Enhancing Anaerobic Treatment of Wastewaters Containing Oleic Acid

Ching-Shyung Hwu

Promotor: dr. ir. G. Lettinga
bijzonder hoogleraar in de anaërobe zuiveringstechnologie
en hergebruik

Co-promotor: dr. ir. J. B. van Lier
projectleider bij de vakgroep Milieutechnologie

Ching-Shyung Hwu

Enhancing Anaerobic Treatment of Wastewaters Containing Oleic Acid

Proefschrift
ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van de Landbouwuniversiteit Wageningen,
dr. C. M. Karssen
in het openbaar te verdedigen
op woensdag 10 september 1997
des namiddags te 16.00 uur in de Aula

CIP-DATA KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Hwu, Ching-Shyung

Enhancing anaerobic treatment of wastewaters containing oleic acid
/ Ching-Shyung Hwu. - [S.l. : s.n.].
Thesis Landbouwuniversiteit Wageningen. - With ref. - With summary in Dutch.
ISBN 90-5485-733-1
Subject headings: wastewater / anaerobic treatment / long-chain fatty acids

Propositions

1. The kinetic constants of oleic acid reported by Novak and Carlson are not reliable because COD removal is not an appropriate measure of oleic degradation.

   Novak, J.T. and Carlson, D.A. (1970). The kinetics of anaerobic long chain fatty acid degradation. *J. Water Pollut. Control Fed.*, **42**, 1932–1943.

2. Contrary to the findings of Beccari *et al.*, oleic acid can be degraded without the addition of glucose.

   Beccari, M., Bonemazzi, F., Majone, M. and Riccardi, C. (1996). Interaction between acidogenesis in the anaerobic treatment of olive oil mill effluents. *Water Res.*, **30**, 183–189.

3. Compared to the excellent treatment of lauric acid achieved in EGSB reactors, the lower performance obtained for oleic acid in these systems reflects the complexity of anaerobic treatment of wastewaters containing long-chain fatty acids.

4. Wastewaters containing oleic acid can be treated without the problems of microbial inhibition and sludge flotation provided that the proper sludge and bioreactors are employed.

   Chapter 6, this thesis.

5. A penny to the poor means more than a million to the rich.

6. Free trade has rules; the economic powers make the rules.

7. Communism is to let everyone have a hand in the pie; socialism is to let everyone have a pie in the hand.

8. A Jack of all trades is master of none.

9. An agreement is something you have to pay back and a favor is something you want to pay back.

10. This flat country has good living quality except for living in a flat.

Propositions belonging to the thesis "Enhancing Anaerobic Treatment of Wastewaters Containing Oleic Acid."

Ching-Shyung Hwu
Wageningen, 10 Sep. 1997

To my parents,
my wife Ching-Fen
and
my son Alfons

Without their full support and fine patience,
this thesis would never have been completed.

Acknowledgments

A single pillar can not make a skyscraper.

First of all I would like to thankfully acknowledge my "promotor", Gatze Lettinga, for giving me the great opportunity to study here and for suggesting me the "very very interesting" topic which has turned out to be this thesis. I would also like to thank Jules B. van Lier for his just-in-time act as my "co-promotor". Their guidance has made the thesis well worth reading.

I must express my sincere gratitude to my former supervisor, Professor Szu-Kung Tseng, for his understanding when I dropped the third-year Ph.D. study in Taiwan and for his continuing support during the study here in Holland. I am very grateful to Professor Nyuk-min Chong. As a teacher and a friend of mine, he encouraged me to go abroad to have a global view.

Experiments performed by students in Taiwan as well as in the Netherlands have contributed to several Chapters in the thesis. Without their contribution, the thesis would have had less to show. I would like to show my appreciation to them for their excellent work: Chung-Yu Yuan (Taiwan), Zoltán Kulik, Gerton Molenaar, Jochem Garthoff, and Bram van Beek.

I would like to express my heartfelt thanks to Piet Lens for his inspiring in doing a Ph.D. study and for his valuable views and comments on many articles described in the thesis. I feel equally grateful to Robbert Kleerebezem, my "co-copromotor", for frequently helpful discussion and voluntary translation of the Dutch summary.

Many key problems occurred during the study were solved through the provision of relevant references, data, and techniques from Marc Boncz, Brian Donlon, Jim Field, Jin-Hsiang Lo (Taiwan), Caroline Plugge, Wendy Sanders, Dave van Bergen, Miriam van Eekert, Anita van Langerak, Boudewijn van Veen, Adrie Veeken, and Grietje Zeeman, to whom I would record here my grateful acknowledgments.

Many thanks are to our diligent secretaries, Heleen Vos, Liesbeth Kesaulya-Monster, and Gerda de Fauw, for their daily assistance in the past four years. I am also grateful to the members of our perfect technical team at the department: Jo Ackerman-Jacobs, Ilse Bennehey, Sjoerd Hobma, Rob Roersma, Johannes van der Laan, and Bert Willemsen.

It has been very pleasant to work here because the delightful atmosphere I have shared with Harry Bruning, Laetitia Commandeur, Chiel Cuypers, Vinnie de Wilde, Tim Grotenhuis, Look Hulshoff Pol, Sergei Kalyuzhnyi, Bram Klapwijk, Sjon Kortekaas, Katarzyna Kujawa, Marjo Lexmond, Greg Malina, Adriaan Mels, Francisco Omil, Ronaldo Petruy, Henk Rensink, Wim Rulkens, Lucas Seghezzo, Richard Tichy, Joost van Buuren, Renze van Houten, Paul Versteeg, Wang Kaijun, Yang YiPing and Frank v/d Zee. I also thank all other past and present colleagues and students working in the Biotechnion.

It has been a great pleasure to stay for such a long time in this country because the cordial friendships I have experienced. I greatly appreciate Håkan Blanck, Antonio Brito, Jian Chen, Shu-Hsien Chu, Yun-Yi Chu, Lourdinha Florencio, Chin-Ho Hsiao, Chia-Ming Hsiung, Mario Kato, Lam Man Shing, Jaing-Nan Lin, Joost Mortier, Bram Mortier, Margot Mortier, Charles C. T. Peng, Elías Razo-Flores, Rebeca Rentería Hernández de Ramírez, Isabel Ruiz, Ronald Schoester, Armelle Simon, Pascal Weijters, and Wong Chew Siong.

Last but not least, I am deeply indebted to all my families and friends in Taiwan, China and the States, who have been concerned about the three persons in Wageningen, Nederland.

Financial support to the author himself and to the research described in this thesis from the Ministry of Education, Taiwan, ROC, is acknowledged. Special thanks are to Dr. Fang Sheng-Shyong and Mr. Yang I Shang at the Cultural Division in the Taipei Representative Office in Belgium, who ensured the continuous input of the funds.

Contents

1

Anaerobic Treatment of Wastewaters Containing Long-Chain Fatty Acids: Introduction

1

Anaerobic Treatment of Wastewaters Containing Long-Chain Fatty Acids: Introduction

CHARACTERISTICS AND SOURCES OF WASTEWATERS CONTAINING LONG-CHAIN FATTY ACIDS

Fatty acids are products of lipid hydrolysis which is catalyzed by exoenzymes called lipases. Triacylglycerols are the most abundant family of lipids and the major components of depot or storage lipids in plant and animal cells. Fig. 1.1 structurally represents the hydrolysis of triacylglycerol to fatty acids and glycerol. The reaction does not reduce the chemical oxygen demand (COD) of the reactant, but pass it to the products. Fatty acids in biological systems usually contain an even number of carbon atoms, typically between 14 and 24 (Stryer, 1995). For convenience we term the fatty acids with 12 and more carbons as long-chain fatty acids (LCFA) throughout this thesis. Approximately 93–96% of the lipid COD would be passed to LCFA and the remaining to glycerol. Moreover, because lipid hydrolysis proceeds rapidly in anaerobic digestion (Heukelekian and Mueller, 1958; Hanaki *et al.*, 1981; Angelidaki and Ahring, 1992), it is thus reasonable to expect that LCFA will prevail in lipid-containing wastewaters.

Various concentrations of lipids can be found in both domestic sewage and industrial wastewaters. Although domestic sewage generally contains about 40–100 mg lipids/l (Forster, 1992; Quéméneur and Marty, 1994), it is industrial wastewaters that are of greater concern when considering the higher lipid concentrations in discharged effluents. Typical industries that generate lipids-

containing wastewaters are dairy, edible oil and fat refinery, slaughterhouse and meat-processing, rendering and wool scouring (references refer to Table 6.4 in Chapter 6). As mentioned above, we regard these wastewaters as the LCFA-containing wastewaters in this thesis.

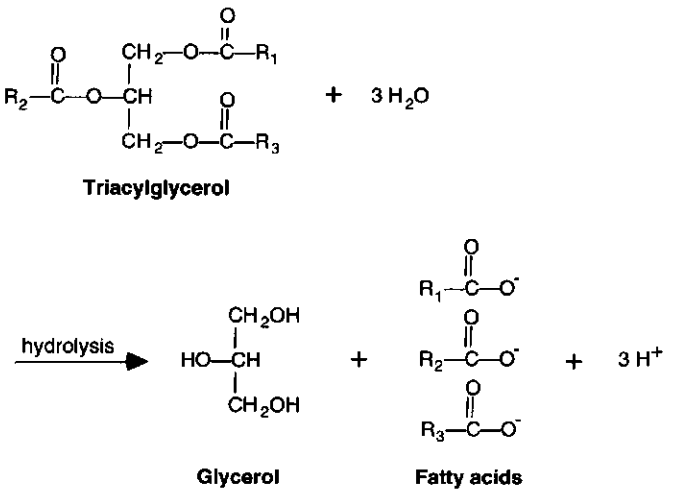


Fig. 1.1 Hydrolysis of triacylglycerol.

LCFA vary in chain length and degree of saturation. Table 1.1 summarizes some naturally occurring LCFA and Table 1.2 presents the LCFA compositions in lipid-containing raw materials and wastewaters. It is very likely that the major constituents in a raw material are also present in the wastewater of its production process. Obviously, palmitic (hexadecanoic) acid and oleic (*cis*-9-octadecenoic) acid, respectively, is the most abundant saturated and unsaturated LCFA.

LCFA have interesting chemical properties because they contain a highly hydrophobic and a highly hydrophilic moiety, referred to as the carboxylic (head) and aliphatic (tail) groups. Due to the amphiphilic structure, LCFA are surface active and thus behave similarly as synthetic surfactants in aqueous systems. In fact, LCFA are ionized in neutral pH environments, e.g., in a bioreactor; and so it is appropriate to refer to them according to their carboxylate form: for instance, oleate and *cis*-9-octadecenoate instead of oleic and *cis*-9-octadecenoic.

Table 1.1 Some naturally occurring LCFA

Number of carbons	Number of double bonds	Common name	Systematic name	Abbreviation	*Structural formula*
saturated					
12	0	Lauric	Dodecanoic	$C_{12:0}$	$CH_3(CH_2)_{10}COOH$
14	0	Myristic	Tetradecanoic	$C_{14:0}$	$CH_3(CH_2)_{12}COOH$
16	0	Palmitic	Hexadecanoic	$C_{16:0}$	$CH_3(CH_2)_{14}COOH$
18	0	Stearic	Octadecanoic	$C_{18:0}$	$CH_3(CH_2)_{16}COOH$
20	0	Arachidic	Eicosanoic	$C_{20:0}$	$CH_3(CH_2)_{18}COOH$
22	0	Behenic	Docosanoic	$C_{22:0}$	$CH_3(CH_2)_{20}COOH$
24	0	Lignoceric	Tetraacosanoic	$C_{24:0}$	$CH_3(CH_2)_{22}COOH$
unsaturated					
16	1	Palmitoleic	*cis*-9-Hexadecenoic	$C_{16:1,\ cis\text{-}9}$	$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$
18	1	Oleic	*cis*-9-Octadecenoic	$C_{18:1,\ cis\text{-}9}$	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$
18	2	Linoleic	*cis*-9,12-Octadecadienoic	$C_{18:2,\ cis\text{-}9,12}$	$CH_3(CH_2)_4(CH{=}CHCH_2)_2(CH_2)_6COOH$
18	3	Linolenic	*cis*-9,12,15-Octadecatrienoic	$C_{18:3,\ cis\text{-}9,12,15}$	$CH_3CH_2(CH{=}CHCH_2)_3(CH_2)_6COOH$
20	1	Gadoleic	*cis*-9-Eicosenoic	$C_{20:1,\ cis\text{-}9}$	$CH_3(CH_2)_9CH{=}CH(CH_2)_7COOH$
20	4	Arachidonic	*cis*-5,8,11,14-Eicosatetraenoic	$C_{20:4,\ cis\text{-}5,8,11,14}$	$CH_3(CH_2)_4(CH{=}CHCH_2)_4(CH_2)_2COOH$
22	1	Erucic	*cis*-13-Docosenoic	$C_{22:1,\ cis\text{-}13}$	$CH_3(CH_2)_4(CH{=}CHCH_2)_4(CH_2)_2COOH$

Table 1.2 Compositions (% of total LCFA) of LCFA commonly found in raw materials and wastewaters

Common name	beef tallow[a]	chicken fat[a]	lard[a]	cocoa butter[a]	palm oil[a]	cotton seed oil[a]	ground nut oil[a]	soya bean oil[a]	olive oil[a]	linseed oil[a]	whole milk[b]	raw sewage[c]	domestic wastewater[d]
saturated													
Lauric	1.0		1.0								7		
Myristic	2.6	1.4	2.4		1.4	1.4		1.0			6		2.2
Palmitic	28.1	21.0	26.2	26.7	42.9	25.7	16.2	11.0	14.3	7.1	21	27.6	16.4
Stearic	20.0	4.3	13.3	32.9	4.8	2.9	2.4	4.8	2.4	3.3	6	16.7	8.1
Arachidic	0.5	0.5	0.5	1.4	0.5		5.2	0.5	0.5				0.7
unsaturated													
Palmitoleic	3.8	6.7	3.8	0.5	0.7	1.0	0.8		1.4		2		0.9
Oleic	37.6	42.4	43.3	33.8	39.0	15.2	41.0	21.9	71.4	15.2	39	48.3	30.5
Linoleic	2.9	20.0	7.1	4.3	10.0	51.9	32.9	49.0	5.5	11.4	13	5.1	29.2
Linolenic	0.5	1.4	0.5			1.0	1.0	7.6		57.1			1.0

Superscripts indicate references:
[a]Taylor, 1965; [b]Hanaki *et al.*, 1981; [c]Viswanathan *et al.*, 1962; [d]Quéméneur and Marty, 1994.

BIODEGRADATION OF LCFA

Degradation Pathway

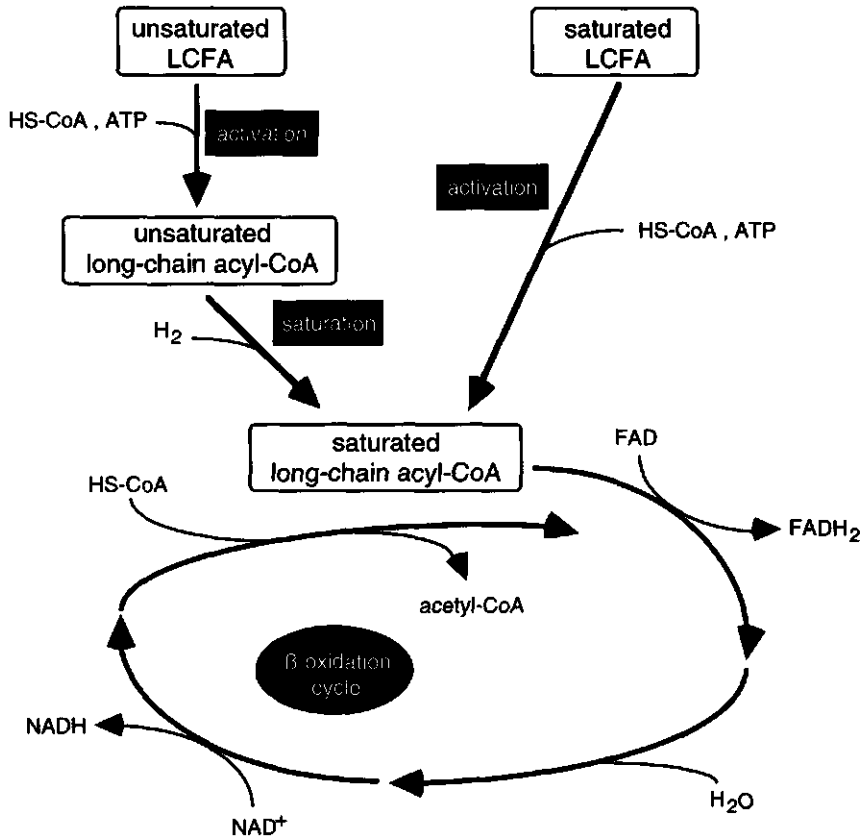


Fig. 1.2 Degradation pathway of LCFA, after Novak and Carlson (1970) and Nunn (1986).

