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EFFECT OF TEMPERATURE SHOCK TO THERMOPHILIC GRANULES

IVAN W. C. LAU and HERBERT H. P. FANG*

Environmental Engineering Research Centre, Department of Civil and Structural Engineering, The University of Hong Kong, Pokfulam Road, Hong Kong

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Abstract—Effects of temperature shock to the activity of thermophilic biogranules in two upflow anaerobic sludge blanket (UASB) reactors were investigated. Treating wastewater containing 9450 mg litre^{-1} of sucrose, equivalent to 10000 mg litre^{-1} of COD (chemical oxygen demand) at 55°C and 24 h of hydraulic retention time (corresponding to a loading rate of 10 g-COD litre^{-1} day^{-1}, both reactors consistently removed 85–90% of COD. The temperature in Reactor-I was raised to 65°C for 8 days, while that in Reactor-D was lowered to 37°C for 16 days. The temperature shocks significantly reduced the reactors' performance: COD removal efficiencies were lowered to 60% for Reactor-I and to only 40% for Reactor-D. Sludge yields during temperature shock were lowered by 25%. In addition, the temperature shock also caused severe biomass washout, lowering of pH and accumulation of fatty acids, in particular propionate. However, both reactors were able to fully recover their efficiencies within 18 days, after the pH was rectified by the addition of alkaline and the temperature was re-adjusted to the normal 55°C. Results of specific methanogenic activity (SMA) tests show that acetotrophic methanogens were not as sensitive to the temperature shock as other bacteria. © 1997 Elsevier Science Ltd

Key words—anaerobic, biogranule, shock, SMA, temperature, thermophilic, UASB

INTRODUCTION

The upflow anaerobic sludge blanket (UASB) process has been successfully commercialized in the past decade for the treatment of various wastewaters (Lettinga and Hulsoff Pol, 1991; Fang *et al.*, 1994a), including those containing starch (Fang and Kwong, 1995), aromatic pollutants (Li *et al.*, 1995; Fang *et al.*, 1996), proteins (Fang *et al.*, 1994b), fatty acids (Fang *et al.*, 1995), etc. Most of these treatment processes were operated at mesophilic condition; only a few studies have been conducted for the UASB treatment of wastewater under thermophilic condition (Wiegant 1985; Souza *et al.*, 1992; Van Lier *et al.*, 1992; Shi and Forster, 1993; Fang and Lau, 1996). Under thermophilic conditions, pollutants could be degraded at higher rates and pathogens could be killed more effectively. Furthermore, for those industrial wastewaters discharged at elevated temperatures, cost saving from cooling could also be substantial.

Anaerobic processes are commonly believed to be sensitive to the sudden change of environmental condition (Speece, 1983). The effect of a temperature shock depends on the temperature induced, exposure time and bacterial composition of sludge (Van Lier *et al*, 1990, 1996; Visser *et al.*, 1993; Obaya *et al.* 1994). In anaerobic reactors operated under mesophilic conditions, temperature shock could result in unstable performance and in some extreme cases complete failure (Lescure *et al.*, 1988; Van Lier *et al.*, 1990). As a result, careful temperature control is essential for anaerobic treatment of industrial wastewater. However, there is little information in literature on the effect of temperature shock on the performance of anaerobic thermophilic reactors. It is, thus, of practical interest to investigate such an effect.

MATERIALS AND METHODS

Two 2.8-litre UASB reactors with an internal diameter of 84 mm and a height of 500 mm were used in this study. Five evenly distributed sampling ports were installed over the height of the column. Total biomass in the reactor was estimated based on the profile of the volatile suspended solids (VSS) of the samples taken from these ports. On the top of each reactor was a gas–liquid–solid separator with an internal diameter of 114 mm and a height of 250 mm, making a filled volume of 2.0 litres. Volumetric loading was calculated basing on the reactor volume alone, excluding volume of the separator. The reactor was water- jacketed and operated at a constant temperature of 55°C, except during the period of sudden temperature change.

