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Anaerobic Lipid Degradation Through Acidification and Methanization

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In biological wastewater treatment, high lipid concentrations can inhibit the activity of microorganisms critical to the treatment process and cause undesirable biomass flotation. To reduce the inhibitory effects of high lipid concentrations, a two-phase anaerobic system, consisting of an anaerobic sequencing batch reactor (ASBR) and an upflow anaerobic sludge blanket (UASB) reactor in series, was applied to synthetic dairy wastewater treatment. During 153 days of operation, the two-phase system showed stable performance in lipid degradation. In the ASBR, a 13% lipid removal efficiency and 10% double-bond removal efficiency were maintained. In the UASB, the chemical oxygen demand (COD), lipid, and volatile fatty acid (VFA) removal efficiencies were greater than 80%, 70%, and 95%, respectively, up to an organic loading rate of 6.5 g COD/l/day. No serious operational problems, such as significant scum formation or sludge washout, were observed. Protein degradation was found to occur prior to degradation during acidogenesis.

Keywords: Two-phase, anaerobic digestion, LCFA

Lipids are the major organic component in wastewaters from many industrial sites including dairy production facilities, edible oil refineries, slaughterhouses, and wool scouring factories. Through digestion by anaerobic bacteria, lipids can be degraded *via* hydrolysis and β-oxidation to acetate and hydrogen, which in turn are converted to methane [17]. A large amount of CH_4 can be produced from lipids because they are highly reduced organics. Theoretically, 1 g of glycerol trioleate ($C_{57}H_{104}O_6$), a common lipid in nature, can be converted to 1.08 l of methane at standard temperature and pressure (STP), whereas 1 g of glucose ($C_6H_{12}O_6$) can be converted to only 0.37 l. However, lipid accumulation during attached-growth anaerobic processes tends to cause operational problems such as clogging, scum formation, and washout of active biomass due to the low solubility of lipids and their adsorption onto the surface of sludge or media [10]. Furthermore, long-chain fatty acids (LCFAs), the intermediate step in lipid biodegradation, are toxic to bacterial cells. The mechanism of LCFA toxicity is related to their adsorption onto cell walls, which affects the cell's transport and protective functions. LCFAs are particularly toxic to hydrogen-producing acetogenic bacteria [8] and methanogens [20]. It has been recommended that the chemical oxygen demand (COD) due to lipids in wastewater should not exceed half of the total COD to prevent operational failure of anaerobic digestion systems [24]. Safe operation has only been guaranteed for organic loading rates below 7.5 g COD/l/day in upflow anaerobic sludge blanket (UASB) reactors.

Generally, phase separation has been widely studied and applied in anaerobic digestion [27]. It can improve operational performance throughout microbial separation [12]. Lipid-degrading microorganisms can be classified as acidogenic and methanogenic groups. Previous studies on lipid-degrading bacteria have found the acidogens such as *Syntrophomonas wolfei* [18], *Syntrophomonas saponavida* [25], *Syntrophospora bryantii* [31], and *Thermosyntropha lipolytica* [28]. Reported methanogens include *Methanobacterium* [2], *Methanosarcina*, *Methanococcus* [11], *Methanobacteriales*, and *Methnococcales* species [16]. The main potential advantages expected from a two-phase anaerobic digestion are better control of both acidogenic and methanogenic reactors, higher suspended solid removal efficiency and a higher specific methanogenic activity in the second reactor [7]. Moreover, potentially toxic compounds for methanogens such as long-chain fatty acids can either be removed or detoxified in an acidogenic reactor [15]. Kim *et al.* [11] operated a two-phase system composed of a continuously stirred tank reactor (CSTR) and an upflow anaerobic sludge blanket reactor, as well as a single-phase system composed of only a UASB reactor. Both systems were fed

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with synthetic wastewater containing major LCFAs and glucose. Enhanced performance was observed for the two-phase system, with increased degradation (19%–30%) and saturation (10%–12%) of LCFAs during acidogenesis. However, a CSTR cannot maintain high sludge activity owing to the relatively low hydraulic retention time (HRT) of this reactor design. Furthermore, previous studies have found that lipids adsorbed onto the surface of sludge are gradually mineralized [21, 26]. This implies that internal sludge retention would be beneficial for the acidogenesis of lipid-containing wastewaters.

Another factor influencing the treatment of wastewater with high lipid content is the effect of other slowly degradable components. Wastewaters from the foodstuff industry largely consist of lipids, proteins, and carbohydrates. Among the three major components, lipids and proteins are known to be degraded slower than carbohydrates [30]. However, the mutual effect of lipids and proteins is not yet clearly understood [5].

This study focuses on lipid degradation in dairy processing wastewater. A two-phase anaerobic system composed of an anaerobic sequencing batch reactor (ASBR) and a UASB was operated for 153 days by controlling the HRT and organic loading rate. In addition, a batch assay for acidogenesis was conducted to determine the effect of protein concentration on lipid degradation.

Materials and Methods

Reactor Operations

An ASBR and a UASB connected in series were operated at 35°C. The actual volumes of the ASBR and UASB were 16.7 l and 50.0 l, respectively. As shown in Fig. 1, a reservoir was designed to compensate for the volume difference between the ASBR and UASB. After an acclimation period, the HRT was changed to investigate the effect of organic loading rate on the operation of the system. Operation of the ASBR consisted of filling (10 min), reacting (140 min), settling (20 min), and decanting (10 min). During the reaction period, the reactor was mixed at a rate of 60 rpm. Activity in the reservoir was followed by use of an acidogenic reactor, in which only mixing (60 rpm) was conducted.