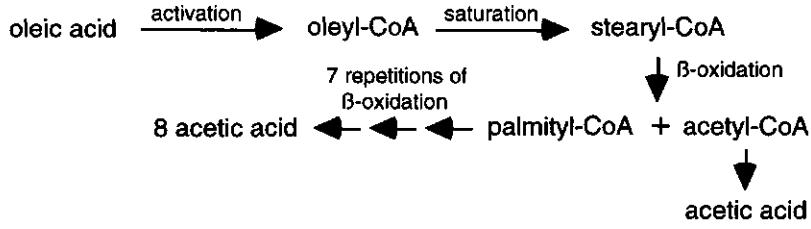
Following the liberation of LCFA from lipids, even-numbered LCFA may be further degraded to acetate and hydrogen. For example, 9 moles acetate and 15 moles hydrogen are formed via the degradation of 1 mole oleate:

$$\text{oleate} + 16\ H_2O \longrightarrow 9\ \text{acetate} + 15\ H_2$$

The LCFA degradation is generally referred to as β-oxidation, as they are degraded by oxidation at the β-carbon. The sequential removal of two-carbon units

characterizes the end-product of β-oxidation, viz., acetate. Although Jeris and McCarty (1965) proposed that both β-oxidation and ω-oxidation can take place, β-oxidation has been confirmed as the principal mechanism by experiments using ^{14}C labeled palmitic (Jeris and McCarty, 1965; Nuck and Federle, 1995) and oleic acid (Weng and Jeris, 1976), and by evidences of end-products produced under mesophilic conditions (Novak and Carlson, 1970; Hanaki *et al.*, 1981; Rinzema, 1988) and thermophilic conditions (Angelidaki and Ahring, 1995).

In fact, β-oxidation is not the sole reaction involved in the whole degradation pathway of LCFA. Even the oxidation itself consists of 4 sequential reactions. Also the whole pathway is slightly different between saturated and unsaturated LCFA (Fig. 1.2). Both saturated and unsaturated LCFA are first activated by acyl-CoA synthetase in cytoplasmic membrane. The saturated long-chain acyl-CoA then directly enters the cyclic reaction known as β-oxidation, while the unsaturated long-chain acyl-CoA needs to be saturated (hydrogenated) before entering β-oxidation. Through the oxidation, each time a two-carbon shortened fatty acyl-CoA molecule reenters the degradation cycle without further activation. Although the pathway was studied by using *E. coli.*, the basic features are substantially similar to the β-oxidation pathways present in other microorganisms. This pathway is a classic example of the oxidation of a series of homologous substrates through a series of homologous intermediates (Nunn, 1986). The degradation pathway is illustrated by using oleate as an example:



Transport of LCFA into Cytoplasm

In contrast to lipid hydrolysis which occurs in the bulk, LCFA degradation begins by the transport into the microbial cell. Prior to β-oxidation, LCFA must enter the cell via uptake systems which vectorially translocate them across the membrane. Nunn (1986) reviewed several genetic and biochemical studies and

stated *"LCFA are initially adsorbed to FLP, which functions as an outer membrane receptor and, possibly, a pore. Once the LCFA are transferred across the outer membrane, they are somehow transferred across the cytoplasmic membrane to the peripheral membrane-bound acyl-CoA synthetase, where they are activated and released into the cell."* Fig. 1.3 depicts the membrane-associated transport in *E. coli* K-12. Direct evidence of LCFA transport in anaerobic microorganisms is, however, still absent.

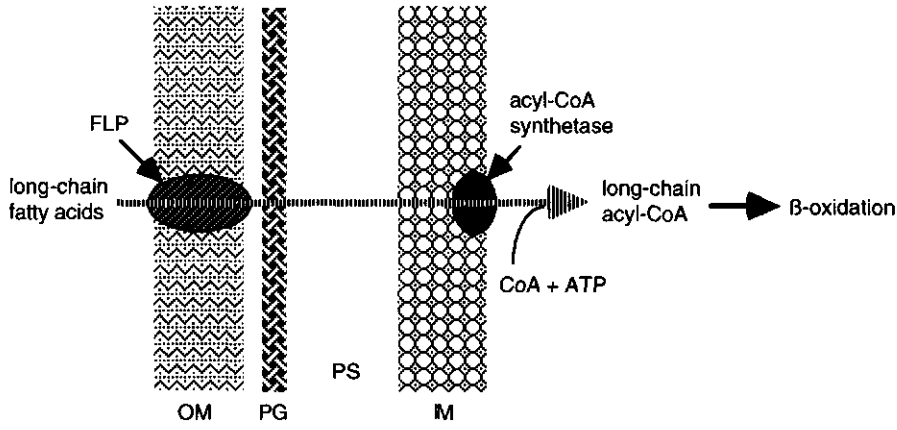


Fig. 1.3 Model of LCFA transport into *E. coli*. LCFA transverse the outer membrane via the membrane protein FLP which is the possible receptor. OM: outer membrane; PG: peptidoglycan; PS: periplasmic space; IM: inner membrane. Adapted from Nunn (1986).

The LCFA of which bacteria take up are their undissociated free form (Ratledge, 1994). The fraction of the undissociated free LCFA is pH dependent. The concentration of the undissociated acids is of key significance in the inhibitory properties towards microorganisms (Prince, 1959). It is import in all microorganisms that free fatty acids within the cytoplasm are effectively eliminated. The acyl-CoA synthetase therefore fulfills a dual role: it activates the fatty acid, prior either to its transfer into cell lipids or to its oxidative degradation, and also effectively detoxifies the fatty acid as the CoA esters are not inhibitory (Ratledge, 1994).

Syntrophic Acetogenic LCFA Degrading Bacteria

In anaerobic digestion LCFA are converted to acetate and hydrogen by proton reducing, acetogenic bacteria (Roy *et al.*, 1986; Lorowitz *et al.*, 1989). This

conversion is thermodynamically unfavorable unless the H_2 partial pressure remains very low, e.g., 10^{-4} atm. The effective removal of hydrogen is possible when hydrogen-utilizing bacteria are prevailed. Thus the LCFA acetogenic bacteria are generally cultivated in syntrophic cocultures usually with hydrogenotrophic methanogens (Roy *et al.*, 1986; Lorowitz *et al.*, 1989; Angelidaki and Ahring, 1995; Svetlitshnyi *et al.*, 1996) and sometimes with sulfate-reducing bacteria (Roy *et al.*, 1986; Lorowitz *et al.*, 1989; Gibson, 1990). However, under relatively high sulfate concentrations (> 2 g SO_4^{-2} /l), a sulfate-reducing bacterium is capable of degrading LCFA using sulfate as electron acceptor (Cord-Ruwisch and Garcia, 1985).

Regarding syntrophic acetogens, it is somehow surprising that until now there have been described only two mesophilic and two thermophilic bacteria with the ability to degrade fatty acids higher than lauric. Table 1.3 summarizes the characteristics of the above acetogens which can β-oxidize LCFA.

Table 1.3 Characteristics of syntrophic, acetogenic, LCFA degrading bacteria

Organism	LCFA used[a]	Syntrophic partner	Growth temp. (°C)	Growth pH	Maximum growth rate (d^{-1})	Reference
Syntrophomonas sapovorans	la, my, pa, st, ol, li	*Methanospirillum hungatei*	25–45 35–37[b]	6.3–8.1 7.3[b]	0.6[c]	Roy *et al.*, 1986
Syntrophomonas wolfei subsp. *saponavida*	la, my, pa, st	*Desulfovibrio* sp.	37	7.2	0.4[c]	Lorowitz *et al.*, 1989
Thermosyntropha lipolytica	la, my, pa, st, ol, li	*Methanobacterium* sp.	52–70 60–66[b]	7.2–9.5[d] 8.1–8.9[b,d]	7[e]	Svetlitshnyi *et al.*, 1996
short rod thermophile	pa, st, ol	*Methanobacterium thermoautotrophicum*	55[b]	7	0.3[f]	Angelidaki & Ahring, 1995

[a]la: lauric; my: myristic; pa: palmitic; st: stearic; ol: oleic; li: linoleic
[b]optimum value(s)
[c]determined on butyrate medium
[d]pH determined at 25°C
[e]determined on olive oil
[f]determined on stearate

Rate-Limiting Step in LCFA Anaerobic Degradation

Since there are several steps involved in the acetogenesis of LCFA, realization of the rate-limiting step may facilitate the degradation rate and improve the treatment efficiency. Novak and Carlson (1970) conducted a kinetic study on LCFA degradation and concluded that LCFA are readily available for microorganisms, regardless of their low solubility, and are rapidly transported through the cell wall. Hence the access to LCFA by the microorganisms probably is not limited by solubility considerations.

Novak and Carlson (1970) observed an accumulation of palmitic acid in the degradation of oleic and linoleic acid while not any intermediates were found for myristic, palmitic and stearic degradation. Conclusively, β-oxidation is the rate-limiting step for unsaturated LCFA degradation while activation (addition of acyl-CoA) is the rate-limiting step for saturated LCFA. In contrast, Rinzema *et al.* (1994) reported that β-oxidation is the rate-limiting step for a saturated, medium-chain fatty acid (capric, $C_{10:0}$). However, despite the report by Novak and Carlson (1970), literature relevant to LCFA anaerobic degradation has not clearly differentiated all steps involved in the degradation pathway. Consequently, the indiscriminate β-oxidation has been generally regarded as the rate-limiting step.

SOME POSSIBLE FACTORS AFFECTING LCFA DEGRADATION

Many environmental and biological factors relevant to the β-oxidation may affect the LCFA degradation. We herein review 4 important factors that are most frequently reported on the degradation of lipids/LCFA: temperature, pH, cosubstrate and methanogens.

Temperature

Anaerobic digestion process can be operated optimally within two temperature ranges: mesophilic (25–40°C) and thermophilic (> 45°C) (Van Lier, 1995). Regarding anaerobic treatment of LCFA-containing wastewaters, most work has been conducted at the mesophilic range rather than at the thermophilic range of temperature (see the references in Table 6.4). Very limited comparisons have been made between the two temperature ranges. Borja *et al.* (1995)

investigated the anaerobic digestion of olive mill effluent in mesophilic and in thermophilic processes. They found that the rates of methane conversion, COD removal and substrate uptake are higher under thermophilic conditions than under mesophilic conditions. Since oleic and palmitic acid are largely prevailing in olive oil (Table 1.2), thermophilic conditions may very likely benefit the treatment of the two compounds.

Until now, temperature effects on LCFA degradation rates have not been simultaneously compared. Although some maximum growth rates of LCFA β-oxidizers have been established at both temperature ranges, the two rates obtained under thermophilic conditions are somehow conflicting (Table 1.3). This conflict disables the applicability of the data from the comparison of the growth rates between the two temperature ranges.

Many studies conducted under the two temperature conditions were focused on the acetate/hydrogen removal rates. The data reported on these papers might be useful for the selection of an appropriate temperature range, considering that the faster removal of the end products of LCFA degradation may favor the degradation. In a review article Zinder (1990) summarized that thermophilic methanogens grow 2–5 times more rapidly compared to their mesophilic homologues. For hydrogenotrophic methanogens a factor of 10 can even be reached. Besides, according to thermodynamic calculations, higher temperature may broaden the possibility of hydrogen concentration-dependent reactions (Lee and Zinder, 1988). These findings collectively may imply that a higher rate methanogenesis, which favors the removal of the end products, can be attained under thermophilic conditions. Apart from these investigations (refer to references in Zinder's review) using axenic cultures, Van Lier *et al.* (1996) further demonstrated that acetate is more rapidly removed by thermophilic than by mesophilic anaerobic sludges. Temperature effects on thermodynamics, gas solubility, mass transfer and process stability related to anaerobic treatment are recently reviewed by Van Lier (1995).

Besides the significant effect on substrate conversion, temperature may also affect the inhibition of microorganisms. Addition of Tween 80, consisting of oleic and palmitic acid, to a thermophilic bacterium decreases the optimal growth temperature (Thies *et al.*, 1994). This indicates a change in the membrane composition of the thermophile, which eventually leads to cytolysis. The authors attributed the lytic effect to the presence of LCFA. It is beyond controversy that

the biodegradation can only proceed when serious inhibition, e.g., cell lysis, is not prevailing. Thus prior to the selection of a treatment temperature, the LCFA toxicity (described below) at different temperature ranges should be taken into consideration. No literature, however, had been available.

pH

Anaerobic bioreactors are operated at neutral pH ranges because most methanogens have pH optima near neutrality (Jones *et al.*, 1987). Deviations from this optimum, if not introduced with the influent substrate, are usually resulted from excess production and accumulation of acidic or basic conversion products such as organic fatty acids or ammonia, respectively (Pohland, 1992). It has long been known that high concentrations of fatty acids can enhance the inhibitory effect of low pH on methanogenesis in anaerobic bioreactors (McCarty, 1964). Komatsu *et al.* (1991) found that oleic acid exerts higher inhibition on methanogenesis from acetate at lower pH values. They also observed that both β-oxidation and methane production are favored at pH = 7.8 rather than at pH = 7.05. With respect to LCFA-containing wastewater, Beccari *et al.* (1996) noted that both acidogenesis and methanogenesis in the treatment of olive mill effluent are much better at pH = 8.5 than at pH = 6.0.

Exergonic Cosubstrate

Microbial uptake of LCFA is an energy-required process (Nunn, 1986). The overall standard free energy change, $\Delta G^{o'}$, of the palmitic oxidation is calculated as + 345.6 kJ/mol, indication this reaction is a very endergonic reaction. The $\Delta G^{o'}$ values of glucose fermentation, depending on different products formed, range from – 457.5 to – 610.5. Addition of an exergonic substrate like glucose thus may favor the uptake and further enhance an endergonic reaction, viz., β-oxidation. This explains the findings of Beccari *et al.* (1996) that oleic (3 g COD/l) is not degraded without the supplement of glucose (6 g COD/l).

Methanogenic Bacteria

As early as in 1958, Heukelekian and Mueller postulated that LCFA are not degraded during the acid-forming phase where methane is not produced. This early finding has already highlighted the role of methanogens in LCFA degradation. As β-

oxidation of LCFA is an acetogenic reaction that is performed by obligate proton-reducing bacteria, the prevailing of hydrogen-scavengers is of importance in the degradation. The syntrophic phenomenon of interspecies hydrogen transfer has been reviewed by Schink (1992) and Stams (1994).

PROBLEMS IN ANAEROBIC TREATMENT OF LCFA-CONTAINING WASTEWATERS

McCarty (1964) reported that a successful treatment of LCFA-containing wastes is possible, provided that considerably long hydraulic retention times (HRT), e.g., 10–40 days, are applied in traditional anaerobic bioreactors. The occurrence of failure was reported on the treatment of dairy waste in an industrial-scale upflow anaerobic sludge bed (UASB) reactor (Samson *et al.*, 1985), and on the treatment of lauric acid in lab-scale UASB reactors (Rinzema *et al.*, 1989). The problematic LCFA treatment is attributed to two causes:

(i). occurrence of flotation/washout of granular sludge and fatty matter at low loadings (Rinzema *et al.*, 1989), and
(ii). LCFA inhibition of anaerobic microorganisms at millimolar concentrations (Koster and Cramer, 1987).

Mostly the insufficient treatment results of LCFA-containing wastewaters were attributed to the toxicity of LCFA (Hanaki *et al.*, 1981; Angelidaki and Ahring, 1992; Hamdi, 1992; Perle *et al.*, 1995; Hamdi, 1996). For toxicology of LCFA we refer to the comprehensive review of Rinzema (1988). Regarding the degree of individual LCFA toxicity, oleic acid can be considered as the most problematic since it exerts very high inhibitory effect and is the most abundant in wastewaters (in combination with data in Demeyer and Henderickx, 1967; Galbraith *et al.*, 1971; Koster and Cramer, 1987; and Table 1.2 in this Chapter).

Sorption of LCFA onto cell surface was indicated as the mechanism of inhibition (Galbraith *et al.*, 1971) and that onto granular sludge was speculated as the reason of sludge flotation/washout (Lettinga and Hulshoff Pol, 1992). As salts of LCFA are used as detergents in textile industry, studies on LCFA sorption are conducted by using textile fibers as adsorbents. Weatherburn *et al.* (1950) and Meader and Fries (1952) concluded that the LCFA sorption onto fibers can be

regarded as a chemical adsorption process. Similar results were also found by using nickel and platinum catalysts (Smith and Fuzek, 1946). These results have, however, to be re-evaluated in anaerobic treatment processes, especially in UASB reactors. This is because the sorption behavior can be different when granular sludge serves as the adsorbent.

It has been reported that, in anaerobic digestion of oleic and myristic acid, an 84% COD removal was achieved while no methane production was observed (Cánovas-Diaz *et al.*, 1992). Very likely the authors obtained an adsorptive removal of the LCFA-COD. Because the occurrence of LCFA in anaerobic reactors, it has to be taken into consideration that the COD removed was not only due to the metabolic activity of active biomass but also to the adsorption onto the sludge.

