}O_{11} + H_2O \rightarrow 2CH_3(CH_2)_2COOH + 4H_2 + 4CO_2 \quad (2)$$

According to the stoichiometries of reactions (1) and (2), the theoretical hydrogen yields are 0.57 l/g for acetate and 0.29 l/g for butyrate. The yield of 0.27 1/g found in this study were closer to the theoretical yield for butyrate, which accounted for 67% of organic products in the effluent.

Production of VFA and alcohols

Table 1 lists the distribution of the key VFA and alcohols in the effluents at various HRT. It shows that butyrate and acetate were the two most abundant species in the effluent. Increasing HRT from 4.6 h to 28.6 h (or sucrose concentration from 4,800 mg/l to 29,800 mg/l) resulted in an increase of butyrate in effluent from 58.7% to 68.4%. Acetate accounted for 14.3% of total TOC in effluent at HRT 4.6 h (sucrose 4,800 mg/l), and increased to 25.2% at HRT 13.7 h (sucrose 14,300 mg/l). Further increase of HRT and sucrose concentration decreased the acetate concentration in the effluent to 10.2%.

A small amount of caproate was also found in the effluent, increasing from 0.3% to 3.7% with the increase of HRT and sucrose concentration. Other constituents in the effluent included ethanol (0.9–1.4%) and propionate (0.4–0.9%), plus methanol, propanol, butanol, i-butyrate, i-valerate, valerate and i-caproate. The suppression of propionate and alcohol production was due to the pH value (Inanc *et al.*, 1999; Lay, 2000).

Carbon balance and sludge yield

Carbon in the influent was converted into biomass, carbon dioxide in the biogas, plus those in the effluent. Table 2 summarizes the overall carbon mass balance. The TOC of influent was calculated from the sucrose concentration, whereas those of the effluent were measured. The inorganic carbon (IC) in the influent was attributed by the added $NaHCO_3$, and the IC content in the effluent was the sum of CO_2 (aq), HCO_3^- and CO_3^{2-}, calculated from the partial pressures of carbon dioxide, according to Henry's Law, and the dissociation constants of H_2CO_3 and HCO_3^-. The carbon content in the biomass was calculated assuming the composition of $C_5H_7O_2N$. Table 2 shows that the overall carbon balance was 98–107%.

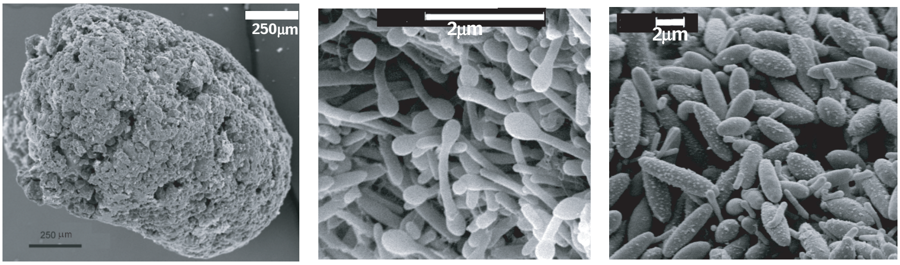
The sludge yield, averaging 0.20 g-VSS/g-sucrose, decreased with HRT and sucrose concentration. The yields ranged from 0.27 g-VSS/g-sucrose at HRT 4.6 h (sucrose

Table 1 Product distribution (on carbon basis) in effluent

HRT (h)	TOC (C-mg/l)	Butyrate (%)	Acetate (%)	Caproate (%)	Ethanol (%)	Propionate (%)
4.6	1,168	58.7	14.3	0.3	1.1	0.4
6.8	1,779	60.1	15.6	0.3	0.9	0.4
10.0	2,689	62.0	21.8	0.7	1.6	0.6
13.7	4,150	67.0	25.2	1.4	1.1	0.9
17.1	5,070	67.6	20.1	1.4	1.2	0.8
28.6	8,610	68.4	10.2	3.7	1.4	0.6

Table 2 Carbon balance for one litre of wastewater

HRT	Influent		Effluent				
	TOC (mg)	IC (mg)	TOC (mg)	IC (mg)	Biogas (CO_2) (mg)	Biomass (mg)	Recovery (%)
4.6	2,012	150	1,168	103	284	679	103
6.8	3,002	150	1,779	116	433	962	104
10.0	4,378	150	2,689	113	632	1,019	98
13.7	6,020	150	4,150	113	1,022	1,302	107
17.1	7,494	150	5,070	116	1,226	1,585	105
28.6	12,630	150	8,610	120	1,817	2,094	99



(a) (b) (c)

Figure 2 SEM images of (a) a typical hydrogen-producing acidogenic granule, (b) spore-forming bacteria, and (c) fusiform bacilli

4,800 mg/l) to 0.13 g-VSS/g-sucrose at HRT 28.6 h (sucrose 29,800 mg/l). At the optimum conditions, i.e. HRT 13.7 h and sucrose 14,300 mg/l, the yield was 0.16 g-VSS/g-sucrose.

Characteristics of granular sludge

The granule had an average diameter of 1.6 mm and a density of 1.038 g/ml. It exhibited a settling velocity of 50 m/h, which was comparable to higher reported velocities for the methanogenic granules (Ross, 1984).

The hydrogen-producing acidogenic granules were creamy white in color, due to suppression of sulfate reducing activity at pH 5.5.

Figure 2 illustrates the SEM images of the hydrogen-producing acidogenic granules sampled at HRT 13.7 h (sucrose 14,300 mg/l). Figure 2a illustrates that the acidogenic granules had porous and multiple cracks on the surface, which is quite different from that of the methanogenic granules. These pores were likely to facilitate the passage of nutrients and substrate as well as the release of hydrogen, which has a very limited solubility of 1.58 mg/l in water. Figures 2b and 2c illustrate that hydrogen producing bacteria were mostly composed of the spore-forming, rod-shaped bacteria and fusiform bacilli. The dominant species might be *Clostridium*, as observed in an earlier study (Fang *et al.*, 2001).

Conclusions

Hydrogen was produced by granulated acidogenic sludge at 26°C, pH 5.5 treating a sucrose-rich wastewater at the constant loading rate of 25 g-sucrose/(l·day), but at various HRT (4.6–28.6 h) and sucrose concentrations (4,800–29,800 mg/l). Results show that the gas composition was not significantly influenced by HRT or sucrose concentration. The biogas consistently contained 57–68% hydrogen, and the maximum hydrogen yield of 0.27 l/g-sucrose occurred at HRT 13.7 h and 14,300 mg/l of sucrose in wastewater. The predominant acidified products in effluent were butyrate (58.7–68.4% on carbon basis) and acetate (10.2–25.2%). SEM micrographs illustrated that the granules were highly porous with multiple cracks on the surface. They were mostly composed of spore-forming, rod-shaped bacteria and fusiform bacilli.

Acknowledgement

The authors wish to thank the Hong Kong Research Grants Council for the financial support of this project (HKU 7004/98E).

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