Seed and Substrate

Seed sludge used in the ASBR was taken from the municipal sewage treatment plant in Daejeon, Korea and was acclimated prior to operating the bench-scale system. The pH and volatile suspended solid (VSS) concentration of the sludge were 7.6 and 8.5 g/l, respectively, when the initial VSS concentration in the reactor was held constant at 6.0 g/l. A synthetic wastewater containing glucose was fed during the acclimation period. Once constant production of methane gas was observed, the feed was switched to synthetic dairy wastewater. Granular sludge used in the UASB was derived from treated brewery wastewater originating in Cheongwon, Korea. Before treating the synthetic dairy wastewater, the sludge was maintained under the acclimation conditions described above. A total of 19 l of sludge granules was added, which filled approximately 38% of the UASB volume. The pH and VSS concentration of the seed sludge were 7.5 and 36.5 g/l, respectively. After inoculation, the initial VSS was started at 18.3 g VSS/l. The specific methanogenic activity for the seed granules was 0.22 g COD/g VSS/day and 0.27 g COD/g VSS/day for acetate and propionate, respectively.

The substrate was made by using milk, which was diluted to replicate the characteristics of dairy wastewater. The ratio of milk to water was determined by the COD concentration of dairy wastewater. The COD concentration of the synthetic dairy wastewater was around 4,000 mg/l. Commercial milk from a grocery shop was used, and was diluted with tap water. Although dairy wastewaters are known to have a range of CODs, there was no significant variation in the influent COD concentration in this study. As the HRT was reduced from 60 to 20 h, the organic loading rate increased from 1.6 g COD/l/day to 4.8 g COD/l/day.

The dairy wastewater showed a range of pH values, depending on the characteristics of the detergent applied. In this study, alkali treatment was conducted to control pH and to hydrolyze the wastewater's lipid component before use of the two-phase anaerobic

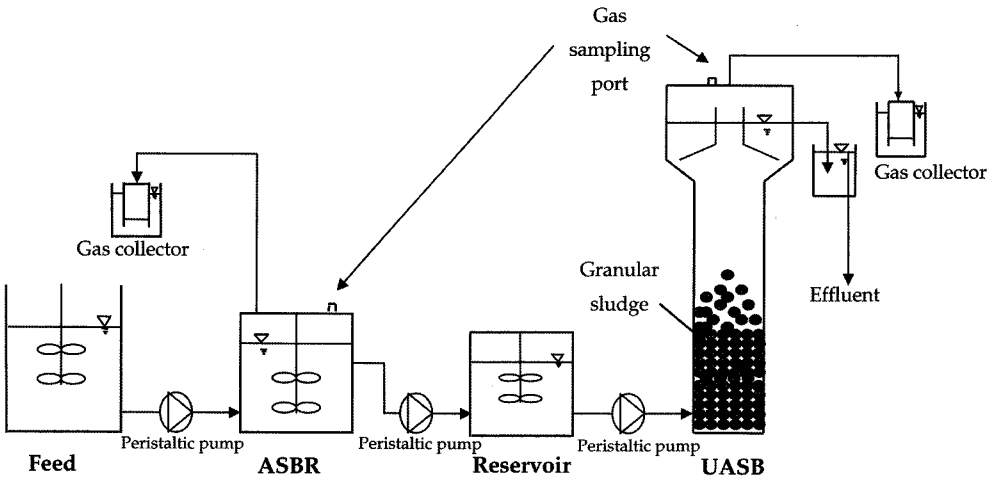

Fig. 1. Schematic diagram of the two-phase anaerobic process.

treatment system. Briefly, 5 ml of 6 N KOH was added to each liter of milk and stirred for 20 min. The volume was then increased to 50 l by dilution with tap water. Two ml of 6 N HCl was injected into the diluted wastewater to decrease the pH to 7–8. $NaHCO_3$ was mixed with the diluted wastewater as a buffer. The alkalinity was maintained at more than 3 g/l as $CaCO_3$. Around 10% double-bond reduction was achieved in pretreatment.

Analyses

The biogas production was measured by the water displacement method. The biogas collected in the gas collector was sampled using glass syringes, and gas composition was analyzed using a gas chromatography unit (Gow Mac series 580, U.S.A.) with a thermal conductivity detector and two columns. Methane and carbon dioxide were detected using a column packed with Porapak Q (80/100 mesh). The temperatures of the injector, detector, and column were kept at 80, 90, and 50°C, respectively. Helium was used as a carrier gas. VFA was quantified using a high-performance liquid chromatography unit (Spectrasystem P2000, U.S.A.) with an ultraviolet (210 nm) detector and an Aminex HPX-97H (300 mm×7.8 mm) column after pretreatment with a 0.45-μm membrane filter. For the mobile phase, 0.005 M H_2SO_4 was used. Measurements of VSS and pH were performed according to standard methods [3]. COD was measured using the dichromate reflux method.

For the lipid analysis [1], the pH of the sample was decreased to less than 2 by addition of 6 N HCl. To extract the lipid components, *n*-hexane was mixed with the sample in a separation funnel. The sample and the *n*-hexane were homogenized through 2–3 min of vigorous shaking. Organic and aqueous layers separated after 20–30 min of resting time. The *n*-hexane layer was transferred to an Erlenmeyer flask and evaporated at 90°C for 5–6 h, leaving only lipids in the flask. The residue in the flask was measured with the same closed reflux titrimetric method used for the COD analysis.