OUTLINE OF THIS THESIS

The objectives of this research are to gain more science and engineering insights regarding the anaerobic treatment of oleic-containing wastewaters and to solve the above-mentioned treatment problems. Experimental approach was initially focused on toxicity (Chapters 2–3), then on sorption (Chapter 4) and finally on reactor technology (Chapters 5–6).

Chapter 2 describes the oleic toxicity to anaerobic sludges from various origins. Effects of three biological factors, viz., sludge origin, methanogenic activity and sludge adaptation, and a physical factor, viz., specific surface area of sludge, on the toxicity were investigated under mesophilic conditions.

Chapter 3 compares oleic toxicity to granular and suspended sludges at mesophilic and thermophilic ranges of temperature. Also the anaerobic biodegradability of granular sludge was compared at the two temperature ranges.

Chapter 4 deals with the complicated sorption behaviour of LCFA (oleate and a mixture of three LCFA) in batch and continuous UASB reactors. The sorption phenomena on active and inactivated granular sludges were characterized. Relationships between LCFA adsorption and sludge flotation as well as inhibition are demonstrated.

Chapter 5 compares the effects of reactor hydrodynamics, thermophilic and mesophilic temperatures, and addition of cosubstrate on biological and physical performance of expanded granular sludge bed (EGSB) reactors.

Chapter 6 explores the optimum operational parameters of thermophilic reactors supplemented with cosubstrates. Effects of recirculation of washed out biomass to the reactor were also investigated. Strategies to enhance the treatment are discussed.

At last, in Chapter 7, based on experimental results obtained comprehensive suggestions for optimizing the anaerobic treatment of LCFA-containing wastewaters are proposed as the Summary of this thesis.

REFERENCES

Angelidaki, I. and Ahring, B.K. (1992). Effects of free long-chain fatty acids on thermophilic anaerobic digestion. *Appl. Microbiol. Biotechnol.*, **37**, 808–812.

Angelidaki, I. and Ahring, B.K. (1995). Establishment and characterization of anaerobic thermophilic (55°C) enrichment culture degrading long-chain fatty acids. *Appl. Environ. Microbiol.*, **61**, 2442–2445.

Beccari, M., Bonemazzi, F., Majone, M. and Riccardi, C. (1996). Interaction between acidogenesis and methanogenesis in the anaerobic treatment of olive oil mill effluents. *Water Res.*, **30**, 183–189.

Borja, R., Martín, A., Banks, C.J., Alonso, V. and Chica, A. (1995). A kinetic study of anaerobic digestion of olive mill wastewater at mesophilic and thermophilic temperatures. *Environ. Pollut.*, **88**, 13–18.

Cord-Ruwisch, R. and Garcia, J.L. (1985). Isolation and characterization of an anaerobic benzoate-degrading spore-forming sulfate-reducing bacterium, *Desulfotomaculum sapomandens* sp. nov. *FEMS Microbiol. Lett.*, **29**, 325–330.

Cánovas-Diaz, M., Sánchez-Roig, M.J. and Iborra, J.L. (1992). Myristic and oleic acid degradation by an acclimated anaerobic consortia: synergistic behaviour. In: *Proc. 6th EC Conf. Biomass for Energy, Industry and Environment*, Grassi, G., Collina, A. and Zibetta, H. (Eds.), Athens, Greece, Apr. 1991, Elsevier, UK, pp. 580–584.

Demeyer, D.I. and Henderickx, H.K. (1967). The effects of C_{18} unsaturated fatty acids on methane production in vitro by mixed rumen bacteria. *Biochim. Biophys. Acta*, **137**, 484–497.

Forster, C.F. (1992). Oils, fats and greases in wastewater treatment. *J. Chem. Technol. Biotechnol.*, **55**, 402–404.

Galbraith, H. and Miller, T.B., Paton, A.M. and Thomson J.K. (1971). Antibacterial activity of long chain fatty acids and the reversal with calcium, magnesium, ergocalciferol and cholesterol. *J. Appl. Bacteriol.*, **34**, 803–813.

Gibson, G.R. (1990). A review: Physiology and ecology of the sulphate-reducing bacteria. *J. Appl. Bacteriol.*, **69**, 769–797.

Hamdi, M. (1992). Toxicity and biodegradability of olive mill wastewaters in batch anaerobic digestion. *Appl. Biochem. Biotechnol.*, **37**, 155–163.

Hamdi, M. (1996). Anaerobic digestion of olive mill wastewaters. *Process Biochem.*, **31**, 105–110.

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

Heukelekian, H. and Mueller, P. (1958). Transformations of some lipids in anaerobic sludge digestion. *Sew. Ind. Wastes*, **30**, 1108–1120.

Jeris, J.S. and McCarty, P.L. (1965). The biochemistry of methane fermentation using ^{14}C tracers. *J. Water Pollut. Control Fed.*, **37**, 178–192.

Jones, W.J., Nagel, Jr., D.P. and Whitman, W.B. (1987). Methanogens and the diversity of archaebacteria. *Microbiol. Rev.*, **51**, 135–177.

Komatsu, T., Hanaki, K. and Matsuo, T. (1991). Prevention of lipid inhibition in anaerobic processes by introducing a two-phase system. *Water Sci. Technol.*, **23**(Kyoto), 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.

Lee, M.J. and Zinder, S.H. (1988). Hydrogen partial pressures in a thermophilic acetate-oxidizing methanogenic coculture. *Appl. Environ. Microbiol.*, **54**, 1457–1461.

Lettinga, G. and Hulshoff Pol, L.W. (1992). UASB process design for various types of wastewaters. In: *Design of Anaerobic Processes for the Treatment of Industrial and Municipal Wastes*, Malina, Jr., J.F. and Pohland F.G. (Eds.), Technomic Publ., Basel, Ch. 3, pp. 119–146.

Lorowitz, W.H., Zhao, H. and Bryant, M.P. (1989). *Syntrophomonas wolfei* subsp. *saponavida* subsp. nov., a long-chain fatty-acid-degrading, anaerobic,

syntrophic bacterium; *Syntrophomonas wolfei* subsp. *wolfei* subsp. nov.; and emended descriptions of the genus and species. *Intl. J. Syst. Bacteriol.*, **39**, 122–126.

McCarty, P.L. (1964). Anaerobic waste treatment fundamentals. Part III: Toxic materials and their control. *Public Works*, **95**(Nov.), 91–94.

Meader, Jr., A.L. and Fries, B.A. (1952). Adsorption in the detergent process. *Ind. Eng. Chem.*, **44**, 1636–1648.

Novak, J.T. and Carlson, D.A. (1970). The kinetics of anaerobic long chain fatty acid degradation. *J. Water Pollut. Control Fed.*, **42**, 1932–1943.

Nuck, B.A. and Federle, T.W. (1995). Batch test for assessing the mineralization of ^{14}C-radiolabeled compounds under realistic anaerobic conditions. *Environ. Sci. Technol.*, **30**, 3597–3603.

Nunn, W.D. (1986). A molecular view of fatty acid catabolism in *Escherichia coli*. *Microbiol. Rev.*, **50**, 179–192.

Perle, M., Kimchie, S. and Shelef, G. (1995). Some biochemical aspects of the anaerobic degradation of dairy wastewater. *Water Res.*, **29**, 1549–1554.

Pohland, F.G. (1992). Anaerobic treatment: fundamental concepts, applications, and new horizons. In: *Design of Anaerobic Processes for the Treatment of Industrial and Municipal Wastes*, Malina, Jr., J.F. and Pohland F.G. (Eds.), Technomic Publ., Basel, Ch. 1, pp. 1–40.

Prince, H.N. (1959). Effect of pH on the antifungal activity of undecylenic acid and its calcium salt. *J. Bacteriol.*, **78**, 788–791.

Quéméneur, M. and Marty, Y. (1994). Fatty acids and sterols in domestic wastewaters. *Water Res.*, **28**, 1217-1226.

Ratledge, C. (1994). Biodegradation of oils, fats and fatty acids. In: *Biochemistry of Microbial Degradation*. Anon. (Ed.), Kluwer Academic Publ., Dordrecht, Ch. 4, pp. 89–141.

Rinzema, A. (1988). *Anaerobic Treatment of Wastewater with High Concentrations of Lipids or Sulfate*. Ph.D. thesis, Wageningen Agricultural University, Wageningen, The Netherlands.

Rinzema, A., Alphenaar, A. and Lettinga, G. (1989). The effect of lauric acid shock loads on the biological and physical performance of granular sludge in UASB reactors digesting acetate. *J. Chem. Technol. Biotechnol.*, **46**, 257–266.

Rinzema, A., Boone, M., van Knippenberg, K. and Lettinga, G. (1994). Bactericidal effect of long chain fatty acids in anaerobic digestion. *Water Environ. Res.*, **66**, 40–49.

Roy, F., Samain, E., Dubourguier, H.C. and Albagnac, G. (1986). *Syntrophomonas sapovorans* sp. nov., a new obligately proton reducing anaerobe oxidizing

saturated and unsaturated long chain fatty acids. *Arch. Microbiol.*, **145**, 142–147.

Samson, R., Van den Berg, B., Peters, R. and Hade, C. (1985). Dairy waste treatment using industrial-scale fixed-film and upflow sludge bed anaerobic digesters: design and start-up experience. In: *Proc. 39th Purdue Industrial Waste Conference*, Bell, J.M. (Ed.), Butterworth Publ., Boston, pp. 235–241.

Schink, B. (1992). Syntrophism among prokaryotes. In: *The Prokaryotes*. Balows, A., Trüper, H.G., Dworkin, M., Harder, W. and Schleifer, K.H. (Eds.), Springer Verlag, New York, pp. 276–299.

Smith, H.A. and Fuzek, J.F. (1946). The adsorption of fatty acids on nickel and platinum catalysts. *J. Amer. Chem. Soc.*, **68**, 229–231.

Stams, A.J.M. (1994). Metabolic interactions between anaerobic bacteria in methanogenic environments. *Antonie van Leeuwenhoek*, **66**, 271–294.

Stryer, L. (1995). *Biochemistry*. 4th edn., W.H. Freeman & Co., New York, Ch. 24, pp. 603–628.

Svetlitshnyi, V., Rainey, F. and Wiegel, J. (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 methanogenic archaeum. *Intl. J. Syst. Bacteriol.*, **46**, 1131–1137.

Taylor, R.J. (1965). *The Chemistry of Glycerides*, Unilever Ltd., England.

Thies, E., Jenkins, T. and Stutzenberger, F. (1994). Effects of the detergent Tween 80 on *Thermomonospora curvata*. *World J. Microbiol. Biotechnol.*, **10**, 657–663.

Van Lier, J.B. (1995). *Thermophilic Anaerobic Wastewater Treatment; Temperature Aspects and Process Stability*. Ph.D. thesis, Wageningen Agricultural University, Wageningen, The Netherlands.

Van Lier, J.B., Sanz Martin, J.L. and Lettinga, G. (1996). Effect of temperature on the anaerobic thermophilic conversion of volatile fatty acids by dispersed and granular sludge. *Water Res.*, **30**, 199–207.

Viswanathan, C.V., Meera Bai, B. and Pillai, S.C. (1962). Fatty matter in aerobic and anaerobic sewage sludges. *J. Water Pollut. Control Fed.*, **34**, 189–194.

Weatherburn, A.S., Rose, G.R.F. and Bayley, C.H. (1950). The sorption of soap by textile fibers. *Can. J. Res.* **28F**, 51–61.

Weng, C.-N. and Jeris, J.S. (1976). Biochemical mechanisms in the methane fermentation of glutamic and oleic acids. *Water Res.*, **10**, 9–18.

Zinder, S.H. (1990). Conversion of acetic acid to methane by thermophiles. *FEMS Microbiol. Rev.*, **75**, 125–138.

2

Comparative Toxicity of Oleic Acid to Anaerobic Sludges from Various Origins

A modified version of this Chapter has been published as:

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

2

Comparative Toxicity of Oleic Acid to Anaerobic Sludges from Various Origins

ABSTRACT

Seven anaerobic sludges were screened in order to obtain the most suitable methanogenic inoculum for the anaerobic treatment of wastewaters containing long-chain fatty acids (LCFA). The selection was made on the basis of the toxicity of a model compound, oleate, to acetoclastic methanogens in different sludges. The effects of three biological factors, sludge origin, specific acetoclastic methanogenic activity and sludge adaptation to lipids, and a physical factor, specific surface area of sludge, on the degree of toxicity were investigated and compared. Values of the fifty percent inhibition concentration (IC_{50}) of oleate obtained from 40°C batch toxicity tests ranged from 0.26 to 3.34 mM for the various sludges examined. It was found that the toxicity of oleate to anaerobic sludges did not depend on the three biological factors. Instead, it was closely correlated to the specific surface area of sludge. Suspended and flocculent sludges, which have higher specific surface area, suffered much more from inhibition than granular sludges. The results presented in this Chapter therefore indicate to use granular sludges as appropriate inocula for reactors treating lipids (fats, oils and greases) wastewaters, to decrease the toxic impact from their hydrolysis products—LCFA.

INTRODUCTION

Lipids, characterized as fats, oils and greases, are one of the major organic materials in wastewater (Yang and Anderson, 1993; Raunkjær *et al.*, 1994). Various concentrations of lipids are widely found in both domestic sewage and industrial wastewaters. Although domestic sewage typically contains about 40–100 mg/l lipids (Forster, 1992; Quéméneur and Marty, 1994), industrial wastewaters are of greater concern when considering the higher lipid concentrations in discharged effluents. Typical industries which generate lipids-containing wastewaters were extensively reviewed by Rinzema (1988). The tremendous industrial productivity is accompanied with huge quantities of effluent and, consequently, may lead to environmental deterioration.

Most of the discharges containing lipids would have received some form of preliminary physico-chemical treatment before entering to the biological stages, e.g., trapping, intercepting and floating separation. Although these processes may reach an effective removal efficiency up to 90% of the lipids, there still remains some practical impediments, due to the small amount of partially unremoved emulsified and/or colloidal lipids, to conduct either anaerobic or aerobic biological treatment of lipids-containing wastewaters (Forster, 1992; Perle *et al.*, 1995). Previous investigations demonstrated that the conversion (hydrolysis) of lipids to long-chain fatty acids (LCFA) and glycerol is not the rate limiting step for anaerobic digestion (Heukelekian and Mueller, 1958; Hanaki *et al.*, 1981; Rinzema *et al.*, 1994). However, LCFA are well-known inhibitors of various anaerobic micro-organisms at millimolar concentrations (Koster and Cramer, 1987) and, consequently, cause some serious problems in anaerobic treatment systems (Rinzema, 1988; Angelidaki and Ahring, 1992; Rinzema *et al.*, 1994).

Naturally occurring LCFA are mostly β-oxidized (Weng and Jeris, 1976; Nuck and Federle, 1995) to acetic acid and hydrogen which in turn are further converted to methane gas. Both methanogens and acetogens, the two consortia responsible for LCFA mineralization, suffer greatly from LCFA inhibition (Roy *et al.*, 1986). Demeyer and Henderickx (1967) and Galbraith *et al.* (1971) reported that adsorption of the surface active LCFA onto the cell wall/membrane leads to the damage of transport function or protective function, which is supposed to be the mechanism of LCFA toxicity. The effect of LCFA toxicity depends on the concentration but not on the concentration:biomass ratio (Koster and Cramer,

1987; Angelidaki and Ahring, 1992; Rinzema *et al.*, 1994). Yet, to date the toxicity of LCFA has not been investigated relative to the origin of sludges.

Research has shown that success was uncertain when an anaerobic sludge acclimated to one wastewater was used as an inoculum for treating another types of wastewater (Crawford and Teletzke, 1986; Morgan *et al.*, 1990; Yang and Anderson, 1993). Significant differences in reactor performance were observed when using different seed sludges for the treatment of toxic wastewaters (Hendriksen and Ahring, 1992; Peng *et al.*, 1994). Both from the research as well as the practical point of view, it is essential to select a suitable seed sludge prior to the start-up of the treatment of LCFA-containing wastewaters. The investigations in this Chapter dealt with anaerobic toxicity tests as the preliminary screening assay for the purpose of start-up.

We selected LCFA toxicity to acetoclastic methanogens in view of their big metabolic importance (Gujer and Zehnder, 1983) and the high sensitivity to inhibition (Yang and Speece, 1986). Among the LCFA, lauric acid and oleic acid have been reported to be versatile inhibitors (Galbraith *et al.*, 1971; Koster and Cramer, 1987). However, we selected the use of merely sodium oleate as the model compound in this study for another three reasons: (i) the most abundant amount among all fatty acids in wastewaters (Viswanathan *et al.*, 1962; Komatsu *et al.*, 1991), (ii) well-understood biochemical degradation pathway (Weng and Jeris, 1976), and (iii) its good solubility as a sodium salt. All the experiments were conducted at 40°C because this temperature results in the highest conversion rates (Van Haandel and Lettinga, 1994) and maximal growth rate of methanogens (Lettinga, 1995) within the mesophilic range. In the context of this study to compare sludges from different origins, we studied the effects of three biological factors, i.e., sludge origin, specific acetoclastic methanogenic activity and sludge adaptation to lipids, and one physical factor, i.e., specific surface area of sludge, on the extent of toxicity to acetoclastic methanogens.

