High-rate Anaerobic Treatment Processes

PREVENTION OF LIPID INHIBITION IN ANAEROBIC PROCESSES BY INTRODUCING A TWO-PHASE SYSTEM

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ABSTRACT

The inhibitory effect of lipids and prevention of this inhibition in a two-phase anaerobic process were examined using laboratory-scale reactors and batch experiments. Lipids were satisfactorily degraded in a two-phase anaerobic filter while in a single-phase system, inhibition resulted in poor lipid degradation. Unsaturated long-chain fatty acids (LFAs) had a greater inhibitory effect than saturated LFAs. Methane production as well as beta-oxidation (degradation of saturated LFAs) were inhibited by unsaturated LFAs. The saturation of unsaturated LFAs was not inhibited, and palmitate (C16:0) was accumulated in the degradation of oleate (C18:1) or linoleate (C18:2). Greater inhibition was observed at low pH values. Continuous operation of a suspended-growth acidogenic reactor showed that hydraulic retention times (HRTs) of no less than 8 hours were necessary to mitigate the inhibition in a two-phase process. The fact that saturation of oleate occurred at HRTs no less than 8 hours suggests that the saturation of unsaturated LFAs in an acidogenic reactor is essential in the prevention of lipid inhibition in two-phase anaerobic processes.

KEYWORDS

Anaerobic process; two-phase system; lipid; long-chain fatty acids; inhibition; saturation.

INTRODUCTION

Anaerobic processes have been used for many years, and their applications are now being extended to the treatment of weak wastewaters such as domestic sewage. This has been made possible by the development of so-called advanced anaerobic processes including anaerobic filters, upflow anaerobic sludge blanket (UASB) reactors, fluidized bed reactors, and stationary fixed-film reactors. However, expansion of the applications of anaerobic processes has been limited by certain drawbacks of which the most important is poor stability. Process stability can be easily lost due to the presence of inhibitory compounds or changes in the environment. Heavy metals, cyanide, and some organic compounds are known to be inhibitory, but they seldom occur in ordinary wastewaters. However, lipids are potentially inhibitory compounds, which environmental engineers often encounter in anaerobic processes. Lipids can be degraded in biological processes, and they do not cause serious inhibition in aerobic processes, but they sometimes seriously affect anaerobic processes.

Hanaki et al. (1981) showed that the inhibitory effect of lipids in anaerobic processes can be attributed to long-chain fatty acids (LFAs). This inhibition can cause retardation in methane production and in the degradation of the LFAs them-
selves. Batch studies (Hanaki et al., 1987) suggested that a two-phase anaerobic process, consisting of an acidogenic reactor and a methanogenic reactor, would be effective in preventing this inhibition. Lipids are not degraded to acetate in the acidogenic reactor, although the hydrolysis of neutral fats can take place. The reason why the two-phase process can prevent lipid inhibition is unclear, and should be elucidated to enable better operation of the process. The purpose of this study was to examine which functions in the acidogenic reactor were essential to prevent the inhibition, and to examine the degradation of various LFAs.

MATERIAL AND METHODS

Upflow Anaerobic Filters

Laboratory-scale anaerobic filters (diameter = 10 cm; height = 60 cm; working volume = 5.0 l) were installed in a 20°C constant temperature room. 'Net ring type' plastic media (2.7 cm long) were packed in the filters. The specific surface area was 206 m²/m³, and the void fraction was 89%. The filters were operated either as single-phase or two-phase systems. Figure 1 shows the experimental set-up of the two-phase system. An acidogenic reactor with a volume of 1.0 l was installed prior to the anaerobic filter. This acidogenic reactor was of the suspended-growth type, and was continuously mixed with a magnetic stirrer. The substrate, which was stored in a refrigerator, was continuously fed, via a pump, to the acidogenic reactor, and the mixed liquor which overflowed from this was introduced into the bottom of the filter. The overall hydraulic retention time (HRT) was 2 days in both the single-phase and the two-phase systems. The filter was expected to operate as a methanogenic reactor because the biomass residence time was kept sufficiently long. The acidogenic reactor was omitted in the single-phase mode and the substrate was fed directly to the filter. Artificial complex substrates were used in this experiment. Baby milk or skimmed milk having concentrations of 1500 mg COD/l were used. The baby milk was composed of 44% carbohydrates, 12% proteins, and 44% lipids, on the basis of COD. The skimmed milk was composed of 52% carbohydrates, 46% proteins, and 2% lipids. The main carbohydrate in these substrates was lactose. The main difference between these two substrates was that the baby milk contained a rather high percentage of lipids while the skimmed milk contained almost no lipids. The substrates used were supplemented with ammonium bicarbonate to give a C/N ratio of about 7:1. Phosphate salts were also added to provide nutrients as well as buffering capacity.