The unsaturated fatty acids were measured using iodine values, with a solution of iodine monobromide in glacial acetic acid [9]. Ten ml of sample was mixed with 1 ml of iodine monobromide in an Erlenmeyer flask. The flask was placed in the dark for 20–30 min. Ten ml of 1 N potassium iodide was then added to the flask and stirred. A 0.01 N solution of thiosulfate was titrated until the color of the solution in the flask changed to a light yellow. A starch solution was added as an indicator. The thiosulfate solution was titrated until the color disappeared. The titration volume was used to calculate the concentration of unsaturated bonds (mol/l) as in Eq. (1).

$$\text{Unsaturated degree (mol/l)} = \frac{\text{Titration}_{\text{blank}} - \text{Titration}_{\text{sample}}}{2} \times \frac{\text{Normality}_{\text{thio-sulfate}}}{\text{Volume}_{\text{sample}}} \quad (1)$$

For the protein analysis, soluble protein concentration was estimated by the Lowry method [13]. NH_4-N was analyzed using standard methods. The chromatropic acid method was used to analyze the total nitrogen. HS-TN(CA)-L (Humas Co., Ltd) and an HS2000 wastewater quality analysis kit were used to measure total nitrogen concentrations in the samples.

Acidogenic Batch Assays

Batch assays were conducted to determine the extent of protein degradation and the effect of protein degradation on lipid hydrolysis. Sludge taken from the ASBR in the two-phase anaerobic digestion was used as seed for these assays, whereas mixtures of synthetic dairy wastewater and casein were used as a substrate. Casein was selected as the representative protein present in dairy wastewater, which consists mainly of milk [29]. Four track studies were carried out at different casein concentrations to determine the total concentrations of lipids, double bonds, total nitrogen, soluble proteins, VFAs, NH_4^+-N, as well as the pH, gas production rate, and composition analysis for each of the four casein concentrations studied.

RESULTS AND DISCUSSION

Operation of Two-Phase Anaerobic Digestion

Continuous operation using two-phase anaerobic digestion was conducted for 153 days. During the operation, the organic loading rate was changed 4 times. Phases 1, 2, 3, 4, and 5 were divided in accordance with the change of organic loading rate.

During the first 12 days, acclimation was carried out with glucose feeding. After acclimation, synthetic dairy wastewater was fed into the two-phase anaerobic digestion

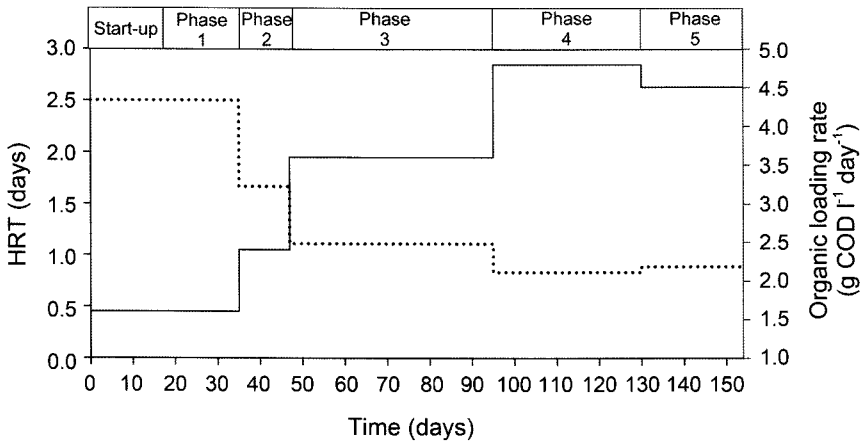

Fig. 2. HRT and organic loading rate in a two-phase anaerobic digestion system: (···) HRT; (–) loading rate.

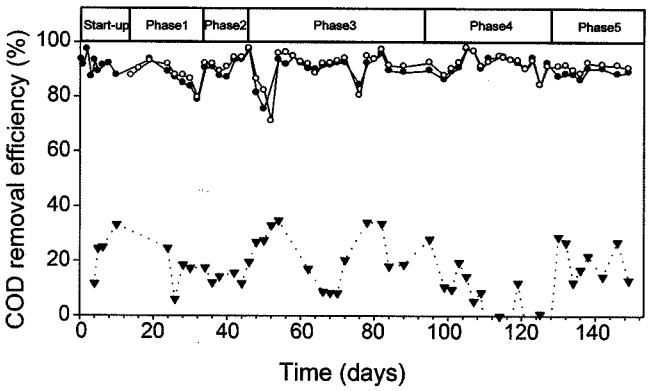

Fig. 3. COD removal efficiency in the system: (●) UASB; (▼) ASBR; (○) total.

system. The feed concentration was then held at 4 g COD/l of synthetic dairy wastewater through the end of the experiment. The organic loading rate was increased by decreasing the HRT.

COD removal efficiency is shown in Fig. 3. Overall COD removal performance was found to be stable over the 153 days of operation. In the ASBR, COD removal showed fluctuations. During phase 4, COD removal in the ASBR ceased. This may reflect the fact that lipids accumulating in the ASBR may have been able to flow out as the organic loading rate increased. In spite of unstable COD removal rates in the ASBR, UASB performance was not noticeably affected. During phase 5, as the organic loading rate was decreased, some recovery of COD removal rates in the ASBR was observed.

Fig. 4 shows VSS variation in the ASBR during continuous operation. Because no artificial method was adopted to control the volume of VSS, it was allowed to fluctuate naturally. There was a high degree of VSS fluctuation during phase 3, whereas the VSS concentration increased continuously in phase 2 and decreased in phase 4. The VSS concentration increased again in phase 5 owing to the decreased organic loading rate during this phase. After phase 3, VSS concentrations seemed to be stable. Increasing the organic loading rate in phase 4 caused the residues discharged from the ASBR to affect the rate of removal of COD. The total COD removal after phase 2 decreased sharply, as the VSS in the ASBR was decreased. The relationship between the volume of VSS and the extent of COD removal was found to be proportionate.