Each reactor was seeded with about 0.8 litres of thermophilic biogranules obtained from a previous study on the start-up of thermophilic UASB reactors at 55°C (Fang and Lau, 1996). Synthetic wastewater used in this study was composed of 9500 mg litre^{-1} of sucrose,

*Author to whom all correspondence should be addressed [Fax: 852-2559-5337].

equivalent to 10000 mg litre^{-1} of COD, plus nutrients, alkalinity and trace elements, based on the formulation used previously (Fang and Chui, 1993). Throughout this study, the hydraulic retention time was 24 h, and thus the chemical oxygen demand (COD) loading rate was kept at a constant 10 g-COD litre^{-1}day^{-1}. The wastewater had a constant pH value of 8.0.

Both reactors were first operated at 55°C for 30 days to establish a performance base line. The temperature in one reactor (Reactor-I) was then increased to 65°C and kept at that temperature for 8 days, before returning to the normal temperature of 55°C. The temperature of the other reactor (Reactor-D), on the other hand, was decreased to 37°C for 16 days before returning to normal temperature. Both reactors were then kept at the normal condition until the experiment ended on day 75.

Throughout this study, the biogas production rate and composition and the mixed liquor pH were measured daily. The methane content in the biogas was measured by a gas chromatograph (Hewlett Packard, model 5890A) 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 and detector temperatures were respectively kept at 130 and 200°C, while column temperature was increased from 90 to 110°C.

Effluent qualities, such as COD, volatile fatty acids (VFA), VSS and total suspended solids (TSS) were measured normally about twice a week, except during the temperature shock period they were measured daily. Measurements of COD, VFA, VSS and TSS followed the analytical procedures of the *Standard Methods* (APHA, 1985). The composition of VFA was measured by another gas chromatograph (Hewlett Packard, model 5890A), equipped with a 10 m × 0.53 mm HP- FFAP fused-silica capillary column and a flame ionization detector (FID), using helium as the carrier gas. Injector and detector temperatures were 200 and 250°C, respectively. The fluid sample was filtered through a 0.45-μm membrane filter and acidified to pH 2 with concentrated formic acid prior to injecting into the column using the fast injection technique. The initial temperature of the column was 80°C for 5 min followed with a ramp of 10°C min^{-1} and a final temperature of 130°C for 4 min. VFA standards (Supelco, Bellefonte, PA) were used for the calibration of the FID.

Biogranules were sampled from both UASB reactors three times for specific methanogenic activity (SMA) measurements: first, on day 30, immediately before the temperature shock; then at the end of the shock (i.e. day 38 for Reactor-I, and day 46 for Reactor-D); and, finally, at the end of this study on day 75. The SMA of each biogranule sample was measured in duplicate, using 157- ml serial vials following the method of Owen *et al.* (1979) modified by Dolfing and Bloemen (1985). Sucrose and acetate, respectively, were used as the sole substrate. Each vial was capped with butyl rubber, after adding about 100 mg of biogranules and 100 ml of feed solution containing 300 mg-COD of substrate, plus nutrients and then submerged in a 55°C shaking water-bath. The biogas production was periodically monitored by a syringe, and the methane content by the gas chromatograph. The exact amount of biomass in each vial was measured (as VSS) at the end of each test.

RESULTS AND DISCUSSION

Figures 1 and 2, respectively, illustrate the overall performances of Reactors-I and -D.

Biomass washout and VSS in reactors

Prior to the temperature changes, both reactors performed steadily with 650–850 mg litre^{-1} of VSS in the effluent. Immediately after the temperature increase to 65°C on day 30, the VSS level in the effluent of Reactor-I increased drastically to 1600 mg litre^{-1}, resulting in severe washout of biomass (Fig. 1a). However, the effluent VSS gradually decreased to the original level by day 38; the reduction of biomass washout was partly due to the decrease of biogas production (Fig. 1b). The degree of biomass washout became steady after the temperature returned to 55°C on day 38. Although the effect of temperature increase on biomass washout appeared to be temporary, over 30% of biogranules were washed out within 8 days. Based on the reactor VSS profile, total biogranules inside Reactor-I was estimated to drop from 44 g VSS on day 30 to only 29 g VSS by day 38. Similar observations on the washout of biogranules was also reported in a previous start-up study of thermophilic UASB reactors (Fang and Lau, 1996) when the reactor temperature step-increased from 37 to 55°C. The washout could probably be due to the disintegration of biogranules. Wiegant (1985) speculated that the temperature increase accelerated the decay of fermentative microorganisms; because fermentative microorganisms were believed to be responsible for the structure of granules, their accelerated decay, thus, resulted in the disintegration of biogranules and, hence, the biomass washout. After the temperature returned to 55°C, the amount of biogranules inside Reactor-I slowly increased to 32 g VSS by the end of the experiment on day 75.