Batch Experiments

Glucose, sodium acetate, and various LFAs were used as substrates, with the LFAs being used in the form of their soluble sodium salts. Oleate (C18:1) was mainly used, since this is the most common LFA in wastewater, and myristate (C14:0), palmitate (C16:0), stearate (C18:0), and linoleate (C18:2) were also used. The stearate used was not of high purity, and contained about 30% of palmitate. Seed sludge was cultivated, using the baby milk as the substrate, for over six
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months. Digested sludge from Shibaura Sewage Treatment Plant, Tokyo, Japan, was acclimated with this substrate in a semi-continuous reactor at a constant temperature of 37°C. The substrate was dosed once a day using a fill-and-draw mode. The detention time and organic loading rate were 40 days and 0.3 g COD/l·day, respectively. The pH was in the range 7.0 to 7.2. The seed sludge contained about 350 mg/l of MLVSS (mixed liquor volatile suspended solids) and it could convert the dosed baby milk to methane almost completely within a day. Lipids in the baby milk were in the form of neutral fat of which the main LFAs were oleate (39%), palmitate (21%), and linoleate (13%). The anaerobic degradation of each substrate was examined by batch experiments at 37°C using 120-ml serum vials. The seed sludge (40 to 50 ml) and the same volume of the substrate were placed into each vial. The gas phase of the vial was replaced by nitrogen gas and the vial was tightly sealed with a rubber cap. The vials were incubated without agitation. The amount of gas production was measured by inserting the needle of a glass syringe, and gas composition was also determined. The mixed liquor (2 to 5 ml) was sampled using a syringe and then analysed. The progress of the reactions was examined by observing the changes in a single vial.

Acidogenic Reactors

The set-up of the continuous acidogenic reactors was almost the same as that of the acidogenic reactor in the anaerobic filter system, except that these reactors were operated at 37°C. Glucose with oleate was used as the substrate, and the concentrations were 1000 mg COD/l and 1250 mg COD/l, respectively. The substrate was supplemented with ammonium bicarbonate, to give a C/N ratio of about 5:1. Phosphate salts were also added. Two reactors were used, operated at different pH values, namely 6.0 ± 0.1 (Reactor A1) and 7.0 ± 0.1 (Reactor A2). The reactors were initially operated at an HRT of 18 hours, and this was shortened stepwise to 4 h. After the reactors had been operating for at least one week under each set of conditions, steady-state data on acidogenesis were obtained. Another series of experiments was carried out at HRTs of 12 and 8 h, with a substrate consisting of 1250 mg COD/l of oleate alone. In addition to these experiments, the degradation of the mixed liquor from the acidogenic reactors under each set of conditions and the degradation of unacidified substrate were compared by batch experiments, in order to evaluate the effect of phase separation. The degradation of the mixed liquor from the acidogenic reactors by the seed sludge represented the reaction in the methanogenic reactor of a two-phase system, while the direct degradation of substrate by the seed sludge represented a single-phase system.

