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FORMATION OF INTERIM BY-PRODUCTS IN METHANOGENIC DEGRADATION OF BUTYRATE

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Abstract—The formation of interim by-products during the methanogenic degradation of butyrate was monitored and analyzed in this study. Two series of experiments were conducted at various butyrate concentrations and under the influence of increased partial pressure of hydrogen (P_{H_2}). In all batches, acetate was found most abundant in the mixed liquor, accounting for over 52–83% of carbon in the original butyrate. This confirms that the degradation of butyrate was through acetate by β-oxidation, and the rate of butyrotrophic acetogenesis was considerably higher than that of acetotrophic methanogenesis. Assuming the degradation followed the Monod equation, the maximum-rate constant μ_{max} was found in the range of 3.4–6.0 mg (mg VSS d)$^{-1}$ and the half-rate concentration K_s was 700–1150 mg l^{-1}. Other interim by-products included hydrogen (up to $10^{-3.8}$ atm), propionate (up to 25 mg l^{-1}), *i*-butyrate (up to 780 mg l^{-1}), and several higher-molecular-weight carboxylic acids (up to 21 mg l^{-1}). All these by-products became fully degraded eventually after reaching the maximum levels, except propionate which remained at the peak concentration. This suggests that the butyrate-degrading sludge was incapable of degrading propionate. Addition of formate to the feed solution increased the P_{H_2}, which enhanced the formation of propionate but did not affect butyrate degradation. The effect of P_{H_2} on propionate was in accordance with the chemical energy analysis. The chemical energy analysis also suggests that the formation of caproate, one of the higher-molecular-weight acids, was independent of the increase of P_{H_2}. © 1999 Elsevier Science Ltd. All rights reserved

Key words—butyrate, by-products, caproate, formate, free-energy, hydrogen, methanogenesis, propionate, valerate

INTRODUCTION

Butyrate is one of the major intermediates in the anaerobic degradation of complex organic pollutants (Gujer and Zehnder, 1983). These pollutants are at first hydrolyzed and converted by acidogens to volatile fatty acids (VFA), which are then further converted to acetate and CO_2/H_2 by acetogens. Ultimately, both acetate and CO_2/H_2 are converted by methanogens to methane. Harper (1989) estimated that in the anaerobic treatment of a soft-drink wastewater about 60% of the glucose was converted to methane through butyrate. On the other hand, Henson *et al.* (1986) reported that a high concentration of butyrate could inhibit methanogenesis. The concentration buildup of butyrate could result from perturbations of process conditions, such as loading rate and/or substrate concentration. The buildup of butyrate is also often accompanied by the increase of partial pressure of hydrogen, one of the key intermediates, and the lowering of pH (McCarty and Smith, 1986; Harper, 1989).

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A number of mesophilic butyrate-degrading bacteria have been identified so far, including *Syntrophomonas wolfei* (McInerney *et al.*, 1981), *Syntrophomonas sapovorans* (Roy *et al.*, 1986), *Syntrophospora bryantii* (Stieb and Schink, 1985; Zhao *et al.*, 1990). Anaerobic treatment of butyrate-containing wastewater has been studied in various processes, such as upflow anaerobic sludge blanket (UASB) (Fang *et al.*, 1995a,b) and fluidized-bed (Zellner *et al.*, 1991). In most of the wastewater studies, treatment efficiency is expressed by the removal of overall COD (chemical oxygen demand), which is the measurement of organic content. Little attention has been paid to the formation of interim by-products during the anaerobic degradation process.

This study was conducted to investigate the interim by-product formation of a butyrate-degrading sludge under various conditions, including increased concentrations of butyrate and formate. Formate was chosen in this study, instead of hydrogen, for several reasons: (1) hydrogen has a limited mass transfer rate from the gas phase to water (Harper and Suidan, 1991), whereas formate is a liquid completely soluble in water; (2) many hydrogenotrophic methanogens can also use formate as

substrate; according to Boone and Whitman (1988), out of the 43 species of methanogens identified 19 can use either formate or H_2/CO_2 as substrate; (3) formate and H_2/CO_2 are of similar levels of energy of formation (Thauer *et al.*, 1977), and can be transferred rather freely from one to another through enzymatic reactions.