MATERIALS AND METHODS

Biomass

Anaerobic sludges from bench-, pilot- and full-scale, mesophilic and thermophilic, adapted and non-adapted, and granular type and suspended/flocculent form were collected for this study. The characteristics of the anaerobic sludges used in this study are listed in Table 2.1. Prior to starting the experiment, all sludges had been stored at 4°C in gas tight plastic containers for a time period less than 2 weeks. After reactivation (see below), some sludge Y was placed in a test tube containing a few glass beads (inner diameters 3–5 mm). Subsequently, the tube was vigorously vortexed for 5 min. The crushed sludge Y is denoted by Yc.

Table 2.1 Sources and characteristics of anaerobic sludges used for oleate toxicity test

Source of sludge	Aviko	Borculo	Agrico	own lab.	own lab.	own lab.	own lab.
Code used in this study	A	B	C	D	E	Y	Yc
Wastewater type	potato	whey	potato	synthetic[a]	synthetic[a]	milk fat	milk fat
Reactor type	UASB	UASB	IC[b]	USSB[b]	USSB[b]	EGSB[c]	EGSB[c]
Reactor volume	1700 m^3	590 m^3	110 m^3	5 l	5 l	200 l	200 l
Reactor temperature[d]	M	M	M	T	T	M	M
Sludge appearance	granular	granular	granular	granular	flocculent	granular	suspended
Total solids (%, w/w)[e]	9.58	12.68	10.96	20.95	11.26	10.49	1.83
Volatile solids (%, w/w)[e]	7.52	9.14	8.75	9.06	2.17	9.31	1.58

[a]synthetic wastewater composed of sucrose-VFA (volatile fatty acids) mixture treated in upflow staged sludge bed (USSB) reactor (Van Lier *et al.*, 1994)
[b]internal circulation reactor
[c]expanded granular sludge bed
[d]M = mesophilic; T = thermophilic
[e]sludge content (elutriated or settled) used in toxicity test

Media

The basal medium used for the reactivation of sludges and for the toxicity assay as well was composed of (in mg/l final concentration) NH_4Cl (280), K_2HPO_4 (250), $MgSO_4 \cdot 7H_2O$ (100), $CaCl_2 \cdot 2H_2O$ (10) and yeast extract (100). The medium was made up in demineralized water and buffered by adding 5 g/l $NaHCO_3$. One millilitre of a trace element solution (Zehnder *et al.*, 1980) was added per litre of medium. All media were pH adjusted to 7.0 ± 0.1 by adding few drops of HCl.

Reactivation

Because the test sludges were obtained from various origins and with different temperatures, reactivation of the sludges at 40°C under the same condition was necessary to attain a compatible methanogenic activity of each sludge. Prior to use, each sludge was reactivated in a 1 l serum bottle by fed-batch incubation using 5 g COD/l acetate except for sludge Yc, which was directly prepared from sludge Y. Bottles for reactivation were intermittently shaken. Residual concentrations of acetate was monitored to determine the acetoclastic methanogenic activity. The reactivation of a sludge was terminated when the activity reached 0.26 ± 0.09 g COD per g volatile solids (VS) per day. The reactivated sludge was to be used for toxicity test (see below) as seed material.

Acute Toxicity Test

In the present investigations we used a conceptual approach and experimental design which deviated slightly from the procedures reported by Koster and Cramer (1987) and Sierra-Alvarez and Lettinga (1991). Reactivated granular sludges were elutriated to remove floating matter and fine particulates. On the average 7.5–9.3% (w/w) VS contents (actual compositions given in Table 2.1) were obtained per gram of those elutriated wet sludges. Sludge Y was crushed to prepare Yc by a mixer under an anaerobic atmosphere. Both E and Yc sludges were repeatedly washed and settled for five times. The sediment with ca. 2% (w/w) VS content was then pipetted into serum bottles. The bottle had 136 ± 1 ml working volume and was equipped with a butyl rubber septum and aluminum screw cap. Such treatments (elutriation or wash and settling) minimized the background methane production during the experiments. A sludge concentration of ca. 2 g VS/l was applied for each test throughout the whole study. The final liquid volume, including sludge (2 g VS/l), basal medium, acetate (2.5 g COD/l), and sodium oleate solution, was 25 ml. The 2.5 g COD/l acetate was used to revive the anaerobicity and the methanogenic activity, which possibly had been changed during sludge elutriation.

Subsequently, the headspace of the reactors was flushed with N_2/CO_2 gas (70/30, v/v) for 3 min. Reactors were then placed in a reciprocating shaker water-bath with temperature controlled at 40°C and stirred at approximately 50 strokes. After 1 day of incubation, varying concentrations of oleate (smaller amounts for E and Yc sludges) were fed using syringes to all reactors except for the control. The control was injected with the same volume of demineralized

water. After overnight exposure to the toxicant, all bottles were fed with 1 g COD/l acetate to assay the methanogenic activity. The headspace was flushed and the bottles were reincubated for 1 hour. Then the methane composition in the headspace of every reactor was determined intermittently (1.5 h in general) over an 8 h time period. Preliminary experiments conducted over a time course of 125 h revealed the absence of a lag phase (data not shown). Consequently, a period of 8 h was appropriate for gaining the maximum specific acetoclastic methanogenic activity. The maximum methanogenic activity was obtained by computing from the slope of the curve of cumulative methane production against time course. The relative activity formed in the toxicity experiment was expressed as a percentage of the control activity. Toxicant concentrations causing 50% relative activity loss are defined as the IC_{50} values. All the experiments were conducted in duplicate except for the control in each batch, for which triplicate or quintuplicate samples were used.

Analyses

Physical characteristics of the seven sludges such as particle numbers, surface area and size distribution were analyzed by an image analyzer (Applied Imaging, Tyne and Wear, England). The scanner was set such that only particle diameters exceeding 10 μm would be scanned and analyzed. Two dimensional data were converted into three dimensional data assuming that all sludge particles were spherical. Sludge samples received the same treatment as that for toxicity tests were taken in quintuplicate for image analysis.

The methane content in the gas samples was determined, as previously described (Sierra-Alvarez and Lettinga, 1991). The VFA was determined by gas chromatography. The chromatograph (HP 5890A, Palo Alto, USA) was equipped with a 2 m X 4 mm glass column, packed with Supelcoport (100–120 mesh) coated with 10% Fluorad FC 431. Operating conditions were: column, 130°C; injection port, 200°C; flame ionization detector, 280°C. Nitrogen saturated with formic acid at 20°C was used as carrier gas at 30 ml/min. Samples for VFA determination were centrifuged at 16000 *g* for 3 min. The supernatant was analyzed with an injection volume of 10 μl. The total solids (TS) and VS were measured according to standard methods (APHA, 1992).

Chemicals

Sodium oleate was purchased from BDH, London, England. All chemicals were of analytical grade.

RESULTS AND DISCUSSION

An overnight exposure to oleate was chosen according to findings of Koster and Cramer (1987) that the start of the degradation of LCFA will not within a day. In this way we ruled out the prevalence of oleate degradation in our toxicity assessment experiments; the methane production merely originated from the acetate, and all inhibition could be attributed to the oleate and not to any of its degradation products.

The oleate toxicity to anaerobic sludges from various origins is expressed as IC_{50} values. Table 2.2 summarizes the experimental data found for the oleate IC_{50} values from our toxicity tests together with the sludge activity of each control during the toxicity tests and the specific surface area of sludges used for the tests. According to the IC_{50} values, it is obvious that the granular type of sludges were less susceptible to the toxic effects of oleate than the suspended (Yc) and flocculent (E) type of sludges. The sludge from the pilot-scale (200 l) reactor treating milk fat (sludge Y) was the most resistant to oleate toxicity. As shown in Table 2.2, the order of the susceptibility to oleate toxicity at 40°C is: Yc > E > D ≥ B > C > A >Y. Possible biological and physical factors affecting the order are discussed below. To date, according to our surveys, these are the first results of LCFA toxicity investigated at 40°C. Since this temperature results in high conversion rates within the mesophilic range, the data presented here are of importance for anaerobic treatment of LCFA-containing wastewaters.

Table 2.2 Summary of the experimental results obtained in this study

Sludge code	A	B	C	D	E	Y	Yc
Oleate IC_{50} (mM)	2.27	1.78	2.01	1.75	0.53	3.34	0.26
Activity* (g CH_4-COD/g VS·d)	0.47	0.83	1.08	0.40	0.42	0.77	0.69
Specific surface area (x 10^5 mm^2/g TS)	0.59	0.56	0.67	0.21	2.55	0.43	7.37

*acetoclastic methanogenic activity of control (without receiving oleate), determined during the toxicity test

Biological characteristics of the sludge may represent important factors influencing the degree of toxicity of numerous toxicants. Therefore the effects of

sludge origin, adaptation and activity on oleate toxicity to methanogens were investigated. Fig. 2.1 shows the relative methanogenic activity of sludge D, E, Y and Yc exposed to various oleate concentrations. It should be noted that sludges D and E and sludges Y and Yc were of the same origin: sludges Y and Yc were taken from a pilot-scale EGSB reactor treating milk-fat, and sludges D and E originated from a bench-scale USSB treating synthetic wastewater (cf. Table 2.1). As the microbial composition can be regarded as the same, one might expect that sludge from the same origin should have similar susceptibility to one toxicant. To the contrary, both the IC_{50} values for sludges D and E and those for sludges Y and Yc are significantly different. Moreover, although the milk-fat-acclimated sludge Y was the least sensitive to oleate toxicity, its homologue sludge Yc was the most sensitive among the sludges tested. These disordered behaviour and dramatically different responses, i.e., one order of magnitude, imply that LCFA toxicity is irrelevant to both sludge adaptation and origin.

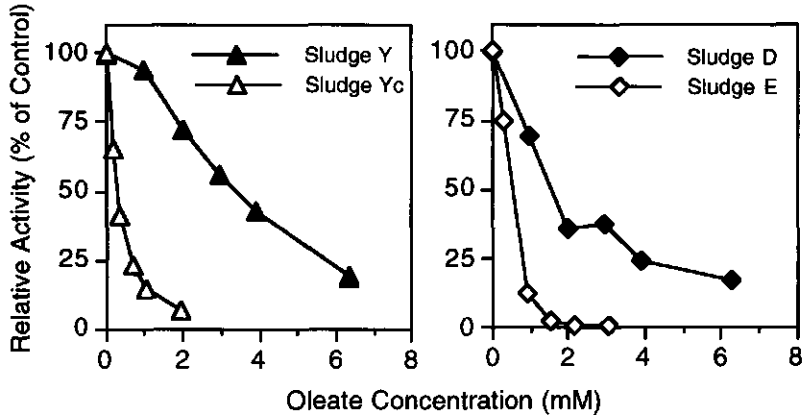


Fig. 2.1 Toxicity of oleate to (a) granular sludge (Y) and suspended sludge (Yc) from mesophilic origin; (b) granular sludge (D) and flocculent sludge (E) from thermophilic origin.

Furthermore, Fig. 2.2 shows a plot of the specific methanogenic activity on acetate of each sludge against its oleate IC_{50} value. The correlation coefficients (r) and the best fit curves have been obtained by applying Curve Fit command (using the linear least-squares regression method) in a computer software (CA-Cricket Graph III). Apparently a clear correlation between the oleate toxicity and the sludge methanogenic activity does not exist (r = 0.205, including data from Yc; r = 0.474, excluding). The irrelevance can be attributed to the unique inhibition mechanism of LCFA that associates with cell membrane/wall

(Demeyer and Henderickx, 1967; Galbraith *et al.*, 1971). Unlike most microbiological inhibitors, LCFA bears its toxic behavior similar to bactericidal effect of detergents (Asther and Corrieu, 1987; Thies *et al.*, 1994) which eventually induce cytolysis. Hence, it is not surprising that the toxicity of these surface active compounds (e.g., LCFA) is not closely correlated with the substrate conversion rate (e.g., methanogenic activity) in this study.

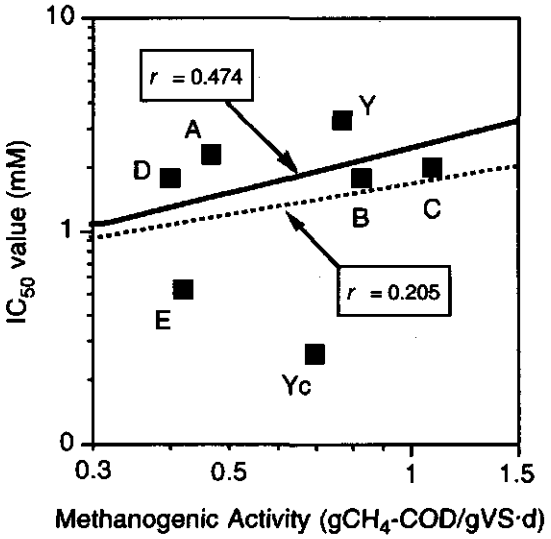


Fig. 2.2 Relation between specific methanogenic activities and IC_{50} values of anaerobic sludges tested. The bold line (—) represents the computer fitted curve which excludes the data of Yc. The dotted line (····) includes Yc. Letters next to filled squares indicate the codes of sludges which can be found in Table 2.1.

In addition to the above-discussed biological conditions, some physical effects, e.g., particle size of sludge and experimental temperature, represent other important factors that could possibly influence the degree of toxicity. Fig. 2.3 represents the correlation between specific surface areas and oleate IC_{50} values of the seven tested sludges. The computer fitted curve (r = 0.988, excluding D) indicates that all data indeed fit very well with the tendency except for sludge D. The toxicity of oleate clearly increases with a concomitant increase of the specific sludge surface area. It should be noted that the suspended (Yc) and the flocculent (E) sludges have higher specific surface areas than those of other granular sludges (Table 2.2). Thus they received higher oleate toxicity than did others. A visual examination of the appearance of sludges revealed that sludge D

had distinctly different size distribution from the other granular sludges. As an example, Fig. 2.4 illustrates the profile of size distribution of sludge D, Y and Yc. Sludge D was composed of more small (e.g., less than 0.5 mm) particles and less big (e.g., 1.5–2.0 mm) granules than was Y. Suspended sludge Yc was composed mainly of fine particles. These different compositions reinforce the correlation between the surface area and the oleate toxicity and also explain the disagreement of the toxicity towards sludge D with other sludges' data.

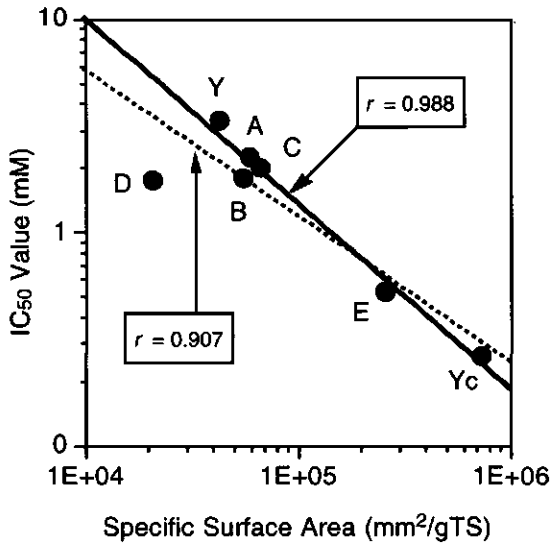


Fig. 2.3 Correlation of specific surface areas and IC_{50} values of anaerobic sludges tested. The bold line represents the computer fitted curve which excludes the data from sludge D. The dotted line is the regression including D.

Koster and Cramer (1987) performed oleate toxicity tests at 30°C using the same biomass origin as sludge A in the present study. They attributed the huge different IC_{50} levels between their study (4.35 mM) and Hanaki *et al.* (1983) (0.015 mM) to the abatement effect of calcium and magnesium ions, viz., precipitation of oleate by these bivalent ions. However, the only significant amount of cation applied in the former study was 1.75 meq/l calcium in contrast to nil used in the latter study. It therefore is questionable that such a small amount of calcium would lead to such an enormous difference. In our opinion, there were at least two further factors contributing to the big difference, i.e., surface area of sludge and experimental temperature, on which Koster and Cramer (1987) did not discuss in their paper. Hanaki *et al.* (1983) carried out

their experiment at 37°C using digested, flocculent sludge from a domestic sewage treatment plant. The recent results in our laboratory showed that temperature also plays an important role in the gravity of the methanogenic toxicity of detergent-like compounds: the higher temperature leads to the higher oleate toxicity (Chapter 3; Hwu and Lettinga, 1997). Hence, the combination of the higher temperature as well as higher surface area probably resulted in a additive effect, leading to the very low oleate IC_{50} value, i.e., very high toxicity to the sludge, in the study of Hanaki *et al.* (1983). Our IC_{50} value to sludge A (2.27 mM) equals half of that found by Koster and Cramer, which clearly reflect the temperature effect on oleate toxicity.