Analytical Methods

Gas composition and volatile fatty acids (VFAs) were determined by gas chromatography with TCD and FID, respectively. In the anaerobic filter experiments, carbohydrates were determined by the anthrone method and proteins by dye-binding assay. Lipids were first extracted with chloroform and methanol and total lipids were then determined by COD after evaporating the organic solvents. In the batch experiments, LFAs were extracted with n-hexane and isopropanol, and each LFA was determined by FID gas chromatography. In the acidogenic reactor experiments, proteins were measured for estimating biomass, because the analytical values of MLVSS may include LFAs adsorbed by the sludge. The Lowry-Folin method was used for the analysis of proteins and the value obtained was converted to that of MLVSS. Glucose was determined by the anthrone method. In some cases, the mixed liquor was centrifuged (10 minutes at 4000 rpm) to separate the solids from the supernatant. The progress of substrate degradation was evaluated by cumulative methane production and cumulative net acid production on a COD basis. The latter was the sum of the former and the intermediates (accumulated VFAs and hydrogen). In the degradation of LFAs, the latter is achieved largely by beta-oxidation.

RESULTS AND DISCUSSION

Treatment Results from Upflow Anaerobic Filters

The filters had been kept in operation for more than 2 years using the same weak synthetic wastewater (200 mg COD/l) before they were fed with the milk.
substrate (1500 mg COD/l). The filters were operated for at least 46 days under each set of conditions. The COD of the effluent was measured every two or three days. Table 1 shows the average values obtained for COD removal and methane conversion on the basis of influent COD.

<table>
<thead>
<tr>
<th>Table 1 COD Removal and Methane Conversion Relative to Influent COD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baby milk</strong></td>
</tr>
<tr>
<td><strong>COD removal, %</strong></td>
</tr>
<tr>
<td><strong>Methane conversion, %</strong></td>
</tr>
</tbody>
</table>

COD removal. The COD removal percentage was as high as 85 to 92%, even at the short HRT of 2 days. Effluent COD fluctuated little during the long operation periods under all conditions. A comparison between the results obtained with the two substrates indicates that the COD removal percentage was slightly higher with baby milk compared to skimmed milk. There was no significant difference between the single-phase system and the two-phase system.

Methane conversion. The conversion of the influent COD to methane represents the fate of organic matter in anaerobic processes, and this index is often used for estimating the true stabilization of the organic matter. This value should be stoichiometrically equal to that of COD removal, but in practice it is lower than this because of the conversion of part of the COD removed to biomass and the escape of some methane gas in dissolved form in the effluent. In the case of weak wastewater, the relative contribution of the latter is high. The methane conversion is normally lower than the COD removal by about 15% in anaerobic processes treating medium-strength wastewater, as in the present study. In the present study, it is remarkable that the single-phase system had a much lower methane conversion than the two-phase system when baby milk was degraded, as shown in Table 1. However, no significant difference was found between the two systems with respect to the degradation of skimmed milk. These results suggest that some fraction of the baby milk was degraded in the two-phase system which was not degraded in the single-phase system. The COD removal data indicate no difference between the two systems. This means that, in the single-phase system, this fraction of the baby milk was possibly removed by entrapment in the filter but it was not degraded. The most probable component for this fraction is the lipid fraction of the baby milk. The fact that phase separation is essential to degrade the lipid fraction confirms the effectiveness of the two-phase process in terms of preventing lipid inhibition. Similar results have been obtained in anaerobic filters degrading cafeteria wastewater containing about 30% lipids on a COD basis (Hanaki et al., 1990). Poor lipid degradation in the single-phase system might cause clogging problems during long-term operation.

Degradation in the two-phase system. The degradation of the three major components, namely carbohydrates, proteins, and lipids, of baby milk and skimmed milk in the two-phase system is shown in Fig. 2. The HRT of the acidogenic reactor was 8 h. The carbohydrates in the baby milk and the skimmed milk were degraded almost completely in the acidogenic reactor, because these were mainly easily-biodegradable lactose, and VFAs were produced. Proteins were degraded both in the acidogenic reactor and the methanogenic filter, but mainly in the latter. However, almost no degradation of baby milk lipids took place in the acidogenic reactor, although most of these were decomposed in the methanogenic filter. Most of the degraded COD was recovered as methane gas in the degradation of both the baby milk and the skimmed milk.