Biomass washout was less severe in Reactor-D. After the temperature decreased to 37°C on day 30, the VSS level in the effluent of Reactor-D immediately increased to 1100 mg litre^{-1} (Fig. 2a). The biomass washout was soon stopped, as the VSS in the effluent was reduced to 500 mg litre^{-1} in 3 days partly as a result of reduced biogas production (Fig. 2b). Even so, about 14% of biomass was washed out from Reactor-D. Total biogranules inside Reactor-D was reduced from 56 g VSS on day 30 to 48 g VSS by day 46; however, it was later increased to 66 g VSS by day 75 as the temperature returned to the normal 55°C. Throughout the study, the TSS/VSS ratio for the washed-out biomass ranged from 1.14 to 1.18.

Soluble COD removal

In anaerobic degradation, complex organic pollutants (as measured by COD) are converted by microorganisms into methane and carbon dioxide, plus bacterial cells. The COD-equivalent for carbon dioxide is nil. A previous study (Fang and Lau, 1996) reported that, in the thermophilic degradation of sucrose and milk, 85.9% of COD in wastewater was converted to methane and only 14.1% was converted to cells. Figures 1(b) and 2(b) illustrate, respectively, the methane production in both Reactors. Figures 1(c) and 2(c) illustrate the corresponding COD removal in the reactors. Comparisons between