MATERIALS AND METHODS

Enrichment culture

The anaerobic butyrate-utilizing sludge was enriched in a 5.0-l water-jacketed chemostat reactor at 37°C. The reactor was seeded with the anaerobic sludge obtained from a UASB reactor (Fang and Chui, 1993) treating dairy wastewater. The granular sludge was disintegrated using a Waring blender before the seeding. Feed solution for the chemostat reactor contained 5500 mg l^{-1} of butyrate, equivalent to 10000 mg l^{-1} of COD, as the sole substrate. The solution also comprised nutrient and trace metals, plus bicarbonate as the buffering chemical, following a formula used in previous studies (Kwong and Fang, 1996) with the exception of excluding sulfate in the formulation. The content in the reactor was completely mixed by recirculating the biogas using a compressor. The hydraulic retention time was 10 days. The reactor was operated for over 90 days to ensure reaching a steady state before sludge was sampled for the batch tests. During the period of day-60 to day-90, the VSS (volatile suspended solids) average 361 mg l^{-1} in the reactor, and the average COD removal was 91%. The sludge yield was thus estimated as 0.040 mg VSS mg COD^{-1}, which is comparable to the literature data of 0.037 mg VSS mg COD^{-1} (Fang *et al.*, 1995b) and 0.047 mg VSS mg COD^{-1} (Lawrence and McCarty, 1969). The mixed liquor on average contained 150 mg l^{-1} of residual butyrate and 610 mg l^{-1} of acetate with a pH ranging from 7.6 to 7.8. There were no fatty acids detected other than residual butyrate and acetate. The partial pressure of hydrogen (P_{H_2}) in the biogas was 10^{-5} atm.

Batch experiments

Two series of batch experiments on the by-product formation of butyrate-degrading sludge were conducted in 120 ml glass serum vials. The first series was to investigate the effect of butyrate concentration. Five batches were run using butyrate as the sole substrate at 500, 900, 3000, 6000 and 9000 mg l^{-1}, respectively. The second series was to investigate the effect of increased P_{H_2} by using formate as the co-substrate for the degradation of butyrate. Two batches were run using feed solutions containing 800 mg l^{-1} of butyrate plus 3000 mg l^{-1} of formate in one, and 960 mg l^{-1} of butyrate and 6000 mg l^{-1} of formate in the other. Formulation of the nutrient and trace elements for the feed solution followed that used in several previous studies (Jia *et al.*, 1996). The nutrient stock solution was sparged with nitrogen to strip off any dissolved oxygen.

The butyrate-degrading sludge from the chemostat reactor was used to seed each batch reactor. About 200 ml of mixed liquor was obtained from the chemostat reactor. After centrifugation, the biomass was washed with the stock nutrient solution. After another centrifugation, the biomass was re-suspended in 800 ml of fresh stock nutrient solution. Each 120 ml vial was added with 60 ml of this solution containing 70 mg l^{-1} of protein, equivalent to 90.3 mg l^{-1} of VSS. Because of dilute concentration, the biomass content in the solution could not be measured directly by the VSS content. In this study, biomass was estimated from the protein concentration of the mixed liquor. Assuming biomass has a chemical formula of $C_5H_7O_2N$, each gram of biomass contains 0.124 g of N and 0.531 g of C. Because the average nitrogen content in protein is 16.0% (Speece and McCarty, 1964; Noguera *et al.*, 1994; Kuo *et al.*, 1996), each gram of protein in the mixed liquor represents 1.29 g (1.29 = 0.160/0.124) of biomass.

After the addition of butyrate and formate and adjusting the pH to 7.9, each vial was submerged in a 37°C shaking water bath. The vigorous shaking motion (35 mm × 125 strokes min^{-1}) ensured complete mixing. At given time intervals, the volume of biogas produced was measured using a syringe, and the contents of the biogas and mixed liquor were analyzed. All sludge handling and solution transfers were conducted inside an anaerobic workstation (Forma Scientific, Model 1029).

Analytical

The protein content in the mixed liquor was measured by the folin method (Lowry *et al.*, 1951), and the COD by the standard methods (APHA, 1989). The biogas contents, including methane, carbon dioxide and hydrogen, were analyzed by a gas chromatograph (Hewlett-Packard, Model Series II) equipped with a thermal conductivity detector, and a 25 m × 0.53 mm CarboPLOT P7 column with a film thickness of 25 μm. Argon was used as the carrier gas at a flowrate of 30 ml min^{-1}. The column was operated at a temperature program of 50°C for 2.5 min and then 110°C for 1.3 min. The injection port and detector were both kept at 180°C. Hydrogen content could be detected down to the 10^{-6} atm level.

Concentrations of VFAs and alcohols were measured by a second gas chromatograph of the same model equipped with a flame ionization detector and a capillary column (Alltech, Econo-Cap FFAP, 30 m in length, 0.53 mm i.d. and 1.2 μm film thickness). The column was operated at a temperature program of 70°C for 4 min and then 140°C for 3 min. The temperature program of injection port and detector were the same, 200°C. Helium was used as the carrier gas at a flow rate of 40 ml min^{-1}. Formate was analyzed by an ion chromatograph (Shimadzu HPLC 10A) equipped with a CDD-6A conductivity detector and a Shim-Pack IC-A3 column. A solution containing 8.0 mM of 4-hydroxybenzoic acid and 3.2 mM of bis-[2-hydroxyethyl]-iminotris-[hydroxyethyl]-methane was used as the mobile phase. The flow rate of the mobile phase was 1.0 ml min^{-1}, the oven temperature 40°C and the detector temperature 43°C. Individual VFA (from C_1 to C_7) could be detected at the 1 mg l^{-1} level, whereas alcohols (from C_1 to C_4) could be detected at the 5 mg l^{-1} level.

