Inhibitory effects of long-chain fatty acids on VFA degradation and β-oxidation

H.-S. Shin*, S.-H. Kim*, C.-Y. Lee and S.-Y. Nam*****

* Department of Civil and Environmental Engineering, Korea Advanced Institute of Science and Technology, 373-1 Guseong-dong, Yuseong-gu, Daejeon, 305-701, Republic of Korea (E-mail: *hangshin@kaist.ac.kr*)

** R&D Team, Institute of Technology, Samsung Construction, 428-5, Gongse-ri, Giheung-eup, Yongin, Gyeonggi-do, 449-900, Republic of Korea

*** Department of Environmental Engineering, Hankyung National University, 67 Seokjeong-dong, Anseong, Gyeonggi-do, 456-749, Republic of Korea

Abstract The inhibitory effects of major long-chain fatty acids (LCFA), which have 16 or 18 carbons, not only on acetate degradation, but also on propionate degradation and β-oxidation were examined in anaerobic serum bottle tests at 35°C with the acclimated granular sludges. A modified Gompertz equation described cumulative methane production to assess the rates of VFA degradation and β-oxidation, which were applied to a simplified noncompetitive model and a simplified substrate inhibition model, respectively. The specific methane production rates on acetate decreased as LCFA concentration increased, which was in good agreement with the noncompetitive inhibition model. Unsaturated oleate (C18:1) and linoleate (C18:2) were more inhibitory than saturated stearate (C18:0) and palmitate (C16:0) on acetate degradation. LCFA inhibition on propionate degradation was similar to that for acetate; however, propionate degradation was less inhibited than acetate degradation. β-oxidation was the rate-limiting step in LCFA degradation in most cases. As LCFA concentration increased, β-oxidation rate reached the maximum value, and then decreased, which confirmed the substrate inhibition of LCFA. Oleate, the most abundant LCFA in wastewater, could be degraded more quickly than saturated LCFA containing the same or even less carbon in spite of relatively high toxicity on acetate degradation.

Keywords β-oxidation; acetate; inhibitory effects; long-chain fatty acids (LCFA); propionate; unsaturated

Introduction

Fats are abundant in various wastewaters from factories such as edible oil refinery, slaughterhouse, wool scouring, and dairy industries. Most fats are readily hydrolyzed to long-chain fatty acids (LCFA) and glycerol in anaerobic digestion. Acetogens degrade LCFA via β-oxidation pathways into acetate and hydrogen which, in turn, are converted into methane by methanogens. However, LCFA have been reported to cause inhibitory effects on the activity of acetogens and methanogens (Hanaki *et al*., 1981).

Most previous kinetic studies on the inhibitory effects of LCFA were conducted on acetate degradation because of their sensitivity to toxicants and metabolic importance (Koster and Cramer, 1987; Hwu *et al*., 1996). However, some researchers suggested that propionate degradation to be inhibited by LCFA was the rate-limiting step in anaerobic digestion of fat containing wastewater (Salminen *et al*., 2001). Furthermore, β-oxidation might be inhibited severely by LCFA themselves (Hanaki *et al*., 1981; Komatsu *et al*., 1991), which caused the accumulation of LCFA in the reactor followed by the scum layer formation and the biomass flotation (Hwu *et al*., 1998; Shin *et al*., 2001).

This work, therefore, aimed to examine LCFA inhibition on not only acetate degradation, but also propionate degradation and β-oxidation in order to provide general kinetic information for the effective anaerobic digestion of fat containing wastewaters.

Water Science and Technology **Vol 47 No 10 pp 139–146**

Materials and methods

Long-chain fatty acids

LCFA used in the present experiment were shown in Table 1. These four LCFA are the major components of neutral fat in several real wastewaters (Hanaki *et al*., 1981; Hwu *et al*., 1998). Before starting the experiment, LCFA were dissolved in the vigorously stirred and hot (75°C) demineralized water containing two equimolar amounts of NaOH.