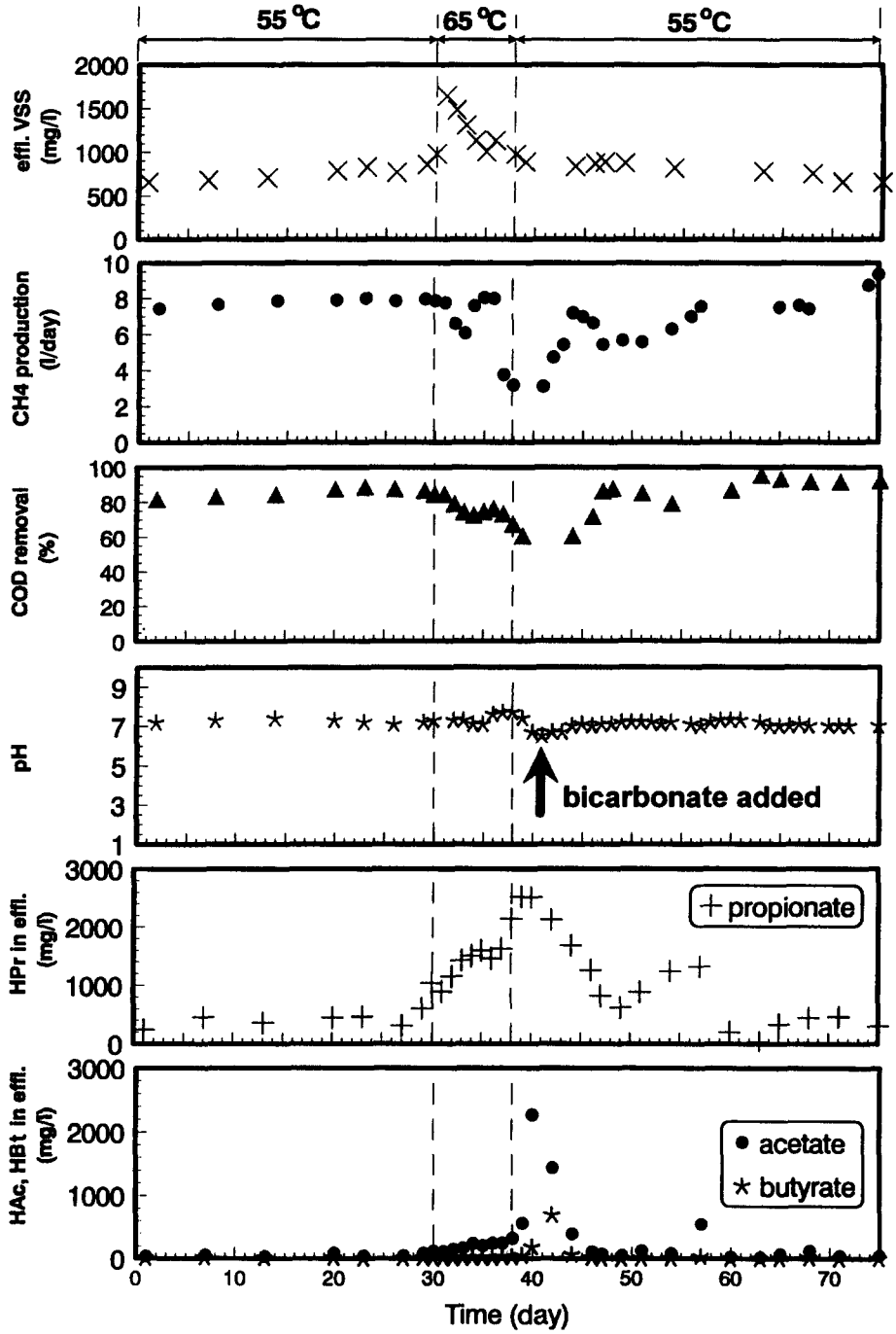

Fig. 1. Performance of Reactor-I: (a) effluent VSS, (b) methane production rate, (c) COD removal efficiency, (d) mixed-liquor pH, (e) propionate in effluent and (f) acetate and butyrate in effluent.

Figs 1(b) and 1(c), and between Figs 2(b) and 2(c), show that the more the methane production the higher the COD removal, as expected.

Figure 1(c) illustrates that, before the temperature increase, Reactor-I removed 85% of COD from the synthetic wastewater at 55°C. After the temperature increased to 65°C on day 30, the COD removal efficiency steadily dropped down to 60% by day 38. After the temperature returned to 55°C on day 39, the COD removal efficiency remained at 60% for 5 days, before recovery began to take place. The efficiency reached 86% by day 47, but it was unstable for the two following weeks and was eventually levelled off at 91%. These results indicate that thermophilic microorganisms in Reactor-I severely lost their methanogenic activity after being exposed to a 10°C increase of temperature. However, they were able to fully regain their activity 8 days after temperature was