In phase 4, COD removal in the ASBR ceased. However, COD removal in the UASB was not affected. The performance of the ASBR is shown in Fig. 5. The double-bond removal dropped to zero in phase 4. Because acidogenesis is the process of reducing double bonds, it is concluded that the function of the ASBR was lost in this stage, even though there was no sign of failure in the early stages of phase 4. After 20 days, no COD removal and no removal of double bonds were observed. Because there was no significant variation in the VSS of the ASBR, the lipids might be discharged to be balanced without degradation as the lipid loading rate was increased. The increase of total lipid concentration in phase 4 supported this assumption.

Performance of the Acidogenic Reactor

The acidification was related to VFA production. Acidification was less affected by the loading rate than were COD and double-bond removal. Relatively constant acidification occurred throughout the continuous operation, with the exception of phase 4. Acidification is one of the main roles of an ASBR designed for use in an acidogenic reactor.

From the performance data of the ASBR, the lipid loading rates appeared to exceed the capacity of the ASBR in phase 4, when the ASBR organic loading rate was 19.2 g COD/l/day (ASBR lipid loading rate was 8.7 g lipids-COD/l/day). In phase 5, when the ASBR recovered, the ASBR organic loading rate was decreased to 15.2 g

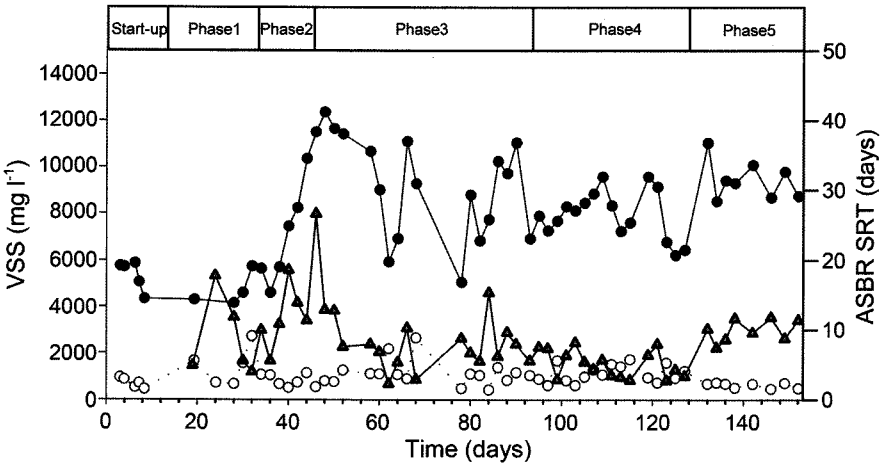

Fig. 4. VSS variations: (●) inside ASBR; (○) ASBR effluent; (△) ASBR SRT.

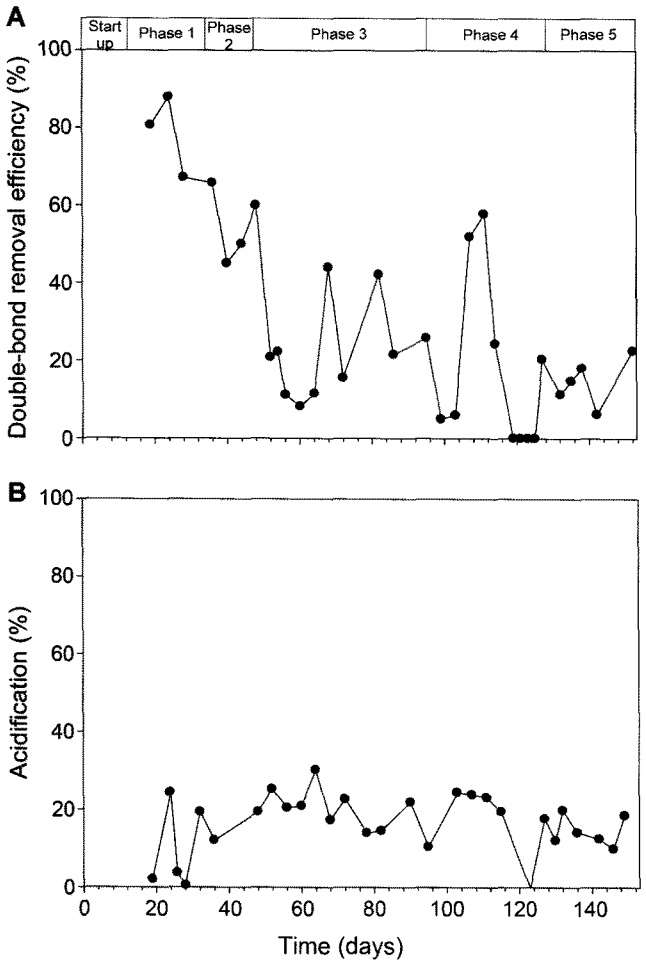

Fig. 5. Performance of ASBR.
A. Double-bond removal efficiency. **B.** Acidification.

COD/l/day (ASBR lipid loading rate was 6.7 g lipids-COD/l/day), which was a condition similar to that in phase 3.

The amount of acidification was calculated using Eq. (2).

$$\text{Acidification (\%)} = \frac{VFA_{ASBR} - VFA_{Feed}}{COD_{Feed} - VFA_{Feed}} \quad (2)$$

The acidogenic reactor was expected to degrade lipids to methane, VFAs, or saturated fatty acids. Use of an acidogenic reactor is known to reduce the toxic effect of lipids in methanogenic reactors [14]. Until the failure occurred, the ASBR operated successfully and as expected. The best lipid removal rate was found in phase 3 (ASBR organic loading rate: 14.4 g COD/l/day; ASBR lipid loading rate: 7.0 g lipids-COD/l/day). The COD balance of the ASBR is shown in Fig. 6. The role of the ASBR was highlighted by the fact that VFA production occurred in all phases. The portion of lipids in the influent COD was about 48%, and these lipids were changed to VFAs. From phase 3 to phase 5, the variation in percentage of lipids followed the operating conditions of the ASBR.