RESULTS AND DISCUSSION

Degradation of butyrate

In the first series of experiments, five batches were run using butyrate as the sole substrate at 500, 900, 3000, 6000 and 9000 mg l^{-1}, respectively. The results of the five batches were similar. Figure 1 illustrates the variations of butyrate and products in the mixed liquor, using the batch degrading 3000 mg l^{-1} of butyrate for exemplification, throughout the experiment, including (a) butyrate and acetate, (b) methane, (c) P_{H_2}, (d) *i*-butyrate, (e) propionate, and (f) *i*-valerate and caproate.

Figure 1a illustrates that, after an acclimation period of about 100 h, butyrate degraded rapidly and became depleted after 200 h. During the period, acetate, which was absent in the original feed

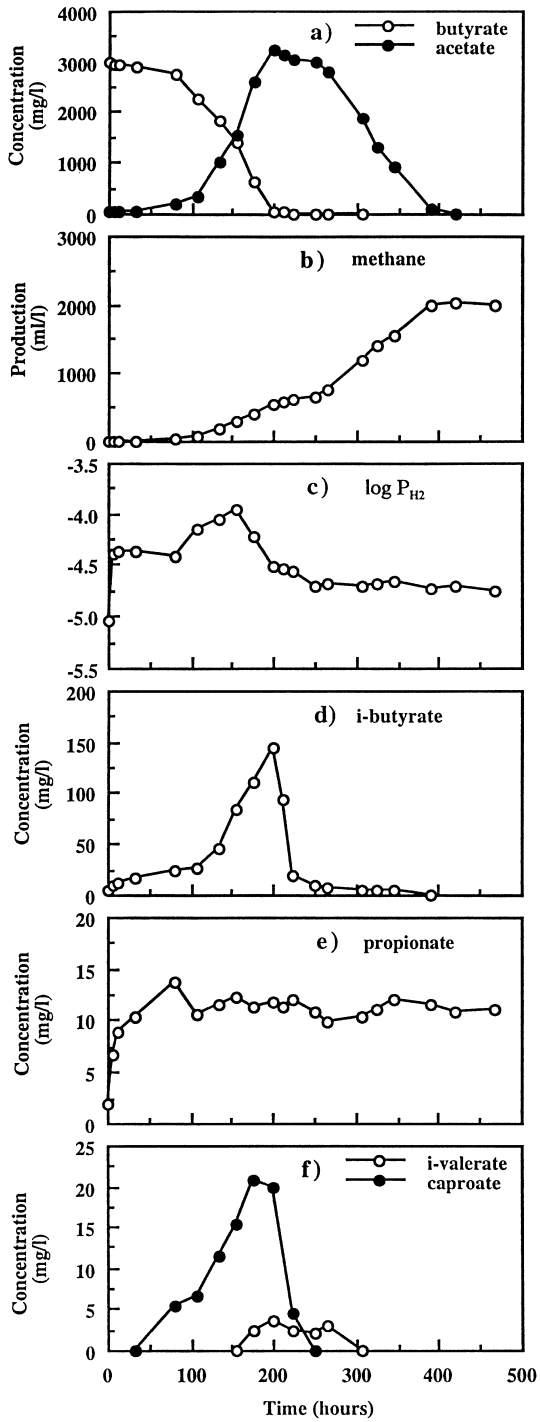

Fig. 1. Concentrations of by-products in batch degradation of 3000 mg l^{-1} of butyrate: (a) butyrate and acetate, (b) methane production, (c) P_{H_2}, (d) *i*-butyrate, (e) propionate, and (f) *i*-valerate and caproate.

solution, increased with the decrease of butyrate. At hour 200, the acetate concentration reached a maximum level of 3225 mg l^{-1}, equivalent to 73% of carbon in the original butyrate. This confirms that acetogenesis through β-oxidation (Gujer and Zehnder, 1983) was the major degradation pathway of butyrate. The by-product acetate was then further converted by methanogens. The significant accumulation of this interim by-product indicates that the rate of butyrotrophic acetogenesis was considerably higher than the subsequent acetotrophic methanogenesis.

Figure 1b illustrates that methane produced from a unit volume of mixed liquor increased in correspondence to the decrease of butyrate and acetate, and the production ceased after hour 420 when acetate became deplete. The mixed liquor pH decreased slightly from the initial 7.9 to 7.4 at hour 200 when the weak butyric acid was converted to stronger acetic acid. It then gradually returned to pH 7.9 by hour 420 when all acetate was converted to methane.