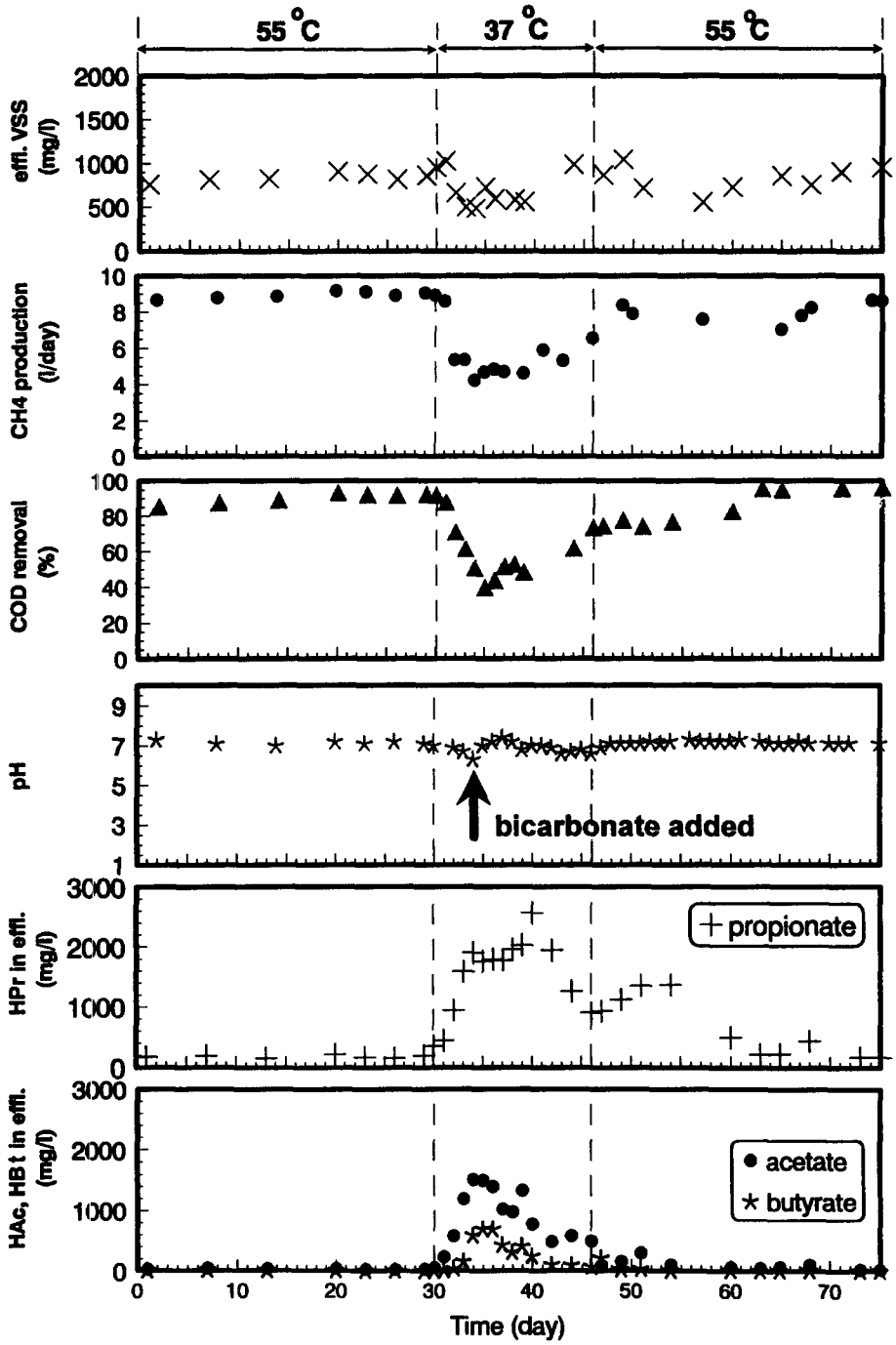

Fig. 2. Performance of Reactor-D: (a) effluent VSS, (b) methane production rate, (c) COD removal efficiency, (d) mixed-liquor pH, (e) propionate in effluent and (f) acetate and butyrate in effluent.

returned to normal. Two studies were found in literature on the effect of temperature increase on mesophilic reactors. Van Lier *et al.* (1990) found that a UASB reactor operated at mesophilic condition (38°C) was unable to regain its bioactivity after a temperature shock at 61–64°C for 5 h. In another study, Lescure *et al.* (1988) found that the COD removal efficiency of mesophilic (36°C) microorganisms in an anaerobic filter was reduced from 85 to 45% after a 7-h temperature shock at 50°C. Comparison of results of the present study with those in literature shows that thermophilic microorganisms appear to be more resilient to temperature increase than mesophilic microorganisms. This is in agreement with a recent report (Van Lier *et al.*, 1996) that thermophilic biogranules had a high thermostability at 45–60°C in a continuous UASB reactor and in batch reactors.

Figure 2(c) illustrates that Reactor-D consistently removed 90% of COD from the wastewater at 55°C before the temperature decrease. Immediately after the temperature decrease to 37°C on day 30, the COD removal efficiency steadily dropped, down to 40% by day 35. Thereafter, the COD removal efficiency gradually increased even when the reactor temperature remained at 37°C, reaching 70% by day 45. After the temperature returned to the normal 55°C on day 46, the efficiency further improved and finally levelled off at 95%. It appeared that thermophilic granules in UASB reactor, after a few days acclimation to the temperature shock, were able to adapt to the lower temperature. The shock of temperature increase had a more severe effect to thermophilic granules than that of temperature decrease.

Sludge yields

Sludge yield in each reactor could be estimated over a period of time from available data, including the change of VSS inside the reactor, total VSS washout and total COD removed over that period. Sludge yields at 55°C were 0.13 g-VSS g-COD^{-1} for Reactor-I (days 39–75) and 0.16 g-VSS g-COD^{-1} for Reactor-D (days 47–75). The corresponding yield values during temperature shock were 0.10 and 0.12 g-VSS g-COD^{-1}, respectively. These results indicate that sludge yields during temperature shock, either an increase or a decrease of temperature, were about 25% lowered than those at the normal 55°C.