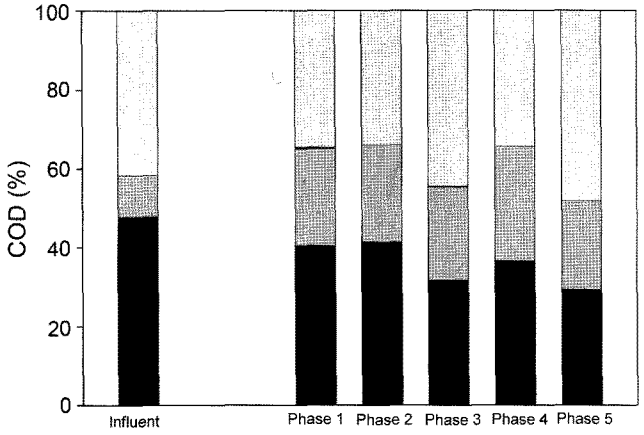

Fig. 6. COD balance in the ASBR (▮: lipid; ▮: VFAs; ▮: CH_4; ▯: others).

Methane Production Through Methanogenesis

CH_4 production was achieved successfully. One indicator of stable production is the CH_4 composition shown in Fig. 7. After the acclimation period, the CH_4 composition was maintained in the range of 60%–70%, as compared with much lower production rates observed during start-up, when glucose was being fed. In terms of biogas recovery, it can be assumed that dairy wastewater has a high potential for biogas production.

CH_4 production was mainly from the UASB rather than from the ASBR. Successful phase separation can be explained by the fact that only a small amount of CH_4 was produced in the ASBR. The majority of CH_4 production was related to the performance of the methanogenic UASB reactor, and the CH_4 production rate (l/l/day) increased proportionally with the increase of the organic loading rate. In Fig. 8B, it can be seen that the measured CH_4 production was dependant on the theoretical CH_4 production, which represents a direct conversion of CH_4 from organic

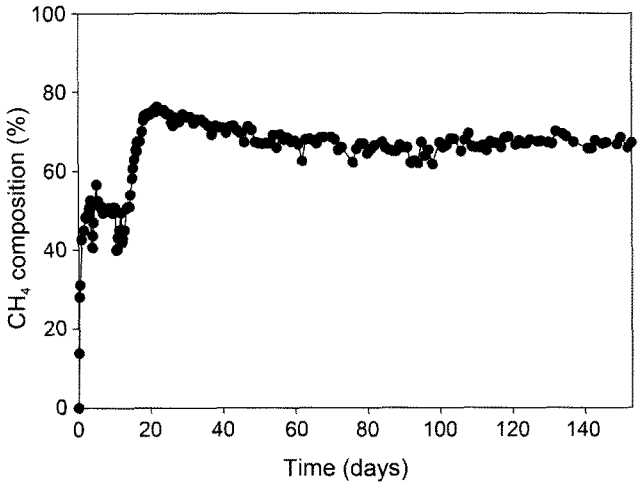

Fig. 7. CH_4 composition in the UASB.

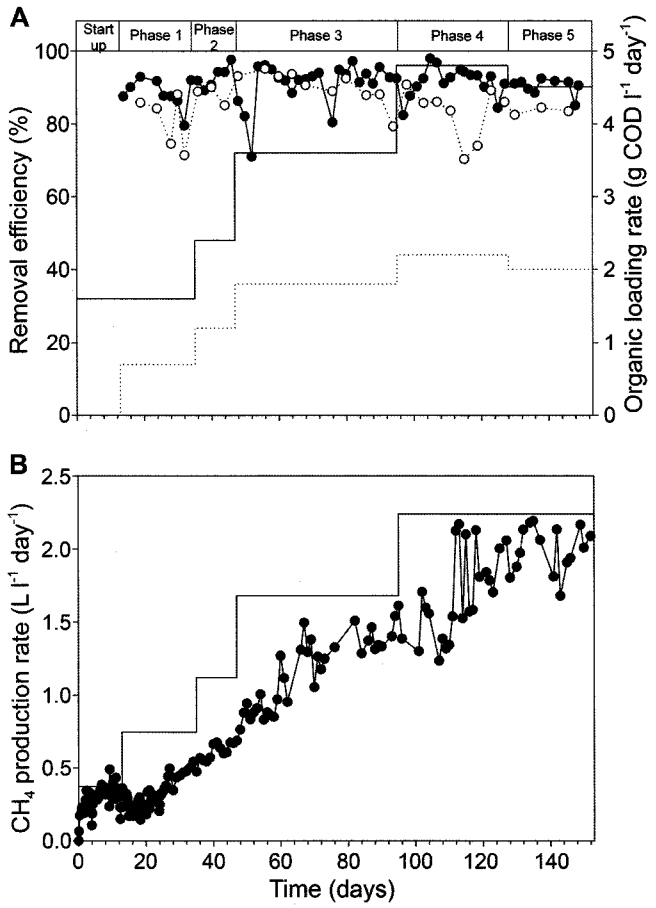

Fig. 8. Removal efficiency and gas production in a two-phase anaerobic digestion system.
A. Performance of COD and lipid removal efficiency: (●) COD removal efficiency; (○) LCFA removal efficiency; (–) COD loading; (···) LCFA loading. **B.** CH_4 production: (●) measured value; (–) theoretical value.