Basal medium

The basal medium used in this experiment contained (in g/L final concentration) KH_2PO_4 (0.54), NH_4Cl (0.53), $MgCl_2 \cdot 2H_2O$ (0.1), $FeCl_2 \cdot 4H_2O$ (0.02). The medium was made up in demineralized water and buffered by adding 5 g/L $NaHCO_3$.

Biomass

The granular sludges were taken from two UASB (upflow anaerobic sludge blanket) reactors treating synthetic wastewater that consisted of LCFA-glucose mixture. The operational conditions of the reactors and the composition of LCFA in the wastewater are shown in Tables 2 and 3, respectively (Shin *et al*., 2001). The sludges were obtained when they were acclimated to LCFA concentration in the present test.

Before starting the experiment, the obtained sludges were elutriated with the basal medium to remove floated matter and fine particulates. The elutriated sludges had VSS concentrations in the range of 27.8–32.4 g/L.

Anaerobic serum bottle tests

Acetate or propionate (2,000 mg COD/L) and LCFA (250–5,000 mg COD/L) were used as substrates. Liquid (90 mL) containing VFA, LCFA and the basal medium was placed in

Table 1 LCFA used in serum bottle tests

LCFA	Carbon length	Number of double bonds	Abbreviation	Formula
Oleate	18	1	C18:1	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$
Linoleate	18	2	C18:2	$CH_3(CH_2)_4(CH=CHCH_2)_2(CH_2)_6COOH$
Palmitate	16	0	C16:0	$CH_3(CH_2)_{14}COOH$
Stearate	18	0	C18:0	$CH_3(CH_2)_{16}COOH$

Table 2 Characteristics of sludges used for serum bottle tests

Sludge code	A	B
Wastewater type	Synthetic wastewater (LCFA-glucose mixture)	
Reactor type	Upflow anaerobic sludge blanket	
Reactor temperature (°C)	35°C	
Reactor volume (L)	10.2	13.8
HRT (d)	2.15	2.9
Influent LCFA (mg COD/L)	250–5,000	
LCFA loading rate (g COD/L/d)	1.16–2.33	0.086–1.72

Table 3 Composition of LCFA in the synthetic wastewater fed to sludges

	Oleate (C18:1)	Linoleate (C18:2)	Palmitate (C16:0)	Stearate (C18:0)
COD ratio (%)	40	10	30	20

160 mL serum bottle. Sludge (10 mL) was then pipetted into the bottle. Subsequently, the headspace of the bottle was flushed with N_2 gas for three minutes and the bottle was then tightly sealed with rubber cap. The bottle was then placed in a reciprocating shaker at 35°C and 100 rpm. The amount of gas production and the composition were measured at predestined intervals. At the same time, 1.5 mL samples from the supernatant were taken to analyze the concentration of VFA. In each test run, the control reactor was not supplied with LCFA.

Analytical methods

Gas production was measured by the displacement of the lubricated plunger of glass syringe. Gas composition was analyzed using a gas chromatograph (Gow Mac series 580) with a thermal conductivity detector and two columns. Methane was detected with a column packed with porapak Q (80/100 mesh), and hydrogen was detected with a column packed with molecular sieve 5A. Helium was used as a carrier gas. VFA (C2–C5) were quantified by a high-performance liquid chromatography (SpectraSYSTEM P2000) with an ultraviolet (210 nm) detector and an Aminex HPX-97H (300 × 7.8 mm) column after pretreatment with 0.45 μm membrane filter. H_2SO_4 of 0.005 M was used as mobile phase.