Mixed-liquor pH

Figure 1(d) illustrates that the pH of mixed liquor in Reactor-I was mostly kept at pH 7.0–7.3. The pH began to drop on day 39 and reached pH 6.5 on day 41, resulting from the accumulation of fatty acids. Figures 1(e) and 1(f) illustrate that the pH decrease was due to the accumulation of VFAs. In order to avoid further pH decrease, 200 ml of saturated sodium bicarbonate solution was added into the reactor on day 45. The pH of mixed liquor since then was able to keep slightly above pH 7.0. Similar pH decrease as a result of a short-term temperature increase was also reported for a mesophilic UASB reactor (Van Lier *et al.*, 1990). Figure 2(d) illustrates that the pH of mixed liquor in Reactor-D was mostly at pH 6.9–7.3. However, the pH began to drop after the temperature decrease to 37°C. On day 34 the pH reached 6.3, and 200 ml of saturated sodium bicarbonate solution was added. Thereafter, the pH gradually returned to pH 7.0. The pH decrease was also resulted from the increase of VFA (Figs 2e and 2f).

VFAs in the effluent

Figures 1(e) and 2(e) illustrate that a temperature shock, either an increase of temperature or a decrease, resulted in an immediate increase of propionate concentration. In both reactors, propionate concentration in the effluent increased from less than 400 mg litre^{-1} at normal 55°C to the level of 2500 mg litre^{-1} in about 10 days. After the addition of sodium bicarbonate to control the pH and the return of temperature to 55°C, the propionate concentration gradually lowered to the original level in both reactors. Figure 1(f) illustrates that the 10°C temperature increase in Reactor-I also resulted in increases of acetate and butyrate concentration; but the initial increases were not as drastic as propionate. Acetate increased from 50 mg litre^{-1} at normal 55°C to 300 mg litre^{-1} at day 38, and butyrate increased only slightly, from 5 to 20 mg litre^{-1}. However, as the pH decreased during days 39–41, the acetate concentration increased drastically to 2200 mg litre^{-1}, coupling with the increase of butyrate to 650 mg litre^{-1}. However, Fig. 2(f) also illustrates that the 18°C temperature decrease had an immediate effect on the effluent concentrations of acetate and butyrate. Acetate increased from 50 mg litre^{-1} at normal 55°C to the level of 1400 mg litre^{-1} within 4 days, while butyrate increased from 5 to 700 mg litre^{-1} in the same period. In both reactors, the maximal acetate and butyrate concentrations in effluent coincided with the minimal pH value.

Under anaerobic condition, carbohydrates are first converted by acidogens into fatty acids, such as propionate and butyrate. These two intermediates are then further converted by acetogens into acetate and hydrogen, following reactions (1) and (2). Finally, acetate is converted by acetotrophic methanogens, such as *Methanothrix* (Zehnder *et al.*, 1980), into methane, following reaction (3); and H_2/CO_2 is converted by hydrogenotrophic methanogens, such as *Methanobrevibacter* (Mah and Smith, 1981), following Reaction (4).

Butyrate-utilizing acetogenesis

$$CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + 2H_2 + H^+ \quad \Delta G^{0\prime} = +48.1\ \text{kJ} \quad (1)$$

Propionate-utilizing acetogenesis

$$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + 3H_2 + HCO_3^- + H^- \quad \Delta G^{0\prime} = +76.1\ \text{kJ} \quad (2)$$

Acetotrophic methanogenesis

$$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^- \quad \Delta G^{0\prime} = -31.0\ \text{kJ} \quad (3)$$

Hydrogenotrophic methanogenesis

$$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2 \quad \Delta G^{0\prime} = -135.4\ \text{kJ} \quad (4)$$

where $\Delta G^{0\prime}$ represents the change of standard Gibbs free energy at pH7. Judging from the positive $\Delta G^{0\prime}$ values, reactions (1) and (2) are infeasible under normal conditions. Thus, both reactions cannot take place unless the products are kept at very low concentrations. Failing to lower the concentration of

either hydrogen or acetate would inhibit reactions (1) and (2), resulting in accumulation of butyrate and propionate. The build-up of fatty acids would lower the pH and suppress the methanogenesis reaction and, ultimately, cause system failure. There are abundant microscopic evidences (Harper, 1989; Schmidt and Ahring, 1993; Fang *et al.*, 1995) showing that butyrate-degrading and propionate-degrading acetogens syntrophically associated with hydrogenotrophic and acetotrophic methanogens inside UASB biogranules (Boone and Bryant 1980), so that hydrogen and acetate produced by the acetogens are readily consumed by the methanogens.

