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SUBSTRATE DEGRADATION OF PROPIONATE-UTILIZING SLUDGE

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ABSTRACT

A propionate-utilizing sludge was enriched in a chemostat reactor for over 90 days. Its methanogenic degradation characteristics were investigated in batch reactors using various substrates. The suspended-growth sludge degraded propionate at 0.65 mg-COD (mg-VSS d)$^{-1}$, which is comparable to the rate reported in the literature for the granular sludge in a UASB reactor. The propionate-degrading rate was not affected by the presence of butyrate as a co-substrate initially; but it was lowered substantially as propionate concentration became below 100 mg l^{-1}. The sludge was also able to degrade butyrate at 0.52 mg-COD (mg-VSS d)$^{-1}$, which is about 30% of the rate reported for granular sludge in a UASB reactor treating butyrate as the sole substrate. The butyrate-degrading rate was lowered when propionate was present at high concentrations. In the interim of butyrate degradation, several higher-molecular weight acids were detected in the mixed liquor, including i-butyrate, i-valerate and caproate; valerate was, however, detected only in the batch when the P_{H2} was as high as $10^{-1.8}$ atm. The presence of formate lowered the degrading rates of both propionate and butyrate.

Keywords: Anaerobic, butyrate, degradation, propionate, rate.

INTRODUCTION

Methanogenic degradation of complex organic pollutants in wastewater involves three phases: hydrolysis/acidification, acetogenesis and methanogenesis. Complex pollutants are first hydrolyzed and acidified by acidogens forming volatile fatty acids (VFA), which are then converted to acetate and CO_2/H_2 by acetogens. Finally, both acetate and CO_2/H_2 are respectively converted by methanogens into the ultimate product, methane [1]. Among the VFA, propionate is a key intermediate which has the lowest tolerance level for the anaerobic bacteria [2]. When an anaerobic reactor is overloaded, propionate tends to accumulate in the mixed liquor, resulting in the lowering of pH [3], and is very difficult to remove during recovery [4]. Based on chemical free energy analysis, propionotrophic acetogenesis is thermodynamically unfavorable, unless the by-product hydrogen can be readily removed by hydrogenotrophic bacteria [5]. The hydrogen partial pressure, P_{H2}, must be kept under 10^{-4} atm for the reaction to take place.

Methanogenic degradation of propionate could be carried out effectively in a UASB (upflow anaerobic sludge blanket) reactor [6, 7]; over 97% of propionate was removed in 12 hours of hydraulic retention at 37 °C for COD (chemical oxygen demand) loading rates up to 23 g (l d)$^{-1}$ [8]. Because of the unique design feature of a built-in gas-liquid-solid separator, the UASB process effectively retains microbes in the reactors allowing them to aggregate into granules. The efficacy of the UASB reactor has been partially attributed to the densely packed microstructure of the biogranules [8, 9] which allows the juxtapositioned syntrophic association taking place between propionotrophic acetogens and hydrogenotrophic methanogens [10].

However, microbes also grow in suspension in many other reactors. Because of the difference in the microbial environment, anaerobic sludge grown in suspension would presumably perform differently from those of UASB biogranules. This study was thus conducted to investigate the methanogenic degradation characteristics of a suspended-growth propionate-utilizing sludge using propionate as the sole substrate, and under the influence of butyrate and formate as co-substrates. The capability of degrading butyrate by this sludge was also examined.

MATERIAL AND METHODS

Enrichment of propionate-utilizing culture

The anaerobic propionate-utilizing sludge was enriched at 37 °C in a 5.0-liter water-jacketed chemostat reactor which had a working volume of 3.5 liters. The reactor was seeded with the anaerobic sludge obtained from a UASB reactor [7] treating dairy wastewater. The granular sludge was

disintegrated using a Waring blender before the seeding. Feed solution for the chemostat reactor contained 6600 mg l^{-1} of propionate, equivalent to 10,000 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 [11] with the exception of excluding sulfate from the formulation. The sludge was completely suspended in the reactor by recirculating the biogas using a compressor. The hydraulic retention time was 10 days. The reactor was operated for over 90 days to ensure a steady state was reached before sludge was sampled for the batch tests. During the steady-state period between day-60 and day-90, the effluent with a pH ranging 7.6-7.8 contained on average 550 mg l^{-1} of residual propionate and 240 mg l^{-1} of acetate. There are no organic acids detected except acetate and residual propionate. The average COD removal efficiency was 89%. The P_{H2} in the biogas was 10^{-5} atm.