The P_{H_2} also increased rapidly in the initial stage, as illustrated in Fig. 1c, from the initial $10^{-5.0}$ atm to $10^{-4.0}$ atm by hour 154. It was then gradually levelled off to the original $10^{-5.0}$ atm by hour 468 when the experiment was terminated. Formate, which could be converted from hydrogen through enzymatic reactions, was however undetected.

During the degradation process, a number of interim by-products, besides acetate and hydrogen, were also detected in the mixed liquor. Isomerization is often observed in the degradation of butyrate (Lovley and Klug, 1982; McInerney, 1988; Tholozan *et al.*, 1988; Wu *et al.*, 1996). Such a reaction is catalyzed by isomerase and involves little change of free energy. Figure 1d illustrates that *i*-butyrate increased slowly during the initial acclimation period and then increased rapidly after hour 100. It reached a maximum concentration of 145 mg l^{-1} by hour 200, when nearly all butyrate became depleted, corresponding to 5% of butyrate in feed. The *i*-butyrate was then degraded rapidly to 20 mg l^{-1} by hour 224 and became undetectable by hour 391. The interim accumulation of *i*-butyrate appears to suggest that *i*-butyrate was degraded to acetate at a lower rate than *n*-butyrate.

Propionate increased rapidly reaching a maximum level of 14 mg l^{-1} by hour 81, and levelled off to the 11 mg l^{-1} level when the experiment was terminated by hour 468. It shows that the butyrate-degrading consortia of bacteria were unable to degrade propionate. Similar observations were noted by Fang *et al.* (1995b) that butyrate-degrading biogranules sampled from a UASB reactor were unable to degrade propionate in the SMA (specific methanogenic activity) test (Owen *et al.*, 1979).

Two carboxylic acids of higher molecular weight (MW), *i*-valerate and caproate, were detected in the mixed liquor as the experiment proceeded. The former was first detected at hour 176. It reached a maximum concentration of 4 mg l^{-1} by hour 200, and then gradually diminished, becoming undetectable by hour 307. The latter, on the other hand, appeared first at hour 81, reached a maximum

Table 1. Peak concentrations of by-products and peak times in the degradation of butyrate

	Initial butyrate concentration in fees solution (mg l^{-1})									
	500		900		3000		6000		9000	
Interim by-products	conc. (mg l^{-1})[a]	time (h)	conc. (mg l^{-1})[a]	time (h)	conc. (mg l^{-1})[a]	time (h)	conc. (mg l^{-1})[a]	time (h)	conc. (mg l^{-1})[a]	time (h)
Acetate	384	107	1095	133	3225	200	5680	250	7430	325
P_{H_2}	$10^{-4.4}$	7	$10^{-4.2}$	10	$10^{-4.0}$	154	$10^{-4.0}$	132	$10^{-3.8}$	176
i-Butyrate	41	82	62	133	145	200	375	224	780	420
Propionate	5	32	9	133	14	81	15	106	25	176
Valerate	n.d.	–	n.d.	–	n.d.	–	n.d.	–	5	468
i-Valerate	2	82	3	133	4	200	3	250	6	501
Caproate	10	82	14	133	21	176	17	200	13	501
Heptanoate	n.d.	–	n.d.	–	n.d.	–	n.d.	–	4	501

[a]For P_{H_2} the unit is atm.

concentration of 21 mg l^{-1} by hour 176, and then gradually diminished becoming undetectable by hour 250. There was no alcohol detected in the mixed liquor. Smith and McCarty (1989) reported that when an ethanol/propionate-fed complete-mix methanogenic reactor was overloaded, propanol and higher MW carboxylic acids, such as valerate, caproate and heptanoate were present in the effluent. They attributed this to the increase of P_{H_2} as a result of overloading. However, based on thermodynamic analysis, Jia and Fang (1998) concluded that the formation of these higher-MW acids was not just affected by the increased P_{H_2} alone; it could be resulted from the increased concentration of propionate and butyrate as well.

Effect of butyrate concentration

Experiments treating butyrate at 500, 900, 6000 and 9000 mg l^{-1} yielded similar results. Interim by-products, including acetate, hydrogen, *i*-butyrate, propionate, *i*-valerate and caproate were detected in the mixed liquor. All interim by-products were eventually fully degraded after reaching respective maximum levels, with the exception of propionate which remained at the peak level until the end of the experiments.