**Inhibitory Effect of and Degradation of Various LFAs**

Poor lipid degradation in single-phase anaerobic filters is probably closely related to lipid inhibition. Hanaki et al. (1981) showed that LFAs inhibited both degradation of LFAs themselves and methane production, but the inhibitory effects of individual LFAs have not yet been clarified. The effects of three individual saturated LFAs (myristate, palmitate, and stearate) and two unsaturated LFAs (oleate and linoleate) were investigated using batch experiments in the present study. These five LFAs are the main LFAs which comprise the lipid
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Fraction of wastewater. Table 2 summarizes the conditions of the batch experiments. Acetate was added together with the LFAs to investigate the inhibitory effect on methane production.

Table 2: Batch Experiments on Degradation of Various LFAs

<table>
<thead>
<tr>
<th>Run</th>
<th>Substrate</th>
<th>COD, mg/L</th>
<th>pH range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>initial</td>
<td>minimum</td>
</tr>
<tr>
<td>1</td>
<td>Myristate + Acetate</td>
<td>810 + 510</td>
<td>7.70</td>
</tr>
<tr>
<td></td>
<td>Oleate + Acetate</td>
<td>810 + 510</td>
<td>7.70</td>
</tr>
<tr>
<td></td>
<td>Linoleate + Acetate</td>
<td>850 + 510</td>
<td>7.75</td>
</tr>
<tr>
<td></td>
<td>Acetate alone</td>
<td>510</td>
<td>7.70</td>
</tr>
<tr>
<td>2</td>
<td>Palmitate + Acetate</td>
<td>880 + 520</td>
<td>8.00</td>
</tr>
<tr>
<td></td>
<td>Stearate + Acetate</td>
<td>850 + 520</td>
<td>7.95</td>
</tr>
<tr>
<td></td>
<td>Oleate + Acetate</td>
<td>740 + 520</td>
<td>7.95</td>
</tr>
<tr>
<td></td>
<td>Acetate alone</td>
<td>520</td>
<td>7.90</td>
</tr>
<tr>
<td>3</td>
<td>Oleate</td>
<td>800</td>
<td>7.80</td>
</tr>
<tr>
<td></td>
<td>Oleate (HCl added)</td>
<td>800</td>
<td>7.05</td>
</tr>
</tbody>
</table>

Inhibitory effects of individual LFAs. The results from Run 1 are shown in Fig. 3. The increase of net acid production represents the degradation of the LFAs. Myristate, which is a saturated LFA, did not inhibit the production of methane from acetate. Net acid production from myristate also proceeded without a lag period. The degradation of the other saturated LFAs (palmitate and stearate) in Run 2 was almost the same as that of myristate. Figure 4 shows the methane production in Run 2. These results indicate that the saturated LFAs used in the present study did not cause any inhibition. On the other hand, the unsaturated LFAs severely inhibited methane production from acetate, as shown in Fig. 3. The occurrence of a lag period in net acid production indicates that they also inhibited the degradation of the LFAs themselves. The longer lag period for methane production compared to net acid production suggests that methane production was more strongly inhibited than LFA degradation. In the degradation of linoleate, lag periods in both net acid and methane production were longer than those for oleate. Therefore, linoleate, which has two double bonds in its structure, was more toxic than oleate, which contains one double bond. The VFAs detected during the degradation of all the LFAs were almost entirely acetate, and this indicates that the LFAs were degraded via beta-oxidation, expressed as follows:

\[ \text{CH}_3(\text{CH}_2)_{2m}\text{COOH} + 2m\text{H}_2\text{O} \rightarrow (m + 1)\text{CH}_3\text{COOH} + 2m\text{H}_2 \]

The hydrogen gas content was always lower than the detection limit (0.01%) in all cases. This suggests that the hydrogen produced was immediately converted to methane in the vial.
Galbraith et al. (1971) reported that unsaturated LFAs had stronger inhibitory effects than saturated LFAs in the rumen, and that the toxicity increased with the number of double bonds. The results of the present study are in good agreement with their study. However, Galbraith et al. also reported that myristate had the greatest toxicity of the saturated LFAs. Moreover, Koster and Cramer (1987) reported that myristate was as toxic as oleate to methanogenesis from acetate. The disagreement between the present results and these other studies is probably due to differences in species of organisms, since the inhibitory effects of individual LFAs vary with different organisms. In the rumen, methane is formed exclusively from hydrogen and not from acetate, and beta-oxidation organisms are washed out. The seed sludge used by Koster and Cramer had not been acclimated with lipids, unlike in the present study.