Batch experiments

Four batch experiments were conducted to investigate the degradation characteristics of propionate-degrading sludge. Single substrates, i.e. propionate and butyrate, were used in Batches 1 and 2, respectively. In Batches 3 and 4, propionate and butyrate were used as mixed substrates; formate was also added in Batch 4 to simulate the effect of high P_{H2}. Formate was chosen rather than using hydrogen directly because the former is readily soluble in water and can be freely converted to CO_2/H_2 through enzymatic reactions. Table 1 lists the substrate concentrations in the feed solutions. All batch experiments were conducted in 120 ml serum vials at 37 °C. Nutrient and trace elements were added to the feed solution following the formulation used in several previous studies [12]. The nutrient stock solution was sparged with nitrogen in order to strip off any dissolved oxygen.

The propionate-degrading sludge from the chemostat reactor was used to seed all the batch reactors. About 300 ml of mixed liquor was sampled from the chemostat reactor. After centrifugation, the biomass was washed with the stock nutrient solution. After another centrifugation the biomass was re-suspended in 300 ml of fresh stock nutrient solution. Sixty ml of this solution with a protein content of 131 mg l^{-1} was added to each serum vial. Because of the low concentration, biomass content in the solution could not be measured directly by VSS (volatile suspended solids). It was estimated, instead, from the protein concentration of the mixed liquor. Assuming biomass has a chemical formula of $C_5H_7O_2N$ [13], 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% [14-16], each gram of protein in the mixed liquor represents 1.29 g (1.29 = 0.160/0.124) of biomass. Thus, the biomass added to each serum vial was equivalent to 169 mg l^{-1} of VSS.

Table 1. Substrate concentrations in batch experiments

	Substrate concentration mg l^{-1}		
Batch	propionate	butyrate	formate
1	350	nil	nil
2	nil	290	nil
3	260	230	nil
4	310	260	1500

After adding substrate(s) and adjusting pH to 7.9, each vial was submerged in a 37°C shaking water bath. The vigorous shaking motion (35mm x 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 [17], and the COD according to the Standard Methods [18]. 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 25m x 0.53mm CarboPLOT P7 column with a film thickness of 25 μm. Argon was used as the carrier gas at a flow rate 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 VFA were measured with 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 ID 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 both at 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}, oven temperature was 40°C and detector temperature was 43°C. Individual VFA (from C_1 to C_7) could be detected at the 1 mg l^{-1} level, whereas normal alcohols (from C_1 to C_4) and i-propanol could be detected at the 5 mg l^{-1} level.

RESULTS AND DISCUSSION

Single substrates

In anaerobic degradation, propionate and butyrate are converted by acetogens forming acetate, as shown in the following chemical reactions:

$$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + HCO_3^- + 3H_2 + H^+ \quad (i)$$

$$CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + 2H_2 + H^+ \quad (ii)$$

Acetate and the by-products, HCO_3^- and H_2 are then converted by methanogen to produce the ultimate product, methane. Figure 1 illustrates the degradation of single substrates and the formation of acetate in Batches 1 and 2. Figure 1a illustrates that propionate was degraded linearly from the initial 350 mg l^{-1} to complete depletion by hour-115. The specific degradation rate was 0.018 mg (mg-VSS h)$^{-1}$, corresponding to 0.65 mg-COD (mg-VSS d)$^{-1}$. This rate is comparable to the 0.63 mg-COD (mg-VSS d)$^{-1}$ observed in the UASB reactor also treating propionate as sole substrate [8]. This seems to imply that anaerobic sludges either in granules or in suspended growth would have similar propionate-degrading activity.