Assay methods

The Gompertz equation has been used as a suitable model for describing the methane production in batch tests (Lay *et al.*, 1998). Because two different substrates, VFA and LCFA, were used in a bottle at the same time, a modified Gompertz equation as Eq. (1) was developed here. Using this equation, the specific methane production rate, the maximum methane production and lag-phase time for VFA and LCFA could be calculated from the measured cumulative methane production as shown in Figure 1.

$$M = P_{VFA}\exp\left\{\left[-\exp\left[\frac{R_{\mathrm{VFA}}}{P_{\mathrm{VFA}}}(\lambda_{\mathrm{VFA}}-t)\mathrm{e}+1\right]\right]\right\} + P_{\mathrm{LCFA}}\exp\left\{\left[-\exp\left[\frac{R_{\mathrm{LCFA}}}{P_{\mathrm{LCFA}}}(\lambda_{\mathrm{LCFA}}-t)e+1\right]\right]\right\} \quad (1)$$

where M = cumulative methane production (g COD/g VSS), t = incubation time (days), λ_{VFA} = lag-phase time for VFA (days), P_{VFA} = specific methane production potential for VFA (g COD/g VSS), R_{VFA} = specific methane production rate for VFA (g COD/g VSS/d), λ_{LCFA} = lag-phase time for LCFA (days), P_{LCFA} = specific methane production potential for LCFA (g COD/g VSS), R_{LCFA} = specific methane production rate for LCFA (g COD/g VSS/d), and $e = 2.718281828$.

The effect of LCFA on VFA degradation was estimated using a noncompetitive product inhibition model as Eq. (2) (Levenspiel, 1980).

$$\frac{\mathrm{d}[VFA]}{X\mathrm{d}t} = -\frac{k_{\mathrm{VFA}}[VFA]}{K_{\mathrm{VFA}}+[VFA]}\left(1-\frac{[\mathrm{LCFA}]}{[\mathrm{LCFA}]^*}\right)^n \quad (2)$$

where X = biomass concentration (g VSS/L), k_{VFA} = maximum specific substrate utilization rate for VFA (g COD/g VSS/d), K_{VFA} = half-saturation constant for VFA (g COD/L), $[\mathrm{LCFA}]^*$ = limiting concentration of LCFA for VFA degradation (mg COD/L) and n = constant.

As acetate is converted to methane directly, the specific methane production rate for acetate could be applied to Eq. 2. The specific methane production rate for propionate could be also applied when the intermediates (acetate and H_2) did not accumulate. Because the half-saturation constants for acetate and propionate were low [about 25–250 mg COD/L (Pavlostathis and Giraldo-Gomez, 1991)], [VFA]/(K_{VFA}+[VFA]) could be regarded as 1. It was also assumed that the concentration of LCFA at the specific methane production rate for VFA was the same as the initial concentration.

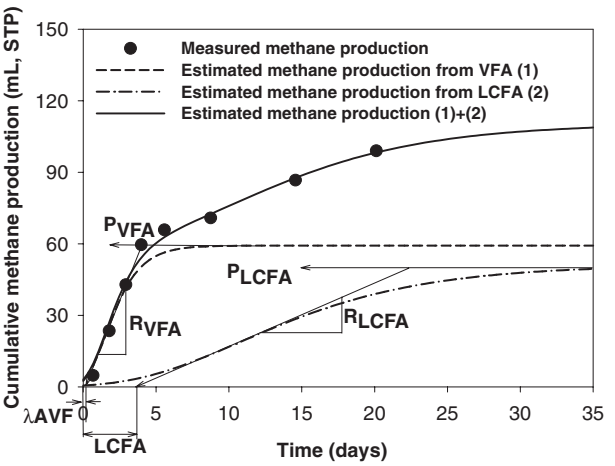

Figure 1 Cumulative methane production for 2,000 mg COD/L stearate with 2,000 mg COD/L acetate using sludge A

Eq. (2) accordingly could be simplified to Eq. (3). LCFA concentration causing 50% relative activity loss was defined as the IC_{50} (50% inhibition concentration).

$$\frac{R_{VFA}}{R_{VFA0}} = (1 - \frac{[LCFA]_i}{[LCFA]^*})^n \quad (3)$$

where R_{VFA0} = specific methane production rate for VFA of the control (g COD/g VSS/d) and $[LCFA]_i$ = initial concentration of LCFA (mg COD/L).