Degradation of unsaturated LFAs. The degradation of oleate and linoleate in Run 1 is shown in Fig. 5. A large part of the LFAs detected was adsorbed by the solids within 2 days, although the LFAs were added in the form of the soluble sodium salt. The main saturated LFAs which accumulated as intermediates were palmitate (during oleate degradation), or palmitate and myristate (during linoleate degradation). Several unsaturated LFAs, such as C14:1, C16:1, and C16:2, were also detected during linoleate degradation, but their concentrations were low (below 20 mg COD/l). However, stearate, which is the saturated LFA having the same number of carbons as oleate or linoleate, was not detected in both cases. Viviani (1970) showed that these unsaturated LFAs were converted by hydrogenation to stearate in the rumen, but the degradation pathway in the present study was probably different from that of the rumen. Novak and Carlson (1970) also reported that palmitate was the main intermediate during the degradation of these unsaturated LFAs in continuous reactors.

Saturation of these unsaturated LFAs proceeded without a lag period and was almost completed within 6 to 8 days, but beta-oxidation of saturated LFAs was
retarded, as shown in Fig. 5. This result indicates that unsaturated LFAs inhibit both beta-oxidation and methane production from acetate, but that their saturation is not a rate-limiting step.

\[\text{Fig. 5. Degradation of unsaturated LFAs in Run 1} \]
\[(A) \text{ oleate; (B) linoleate}\]

**Effect of pH on Inhibition**

The dependency of the inhibitory effect on pH was studied in batch experiments using oleate as the substrate. As the pH in Runs 1 and 2 was relatively high, degradation at a neutral pH was examined by adding hydrochloric acid to one vial, and the results were compared with a control. The experimental conditions are shown in Table 2 (Run 3). Free LFAs in the solids were measured in this experiment. The degradation at each pH is shown in Fig. 6. All steps of the reactions, namely, the adsorption of LFAs by the sludge, beta-oxidation, and methane production, were favored by a high pH (in the control) rather than a neutral pH, although the seed sludge was cultivated at a neutral pH. The saturation of oleate to palmitate also proceeded earlier in the control (data not shown). As beta-oxidation does not take place in the acidogenic phase of anaerobic processes, almost no decrease of lipids occurs in acidogenic reactors. Unsaturated LFAs are expected to be adsorbed by the sludge and to be converted to saturated LFAs in the acidogenic reactor. Rather a high pH was more favorable for both steps than a neutral pH. In addition to this experiment, net acid production from linoleate was examined at three different initial pH values (8.3, 7.7, and 7.1), and it was found that the reaction proceeded earlier at higher pH values. Free LFAs were not measured in this experiment, but their adsorption and saturation probably proceeded earlier at higher pH values. A low pH in acidogenic reactors is not favorable for the initial step of unsaturated LFA degradation. This is probably because a low pH changes LFAs to an insoluble non-ionized form.