Figure 1b illustrates that butyrate was degraded almost linearly, and became depleted by hour-145. The specific degradation rate was 0.012 mg (mg-VSS h)$^{-1}$, corresponding to 0.52 mg-COD (mg-VSS d)$^{-1}$. This rate is about 30% of the 1.70 mg-COD (mg-VSS d)$^{-1}$ observed in the UASB reactor treating butyrate as the sole substrate [19]. It is interesting to note that the sludge was able to degrade butyrate at such a substantial rate despite it being enriched in a chemostat reactor treating only propionate for over 90 days. On the other hand, enriched butyrate-degrading sludge was incapable of degrading propionate in either a UASB reactor [20] or a batch reactor [21].

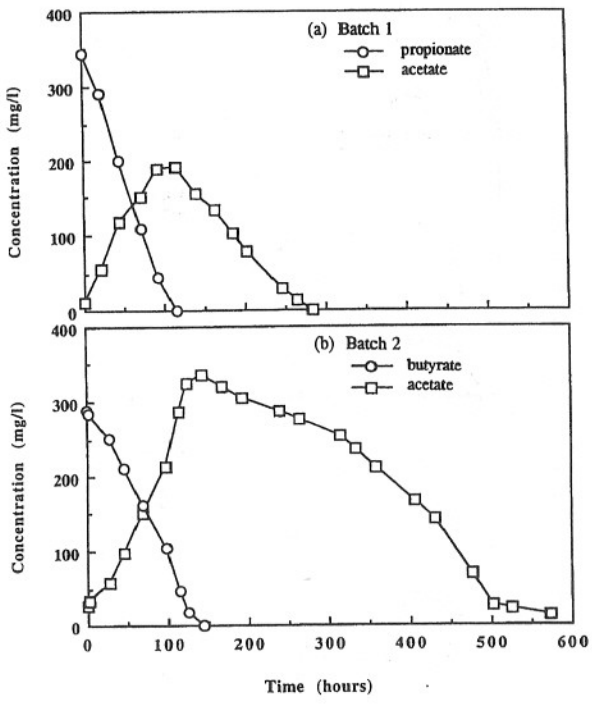

Figure 1. Degradation of propionate and butyrate as individual single substrates, and acetate formation in (a) Batch 1, and (b) Batch 2.

Figures 1a and 1b further illustrate that the concentration of acetate increased with the decrease of propionate and butyrate, respectively, in Batches 1 and 2. This implies that both rates of propiono- and butyro-acetogenesis were substantially higher than the subsequent acetotrophic methanogenesis, resulting in the accumulation of acetate. Concentration of acetate reached the peak levels and began to decrease when propionate and butyrate became depleted. At the peak concentrations, acetate accounted for 45% of carbon in the original propionate, but 85% of that in butyrate. More acetate was accumulated in the interim of butyrate degradation, because two moles of acetate were produced for each mole butyrate (Reaction 1) compared with only one for each propionate (Reaction 2). After propionate or butyrate became depleted in the mixed liquor, acetate was converted to methane at an average rate of 0.18 mg-COD (mg-VSS d)$^{-1}$ in Batch 1 and 0.13 mg-COD (mg-VSS d)$^{-1}$ in Batch 2.

In both Batches 1 and 2, the P_{H2} increased only slightly during the initial hours, but never exceeded $10^{-4.0}$ atm. It returned to the normal $10^{-5.0}$ atm level when the substrates became depleted. Methane was produced corresponding to the degradation of substrate and acetate. Formate and higher molecular-weight (MW) acids were not detected in the mixed liquor during the propionate degradation in Batch 1. However, higher MW acids, including i-butyrate, i-valerate and caproate, were found in the interim of butyrate degradation in Batch 2.