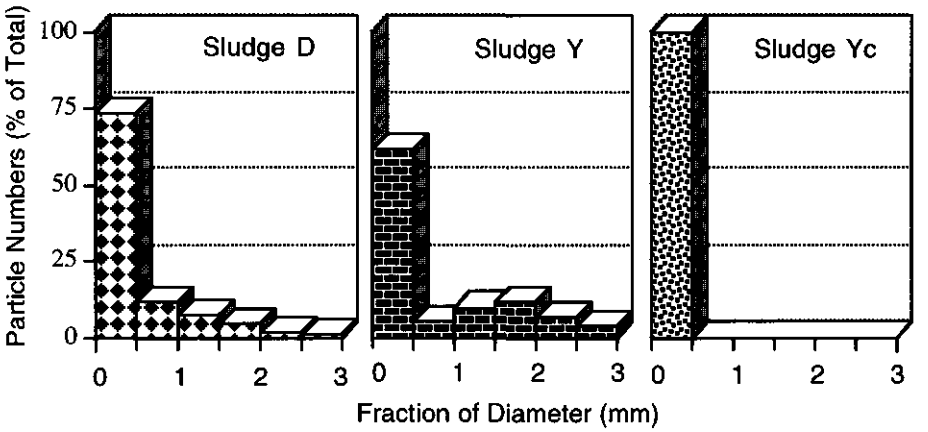


Fig. 2.4 Profile of size distribution showing particle compositions of sludge D (granular sludge from thermophilic origin), Y and Yc (granular and suspended sludge from same mesophilic origin, respectively).

Sam-Soon *et al.* (1991) reported that the anaerobic fermentation of LCFA or lipids will not give rise to the formation of a pelletized sludge in the upflow anaerobic sludge bed (UASB) system. Their report implies that one of the most important advantages of UASB process, e.g., excellent sludge retention due to the formation of granular sludge (Schmidt and Ahring, 1996), will be lost under such circumstances. Granular sludge can be used very beneficially as an inoculum for upflow reactors because of its high specific COD removal rate and good settleability (Lettinga, 1995) and it can be maintained or augmented on wastes that would not allow granulation (Rinzema *et al.*, 1993). This becomes more attractive because in this study we verified that granular sludge is less susceptible to the toxicant. However, regarding the full-scale application the availability of sufficient amount of granular sludge has to be taken into

consideration. To date, over 930 full-scale UASB reactors have been built (Habets, 1997) and more are under construction. This means that granular sludge will become available in near future in increasing amount. The use of granular sludges as inocula for start-up of reactors treating lipids or LCFA wastewaters is, therefore, an appropriate strategy. In this respect it should also be taken into account that adaptability to the LCFA toxicity does not occur when one repeatedly pre-exposed the anaerobes to non-inhibitory LCFA-concentrations (Angelidaki and Ahring, 1992), and Rinzema *et al.* (1994) found that acetotrophic methanogens do not adapt to LCFA. Therefore in selection of seeding material from granular sludges the origin will not be a major matter of consideration. Granule size as well as other physical properties, e.g., settleability would be more appropriate selection criteria regarding the LCFA toxicity investigated in the present study.

CONCLUSIONS

The toxicity of LCFA was investigated by batch experiments at 40°C with seven anaerobic sludges from various origins. According to the results obtained in this work the conclusions that can be drawn are:

LCFA toxicity varies with the type of anaerobic sludges and is more correlated to their physical characteristics, specific surface area and size distribution, than to their biological characteristics.

Selection of inoculum for full-scale reactors treating LCFA- or lipids-containing wastewaters should be based on sludge size rather than on wastewater type. Granular sludge from a full-scale treatment plant is suggested as an appropriate inoculum.

ACKNOWLEDGMENTS

I would like to thank Yiping Yang and the various companies for their kind provision of the anaerobic sludges. I am grateful to Anita van Langerak for her helpful suggestion in computing the results of image analysis. The assistance of Zoltán Kulik in conducting part of image analysis is appreciated.

REFERENCES

Angelidaki, I. and Ahring, B.K. (1992). Effects of free long-chain fatty acids on thermophilic anaerobic digestion. *Appl. Microbiol. Biotechnol.*, **37**, 808–812.

APHA (1992). *Standard Methods for the Examination of Water and Wastewater.* 18th edn. American Public Health Association, Washington, D.C., part 2540D, pp. 2-54–2-57.

Asther, M. and Corrieu, G. (1987). Effect of Tween 80 and oleic acid on liginase production by *Phanerochaete chrysosporium* INA-12. *Enzyme Microb. Technol.*, **9**, 245–249.

Crawford, G.V. and Teletzke, G.H. (1986). Performance of a hybrid anaerobic process. In: *Proc. 41st Purdue Industrial Waste Conference*, Ann Arbor Press, Ann Arbor, Michigan, pp. 196–208.

Demeyer, D.I. and Henderickx, H.K. (1967). The effects of C_{18} unsaturated fatty acids on methane production in vitro by mixed rumen bacteria. *Biochim. Biophys. Acta*, **137**, 484–497.

Forster, C.F. (1992). Oils, fats and greases in wastewater treatment. *J. Chem. Technol. Biotechnol.*, **55**, 402–404.

Galbraith, H. and Miller, T.B., Paton, A.M. and Thomson J.K. (1971). Antibacterial activity of long chain fatty acids and the reversal with calcium, magnesium, ergocalciferol and cholesterol. *J. Appl. Bacteriol.*, **34**, 803–813.

Gujer, W. and Zehnder, A.J.B. (1983). Conversion processes in anaerobic digestion. *Water Sci. Technol.*, **15**(8-9), 127–167.

Habets, L.H.A. (1997). Personal communication. Paques B.V., PO Box 52, 8560 AB Balk, The Netherlands.

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

Hanaki, K., Ishikawa, T. and Matsumoto, J. (1983). Inhibitory and stimulative effects of oleate on methanogenesis from acetate in anaerobic digestion. *Technol. Rep. Tohoku Univ.*, **48**, 123–135.

Hendriksen, H.V. and Ahring, B.K. (1992). Anaerobic dechlorination of pentachlorophenol in fixed-film and upflow anaerobic sludge blanket reactors using different inocula. *Biodegrad.*, **3**, 399–408.

Heukelekian, H. and Mueller, P. (1958). Transformations of some lipids in anaerobic sludge digestion. *Sew. Ind. Wastes*, **30**, 1108–1120.

Hwu, C.-S. and Lettinga, G. (1997). Acute toxicity of oleate to acetate-utilizing methanogens in mesophilic and thermophilic anaerobic sludges. *Enzyme Microb. Technol.*, **21**, in press.

Komatsu, T., Hanaki, K. and Matsuo, T. (1991). Prevention of lipid inhibition in anaerobic processes by introducing a two-phase system. *Water Sci. Technol.*, **23**(Kyoto), 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.

Lettinga, G. (1995). Anaerobic digestion and wastewater treatment systems. *Antonie van Leeuwenhoek*, **67**, 3–28.

Morgan, J.W., Goodwin, J.A.S., Wase, D.A.J. and Forster, C.F. (1990). Effects of using various types of carbonaceous substrate on UASB granules and on reactor performance. *Biol. Wastes*, **34**, 55–71.

Nuck, B.A. and Federle, T.W. (1995). Batch test for assessing the mineralization of ^{14}C-radiolabeled compounds under realistic anaerobic conditions. *Environ. Sci. Technol.*, **30**, 3597–3603.

Peng, D., Zhang, X., Jin, Q., Xiang, L. and Zhang, D. (1994). Effects of the seed sludge on the performance of UASB reactors for treatment of toxic wastewater. *J. Chem. Technol. Biotechnol.*, **60**, 171–176.

Perle, M., Kimchie, S. and Shelef, G. (1995). Some biochemical aspects of the anaerobic degradation of dairy wastewater. *Water Res.*, **29**, 1549–1554.

Quéméneur, M. and Marty, Y. (1994). Fatty acids and sterols in domestic wastewaters. *Water Res.*, **28**, 1217–1226.

Raunkjær, K., Hvitved-Jacobsen, T. and Nielsen, P.H. (1994). Measurement of pools of protein, carbohydrate and lipid in domestic wastewater. *Water Res.*, **28**, 251–262.

Rinzema, A. (1988). *Anaerobic Treatment of Wastewater with High Concentrations of Lipids or Sulfate*. Ph.D. thesis, Wageningen Agricultural University, Wageningen, The Netherlands.

Rinzema, A., Alphenaar, A. and Lettinga, G. (1993). Anaerobic digestion of long-chain fatty acids in UASB and expanded granular sludge bed reactors. *Process Biochem.*, **28**, 527–537.

Rinzema, A., Boone, M., van Knippenberg, K. and Lettinga, G. (1994). Bactericidal effect of long chain fatty acids in anaerobic digestion. *Water Environ. Res.*, **66**, 40–49.

Roy, F., Samain, E., Dubourguier, H.C. and Albagnac, G. (1986). *Syntrophomonas sapovorans* sp. nov., a new obligately proton reducing anaerobe oxidizing

saturated and unsaturated long chain fatty acids. *Arch. Microbiol.*, **145**, 142–147.

Sam-Soon, P.A.L.N.S., Loewenthal, R.E., Wentzel, M.C. and Marais, Gv.R. (1991). A long-chain fatty acid, oleate, as sole substrate in upflow anaerobic sludge bed (UASB) reactor systems. *Water SA*, **17**, 31–36.

Schmidt, J.E. and Ahring, B.K. (1996). Granular sludge formation in upflow anaerobic sludge blanket (UASB) reactors. *Biotechnol. Bioeng.*, **49**, 229–246.

Sierra-Alvarez, R. and Lettinga, G. (1991). The effect of aromatic structure on the inhibition of acetoclastic methanogenesis in granular sludge. *Appl. Microbiol. Biotechnol.*, **34**, 544–550.

Thies, E., Jenkins, T. and Stutzenberger, F. (1994). Effects of the detergent Tween 80 on *Thermomonospora curvata*. *World J. Microbiol. Biotechnol.*, **10**, 657–663.

Van Haandel, A.C. and Lettinga, G. (1994). *Anaerobic Sewage Treatment: a Practical Guide for Regions with a Hot Climate*. John Wiley & Sons, Chichester, Ch. 2, pp. 44–45.

Van Lier, J.B., Boersma, F., Debets, M.M.W.H. and Lettinga, G. (1994). High rate thermophilic anaerobic wastewater treatment in compartmentalized upflow reactors. *Water Sci. Technol.*, **30**(12), 251–261.

Viswanathan, C.V., Meera Bai, B. and Pillai, S.C. (1962). Fatty matter in aerobic and anaerobic sewage sludges. *J. Water Pollut. Control Fed.*, **34**, 189–194.

Weng, C.-N. and Jeris, J.S. (1976). Biochemical mechanisms in the methane fermentation of glutamic and oleic acids. *Water Res.*, **10**, 9–18.

Yang, J. and Speece, R.E. (1986). The effects of chloroform toxicity on methane fermentation. *Water Res.*, **20**, 1273–1279.

Yang, G. and Anderson, G.K. (1993). Effects of wastewater composition on stability of UASB. *J. Environ. Eng. ASCE*, **119**, 958–977.

Zehnder, A.J.B., Huser, B.A., Brock, T.D. and Wuhrmann, K. (1980). Characterization of an acetate-decarboxylating, non-oxidizing methane bacterium. *Arch. Microbiol.*, **124**, 1–11.

3

Anaerobic Toxicity and Degradability of Oleic Acid under Mesophilic and Thermophilic Conditions

This Chapter contains two papers accepted for publication as:

Hwu, C.-S. and Lettinga, G. (1997). Acute toxicity of oleate to acetate-utilizing methanogens in mesophilic and thermophilic anaerobic sludges. *Enzyme Microb. Technol.*, **21**, in press.

Hwu, C.-S., van Lier, J.B. and Lettinga, G. (1997). Anaerobic toxicity and degradability of oleic acid under mesophilic and thermophilic conditions. In: *Proc. Forum for Appl. Biotechnol.*, 25–26 Sep. 1997, Gent, Belgium, in press.

3

Anaerobic Toxicity and Degradability of Oleic Acid under Mesophilic and Thermophilic Conditions

ABSTRACT

Oleic acid, mostly a major derivative of lipid hydrolysis, causes serious problems in biologically anaerobic systems treating fats/oils/greases wastewaters. Toxicity and degradability tests of oleic acid were conducted batch-wise at three temperatures (30, 40 and 55°C) with different anaerobic sludges. Oleate inhibited flocculent sludge more than granular sludge. Its toxicity was irrelevant to the specific methanogenic activity but significantly dependent on temperature. Methanogenesis under thermophilic (55°C) conditions was found to be more susceptible to oleate toxicity than under mesophilic conditions (40 and 30°C). Fifty percent inhibition concentrations of oleate ranged between 0.35–0.79 mM at 55°C, 0.53–2.27 mM at 40°C, and 2.35–4.30 mM at 30°C. Oleate was over 12-fold more toxic to thermophilic flocculent sludge than to mesophilic granular sludge. Physico-chemical surface association between oleate and methanogens played an important role in acute intoxication.

Regarding degradability, complete mineralization of oleic acid to methane was achieved with the specific degradation rates of 124, 41 and 33 mg COD/g VS·d at 55, 40 and 30°C, respectively. Besides the higher degradability achieved, the methanogenic activity recovered more rapidly at 55°C after exposure to high oleate high concentrations, i.e., > 1.97 mM. Considering an appropriate selection

between thermophilic and mesophilic treatment, the contradictory effect of temperature on oleic acute toxicity and degradability is discussed.

INTRODUCTION

Long-chain fatty acids (LCFA) are products of lipid hydrolysis and are well known inhibitors of anaerobic micro-organisms at millimolar concentrations (Koster and Cramer, 1987; Hwu *et al.*, 1996). LCFA exert irreversible and non-adaptable toxic effects on both mesophilic (Rinzema *et al.*, 1994) and thermophilic (Angelidaki and Ahring, 1992) anaerobic digestion. Consequently, LCFA can cause serious problems in anaerobic treatment systems (Rinzema *et al.*, 1989). Koster (1987) reported that LCFA inhibition is a rapid phenomenon: 50% of the methanogenic activity is lost after only 7.5 min of exposure. Recovery of the inhibited methanogenesis can take a few months, which was attributed to growth of the few methanogens that survived the exposure (Rinzema *et al.*, 1994). Lauric ($C_{12:0}$) and oleic ($C_{18:1}$) acids are the strongest inhibitors among the LCFA (Galbraith *et al.*, 1971; Koster and Cramer, 1987). Oleic acid is the most common and abundant LCFA in both wastewaters and sewage (Viswanathan *et al.*, 1962; Komatsu *et al.*, 1991; Quéméneur and Marty, 1994). Lauric acid is, on the other hand, merely a trace constituent in fats/oils/greases raw materials and in LCFA-containing wastewaters. As a result of its toxicity, oleic acid is also indicative of the inhibition of biomass activity during the anaerobic degradation of dairy wastewater (Perle *et al.*, 1995) and is listed as one of the difficult compounds to subject olive mill effluent to anaerobic conversion (Beccari *et al.*, 1996).

Apart from their toxicity, however, LCFA are biodegradable under anaerobic conditions. LCFA are mostly degraded by acetogens via the β-oxidation pathway into acetic acid and hydrogen (Weng and Jeris, 1976), which in turn are further converted into methane gas by methanogens. Considering the most abundant constituent in LCFA-containing wastewaters, however, research on anaerobic digestion rates of oleic acid has been rather limited. Furthermore, although LCFA inhibition affects both methanogens and acetogens (Roy *et al.*, 1986), methanogens are generally the most sensitive anaerobes to toxic compounds (Yang and Speece, 1986). During anaerobic digestion about 70% of total COD flows through acetate to methane (Jeris and McCarty, 1965). Considering both their

sensitivity and metabolic importance, methanogens were investigated as the target micro-organisms of oleate toxicity.

LCFA are present as salts (soaps) in pH neutral ranges thus exert a surface active effect. An increase in temperature will enhance the detergent or surfactant toxicity, resulting in a higher degree of cell lysis of micro-organisms (Asther and Corrieu, 1987; Thies *et al.*, 1994) and higher organisms (Lewis, 1992). By contrast, Borja *et al.* (1995) reported that thermophilic conditions resulted in a 28% higher methane production and an over 2-fold faster substrate uptake rate than mesophilic conditions during anaerobic digestion of olive mill effluent (OME). There are, however, no reports that describe the temperature effects on oleate toxicity or degradation in methanogenic conditions. In anaerobic treatment, both mesophilic and thermophilic methanogenic processes are optimally operated in pH neutral ranges. Hence, it is of importance to understand to what extent temperature affects oleate degradation and oleate toxicity to methanogenesis from acetate. Also long-term evolution after intoxication of sludge by exposure to oleate can be an important implication to practical treatment. Therefore, batch tests were performed to investigate oleic toxicity and degradability, and recovery of sludge activity after intoxication, under mesophilic (30 and 40°C) and thermophilic (55°C) conditions.