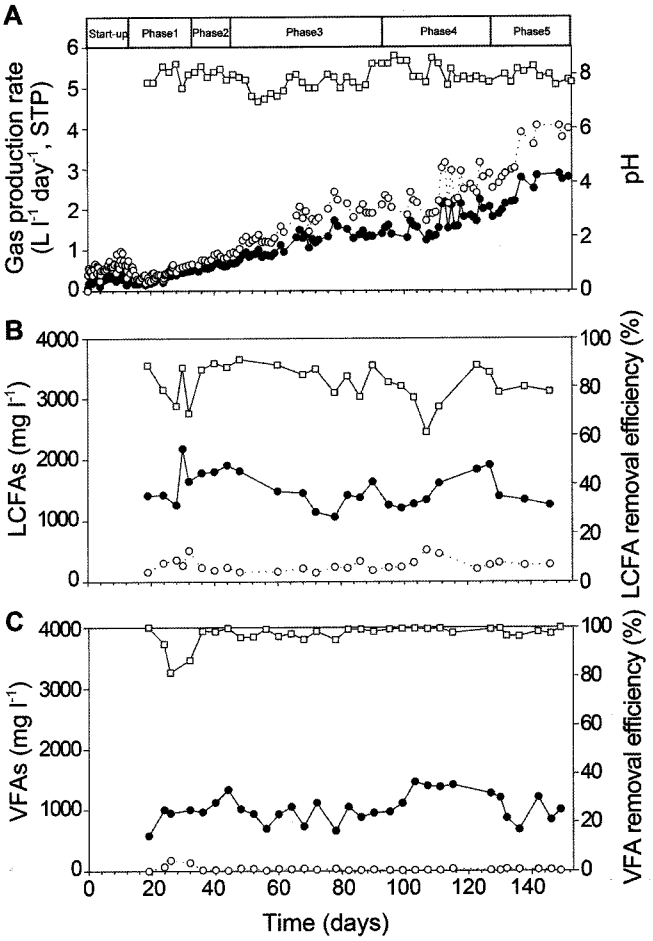

Fig. 9. Performance of UASB.
A. Gas production rate: (●) CH_4; (○) biogas; (□) pH. **B.** Lipid removal: (●) influent lipid concentration; (○) effluent lipid concentration; (□) lipid removal efficiency. **C.** VFA removal: (●) influent VFAs concentration; (○) effluent VFAs concentration; (□) VFA removal efficiency.

loading. As COD and lipid removal were maintained at over 80%, the increase in CH_4 production resulted in stable operation. In spite of the ASBR failure in phase 4, the UASB reactor showed good treatment efficiency. In phase 4, the organic loading rate of the total system was 4.8 g COD/l/day, and the organic loading rate of the UASB reactor was 6.5 g COD/l/day. The rate was slightly higher than the verified range of successful lab-scale operation [28]. This pilot-scale experiment demonstrates the possibility of applying this approach in the field.

The performance of the UASB is shown in Fig. 9. As can be seen in the figure, stable operation was achieved in all phases. No significant problems were observed until the UASB organic loading rate was increased to 6.5 g COD/l/day.

Considerations in the Operation

The designed reactor volume ratio between the ASBR and the UASB was 1:3. After failure in phase 4, the ratio was changed to 1:2.5, and the reactor then operated successfully. In two-phase anaerobic digestion, the organic loading rate and reactor volume ratio were found to be important factors.

Many reports on scum and sludge washout have suggested means of treating wastewater containing lipids [4, 19, 22].

Table 1. Total and lipid COD concentrations in wastewaters.

	Total COD (g COD/l)		Lipid COD (g COD/l)		Lipid (%)
	Range	Mean	Range	Mean	
Dairy wastewater	0.8~9.5	4.5	0.9~2.5	1.5	33
Synthetic wastewater	2.5~4.8	4.0	1.1~2.4	1.9	48

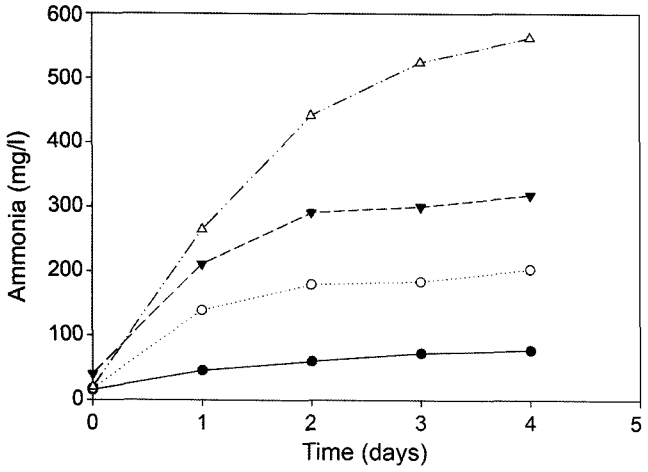

Fig. 10. Ammonia concentrations with regard to casein concentration in acidogenesis: (●) dairy wastewater; (○) dairy wastewater with 0.5 g of casein; (▼) dairy wastewater with 1.0 g of casein; (△) dairy wastewater with 2.0 g of casein.
Dairy wastewater was the substrate used in the continuous operation. The characteristics are shown in Table 1.

In this experiment, scum generation was observed on the surface of the UASB, but it did not increase significantly. The granular sludges washed out slightly after phase 3. Although the height of the sludge bed was slightly decreased, it did not affect the efficiency of dairy wastewater treatment. From this experiment, it was concluded that stable operation was possible with a short HRT (21.3 h), and that land requirements can be reduced in actual field operations through use of a two-phase anaerobic digestion system.