Mixed substrates

Figure 2a illustrates the degradation of both propionate and butyrate by the propionate-utilizing sludge in Batch 3. It shows the sludge preferentially degraded propionate over butyrate at the beginning when both substrates were abundant. During the initial 70 hours, the average degradation rates were 0.014 mg (mg-VSS h)$^{-1}$ for propionate and 0.0037 mg (mg-VSS h)$^{-1}$ for butyrate. However, during the next 70 hours, as propionate concentration was lowered to less than 100 mg l^{-1}, the butyrate-degradation rate increased substantially to 0.014 mg (mg-VSS h)$^{-1}$ while the propionate-degradation rate decreased to 0.0062 mg (mg-VSS d)$^{-1}$. Acetate reached the peak concentration of 425 mg l^{-1}, accounting for 68% carbon in the original substrates, by hour-145. It was subsequently degraded at an average rate of 0.0073 mg (mg-VSS h)$^{-1}$ and became completely depleted by hour-500. Methane was produced

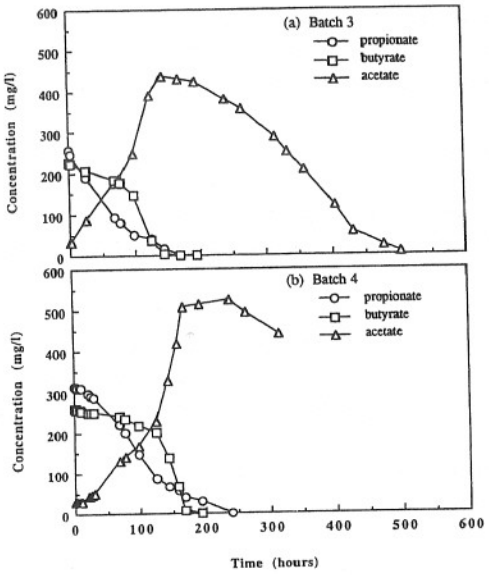

Figure 2: Degradation of propionate and butyrate as co-substrates, and acetate formation in (a) Batch 3, and (b) Batch 4.

corresponding to the degradation of both substrates and acetate. Formate was not detected in the mixed liquor and the P_{H2} in the biogas never exceeded $10^{-4.4}$ atm.

Similar results were observed in Batch 4, as illustrated in Figure 2b, except the addition of formate resulted in the initial increase of P_{H2} to $10^{-1.8}$ atm. But within 25 hours the P_{H2} was soon lowered to the $10^{-4.5}$ atm level. Acetate reached the peak concentration of 523 mg l^{-1} by hour-240, accounting for 68% of carbon in the original propionate and butyrate. The experiment was terminated on hour-310 before acetate became depleted.

Figure 3a illustrates that the presence of co-substrate(s) affected the relative degradation of propionate. Propionate

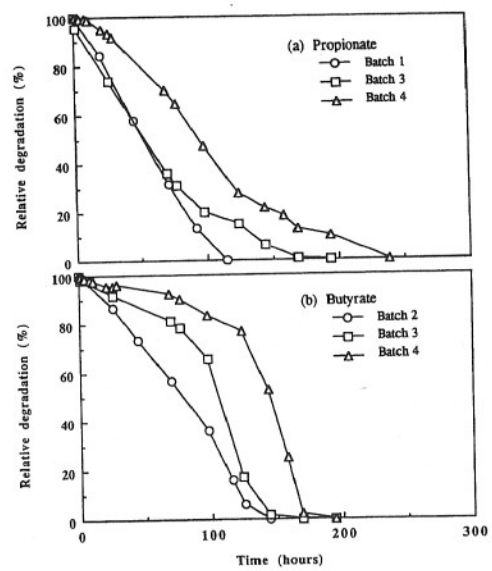

Figure 3. Effect of co-substrate(s) on the relative degradation of (a) propionate, and (b) butyrate.

was degraded at comparable rates in Batches 1 and 3 during the first 70 hours regardless of the presence of butyrate. By hour-70, over 70% of propionate was degraded. Afterwards, the propionate was degraded at a much lower rate. On the other hand, Figure 3b illustrates that the presence of propionate lowered the butyrate degradation rate substantially even during the initial hours. But as propionate concentration in the mixed liquor decreased, the relative degradation rate of butyrate increased substantially after hour-70.