MATERIALS AND METHODS

Oleic Toxicity at Different Temperatures

Anaerobic Sludges

Anaerobic sludges from mesophilic and thermophilic reactors were used in this study (Table 3.1). They originated both from lab- and full-scale installations and were characterized by either a granular or flocculent structure. Before starting the experiments, all sludges had been stored at 4°C in gas-tight plastic containers for a maximum period of three months.

Table 3.1 Characteristics of source of the sludges used for oleate acute toxicity test

Sludge source	Aviko	Borculo	Agrico	own lab.	own lab.
Code used in this Chapter	A	B	C	D	E
Wastewater type	potato	whey	potato	synthetic[a]	synthetic[a]
Reactor type[b]	UASB	UASB	IC	USSB	USSB
Reactor volume	1700 m^3	590 m^3	110 m^3	5 l	5 l
Reactor temperature	30°C	30°C	30°C	55°C	55°C
Sludge structure	granular	granular	granular	granular	flocculent

[a]synthetic wastewater composed of a sucrose-VFA mixture (Van Lier *et al.*, 1994)
[b]UASB: upflow anaerobic sludge bed reactor; IC: internal circulation reactor; USSB: upflow staged sludge bed reactor, sludge taken was a mixture of all stages

Experimental Protocol

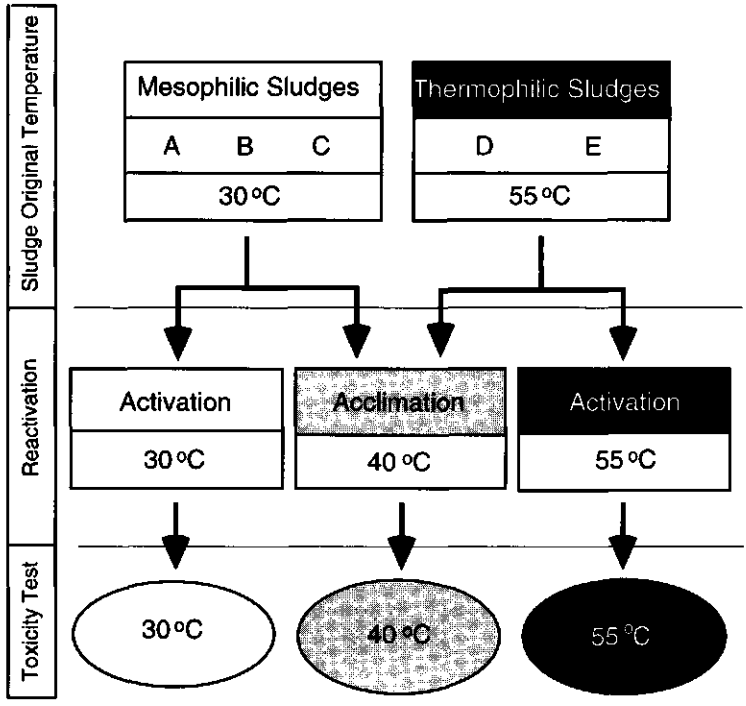


Fig. 3.1 Flowchart showing the progress relationship between sludge sources and toxicity tests at different temperatures.

Except for thermophilic sludge C, sludges which were used at the same temperatures as their source (30 or 55°C) were reactivated only, while those

tested at 40°C were acclimated to that temperature (Fig. 3.1) to attain stable methanogenic activities. The thermophilic sludge C was sampled from a bench-scale (4.4 l) reactor operated at 55°C, treating an LCFA mixture upon finishing the reactor re-start-up (see Chapter 5 for details). Every sludge was activated or acclimated in an intermittently shaken serum bottle (1 l) by fed-batch incubation in the presence of acetate (5 g chemical oxygen demand, COD, per litre). Residual acetate concentrations were monitored until the specific methanogenic activity (SMA) of each sludge reached 0.23 ± 0.1 g COD per g volatile solids (VS) per day.

Acute Toxicity Tests

The reactivated granular sludges (A, B, C and D) were elutriated with tap water to remove non-settling matter and fine particulates. The flocculent sludges (E) were repeatedly washed and settled five times. Sludge (ca. 2 g VS/l) was then placed into serum bottles (136 ± 1 ml), equipped with butyl rubber septa and aluminum screw caps. The pretreatment (elutriation or washing and settling) minimized the background methane production during the experiments. The liquid volume, including sludge and basal medium supplemented with acetate (2.5 g COD/l) was 24 ml. Acetate was used to revive the methanogenic activity, which possibly declined during the sludge pretreatment.

Subsequently, the headspace of the bottles was flushed with N_2/CO_2 gas (70/30, v/v) for 3 min. Bottles were then placed in a temperature controlled (30, 40 or 55°C) reciprocal water-bath shaker (50 strokes per min). After 1 day of incubation, 1 ml of various concentrations of oleate was added to all bottles using syringes, except for the control of each condition which was injected with 1 ml of demineralized water. After an overnight of exposure to oleate, methanogenic activity tests were initiated by feeding the bottles with acetate (1 g COD/l) and flushing their headspace. The bottles were reincubated for 1 h, after which the methane composition in the headspace was determined intermittently (with about 1.5 h intervals) over an 8 h time period. This period was appropriate for gaining the SMA, as no lag period could be observed during 125 h of incubation (data not shown). The SMA was obtained by computing the slope of the cumulative methane production against time divided by the exact amount of biomass. The relative activity (RA) was expressed as a percentage of the control activity:

$$\text{Relative Activity (\%)} = 100\left(\frac{\text{SMA of a tested concentration}}{\text{SMA of control}}\right) \quad (1)$$

All the experiments were conducted in duplicate, except for the control in each condition, which was performed in triplicate or in quintuplicate. All data are reported as the mean values, whose standard deviations were less than 5% of the mean.

Recovery of Inhibited Methanogenic Activity

Long-term monitoring of the inhibitory effect of oleate was carried out to investigate the possibility of any eventual recovery of the lost methanogenic activity due to oleic exposure. Measures of methane production in sludge C at 30 and 55°C conditions were prolonged up to ca. 900 and 300 h, respectively. During these periods, the pressure in the headspace of a serum bottle was balanced to atmosphere with syringes. After quantifying the amount of biogas produced, the methane content in biogas was analyzed.

At certain time instants of incubation, the cumulative methane production in the test bottles was compared with that in control. The ratio (%) was defined as the comparative activity at specific times (CAT):

$$\text{CAT (\%)} = 100\left(\frac{\text{Cumulative methane production in test bottle at time t}}{\text{Cumulative methane production in control bottle at time t}}\right) \quad (2)$$

When methane production in a test bottle was more than in the control, i.e., CAT ≥ 100%, the elapsed time was recorded. The time required for each applied oleate concentration was, theoretically, different and is defined as recovery time in this Chapter.

Oleic Degradability at Different Temperatures

Anaerobic Sludges

Three anaerobic granular sludges were used in oleate degradability tests. The three sludges were of the same origin (Agrico, Wezep, The Netherlands) as described in Table 3.1 but acclimated under different conditions. The 30 and 55°C sludges were sampled from two bench-scale (4.4 l) reactors, operated at respective temperature, treating an LCFA mixture upon finishing the reactor re-start-up (see Chapter 5 for details). After sampling, the two sludges were "reactivated" at their corresponding temperatures in the manner described above. The 40°C sludge was the same as used in the above acute toxicity tests.

Degradability Tests

Sodium oleate was used as the sole carbon and energy source in the batch tests. In each serum bottle (136 ± 1 ml) the initial concentrations were: sludge, 2.5 g VS/l and oleate, 1 g COD/l (equivalent to 1.14 mM). The final liquid volume was about 25 ml. The specific oleate degradation rates were defined as the maximum methane production rates divided by the initial amount of VS determined for each bottle. The methane production was expressed in terms of COD. Theoretical conversion factors of 1 g COD to 421, 401 and 388 ml methane were assumed, respectively, at 55, 40 and 30°C. A ten-day test period, during which a daily measurement was performed, was sufficient to obtain the maximum oleate conversion rates as indicated by a constant amount of methane in the headspace. At least 80% of the added COD was converted to methane during this period. All tests were performed in triplicate. The mean values are used in this Chapter.

Media

The composition of the basal medium (pH 7.0 ± 0.1) was described previously (Hwu *et al.*, 1996). It was supplemented with $NaHCO_3$ (5 g/l) and a trace element solution (1 ml/l) (Zehnder *et al.*, 1980).

Analyses

Procedures for analyzing the methane content were described by Hwu *et al.* (1996). Volatile fatty acid (VFA) concentrations were determined by gas chromatography as previously described in Chapter 2. The VS content of the sludge was determined following the standard method (APHA, 1992).

Chemicals

Sodium oleate used in toxicity tests was purchased from BDH (UK) while that in degradability tests was from Sigma (USA). All chemicals were of analytical grade.

RESULTS

Temperature Effect on Oleic Acute Toxicity

Inhibition concentrations (IC) of oleate to methanogenesis from acetate were derived by plotting the RA versus the oleate concentration, as typically shown in Fig. 3.2 for sludge B. An oleate concentration of 3.7 mM resulted in an 80% loss of the SMA. Generally, IC_{50} values are considered in toxicity studies (Koster and Cramer, 1987; Sierra-Alvarez and Lettinga, 1991) which resemble the concentration of the toxicant causing 50% of inhibition. In this Chapter, the susceptibility of methanogenic sludges to oleate at the three temperatures is compared (Fig. 3.3). Koster and Cramer (1987) reported that oleate is not degraded within the course of the experiment. Therefore, the observed toxicity may not be attributed to inhibition by products or intermediates of oleate degradation. Furthermore, according to Thies *et al.* (1994), an overnight of exposure to oleate is sufficient to obtain designated results of oleate toxicity, viz., changes of compositions of cell membrane, which eventually lead to cell lysis.

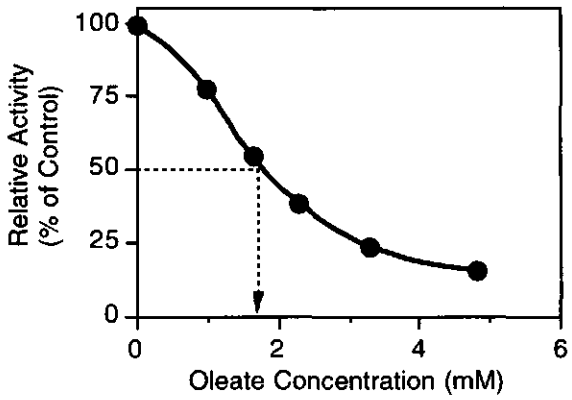


Fig. 3.2 Relative methanogenic activity (% of control) versus oleate concentration of sludge B at 40°C. The arrow indicates the determination of the IC_{50} value of oleate (1.78 mM).

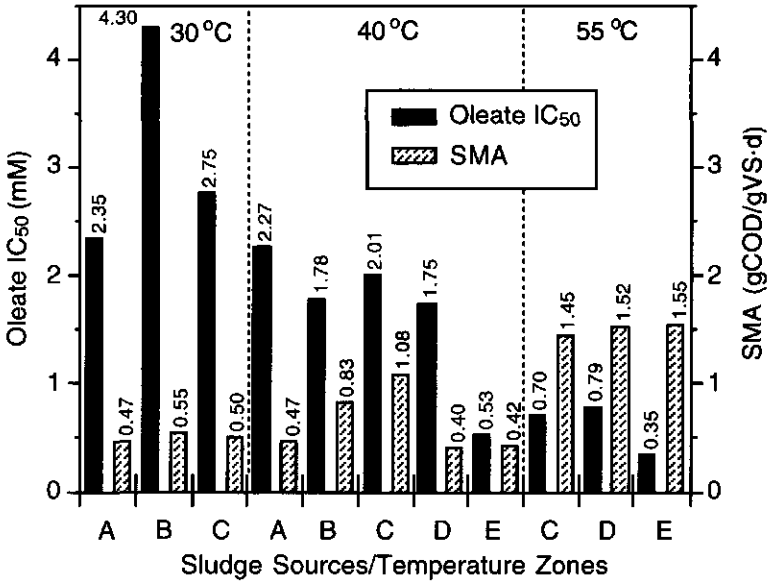


Fig. 3.3 IC_{50} of oleate and SMA showing, respectively, acute toxicity and acetate-utilizing methanogenic activity (without receiving oleate) at different temperatures.

IC_{50} and SMA values of the tested sludges are given in Fig. 3.3. The flocculent sludge E was found to be the most susceptible, either at 40 or 55°C. Oleate was over 12-fold more toxic to thermophilic flocculent sludge E than to mesophilic granular sludge B. In general, a higher inhibitory effect (i.e., lower IC_{50}) of oleate to methanogenesis was observed at higher temperatures. Of the same sludge source, methanogenesis was more susceptible to oleate at elevated temperatures. Except for sludge E, IC_{50} values were about one order of magnitude smaller at 55°C compared to those at 40 and 30°C.

Although the thermophilic sludges had the highest methanogenic activities (Fig. 3.3), they were relatively the most susceptible to oleate toxicity. Comparing SMA and IC_{50} values shows, however, no relation between the methanogenic activity and oleate toxicity (Fig. 3.3). The relation between oleate toxicity and temperature is presented in Fig. 3.4. For the granular sludges, a close relation (correlation coefficient, $r = 0.946$) was established by using a non-linear, least-squares algorithm. Data of sludge E were not included in the regression because of its flocculent structure.

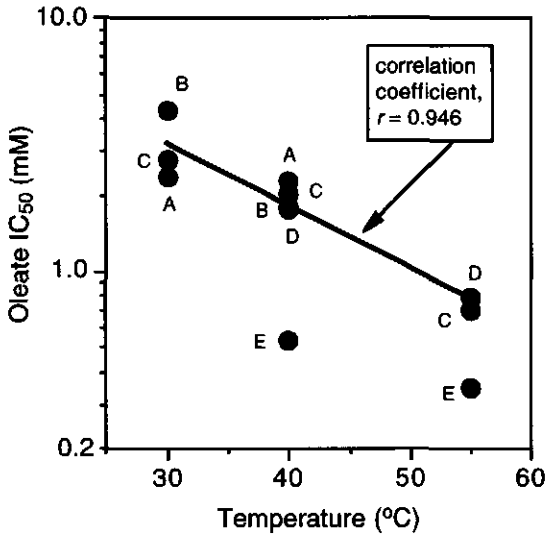


Fig. 3.4 Correlation of oleate toxicity and temperature to anaerobic sludges. The bold line represents the non-linear regression curve from the data of the granular sludges, excluding the flocculent sludge E.

Temperature Effect on Oleic Degradability

At each temperature, acetate was mostly found as the intermediate of oleate degradation. The highest acetate concentration was ca. 70 mg COD/l. Propionate merely appeared in the first 5 days of incubation at 55°C at a relatively low concentration of ca. 20 mg COD/l. Butyrate was always below detection level.

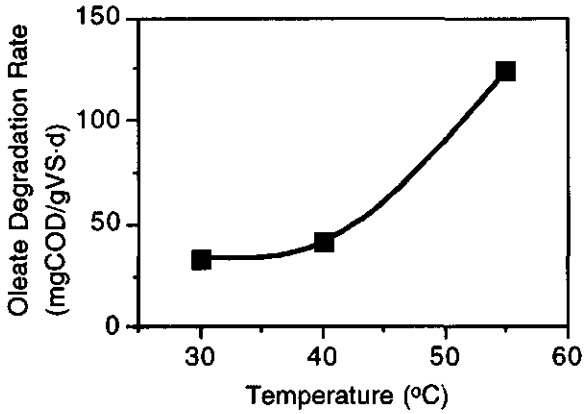


Fig. 3.5 Oleate degradation rates at 30, 40 and 55°C.

After a lag period, occurring on the first day at each temperature, the methane production rate gradually increased. In contrast to the results obtained in the oleate acute toxicity tests, the highest degradation rate was found under thermophilic conditions which was one magnitude higher than under mesophilic conditions (Fig. 3.5).

Recovery of Methanogenic Activity after Oleic Inhibition

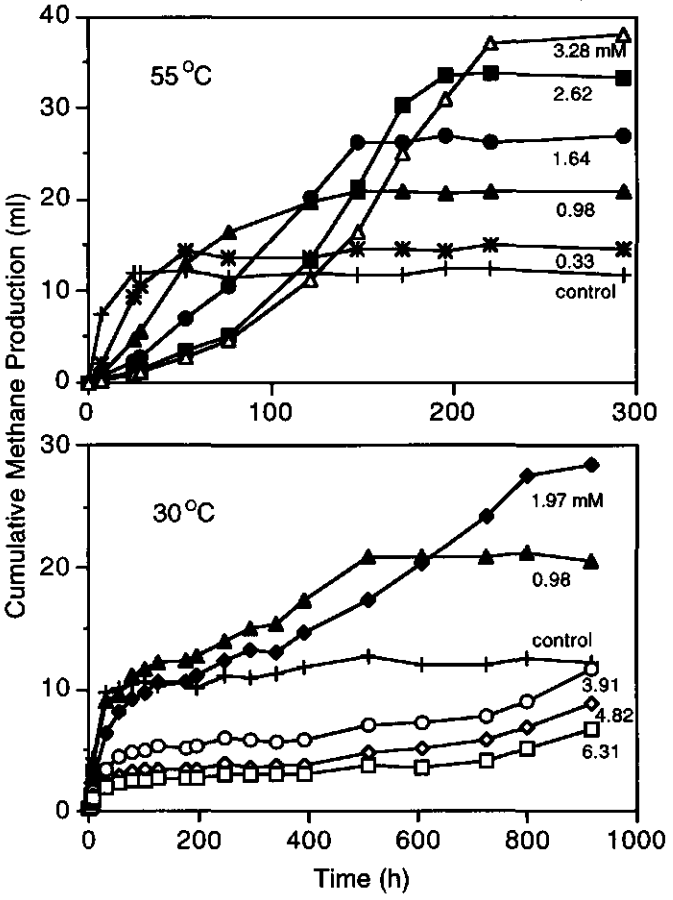


Fig. 3.6 Long-term course of methane production in sludge C at 30 and 55°C.