The effect of LCFA on LCFA degradation was estimated using a noncompetitive product inhibition model as Eq. (4) (Luong, 1987).

$$\frac{d[LCFA]}{Xdt} = -\frac{k_{LCFA}[LCFA]}{K_{LCFA} + [LCFA]}(1 - \frac{[LCFA]}{[LCFA]^*})^m \quad (4)$$

where $[LCFA]^*$ = limiting concentration of LCFA for LCFA degradation (mg COD/L) and m = constant.

Because methane conversion of LCFA was a multi-step reaction and LCFA concentration in the bottle varied during degradation, it was difficult to make a simplified model as Eq. (3). However, Eq. (4) could be transformed to Eq. (5) under two assumptions, such as: (i) the rate-limiting step for the methane conversion of LCFA was the β-oxidation (specific LCFA utilization rate ≅ R_{LCFA}); (ii) the concentration of LCFA at the specific methane production rate for LCFA approximated to the initial concentration of LCFA.

$$R_{LCFA} = \frac{k_{LCFA}[LCFA]_i}{K_{LCFA} + [LCFA]_i}(1 - \frac{[LCFA]_i}{[LCFA]^*})^m \quad (5)$$

where k_{LCFA} = maximum specific substrate utilization rate for LCFA (g COD/g VSS/d) and K_{LCFA} = half-saturation constant for LCFA (g COD/L).

Chemicals

LCFA were of analytical grade (Aldrich Chemical Company, Inc., USA). The other chemicals were of extra pure grade.

Results and discussion

General characteristics in degradation

In average, the maximum methane productions estimated by the modified Gompertz

equation were 86–90% of the theoretical values for VFA and 60–70% for LCFA. The specific methane production rates for acetate of the control were in the range 0.19–0.34 g COD/VSS/d, while those for propionate were in the range 0.23–0.35 g COD/g VSS/d.

If the rate of β-oxidation was faster than that of methanogenesis, VFA concentration would maintain the high value even after the methane equivalent for the initial VFA had been produced. However, in all the tests except some cases for linoleate, after methane production equivalent for 90% of initial VFA, total VFA concentrations were below 100 mg COD/L as shown in Figure 2.

In all the tests, hydrogen contents of gas were below 10^{-3} atm, which meant H_2-consuming methanogens was not inhibited severely by LCFA (Hanaki *et al.*, 1981). In case of propionate addition, acetate concentrations were under 50 mg COD/L, which showed no accumulation of intermediates for propionate degradation in the present experiment.

Effect of LCFA on acetate and propionate degradation

The specific methane production rates for acetate decreased as LCFA concentration increased. This was in good agreement with the noncompetitive inhibition model as shown in Figures 3 and 4.

The IC_{50} values of oleate, which has one double bond, on acetate degradation ranged between 2,700–2,850 mg COD/L, while those of linoleate, which has two double bonds,

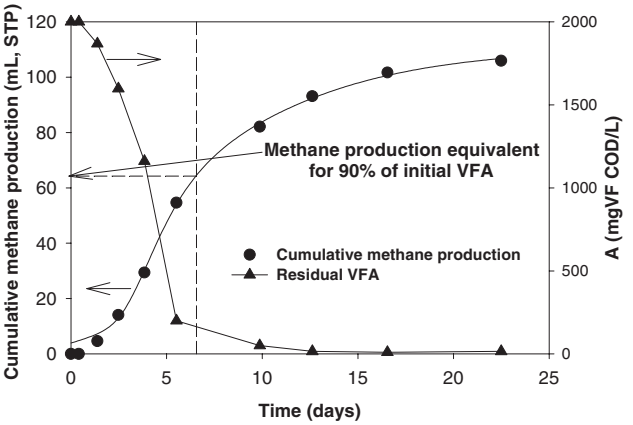

Figure 2 Cumulative methane production and residual VFA concentration for 2,500 mg COD/L oleate with 2,000 mg COD/L acetate using sludge B