Effect of Protein on Lipid Degradation

Batch assays showed that acclimated acidogens from the ASBR preferentially degraded proteins rather than lipids. Ammonia, the intermediate product in protein degradation, was found to increase when initial casein concentration was high (Fig. 10). When casein concentration was increased, lipid degradation was reduced, both in terms of total amount degraded and the saturation degree (Fig. 11). The reason lipid degradation was limited when the loading rate was increased during continuous operation was the priority of biodegradation.

Conclusions

The two-phase anaerobic system tested in this study was found to be effective in treating synthetic dairy wastewater, both in terms of lipid and double-bond removal efficiencies. During ASBR operation, lipid removal efficiency was greater than 13%, and double-bond removal efficiency was greater than 10% at a loading rate up to 19.2 g COD/l/day

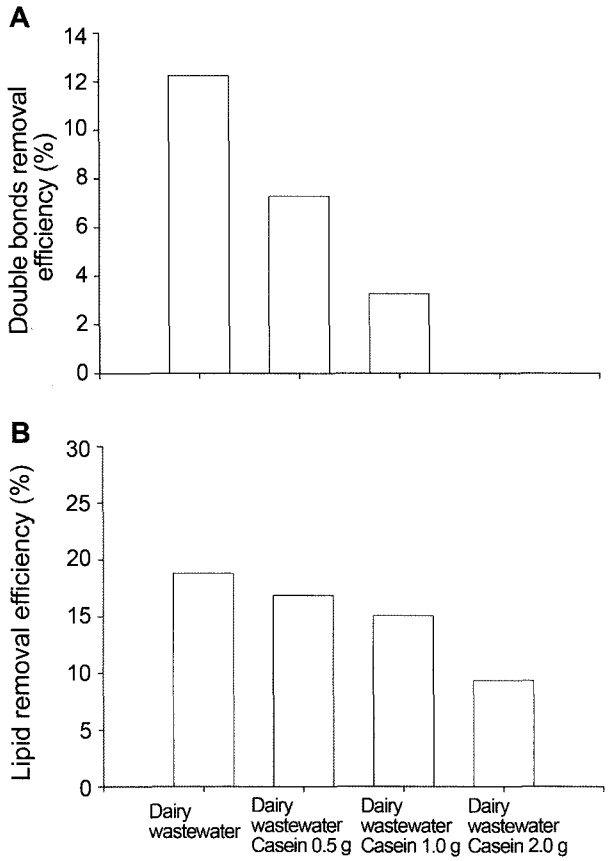

Fig. 11. Effect of casein concentrations on lipid degradation in acidogenesis.
A. Double-bond removal efficiency. **B.** Lipid removal efficiency. Double bond relates to the saturation degree of lipids, meaning carbon is bonded with hydrogen atoms fully; that is, saturation by hydrogen.

(lipid loading rate up to 8.6 g COD/l/day). The maximum lipid removal efficiency was 37% during phase 3 (ASBR OLR: 14.4 g COD/l/day; ASBR LLR: 7.0 g COD/l/day), whereas the maximum double-bond removal efficiency reached 77% during phase 1 (ASBR OLR: 6.4 g COD/l/day; ASBR LLR: 2.8 g COD/l/day). In the UASB, operational problems such as scum formation and sludge washout were insignificant during continuous operation. The maximum CH_4 yield achieved was 0.41 (l/g $COD_{removed}$) during phase 5 (UASB OLR: 5.5 g COD/l/day; UASB LLR: 2.0 g COD/l/day). Throughout the overall period, the COD, lipid, and VFA removal efficiencies were more than 80%, 70%, and 95%, respectively.

Batch assays showed that acclimated acidogens from the ASBR were more efficient at degrading proteins than

Table 2. Average VSS in the ASBR.

Phase	1	2	3	4	5
VSS (mg/l)	5,372	7,973	9,160	7,971	9,132

lipids. High protein concentrations resulted in high acidogenesis of proteins, and relatively low lipid degradation. Instead, protein degradation contributed to an increase in ammonia production.