Harper (1989) estimated that about 40% of the glucose converted to methane through propionate and 60% through butyrate. Propionate is known to have the lowest tolerance level among the VFA for the anaerobic bacteria (Bajpai and Iannotti 1988). Reaction (2) has a higher $\Delta G^{0'}$ value and produces more hydrogen than reaction (1). Thus, degradation of propionate is more sensitive to the build-up of hydrogen than that of butyrate. Propionate is easily accumulated in mesophilic anaerobic digesters during overloading and is difficult to be removed during recovery (McCarty and Mosey, 1991). In addition, it is also the first VFA accumulated in the thermophilic reactor (Wiegant, 1985; Van Lier *et al.*, 1993). Thus, the propionate increase in both reactors could be due to the adverse effect of the temperature shock on hydrogenotrophic methanogens, causing an increase of local hydrogen concentration.

On the other hand, degradation of butyrate produces two acetates (reaction 1), while only one acetate was produced by the degradation propionate (reaction 2). Thus, degradation of butyrate is more sensitive than propionate to the build-up of acetate. This is illustrated in Figs 1(f) and 2(f): the build-up of acetate in effluent after the temperature shock in both reactors was accompanied by the build-up of butyrate.

Specific methanogenic activity

Table 1 summarizes SMA data of biogranules sampled from both reactors using acetate and sucrose, individually, as the sole substrate. Biogranules in each reactor were sampled three times for SMA tests: (a) the day before the temperature shock (day 30), (b) at the end of the temperature shock (i.e. day 38 for those in Reactor-I and day 46 for Reactor-D), and (c) after the biogranules were fully recovered from the shock (day 75).

Table 1. Specific methanogenic activity (SMA) of biogranules

		SMA (g-methane-COD g-VSS^{-1} day^{-1}) of biogranules		
Reactor	Substrate	Day 30	End of shock[a]	Day 75
I	Acetate	1.14	0.73	1.34
I	Sucrose	1.23	0.10	1.07
D	Acetate	0.98	0.84	0.82
D	Sucrose	0.88	0.51	0.82

[a]Day 38 for biogranules from Reactor-I and day 46 for those from Reactor D.

Results in Table 1 show that SMA data at day 75 were in general comparable to those at day 30, indicating that the biogranules had fully regained their bioactivity 30–37 days after the temperature was returned to normal. The SMA data of biogranules at the end of the temperature shock in Table 1 show that a 10°C temperature increase had more severe effect on the SMA of biogranules than a shock of 18°C temperature decrease. Furthermore, the temperature shock had less adverse effect on the acetotrophic methanogens than the others. The SMA of biogranules in Reactor-I using sucrose as substrate was lowered by over 90% as a result of the temperature increase, from an average of 1.15 to 0.10 g-methane-COD g-VSS^{-1}day^{-1}, while those using acetate as substrate was lowered only by 40%, from 1.24 to 0.73 g-methane-COD g-VSS^{-1}day^{-1}. The corresponding decreases of SMA for biogranules in Reactor-D were 40% using sucrose as substrate and 7% using acetate as substrate.

CONCLUSION

A sudden change of temperature, either increase or decrease, resulted in poor COD removal and biomass washout in thermophilic UASB reactors. In addition, it also lowered the pH and caused the accumulation of fatty acids, in particular propionate. However, reactors were able to gradually regain their efficiency as the pH was rectified by alkaline addition and the temperature was returned to the normal 55°C. Results of specific methanogenic activity tests show that activity of biogranules were adversely affected more by the temperature increase than by the temperature decrease. Furthermore, acetotrophic methanogens were not as sensitive as other bacteria to the temperature shock.

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