**Stimulative Effect of Low Concentrations of Oleate**

In order to investigate the inhibitory threshold level of oleate on methane production from acetate, batch experiments with various initial oleate concentrations were carried out. The initial oleate concentrations were adjusted to 0 (control),...
10, 20, 40, 80, 160, and 320 mg COD/l in each vial, and 500 mg COD/l of acetate were added. Figure 7 shows the cumulative methane production after 2 and 4 days, and the residual acetate after 4 days, for each vial. The pH values after 4 days were almost the same in all the vials (in the range 7.45 to 7.52). Vials with oleate up to 40 mg COD/l produced more methane than the control after 4 days, as shown in Fig. 7. The residual acetate in the vials containing oleate up to 20 mg COD/l was also less than that of the control vial. These results indicate that oleate at a low concentration had a small stimulative effect on methane production. Table 3 shows the levels of the major indexes in this experiment. Excess net acid production, which was defined as net acid production in each vial minus that in the control, approximately represents the degradation of oleate. The fact that this value is close to the initial oleate concentration in all cases suggests that oleate was degraded almost completely within 4 days. The addition of oleate at a level of more than 80 mg COD/l had an inhibitory effect on methane production. The addition of oleate at a level of 320 mg COD/l reduced methane production to 63%. This value includes methane production from hydrogen, which, it is assumed, is not inhibited. Therefore, methane production from acetate was reduced to about 45%, assuming that 30% hydrogen and 70% acetate were produced on a COD basis during the degradation of oleate.

![Fig. 6. Effect of pH on degradation of oleate](image)

![Fig. 7. Stimulative effect of low concentrations of oleate](image)

**TABLE 3 Degradation of Oleate at Low Concentrations**

<table>
<thead>
<tr>
<th>Oleate added, mg COD/l</th>
<th>0 (control)</th>
<th>10</th>
<th>20</th>
<th>40</th>
<th>80</th>
<th>160</th>
<th>320</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cumulative CH₄ production on Day 2, mg COD/l</td>
<td>214</td>
<td>226</td>
<td>221</td>
<td>214</td>
<td>196</td>
<td>194</td>
<td>132</td>
</tr>
<tr>
<td>Cumulative CH₄ production on Day 4, mg COD/l</td>
<td>560</td>
<td>620</td>
<td>638</td>
<td>606</td>
<td>545</td>
<td>502</td>
<td>350</td>
</tr>
<tr>
<td>Index of methane production on Day 4*, %</td>
<td>100</td>
<td>111</td>
<td>114</td>
<td>108</td>
<td>97</td>
<td>90</td>
<td>63</td>
</tr>
<tr>
<td>Residual acetate on Day 4, mg COD/l</td>
<td>92</td>
<td>61</td>
<td>81</td>
<td>114</td>
<td>213</td>
<td>344</td>
<td>606</td>
</tr>
<tr>
<td>Excess net acid production on Day 4**, mg COD/l</td>
<td>0</td>
<td>29</td>
<td>67</td>
<td>68</td>
<td>106</td>
<td>194</td>
<td>304</td>
</tr>
</tbody>
</table>

*control = 100; **on the basis on the control

Hanaki et al. (1983) reported that the addition of 12 mg COD/l of oleate reduced methane production from acetate to about 50%, and that a dosage of 24 mg COD/l of oleate stopped methane production completely in batch experiments. The inhibitory threshold level in the present study is approximately 30 times higher than that of their study. This result is probably due to the fact that Hanaki et al. used an acetate enrichment culture where no lipid degrading organisms were present.
as the seed sludge. The present study also suggests that low concentrations of olate have a stimulative effect on methane production from acetate if the sludge is acclimated with lipids.

Role of the Acidogenic Reactor

Batch experiments showed that olate inhibited both beta-oxidation and methane production from acetate, but that its saturation proceeded rapidly. However, if sufficient olate is converted to saturated LFAs in an acidogenic reactor, the inhibition would be eliminated because saturated LFAs have little inhibitory effect. The role of the acidogenic reactor was investigated by continuous operation of acidogenic reactors using glucose with olate as the substrate.

Degradation in the acidogenic reactor. Table 4 gives data on each steady state condition in the acidogenic reactor experiments (the values given are averages of three or four values). The total COD concentration decreased very little under all conditions, since only methane production and hydrogen gas production can reduce the COD of the liquid phase in anaerobic processes. The fact that methane production was observed at HRTs longer than 12 hours indicates that hydrogen-utilizing methanogenic bacteria could grow under these conditions.