Table 1 summarizes the peak concentrations of all the detected interim by-products and the time to reach these concentrations in the first series of experiments. In general, the peak concentration and the time reaching the peak for each by-product increased with the butyrate concentration in the feed solution. Take acetate as an example. The peak concentration of by-product acetate was 384 mg l^{-1} treating 500 mg l^{-1} of butyrate. The corresponding acetate concentrations increased to 1095, 3225, 5680, and 7430 mg l^{-1} when the butyrate concentration in the feed solution increased to

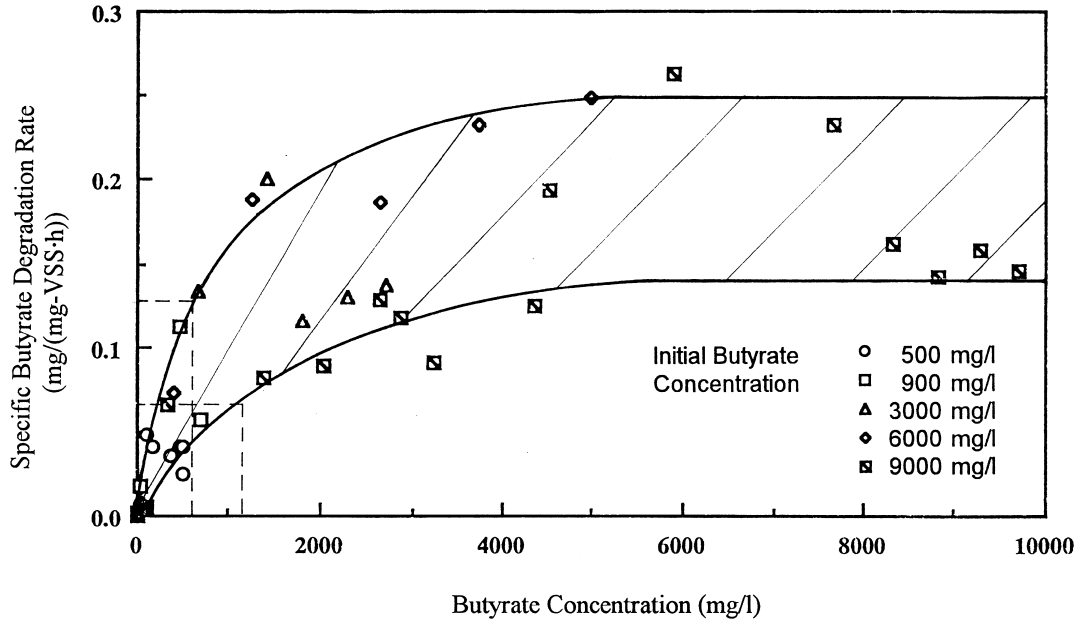

Fig. 2. Specific butyrate degradation rate at various concentrations.

900, 3000, 6000 and 9000 mg l^{-1}. At the peak concentration, the acetate comprised 52–83% of carbons from butyrate. The time to reach the peak concentration increased from 107 h for 500 mg l^{-1} of butyrate to 325 h for 9000 mg l^{-1} of butyrate.

It is interesting to note that valerate and heptanoate, two high-MW carboxylic acids, were not detected in mixed liquor treating 6000 mg l^{-1}, or less, of butyrate. But, they were found in mixed liquor treating 9000 mg l^{-1} of butyrate. The concentrations of valerate and heptanoate peaked at 5 and 4 mg l^{-1}, respectively, after about 500 h. The formation of these two acids could be likely due to the increase of P_{H_2}, which reached a maximum of $10^{-3.8}$ atm which was higher than the maximum of $10^{-4.0}$ atm observed in the other four batches.

Propionate degradability

In all batches, the concentration of propionate reached the maximum level rapidly (about 100 h for the batches treating 9000 mg l^{-1} of butyrate, and less than 10 h for the other four batches), and remained at that level throughout the experiment. Five additional batch experiments were subsequently conducted. The same butyrate-degrading sludge was used to treat propionate (ranging from 500 to 3000 mg l^{-1}) either as a sole substrate or jointly with formate (at either 2000 or 6000 mg l^{-1}) as a co-substrate. In all batches, propionate remained undegradable for over 120 h at the time when the batches were terminated. This further confirms that butyrate-degrading bacterial consortia were incapable of degrading propionate.

Kinetic constants

Figure 2 illustrates the specific butyrate degradation rate at various butyrate concentrations. Microbial degradation kinetics is commonly expressed by the Monod equation (Lawrence and McCarty, 1969),

$$\mu = \mu_{max} S/(K_s + S),$$

where μ is the specific degradation rate and S is the substrate concentration. According to this equation, the degradation kinetics can be characterized by two kinetic constants, i.e. μ_{max} (maximum specific rate) and K_s (half-rate concentration), and has two major characteristics: (1) μ increases linearly with S at low substrate concentrations ($K_s \gg S$), but (2) it levels off to a constant rate, μ_{max}, at high substrate concentrations ($S \gg K_s$). Most data in this study, as plotted in Fig. 2, fall within the range of two Monod curves. The μ_{max} for the degradation of butyrate was in the range from 3.4 to 6.0 mg (mg VSS d)$^{-1}$ (i.e. 0.14–0.25 mg (mg VSS h)$^{-1}$) and the K_s value was in the range from 700 to 1150 mg l^{-1}. For comparison, the corresponding parameters for butyrate degradation in an anaerobic digester were 15.6 mg (mg VSS d)$^{-1}$ for μ_{max} and 5 mg l^{-1} for K_s, respectively (Lawrence and McCarty, 1969). The high K_s value suggests that the rate of butyrate degradation in most reactors (in which the butyrate concentration is $\ll K_s$) would increase linearly with the butyrate concentration.