References

1. Akoh, C. C. and D. B. Min. 2002. *Food Lipid: Chemistry, Nutrition, and Biotechnology*, 3rd Ed. CRC Press, Ohio State University, Columbus, U.S.A.
2. Angelidaki, I. and B. K. Ahring. 1995. Establishment and characterization of an anaerobic thermophilic (55°C) enrichment culture degrading long-chain fatty acids. *Appl. Environ. Microbiol.* **61:** 2442–2445.
3. APHA, AWWA, and WEF. 1998. *Standard Methods for Examination of Waste and Wastewater*, 20th Ed. American Public Health Association, Washington, D.C., USA.
4. Becher, P., D. Koster, M. N. Popov, S. Markossian, G. Antranikian, and H. Markl. 1999. The biodegradation of olive oil and the treatment of lipid-rich wool scouring wastewater under aerobic thermophilic conditions. *Water Res.* **33:** 653–660.
5. Demiral, B., O. Yenigun, and T. T. Onay. 2005. Anaerobic treatment of dairy wastewater: A review. *Proc. Biochem.* **40:** 2583–2595.
6. Gavala, H. N., H. Kopsinis, I. V. Skiadas, K. Stamatelatou, and G. Lyberatos. 1999. Treatment of dairy wastewater using an upflow anaerobic sludge blanket reactor. *J. Agr. Eng. Res.* **73:** 59–63.
7. Guerrero, L., F. Omol, R. Mendez, and J. M. Lema. 1999. Anaerobic hydrolysis and acidogenesis of wastewaters from food industries with high content of organic solids and protein. *Water Res.* **36:** 3281–3290.
8. Hanaki, K. and M. Nagase. 1981. Mechanism of inhibition caused by long-chain fatty acids in anaerobic digestion process. *Biotechnol. Bioeng.* **12:** 1591–1610.
9. Hilp, M. 2002. Determination of iodine values according to Hanus using 1,3-dibromo-5,5-dimethylhydantoin (DBH). *J. Pharm. Biomed. Anal.* **28:** 81–88.
10. Hwu, C. S. 1997. Enhancing anaerobic treatment of wastewater containing oleic acid. Ph.D. Thesis. Wageningen Agricultural University, Wageningen, The Netherlands.
11. Ince, B. K. and O. Ince. 2000. Changes to bacterial community make-up in a two-phase anaerobic digestion system. *J. Chem. Tech. Biotech.* **75:** 500–508.
12. Ince, O. 1998. Performance of a two-phase anaerobic digestion system when treating dairy wastewater. *Water Res.* **32:** 2707–2713.
13. Kamizake, N. K. K., M. M. Goncalves, C. T. B. V. Zaia, and D. A. M. Zaia. 2003. Determination of total proteins in cow milk powder samples: A comparative study between the Kjeldahl method and spectrophotometric methods. *J. Food Compost. Anal.* **16:** 507–516.
14. Kim, S. H., S. K. Han, and H. S. Shin. 2004. Two-phase anaerobic treatment system for fat-containing wastewater. *J. Chem. Tech. Biotechnol.* **79:** 63–71.
15. Komatsu, T., K. Hanaki, and T. Matsuo. 1991. Prevention of lipid inhibition in anaerobic processes by introducing a two-phase system. *Wat. Sci. Tech.* **23:** 1189–1200.
16. Liu, W. T., O. C. Chan, and H. H. P. Fang. 2002. Microbial community dynamics during start-up of acidogenic anaerobic reactors. *Wat. Res.* **36:** 3203–3210.
17. Matsui, T., A. Miura, T. Iiyama, N. Shinzato, H. Matsuda, and K. Furuhashi. 2005. Effect of fatty oil dispersion on oil-containing wastewater treatment. *J. Haz. Mat.* **B118:** 255–258.
18. McInerney, M. J., M. P. Bryant, R. B. Hespell, and J. W. Costerton. 1981. *Syntrophomonas wolfei* gen. nov. sp. nov., an anaerobic syntrophic fatty acid-oxidizing bacterium. *Appl. Environ. Microbiol.* **41:** 1029–1039.
19. Mendes, A. A., E. B. Pereira, and H. F. de Castro. 2006. Effect of the enzymatic hydrolysis pretreatment of lipids-rich wastewater on the anaerobic biodigestion. *Biochem. Eng. J.* **32:** 185–190.
20. Mykhaylovin, O., J. M. Roy, N. Jing, and J. A. Lalman. 2005. Influence of C_{18} long chain fatty acids on butyrate degradation by a mixed culture. *J. Chem. Tech. Biotech.* **80:** 169–175.
21. Pereira, M. A., D. Z. Sousa, M. Mota, and M. M. Alves. 2004. Mineralization of LCFA associated with anaerobic sludge: Kinetics, enhancement of methanogenic activity, and effect of VFA. *Biotechnol. Bioengin.* **88:** 502–511.
22. Perle, M., S. Kimchie, and G. Shelef. 1995. Some biochemical aspects of the anaerobic degradation of dairy wastewater. *Water Res.* **29:** 1549–1554.
23. Ramasamy, E. V., S. Gajalakshme, R. Sanjeevi, M. N. Jithesh, and S. A. Abbasi. 2004. Feasibility studies on the treatment of dairy wastewaters with upflow anaerobic sludge blanket reactors. *Bioresour. Technol.* **93:** 209–212.
24. Rinzema, A., A. Alphenaar, and G. Lettinga. 1993. Anaerobic digestion of long-chain fatty acids in UASB and expanded granular sludge bed reactors. *Proc. Biochem.* **28:** 527–537.
25. Roy, F., E. Samain, H. C. Dubourguier, and G. Albagnac. 1986. *Syntrophomonas sapovorans* sp. nov., a new obligately proton reducing anaerobe oxidizing saturated and unsaturated long chain fatty acids. *Arch. Microbiol.* **145:** 142–147.
26. Salminen, E., J. Einola, and J. Rintala. 2001. Characterization and anaerobic batch degradation of materials accumulating in a digester treating poultry slaughterhouse waste. *Environ. Technol.* **22:** 577–586.
27. Shin, H. S., S. K. Han, Y. C. Song, and C. Y. Lee. 2000. Performance of UASB reactor treating leachate from acidogenic fermenter in the two-phase anaerobic digestion of food waste. *Water Res.* **35:** 3441–3447.
28. Svetlitshnyi, V., F. Rainey, and J. Wiegel. 1996. *Thermosyntropha lipolytica* gen. nov., sp. nov., a lipolytic, anaerobic, alkalitolerant, thermophilic bacterium utilizing short and long-chain fatty acids in syntrophic coculture with a methanegenic archaeum. *Int. J. Syst. Bacteriol.* **46:** 1131–1137.
29. Vidal, G., A. Carvalho, R. Mendez, and J. M. Lema. 2000. Influence of the content in fats and proteins in the anaerobic biodegradability of dairy wastewaters. *Bioresour. Technol.* **74:** 231–239.
30. Yu, H. Q. and H. H. P. Fang. 2001. Acidification of mid- and high-strength dairy wastewater. *Water Res.* **35:** 3697–3705.
31. Zhao, H., D. Yang, C. R. Woese, and M. P. Bryant. 1990. Assignment of *Clostridium bryantii* to *Syntrophospora bryantii* gen. nov., comb. nov. on the basis of a 16S rRNA sequence analysis of its crotonate-grown pure culture. *Int. J. Syst. Bacteriol.* **40:** 40–44.