Both Figures 3a and 3b further illustrate that the presence of formate in Batch 4 lowered the degradation rates of both propionate and butyrate. This could be due to the increase of P_{H2} during the initial hours. The accumulation of by-product hydrogen would have suppressed both acetogenic reactions, as shown in Reactions (1) and (2).

Formation of interim higher-MW acids

There were no higher-MW acids detected during the degradation of propionate in Batch 1. However higher-MW acids, including i-butyrate (up to 15 mg l^{-1}), i-valerate (up to 4 mg l^{-1}) and caproate (up to 10 mg l^{-1}), were found in the mixed liquor of Batches 2-4, as illustrated in Figures 4a-4c.

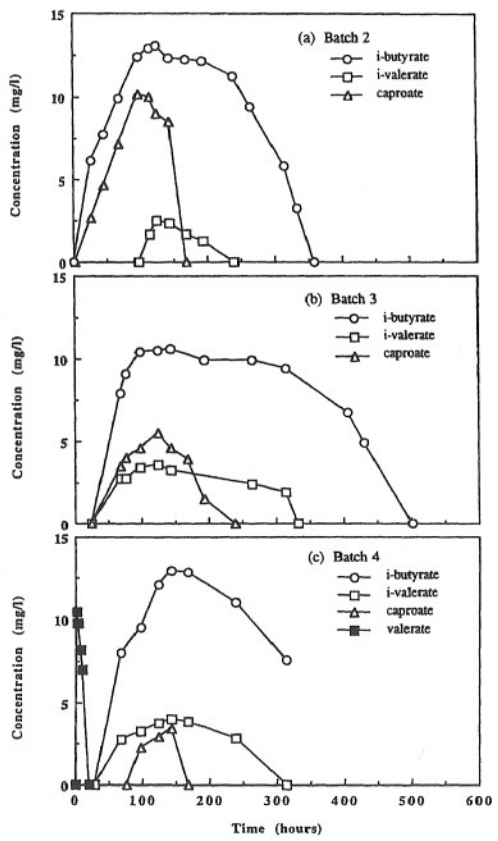

Figure 4. Formation of interim by-products of higher molecular-weight acids in (a) Batch 2, (b) Batch 3, and (c) Batch 4.

The formation of these acids seemed to result from the presence of butyrate in these batches. These acids were also detected, according to a recent study [22], when butyrate was degraded under perturbed loading conditions. Another higher-MW fatty acid, valerate, was only detected during the initial 10 hours in Batch 4 alone, as illustrated in Figure 4c. The formation of valerate probably resulted from the higher P_{H2}, which was up to $10^{-1.8}$ atm, during that period.

CONCLUSION

The suspended-growth methanogenic sludge enriched in a chemostat reactor was able to degrade propionate at 0.65 mg-COD (mg-VSS d)$^{-1}$, comparable to the rate reported in the literature for granular sludge in a UASB reactor. The propionate-utilizing sludge was also able to degrade butyrate at 0.52 mg-COD (mg-VSS d)$^{-1}$, about 30% of the rate reported for granular sludge in a UASB reactor treating butyrate as the sole substrate. The propionate-degrading rate was not affected by the presence of butyrate as a co-substrate initially; but it was lowered substantially as propionate concentration fell below 100 mg l^{-1}. The butyrate-degrading rate was lowered when propionate was present at high concentrations. In the interim of butyrate degradation, several higher-MW acids were formed, including i-butyrate, i-valerate and caproate; valerate was, however, formed only at high P_{H2}. The presence of formate lowered the degrading rates of both propionate and butyrate.

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