Effect of formate as co-substrate

Figure 3 illustrates results observed in the second series of experiments, including the concentration changes of reactants, i.e. butyrate and formate, and two by-products, hydrogen and propionate. Results in Fig. 3a and b indicate that the P_{H_2} increased immediately to the $10^{-2.0}$ atm level after the addition of formate. Formate concentration reduced rapidly, mostly being converted to methane, and became depleted after 50–65 h. During this period, the butyrate concentration remained steady, and gradually decreased only when the P_{H_2} was lowered to the 10^{-5} atm level. This seems to imply that the degradation of butyrate was independent of either formate or P_{H_2}. On the other hand, the concentration

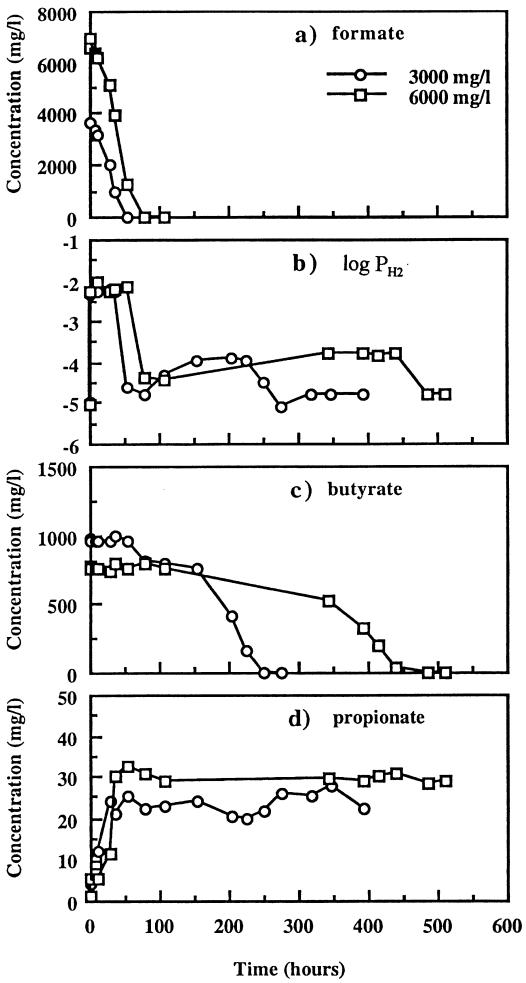

Fig. 3. Concentrations of reactants and products during the degradation of butyrate using formate as co-substrate: (a) formate, (b) P_{H_2}, (c) butyrate, and (d) propionate.

Table 2. Propionate-forming reactions and the changes of standard Gibbs free energy at pH 7

Reactions	$\Delta G^{0\prime}$ (kJ)
A. $CH_3CH_2CH_2COO^- + CO_2 + H_2 \rightarrow CH_3CH_2COO^- + CH_3COO^- + H^+$	−23.37
B. $CH_3COO^- + CO_2 + 3H_2 \rightarrow CH_3CH_2COO^- + 2H_2O$	−71.67
C. $5CO_2 + 11H_2 \rightarrow CH_3CH_2COO^- + CH_3COO^- + 6H_2O + 2H^+$	−261.51
D. $CH_3CH_2CH_2COO^- + 2H_2O \rightarrow CH_3CH_2COO^- + CO_2 + 3H_2$	71.55
E. $2CH_3COO^- + H^+ \rightarrow CH_3CH_2COO^- + CO_2 + H_2$	23.25

$\Delta G^{0\prime}$ represents changes of standard Gibbs free energy at pH 7.

of propionate increased rapidly in the initial hours when P_{H_2} remained at the $10^{-2.0}$ atm level. It reached a maximum level of 25–30 mg l^{-1} and remained at that level for the rest of the experiment. This seems to indicate the formation of propionate was enhanced by the increased P_{H_2}.

The concentration of acetate increased in correspondence to the decrease of butyrate, and gradually decreased as butyrate became depleted, as observed in the first series of experiments. Other than acetate and propionate, there were no other fatty acids, such as *i*-butyrate, and higher-MW acids, detected in the mixed liquor.