Fig. 3.6 shows the prolonged monitoring of methane production in sludge C fed with various oleate concentrations at 30 and 55°C. Addition of a higher oleate concentration caused a longer lag period. Virtually, methane production of each

tested concentration at both temperatures could exceed that of the control, and sooner or later reached a plateau which indicates a complete mineralization of the added substrates, i.e., acetate and oleate.

Fig. 3.7 presents the plots of CAT against oleate concentrations at different time instants. At both temperatures, methane production was inhibited early in the test, but recovery began and increased with elapsed time. The CAT curves reflect this recovery by shifting upward and to the right. The thermophilic sludge was initially more susceptible to oleate inhibition as indicated by more bent curves at 7 h and 25 h. The time required for each concentration to exceed 100% CAT was established as is illustrated in Fig. 3.8. No significant differences in recovery time were found for both temperatures at oleate concentrations < 2 mM. When oleate concentration exceeded 2 mM, thermophilic conditions showed its superiority in exhibiting shorter recovery times of the methanogenic activity. With oleate concentration of 3.28 mM, the recovery time for the thermophilic sludge was 5 days while that for the mesophilic sludge was about a month!

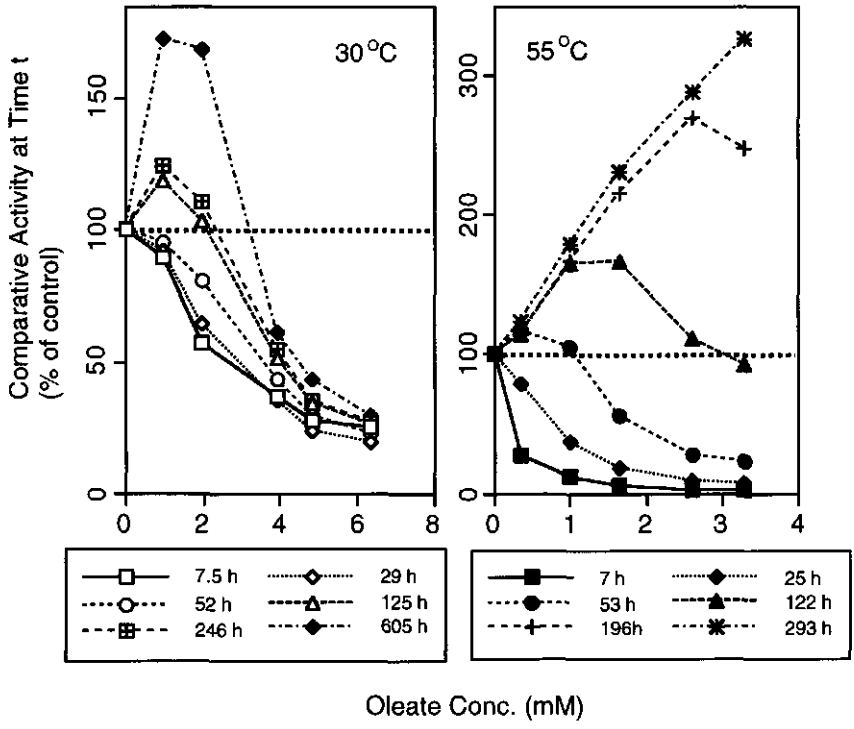


Fig. 3.7 Profile of the comparative activity at different time instants.

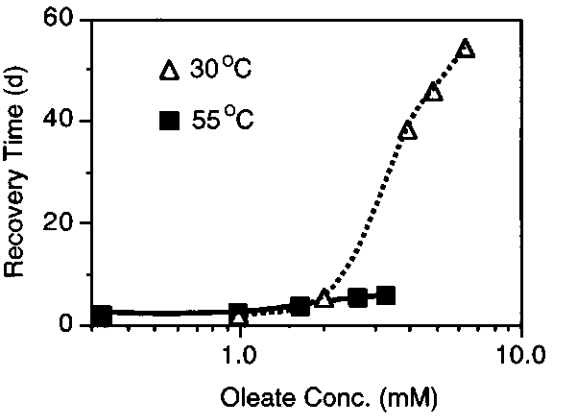


Fig. 3.8 Comparison of the recovery time required after inhibition of oleate at various concentrations.

DISCUSSION

Temperature Effect on Oleate Toxicity

This study shows that increasing operational temperatures aggravate the susceptibility of methanogenesis to acute toxicity of oleate (Figs 3.3 and 3.4). This could be attributed to the composition of cell membranes or the structure of the tested sludges or both.

It is well-known that the compositions of cell membranes of thermophiles and mesophiles are different (Amelunxen and Murdock, 1978). Hence, thermophilic bacteria might have different susceptibility to oleate toxicity compared to mesophilic ones, as the mechanism of LCFA toxicity is the sorption of the surface active LCFA onto the cell membrane (Demeyer and Henderickx, 1967; Galbraith and Miller, 1973a,b). The sorption virtually leads to the damage of transport or protective functions and, consequently, to cytolysis. Moreover, since cell lysis is the ultimate consequence of oleate toxicity, SMA was independent of IC_{50} (Fig. 3.3), as was also found in a previous study (Hwu *et al.*, 1996; Chapter 2).

More specifically, thermophilic cell membranes are composed of more saturated LCFA (SLCFA), whereas mesophilic cell membranes contain more unsaturated LCFA (ULCFA) (Amelunxen and Murdock, 1978). The saturation

degree (ratio of SLCFA/ULCFA), the key factor governing the membrane fluidity, is especially crucial to thermophiles. In fact, this degree can be automatically adjusted by the bacteria themselves upon adaptation to certain variations of the environmental temperature (Russell and Fukunaga, 1990). Thies *et al.* (1994), however, reported that the addition of the detergent Tween 80 (which mainly comprises oleic acid) dramatically changes the fatty acid compositions of thermophilic cell membrane, viz., a 15-fold increase of ULCFA and a 20% decrease of SLCFA. In this study, the combined effect of oleate and temperature might have induced an excessive increase in membrane fluidity and permeability, thus enhancing cell lysis at 40°C and, much more significantly, at 55°C (Fig. 3.4).

Although the compositions of cell membrane of methanogens are based on ether linkages instead of ester linkages (De Rosa *et al.*, 1986), evidence of cytolysis of *Methanococcus vannielii* was found when this culture was exposed to several surfactants (Jones *et al.*, 1977). To date, however, the actual mechanism of oleate toxicity to methanogens is yet not well understood. Nonetheless, due to the observed similar response, one might expect that an equivalent mechanism or a counterpart or both existed in the interactions between oleate and the cell membrane of methanogens.

Hwu *et al.* (1996) showed that oleate toxicity is very closely correlated to the specific surface area of a sludge, with the bigger surface areas suffering from the higher toxicity. Therefore, it is not surprising that sludge E was the most susceptible at either temperature, as this flocculent sludge can be expected to have a relatively larger surface area compared to granular sludges. An increase in temperature aggravated the oleate toxicity, leading to the lowest IC_{50} value established in this study: sludge E, 0.35 mM at 55°C. On the other hand, when compared with mesophilic granules, the strength of thermophilic granules is lower (Quarmby and Forster, 1995) and perhaps they have a more loosely open structure (Macario *et al.*, 1991). Apparently, thermophilic sludges can be more susceptible to toxic compounds than mesophilic ones. Accordingly, degree of oleate acute toxicity to anaerobic sludge is determined by the concentration (Fig. 3.2), operational temperature (this study), and sludge specific surface area (Hwu *et al.*, 1996).

Temperature Effect on Oleate Degradability

A nearly 4-fold faster degradation rate was achieved at 55°C (124 mg COD/g VS·d) than at 30°C (33 mg COD/g VS·d) (Fig. 3.5). Our findings support those found for anaerobic digestion of OME at thermophilic conditions (Borja *et al.*, 1995). According to a review by Van Lier (1995), thermophilic methanogens and acetogens grow 2 to 3 times as rapidly as their mesophilic homologues. The more rapid growth rates may offer the higher degradation rates. In line with this, the occurrence of the lag period of initial methanogenesis at 55°C can be attributed to oleate toxicity, because the applied oleate concentration, 1000 mg COD/l (ca. 1.14 mM), was relatively high as compared with its IC_{50} level (0.7 mM, Fig. 3.3) derived at 55°C. Since the applied oleate concentration (1.14 mM) was below its IC_{50} level (2.75 mM, Fig. 3.3), the lag period with the mesophilic sludge can not be solely attributed to oleate toxicity but more likely to a slow degradation process.

The experimental result that acetate was the main metabolite of oleate degradation agrees with Weng and Jeris (1976) and Nuck and Federle (1996), who reported that β-oxidation is the mechanism of LCFA degradation. Moreover, no accumulation of VFA was observed during the degradation of oleate as the sole carbon and energy source, indicating that β-oxidation is the rate-limiting step in mesophilic (Novak and Carlson, 1970) as well as in thermophilic digestion. It has to be noted that respective oleate-acclimated sludge was inoculated in 30 and 55°C tests but the sludge used at 40°C had not previously been exposed to the substrate. Thus the order of the degradation rates (Fig. 3.5) implies that temperature might have a larger effect than sludge acclimation on anaerobic degradation of the LCFA. Thermodynamically, the temperature effect manifests itself as β-oxidation is an endothermic (energy demanding) reaction under standard conditions.

Although a nearly 4-fold faster degradation rate was achieved at 55°C than at 30°C, the thermophilic degradation rate (0.124 g COD/g VSS·d) obtained in the present study is considerably low compared to the reported maximum specific oleate uptake rate (k), 4 g COD/g VSS·d, estimated at 37°C by Novak and Carlson (1970). The last value is, however, questionable since it was estimated by using COD removal as the measure of substrate degradation. This measure is inappropriate because adsorptive removal of LCFA is prevailing in anaerobic bioreactors (see also Hwu *et al.*, 1997; Chapter 4).

Considering the delicate interactions between toxicity (Fig. 3.4), degradability (Fig. 3.5) and sludge loading (0.4 g oleate-COD/g VS), it is likely that the applied oleate concentration, i.e., 1000 mg COD/l, is the maximum applicable concentration in the anaerobic degradation of oleate. The degradability test showed that, under the applied conditions, oleate can be completely mineralized, though slowly, within 15 days. In contrast, Beccari *et al.* (1996) reported that oleic acid as a sole compound can not be degraded by OME-acclimated sludge within 66 days at 35°C. However, it should be noted that flocculent sludge was exposed to an oleate concentration of 4.2 mM in their study. Based on the results obtained in our present study, it therefore is not surprising that they did not find oleate degradation within 66 days, simply because severe oleate inhibition was prevailed.

Selection of Temperature: Thermophilic or Mesophilic Treatment

Good arguments and sometimes conflicting results for choosing anaerobic wastewater treatment under mesophilic or thermophilic conditions have long been reported (Van Lier, 1995). When confining the target pollutant(s) in wastewaters to oleate/LCFA, to date, anaerobic treatment of wastewaters containing such ingredients is optimally conducted under mesophilic (Sayed *et al.*, 1988; Rinzema *et al.*, 1993; Borja *et al.*, 1995) and thermophilic (Angelidaki *et al.*, 1990) conditions. Notwithstanding, parallel comparison of anaerobic treatment of oleate/LCFA-containing wastewaters at both temperatures is rather limited. The present study provides more insight in temperature effects on both the toxicity and degradability of oleate-containing wastewaters.

The long-term measurements of methane production (Fig. 3.6) indicate that oleate is eventually degradable at the concentration as high as 6.31 mM (ca. 5600 mg COD/l) at 30°C, provided that the contact time between the compound and biomass is sufficient. The pattern of the CAT plots in Fig. 3.7 is characteristic of compounds that degrade readily during the test period or compounds to which the microorganisms adapt easily. However, the latter speculation is obviously of minor importance in the present work since cytolysis is the mechanism of the LCFA toxicity. The later recovery of methane production should be attributed to the growth of microorganisms who survived the toxic exposure, which agrees well with Rinzema *et al.* (1994). Due to higher growth rate of thermophiles, the recovery at 55°C was therefore much faster (Fig. 3.8). The faster recovery of an upset reactor is often a crucial criterion in treatment plants.

Although the experimental results of acute toxicity tests (Fig. 3.4) might suggest a preference of mesophilic treatment, the results of recovery (Fig. 3.8) and degradability (Fig. 3.5) tests would recommend the thermophilic temperature range for anaerobic digestion of oleate. Identification of this controversy depends on the realistic situations dealing with anaerobic treatment of oleate/LCFA. Many industries such as food, slaughterhouse (Couillard *et al.*, 1989), palm oil (Ma and Ong, 1986) and edible oil refining (Eroglu *et al.*, 1990) discharge LCFA-containing wastewater at high temperatures. Under such circumstances, application of thermophilic treatment can thus be economically beneficial. Moreover, if in-reactor oleate concentrations are lower than the IC_{50} level, the thermophilic treatment is more advantageous. Even when considering shock loads which may induce severe treatment upset or failure due to LCFA toxicity, thermophilic treatment still can be an appropriate option. Yet, it might be risky to abruptly imply the batch results to industrial-scale treatment. Hence, further research towards the practical applicability of LCFA toxicity and biodegradation in anaerobic reactors, under both temperature conditions, is required for the ultimate selection of the treatment process temperature.

CONCLUSIONS

Oleic acute toxicity to granular and flocculent sludges and degradation by granular sludge is systematically investigated under mesophilic and thermophilic conditions. The flocculent sludge suffered from more severe inhibition than the granular sludge at all tested temperatures (30, 40 and 55°C). Although the toxicity is significantly higher at the elevated temperatures, both the recovery of methanogenesis after intoxication and the degradation rate are also faster under thermophilic conditions. It looks promising to adopt thermophilic treatment as the option for the anaerobic treatment of LCFA-containing wastewaters. The promising results obtained in batch experiments, however, have to be further evaluated in continuous reactor systems.

ACKNOWLEDGMENTS

I gratefully acknowledge Dr. Piet N. L. Lens for his invaluable comments and suggestions on this work. I thank Zoltán Kulik for his contribution on part of the batch tests.

REFERENCES

Amelunxen, R.E. and Murdock, A.L. (1978). Microbial life at high temperatures: mechanisms and molecular aspects. In: *Microbial Life in Extreme Environments*, D.J. Kushner (Ed.), Academic Press, London, pp. 217–277.

Angelidaki, I., Petersen, S.P. and Ahring, B.K. (1990). Effects of lipids on thermophilic anaerobic digestion and reduction of lipid inhibition upon addition of bentonite. *Appl. Microbiol. Biotechnol.*, **33**, 469–472.

Angelidaki, I. and Ahring, B.K. (1992). Effects of free long-chain fatty acids on thermophilic anaerobic digestion. *Appl. Microbiol. Biotechnol.*, **37**, 808–812.

APHA, AWWA and WEF. (1992). *Standard Methods for the Examination of Water and Wastewater.* 18th edn., American Public Health Association, Washington, DC, part 2540, pp. 2.54–2.57.

Asther, M. and Corrieu, G. (1987). Effect of Tween 80 and oleic acid on liginase production by *Phanerochaete chrysosporium* INA-12. *Enzyme Microb. Technol.*, **9**, 245–249.

Beccari, M., Bonemazzi, F., Majone, M. and Riccardi, C. (1996). Interaction between acidogenesis and methanogenesis in the anaerobic treatment of olive oil mill effluents. *Water Res.*, **30**, 183–189.

Borja, R., Martín, A., Banks, C.J., Alonso, V. and Chica, A. (1995). A kinetic study of anaerobic digestion of olive mill wastewater at mesophilic and thermophilic temperatures. *Environ. Pollut.*, **88**, 13–18.

Couillard, D., Gariépy, S. and Tran, F.T. (1989). Slaughterhouse effluent treatment by thermophilic aerobic process. *Water Res.*, **23**, 573–579.

De Rosa, M., Gambacorta, A. and Gliozzi, A. (1986). Structure, biosynthesis, and physicochemical properties of archaebacterial lipids. *Microbiol. Rev.*, **50**, 70-80.