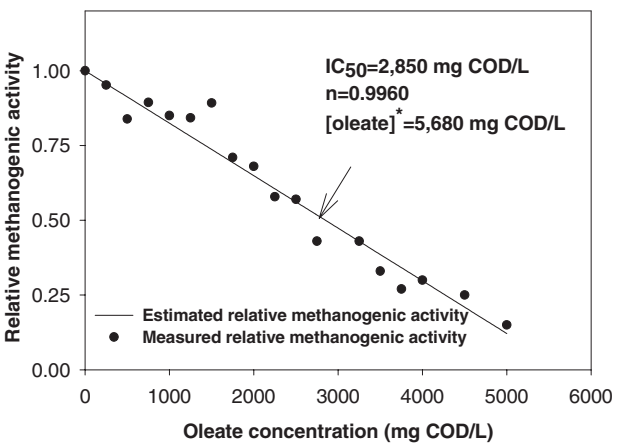

Figure 3 Relative methanogenic activity for acetate versus oleate of sludge A

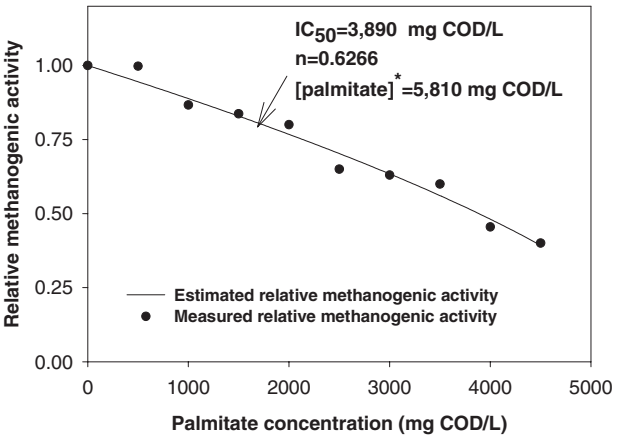

Figure 4 Relative methanogenic activity for acetate versus palmitate of sludge B

were as low as 545–615 mg COD/L as shown in Table 4. The values corresponded well with those reported in the previous literature (Komatsu *et al*., 1991; Hwu *et al*., 1996).

On the other hand, the IC_{50} values of palmitate and stearate, saturated LCFA, were higher than 3,800 mg COD/L. LCFA inhibition on acetate degradation increased as the number of double bonds of LCFA increased, which agreed with previous literatures (Komatsu *et al*., 1991; Angelidaki and Ahring, 1992).

The inhibitory effect of LCFA on propionate degradation was similar to that on acetate degradation as shown in Table 4 and Figure 5. Each IC_{50} value, however, was higher than that for acetate.

Effect of LCFA on β-oxidation

As each LCFA concentration increased, the specific LCFA utilization rate reached a maximum value and then decreased, which were in good agreement with the substrate

Table 4 Inhibitory effect of LCFA on acetate and propionate degradation

VFA	50% inhibition concentration of LCFA (mg COD/L)			
	Oleate (C18:1)	Linoleate (C18:2)	Palmitate (C16:0)	Stearate (C18:0)
Acetate	2,700–2,850	550–620	3,890–4,400	3,800–4,480
Propionate	3,530–3,610	760–1,050	4,310–4,410	4,400–4,410