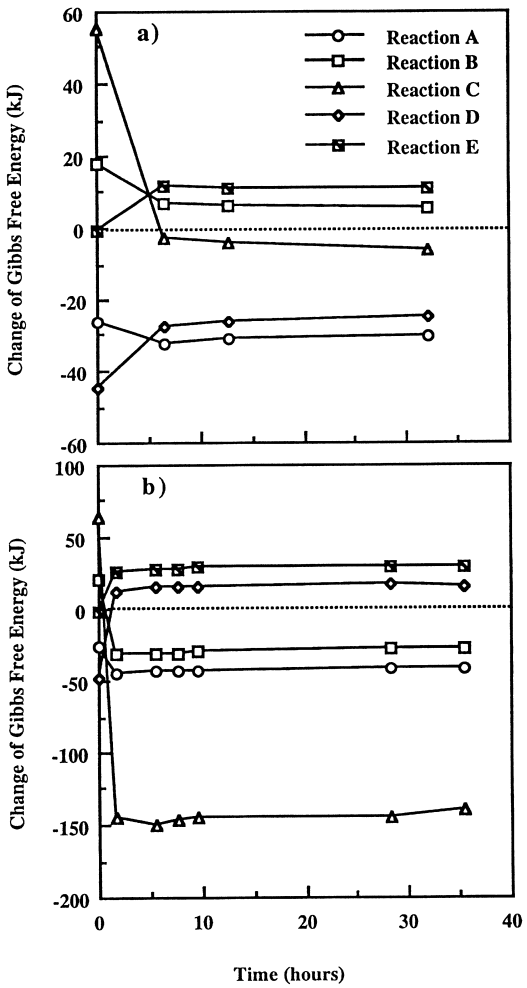

Fig. 4. Changes of standard Gibbs free energy in propionate-forming reactions in experiments using: (a) butyrate as sole substrate, and (b) butyrate and formate as co-substrate.

Thermodynamic analysis of propionate formation

Table 2 list five possible propionate-forming reactions from butyrate, acetate and CO_2, where $\Delta G^{0\prime}$ is the change of Gibbs free energy (Thauer *et al.*, 1977) at pH 7 under standard condition (i.e. all solutes are at the concentration of 1 M, and dissolved gases are having the partial pressure of 1 atm). The actual ΔG during the reaction, however, is dependent on the concentrations of reactants and products. Take reaction (A) for example,

$$\Delta G = \Delta G^{0\prime} + 2.303RT \log [\{\text{propionate}\}\{\text{acetate}\}\{H^+\}/\{\text{butyrate}\} P_{H_2} P_{CO_2}],$$

where R is the universal gas constant, 8.314 J (K mol)$^{-1}$, T is the absolute temperature in K, { } represents the chemical activity, which approximates molarity at low concentrations, and P_{H_2} and P_{CO_2} are partial pressures in atm. At 37°C, the term $2.303RT$ equals 5.934 kJ.

According to chemical thermodynamics, a reaction can take place only if ΔG is negative. Since all terms needed for the ΔG calculation have been measured, the actual ΔG values for the five possible reactions throughout the experiment can be calculated. Figure 4 illustrates the ΔG values for the experiments degrading 3000 mg l^{-1} of butyrate either as a sole substrate (Fig. 4a) or having formate as a co-substrate (Fig. 4b). Plots for the other experiments using different butyrate or formate concentrations gave similar results.

Figure 4a illustrates that, among the five reactions, the ΔG values were consistently negative only in reactions (A) and (D), indicating that only these two reactions were thermodynamically possible. But for the experiments using formate as co-substrate the ΔG of reaction (D) was initially positive, as illustrated in Fig. 4b, indicating that reaction (D) was unlikely. This leaves reaction (A) as the only reaction possible for the formation of propionate. In reaction (A), the formation of propionate requires hydrogen as the electron donor, which is in agreement with the observation that propionate formation was strongly dependent on the P_{H_2}. In all experiments,

propionate increased in the initial hours with the P_{H_2}, and levelled off when the P_{H_2} returned to the normal level in the reactor.