<table>
<thead>
<tr>
<th>Reactor Number</th>
<th>Residual Olate, mg COD/l</th>
<th>Olate, mg COD/l</th>
<th>MLVSS, mg COD/l</th>
<th>Residual VFAs, mg COD/l</th>
<th>Gas production, mg COD/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>12</td>
<td>18</td>
<td>15</td>
<td>233</td>
<td>17</td>
</tr>
<tr>
<td>A2</td>
<td>8</td>
<td>12</td>
<td>15</td>
<td>233</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>8</td>
<td>0</td>
<td>146</td>
<td>10</td>
</tr>
</tbody>
</table>

Glucose was degraded almost completely under all conditions. Almost all the VFAs and biomass were probably derived from the glucose. The COD value of the biomass was calculated by assuming that 1 mg of MLVSS was equal to 1.42 mg of COD. The apparent yield coefficients, namely biomass production from glucose utilized, were in the range 0.15 to 0.29 on a COD basis. The main species of the VFAs produced were acetate and propionate, while butyrate levels were low and neither iso-butyrate nor iso-valerate was detected. On the other hand, olate was only slightly degraded. Saturation of olate occurred to some extent at HRTs no less than 8 hours. The saturated LFA produced was not stearate but palmitate, as observed in the batch experiments. The saturation percentage was defined as the palmitate concentration divided by the substrate olate concentration, and the value of this was in the range 9 to 25%. However, further degradation of palmitate did not occur. This is probably because the bacteria responsible for beta-oxidation were washed out at the short HRT. The saturation percentage was higher at pH 7.0 (Reactor A2) than at pH (Reactor A1). This tendency regarding the effect of pH is the same as was observed in the batch experiments. No saturation of olate occurred at an HRT of 4 h at either pH.

Zoetemeyer et al. (1982), Endo et al. (1983), and Hanaki et al. (1987) independently reported that the species of VFAs produced from glucose or carbohydrates of baby milk shifted with HRT and pH. Butyrate, which is easily degraded in the methanogenic phase, appeared at a high concentration at low pH values around 5.0 or at HRTs shorter than 6 h in these studies. On the other hand, propionate, the degradation of which is believed to be slow in the methanogenic phase, showed the opposite tendency. In the present study, the shift of propionate showed the same tendency. However, for preventing inhibition caused by olate, low pH values are not recommended because these are not favorable for the initial olate degradation step, even if a high concentration of butyrate is produced from glucose.
Effect of phase separation. To investigate the role of acidogenic reactors in the prevention of lipid inhibition, the degradations of mixed liquor from an acidogenic reactor under each set of conditions and unacidified substrate were compared using batch experiments. Figure 8 shows examples of the degradation of unacidified substrate and the mixed liquor from an acidogenic reactor at HRTs no less than 8 hours. Table 5 summarizes the experimental conditions. In the degradation of unacidified substrate, net acid production showed a two-step reaction. The initial step mainly represents the degradation of glucose and the second step mainly represents the beta-oxidation of palmitate (produced from the conversion of oleate). Methane production was inhibited so strongly that a long lag period appeared. It took more than 40 days to complete methane conversion. The initial value of the net acid production in the vials in which the mixed liquor was degraded shows the level of VFA production in the acidogenic reactors. A comparison between the degradations of the unacidified substrate and the mixed liquor (Reactor A2, HRT = 12 h) indicates that net acid production proceeded a little earlier in the latter. On the other hand, the progress of methane production showed a substantial difference. There was no striking lag period in methane production in the degradation of the mixed liquor, and the reaction was completed about 10 days earlier than methane production in the degradation of the unacidified substrate.

![Fig. 8. (A) Degradation of unacidified substrate (B) Degradation of mixed liquor (Reactor A2, HRT = 12 h)](image)

**TABLE 5 Batch Experiments on the Effect of Phase Separation**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>COD, mg/l</th>
<th>pH range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>initial</td>
</tr>
<tr>
<td>Unacidified substrate</td>
<td>2250</td>
<td>7.75</td>
</tr>
<tr>
<td>Mixed liquor from acidogenic reactor A2</td>
<td>2150</td>
<td>7.25</td>
</tr>
</tbody>
</table>