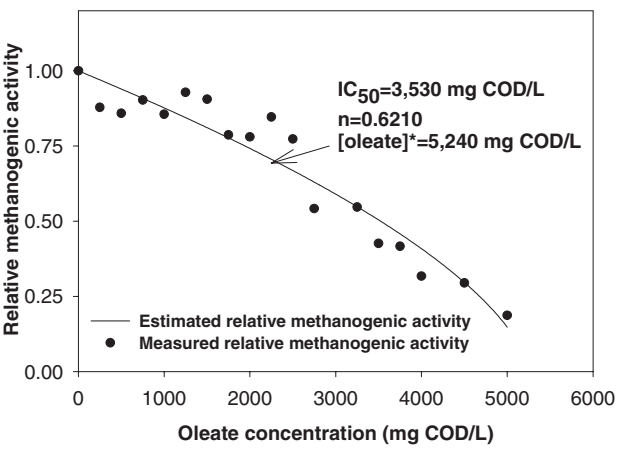

Figure 5 Relative methanogenic activity for propionate versus oleate of sludge A

inhibition model as shown in Figures 6 and 7. It confirmed that LCFA acted as a substrate inhibitor for β-oxidation. The concentrations of LCFA, except linoleate, at maximum degradation rates were in the range of 2,000–3,100 mg COD/L.

Another interesting result was the relatively high degradation rate of oleate. As shown in Table 5, the observed maximum specific rates of oleate were much higher than those of not only stearate of the same carbon length, but also palmitate of less carbon length. Oleate, the most abundant LCFA in the wastewaters, has been regarded as not available for the anaerobic digestion due to its high toxicity to acetoclastic methanogens (Komatsu *et al*., 1991; Beccari *et al*., 1998). The results in this work, however, showed that the assumption would not be always true.

The observed maximum specific rates of linoleate, which severely inhibited the acetate degradation, were also higher than those of steartate. At present, it is not clear what the

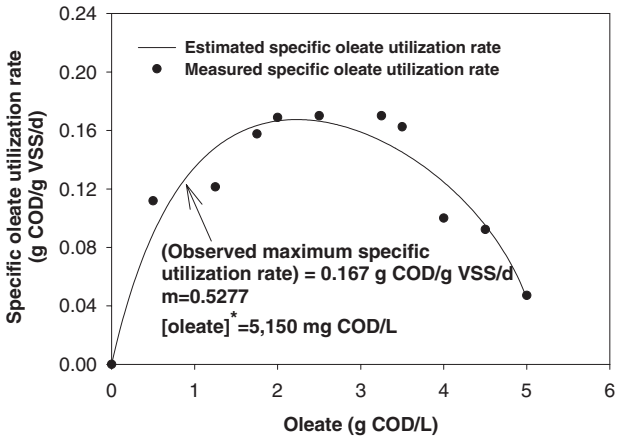

Figure 6 Specific oleate utilization rate versus oleate of sludge B

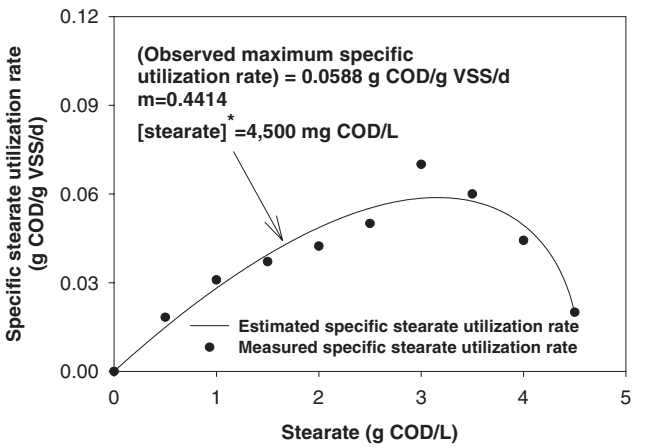

Figure 7 Specific stearate utilization rate versus stearate of sludge A

Table 5 Observed maximum specific LCFA utilization rate

LCFA	Oleate (C18:1)	Linoleate (C18:2)	Palmitate (C16:0)	Stearate (C18:0)
Maximum specific LCFA utilization rate (g COD/g VSS/d)	0.167–0.190	0.0734–0.0739	0.0904–0.0944	0.0587–0.0588

mechanism is for the characteristics of unsaturated LCFA, high toxicity on acetate degradation and fast degradation. However, the high fluidity of unsaturated LCFA might be one possible reason. For sodium salts of LCFA exist in water as colloids due to long carbon chain, the fluidity depends on their melting temperatures (Stryer, 1995). The melting temperatures of stearate and palmitate are higher than 35°C, while those of oleate and linoleate are lower than 35°C due to their double bonds. High fluidity of unsaturated LCFA would enhance the transfer to microorganisms considerably, which affects both the inhibitory effect and degradation rate of LCFA.