Thermodynamic analysis of caproate formation

It has been reported that higher-MW fatty acids could be formed in reactors degrading butyrate, or other lower-MW acids when the reactors were under perturbations. Smith and McCarty (1989) found that when an ethanol- and propionate-fed complete-mix methanogenic reactor was overloaded, propanol and higher-MW acids, such as valerate, caproate and heptanoate, were found in the effluent. They attributed this to the increase of P_{H_2} as a result of overloading. In another perturbation study, Jia and Fang (1998) found that caproate was formed by two possible reactions. In the reaction using H_2 as electron donor, formation of caproate occurred only when the P_{H_2} was greater than $10^{-3.3}$ atm. Caproate could also be formed according to the following reaction independent of hydrogen:

$$2CH_3CH_2CH_2COO^- \longrightarrow CH_3CH_2CH_2CH_2CH_2COO^- + CH_3COO^- \quad \Delta G^{0\prime} = -0.11\ \text{kJ}. \quad \text{(F)}$$

Because in this study P_{H_2} was consistently lower than the threshold level of $10^{-3.3}$ atm in all experiments, caproate was thus probably formed according to reaction (F). Figure 5 illustrates the ΔG of reaction (F) throughout the experiment calculated from the measured fatty acid data in each of the five experiments in the first series. It clearly illustrates that concentration caproate increased when the above reaction was thermodynamically possible ($\Delta G < 0$). Concentration of caproate peaked when $\Delta G = 0$, and began to decrease as caproate formation became thermodynamically impossible ($\Delta G > 0$), due to the reduction of butyrate concentration and the increased concentrations of caproate and acetate. These results support the argument that caproate was likely formed directly from butyrate, as shown in the above reaction, independent of P_{H_2}.

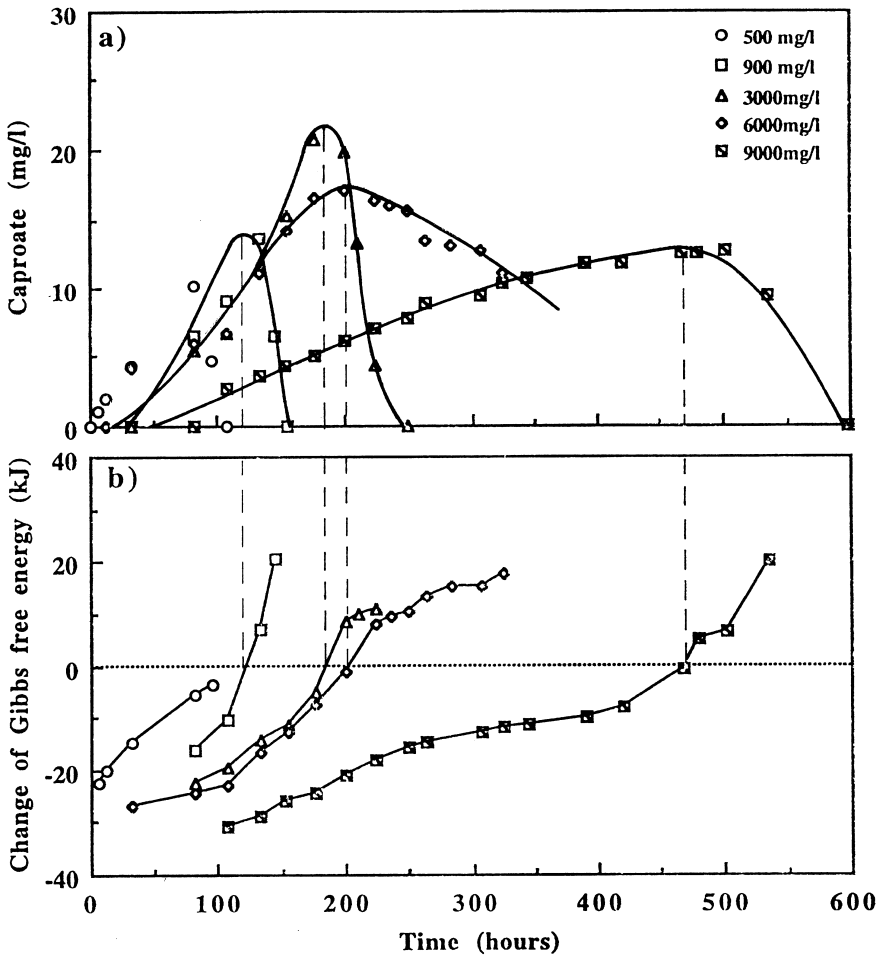

Fig. 5. Changes of (a) caproate concentration, and (b) ΔG for reaction (F) converting butyrate to caproate.

CONCLUSION

Methanogenic degradation of butyrate was through acetate by β-oxidation. The rate of butyrotrophic acetogenesis was considerably higher than that of acetotrophic methanogenesis, as evidenced by the accumulation of considerable quantities of acetate in the interim. At the peak concentration, acetate in the mixed liquor accounted for over 52–83% of carbon in the original butyrate. Other interim by-products included hydrogen, propionate, *i*-butyrate, and several higher-MW carboxylic acids. All these by-products became fully degraded eventually after reaching the maximum, except for propionate which remained at the peak concentration. The butyrate-degrading sludge appeared incapable of degrading propionate. Addition of formate in the feed solution increased the P_{H_2} and enhanced the formation of propionate. The interim formation of caproate was independent of the P_{H_2} increase, according to chemical energy analysis.

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