In the case of the degradation of the mixed liquor from the acidogenic reactor at an HRT of 4 hours, both the retardation in net acid production and the length of the lag period in methane production were almost the same as those of the unacidified substrate in which degradation was examined at the same time (data not shown). The time required for 80% of net acid and methane conversion, based on final net acid production, was calculated for each vial experiment to evaluate quantitatively the effect of phase separation. Figure 9 shows the relationship between HRT in the acidogenic reactor and the required time (an HRT of 0 hours
represents the unacidified substrate). The time required for net acid conversion was nearly constant throughout the range of HRTs investigated. On the other hand, the decrease in the time required for methane conversion with the mixed liquor at HRTs no less than 8 hours indicates that the inhibitory effect of oleate on methane production was mitigated. Under these conditions, the saturation of oleate had occurred in the acidogenic reactor, although the percentage oleate saturation was not very high. The inhibitory effect of oleate on methane conversion was not mitigated in the degradation of the mixed liquor at an HRT of 4 h. Saturation of oleate had not occurred under these conditions, although about 40% of the oleate had been adsorbed by the sludge. The experimental results suggest that adsorption of LFAs by acidogenic bacteria alone was not satisfactory, and saturation of LFAs was essential for mitigation of the inhibitory effect of unsaturated LFAs. Thus, the acidogenic reactor should be operated under those conditions in which saturation of unsaturated LFAs can take place.

![Fig. 9. Relationship between HRT in the acidogenic reactor and the time required for 80% net acid conversion and 80% methane conversion](image)

In the early stages of degradation of both the unacidified substrate and the mixed liquor, propionate and butyrate accumulated besides acetate, and the total concentration of these two VFAs together was 300 to 500 mg COD/l. However, these two VFAs were completely degraded within 20 days in all cases (data not shown). Propionate was not very difficult to degrade in the present study.

Another series of continuous experiments was carried out where the substrate was oleate alone, at a concentration of 1250 mg COD/l, and the HRTs were 12 and 8 h. Saturation of oleate to palmitate also occurred to some extent under these conditions. The saturation percentages at HRTs of 12 hours and 8 hours were 22% and 16%, respectively. The MLVSS concentration was as low as about 20 mg/l on a COD basis. Acetate was detected, at a concentration of about 20 mg/l on a COD basis. This suggests that acetate (C2) is released during saturation of oleate (C18:1) to palmitate (C16:0). Batch experiments were also carried out to evaluate the effect of phase separation. Inhibition was also mitigated by saturation in the two-phase system. The time required for complete methane conversion of unacidified substrate was 38 days, while complete methane conversion of mixed liquor from the acidogenic reactor at HRTs of 12 hours and 8 hours required 24 days and 27 days, respectively.

**SUMMARY AND CONCLUSIONS**

The inhibitory effects of lipids and prevention of inhibition in a two-phase anaerobic process were investigated using laboratory-scale reactors and batch experiments. Lipids were satisfactorily degraded in the two-phase anaerobic filter, while inhibition resulted in poor lipid degradation in the single-phase system. Batch experiments showed that unsaturated long-chain fatty acids (LFAs) had a greater inhibitory effect than saturated LFAs. Methane production from acetate was strongly inhibited and beta-oxidation was moderately inhibited by unsaturated LFAs. Linoleate (C18:2) was more toxic than oleate (C18:1). The saturation of unsaturated LFAs was not inhibited by unsaturated LFAs them-
selves. Palmitate (C16:0), not stearate (C18:0), accumulated in the degradation of oleate or linoleate. Low pH values enhanced the inhibitory effects.

Continuous operation of a suspended-growth acidogenic reactor using glucose with oleate as the substrate showed that HRTs of no less than 8 hours were necessary to mitigate inhibition in the two-phase process. Saturation of oleate occurred to some extent at HRTs no less than 8 hours. Operation with an HRT of 4 hours did not mitigate the inhibition, although acidogenesis from glucose had occurred. These results suggest that the saturation of unsaturated LFAs in an acidogenic reactor is essential in the prevention of lipid inhibition in two-phase anaerobic processes.

REFERENCES