Conclusions

The findings were as follow.

- The inhibitory effect of major LCFA on acetate degradation was noncompetitive.
- Unsaturated LCFA were more toxic than saturated ones on acetate degradation.
- The inhibitory effects of LCFA on propionate degradation were similar to those of acetate, however, propionate degradation was less inhibited than acetate degradation.
- β-oxidation was slower than methanogenesis in most cases and was inhibited by substrate, LCFA.
- Oleate, unsaturated LCFA, could be degraded more quickly than saturated LCFA containing same or even less carbon.

Acknowledgements

This work was supported by the Brain Korea 21 Project and Green Korea 21 Project.

References

Angelidaki, I. and Ahring, B.K. (1992). Effects of free long-chain fatty acids on thermophilic anaerobic digestion. *Appl. Microbiol. Biotechnol.*, **37**, 802–812.

Beccari, M., Majone, M. and Torrisi, L. (1998). Two-reactor system with partial phase separation for anaerobic treatment of olive oil mill effluents. *Water Sci. Tech.*, **38**(4–5), 53–60.

Hanaki, K., Matsuo, T. and Nagase, M. (1981). Mechanism of inhibition caused by long-chain fatty acids in anaerobic digestion process. *Biotech. & Bioeng.*, **23**, 1591–1610.

Hwu, C.S., Dunlon, B. and Lettinga, G. (1996). Comparative toxicity of long-chain fatty acid to anaerobic sludges from various origins. *Water Sci. Tech.*, **34**(5–6), 351–358.

Hwu, C.S., Tseng, S.K., Yuan, C.Y., Kulik, Z. and Lettinga, G. (1998). Biosorption of long-chain fatty acids in UASB treatment process. *Water Res.*, **32**(5), 1571–1579.

Komatsu, T., Hanaki, K. and Matsuo, T. (1991). Prevention of lipid inhibition in anaerobic processes by introducing a two-phase system. *Water Sci. Tech.*, **23**(7–9), 1189–1200.

Koster, I.W. and Cramer, A. (1987). Inhibition of methanogenesis from acetate in granular sludge by long-chain fatty acids. *Appl. Environ. Microbiol.*, **53**, 403–409.

Lay, J.-J., Li, Y.-Y. and Noike, T. (1998). The influence of pH and ammonia concentration on the methane production in high-solids digestion processes. *Water Environ. Res.*, **70**(5), 1075–1082.

Levenspiel, O. (1980). The Monod equation: a revisit and a generalization to product inhibition situations. *Biotech. & Bioeng.*, **22**, 1671–1687.

Luong, J.H.T. (1987). Generalization of Monod kinetics for analysis of growth data with substrate inhibition. *Biotech. & Bioeng.*, **29**, 242–248.

Pavlostathis, S.G. and Giraldo-Gomez, E. (1991). Kinetics of anaerobic treatment, *Water Sci. Tech.*, **24**(8), 35–59.

Shin, H.S., Kim, S.H., Han, S.K., Lim, J. L. and Song, Y.C. (2001). Effect of long-chain fatty acid on organic degradation in high-rate anaerobic digestion. In *Proceeding of 9th World Congress – Anaerobic Digestion 2001 – Part 2*, Antwerpen, Belgium, pp. 111–113.

Salminen, E., Rintala, J., Lokshina, L. Ya. and Vavilin, V.A. (2001). Anaerobic batch degradation of solid poultry slaughterhouse waste, *Water Sci. Tech.*, **41**(3), 33–41.

Stryer, L. (1995). *Biochemistry*, 4th edn, Seoul Foreign Books Publishers Co. Ltd., Seoul, Korea, pp. 604–605.