Demeyer, D.I. and Henderickx, H.K. (1967). The effects of C_{18} unsaturated fatty acids on methane production in vitro by mixed rumen bacteria. *Biochim. Biophys. Acta*, **137**, 484–497.

Eroglu, V., Ozturk, I., San, H.A. and Demir, I. (1990). Comparative evaluation of treatment alternatives for wastewaters from an edible oil refining industry. Water Sci. Technol., 22(9), 225–234.

Galbraith, H., Miller, T.B., Paton, A.M. and Thomson, J.K. (1971). Antibacterial activity of long chain fatty acids and the reversal with calcium, magnesium, ergocalciferol and cholesterol. *J. Appl. Bacteriol.*, **34**, 803–813.

Galbraith, H. and Miller, T.B. (1973a). Effect of metal cations and pH on the antibacterial activity and uptake of long chain fatty acids. *J. Appl. Bacteriol.*, **36**, 635–646.

Galbraith, H. and Miller, T.B. (1973b). Physicochemical effects of long chain fatty acids on bacterial cells and their protoplasts. *J. Appl. Bacteriol.*, **36**, 647–658.

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

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

Hwu, C.-S., Tseng, S.-K., Yuan, C.-Y., Kulik, Z. and Lettinga, G. (1997). Biosorption of long-chain fatty acids in UASB treatment process. *Water Res.*, in press.

Jeris, J.S. and McCarty, P.L. (1965). The biochemistry of methane fermentation using ^{14}C tracers. *J. Water Pollut. Control Fed.*, **37**, 178–192.

Jones, J.B., Bowers, B. and Stadtman, T.C. (1977). *Methanococcus vannielii*: ultrastructure and sensitivity to detergents and antibiotics. *J. Bacteriol.*, **130**, 1357–1363.

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

Koster, I.W. (1987). Abatement of long-chain fatty acid inhibition of methanogenesis by calcium addition. *Biol. Wastes*, **22**, 295–301.

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.

Lewis, M.A. (1992). The effects of mixtures and other environmental modifying factors on the toxicities of surfactants to freshwater and marine life. *Water Res.*, **26**, 1013–1023.

Ma, A.N. and Ong, A.S.H. (1986). Palm oil processing—new development in effluent treatment. *Water Sci. Technol.*, **18**(Singapore), 35–40.

Macario, A.J.L., Visser, F.A., van Lier, J.B. and Conway de Macario, E. (1991). Topography of methanogenic subpopulations in a microbial consortium adapting to thermophilic conditions. *J. Gen. Microbiol.*, **137**, 2179–2189.

Novak, J.T. and Carlson, D.A. (1970). The kinetics of anaerobic long chain fatty acid degradation. *J. Water Pollut. Control Fed.*, **42**, 1932–1943.

Nuck, B.A. and Federle, T.W. (1996). Batch test for assessing the mineralization of ^{14}C-radiolabeled compounds under realistic anaerobic conditions. *Environ. Sci. Technol.*, **30**, 3597–3603.

Perle, M., Kimchie, S. and Shelef, G. (1995). Some biochemical aspects of the anaerobic degradation of dairy wastewater. *Water Res.*, **29**, 1549–1554.

Quarmby, J. and Forster, C.F. (1995). A comparative study of the structure of thermophilic and mesophilic anaerobic granules. *Enzyme Microb. Technol.*, **17**, 493–498.

Quéméneur, M. and Marty, Y. (1994). Fatty acids and sterols in domestic wastewaters. *Water Res.*, **28**, 1217-1226.

Rinzema, A., Alphenaar, A. and Lettinga, G. (1989). The effect of lauric acid shock loads on the biological and physical performance of granular sludge in UASB reactors digesting acetate. *J. Chem. Technol. Biotechnol.*, **46**, 257–266.

Rinzema, A., van Veen, H. and Lettinga, G. (1993). Anaerobic digestion of triglyceride emulsions in expanded granular sludge bed reactors with modified sludge separators. *Environ. Technol.*, **14**, 423–432.

Rinzema, A., Boone, M., van Knippenberg, K. and Lettinga, G. (1994). Bactericidal effect of long chain fatty acids in anaerobic digestion. *Water Environ. Res.*, **66**, 40–49.

Roy, F., Samain, E., Dubourguier, H.C. and Albagnac, G. (1986). *Syntrophomonas sapovorans* sp. nov., a new obligately proton reducing anaerobe oxidizing saturated and unsaturated long chain fatty acids. *Arch. Microbiol.*, **145**, 142–147.

Russell, N.J. and Fukunaga, N. (1990). A comparison of thermal adaptation of membrane lipids in psychrophilic and thermophilic bacteria. *FEMS Microbiol. Rev.*, **75**, 171–182.

Sayed, S.K.I., van der Zanden, J., Wieffels, R. and Lettinga, G. (1988). Anaerobic degradation of the various fractions of slaughterhouse wastewater. *Biol. Wastes*, **23**, 117–142.

Sierra-Alvarez, R. and Lettinga, G. (1991). The effect of aromatic structure on the inhibition of acetoclastic methanogenesis in granular sludge. *Appl. Microbiol. Biotechnol.*, **34**, 544–550.

Thies, E., Jenkins, T. and Stutzenberger, F. (1994). Effects of the detergent Tween 80 on *Thermomonospora curvata*. *World J. Microbiol. Biotechnol.*, **10**, 657–663.

Van Lier, J.B., Hulsbeek, J., Stams, A.J.M. and Lettinga, G. (1993). Temperature susceptibility of thermophilic methanogenic sludge: implications for reactor start-up and operation. *Bioresource Technol.*, **43**, 227–235.

Van Lier, J.B., Boersma, F., Debets, M.M.W.H. and Lettinga, G. (1994). High rate thermophilic anaerobic wastewater treatment in compartmentalized upflow reactors. *Water Sci. Technol.*, **30**(12), 251–261.

Van Lier, J.B. (1995). *Thermophilic Anaerobic Wastewater Treatment; Temperature Aspects and Process Stability*. Ph.D. thesis, Wageningen Agricultural University, Wageningen, The Netherlands.

Van Lier, J.B., Groeneveld, N. and Lettinga, G. (1996). Development of thermophilic methanogenic sludge in compartmentalized upflow reactors. *Biotechnol. Bioeng.*, **50**, 115–124.

Viswanathan, C.V., Meera Bai, B. and Pillai, S.C. (1962). Fatty matter in aerobic and anaerobic sewage sludges. *J. Water Pollut. Control Fed.*, **34**, 189–194.

Weng, C.N. and Jeris, J.S. (1976). Biochemical mechanisms in the methane fermentation of glutamic and oleic acids. *Water Res.*, **10**, 9–18.

Yang, J. and Speece, R.E. (1986). The effects of chloroform toxicity on methane fermentation. *Water Res.*, **20**, 1273–1279.

Zehnder, A.J.B., Huser, B.A., Brock, T.D. and Wuhrmann, K. (1980). Characterization of an acetate-decarboxylating, non-oxidizing methane bacterium. *Arch. Microbiol.*, **124**, 1–11.

4

Biosorption of Long-Chain Fatty Acids in UASB Treatment Process

This Chapter contains two papers published/accepted as:

Hwu, C.-S., Tseng, S.-K., Yuan, C.-Y., Kulik, Z. and Lettinga, G. (1996). Adsorption of long-chain fatty acids onto granular sludges and its effect on upflow anaerobic sludge bed reactor operation. In: *Proc. 1st IAWQ Specialized Conf. on Adsorption in Water Environment and Treatment Processes*, 5–8 Nov. 1996, Shirahama, Wakayama, Japan, pp. 91–98.

Hwu, C.-S., Tseng, S.-K., Yuan, C.-Y., Kulik, Z. and Lettinga, G. (1997). Biosorption of long-chain fatty acids in UASB treatment process. *Water Res.*, in press.

4

Biosorption of Long-Chain Fatty Acids in UASB Treatment Process

ABSTRACT

The occurrence of sludge flotation induced by adsorption of long-chain fatty acids (LCFA) represents one of the most serious deteriorations in modern anaerobic treatment processes. Biosorption of LCFA in the upflow anaerobic sludge bed (UASB) treatment process was investigated using batch tests and continuous reactor runs. Batch experiments were conducted for characterization of the biosorption, using two active and one inactivated (autoclaved) anaerobic granular sludges as sorbents and with a single (oleic acid) or a mixture of LCFA ($LCFA_m$; 50% oleic, 35% palmitic and 15% stearic acid) as sorbate. The $LCFA_m$ showed a faster rate and a larger amount of adsorption onto the granules than oleic acid. Adsorption was followed by desorption in sorption tests using the active sludge granules. Methane production increased significantly, simultaneously (at lower LCFA concentrations) or succeedingly (at higher concentrations) with the desorption. The desorption was mediated by biological activity, i.e., methanogenesis, since it did not occur with inactivated granules and became insignificant with active granules inhibited by $LCFA_m$ at higher concentrations. Increased LCFA concentrations resulted in a greater LCFA adsorption and more serious inhibition of their biodegradation. A dynamic process is proposed to explain the relationship between biosorption, desorption and biodegradation of LCFA by sludge granules. Isothermal studies with oleate showed that the apparent

biosorption could be described by the physical multilayer adsorption theory and the sorption isotherm derived was consistent with the Freundlich model.

The relation between LCFA biosorption and granular sludge flotation was investigated in a UASB reactor fed with $LCFA_m$. Sludge flotation depended on the $LCFA_m$ sludge loading rate rather than on their concentration. The higher the imposed loading, the more severe flotation and the sooner complete flotation of the entire sludge bed occurred. Flotation started when the sludge loading rate exceeded 0.09 g $LCFA_m$-COD/g VSS·d, while complete flotation occurred at loading rates exceeding 0.2 g $LCFA_m$-COD/g VSS·d. These results suggest that in the treatment of LCFA-containing wastewaters by the UASB process sludge bed washout occurs before serious inhibition of methanogenesis takes place.

INTRODUCTION

Wastewaters produced from edible oil refinery, slaughterhouse, wool scouring and dairy products industry contain a high (> 100 mg/l) concentration of lipids (characterized either as fats, oils or greases). In anaerobic wastewater treatment systems, lipids are rapidly hydrolyzed to long-chain fatty acids (LCFA) and glycerol but the degradation (β-oxidation) of LCFA to acetate proceeds slowly (Hanaki *et al.*, 1981). The β-oxidation is the rate-limiting step in anaerobic degradation of capric acid ($C_{10:0}$) (Rinzema *et al.*, 1994) and oleic acid ($C_{18:1}$) (Novak and Carlson, 1970). Moreover, LCFA are strong inhibitors of anaerobic microorganisms (Demeyer and Henderickx, 1967; Prins *et al.*, 1972) and anaerobic granular sludge (Koster and Cramer, 1987; Hwu *et al.*, 1996). In addition, problems with sludge flotation and/or washout in UASB reactors may manifest following shock loads of milk fats (Samson *et al.*, 1985) as well as LCFA (Rinzema *et al.*, 1989). Adsorption of LCFA onto the surfaces of microorganisms has been indicated as the mechanism of inhibition (Galbraith *et al.*, 1971) while that onto granular sludge is speculated as the reason for sludge flotation/washout (Lettinga and Hulshoff Pol, 1992).

Sorption of fatty matter or LCFA proceeds relatively rapidly, viz., sorption equilibria of four LCFA sodium soaps can establish within 30 min (Weatherburn *et al.*, 1950) or 16 h (Meader and Fries, 1952) of contact with textile fibers. Hrudey (1982) pointed out that 80% of the lipids already become adsorbed to aerobic sludge within 20 min. For anaerobic sludge, Hanaki *et al.* (1981) found that LCFA

disappear from the aqueous phase and accumulate in the solid phase within the first 24 h of incubation.

The present knowledge on adsorption and biodegradation of LCFA by granular sludge in UASB reactors is still limited. Adsorption of sodium salts of capric and lauric acid ($C_{12:0}$) by methanogenic granular sludge was investigated, respectively, by Keurentjes and Rinzema (1986) and Koster (1987). However, these two LCFA generally are only present in trace amounts both in raw materials (in upstream processes; Taylor, 1965) and in wastewaters (in pipe-end processes; Viswanathan *et al.*, 1962). As oleic acid is the most abundant LCFA in wastewaters (Viswanathan *et al.*, 1962; Komatsu *et al.*, 1991; Quéméneur and Marty, 1994), it was selected as a model compound in the present study. In addition, a mixture of 35% palmitic ($C_{16:0}$), 15% stearic ($C_{18:0}$) and 50% oleic acid was used to simulate the genuine composition of a local slaughterhouse wastewater (Yuan, 1995). This work was aimed to (i) perform isothermal studies using inactivated UASB granules, (ii) characterize the LCFA biosorption and (iii) determine the effect of biosorption on sludge flotation. Therefore mesophilic (40°C) batch incubations (apparent LCFA biosorption) and experiments with continuous flow UASB reactors (biosorption vs. flotation) were performed. The sorption theory was also approached by conducting isothermal sorption tests at 40°C with oleic acid by use of inactivated sludge granules.

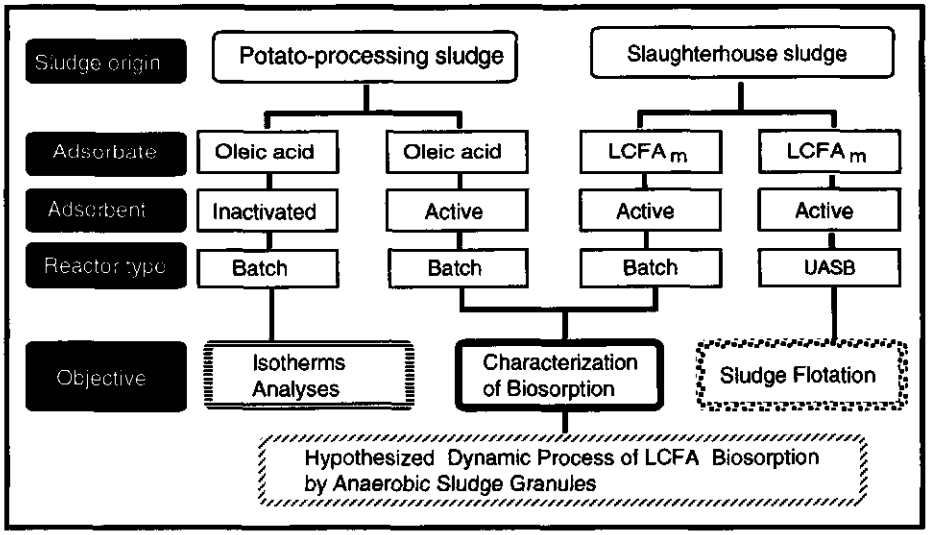


Fig. 4.1 Schematic diagram showing the experimental design protocol (detailed in text).

MATERIALS AND METHODS

Assessing Protocol

The experimental design used in this study is illustrated in Fig. 4.1. LCFA biosorption by anaerobic sludge granules was assessed in batch and continuous reactors using two origins of anaerobic granular sludge with either oleic acid or $LCFA_m$ as sorbate.

Anaerobic Granular Sludges

Granular sludge taken from a 110 m^3 internal circulation reactor treating potato processing wastewater (Agrico, Wezep, The Netherlands) was elutriated and well settled. Size distribution analysis, performed as described previously (Hwu *et al.*, 1996), showed that 87% of the granules had a size between 0.5–2.0 mm in diameter. Active sludge granules were used in the experiments with oleic acid as the sole sorbate. For isothermal sorption tests, these granules were inactivated by autoclaving (121°C, 1.5 bar) for 1 h, followed by a second autoclaving (15 min) on the next day. This treatment was the only one with a nearly 100% inhibitory effect. Other chemical (e.g., $HgCl_2$, NaN_3 and H_2O_2) or physical (gamma-ray irradiation) inactivation procedures did not completely inactivate the granules (unpublished data). For the experiments with $LCFA_m$ as sorbate, sludge granules from a slaughterhouse wastewater treatment plant (Da-Ann, Taipei, Taiwan) were used after elutriation and settling. These granules, examined by microscopy (Nikon Optiphot, Japan), had a size between 0.5–1.0 mm in diameter.

Media

Sodium oleate and a mixture ($LCFA_m$) of sodium salts of 35% palmitic, 15% stearic and 50% oleic acid (on COD basis) were used as sorbates. The $LCFA_m$ simulated the 3 major constituents in the local slaughterhouse wastewater, which represents at least 80% of the total lipid COD. To obtain a compatible ionic strength, solutions of mineral nutrients and trace elements (Hwu *et al.*, 1996) were also provided in experiments with inactivated sludge (isotherm analyses). Media were buffered by $NaHCO_3$ solution (5 g/l). After the addition of sorbates, the pH was immediately adjusted to 7.2 ± 0.1 by adding HCl.

Batch Sorption Tests

Biosorption of oleic acid and $LCFA_m$ was characterized and isotherm analyses were performed with batch-type reactors under anaerobic conditions (Fig. 4.1). Experiments with single LCFA were conducted in glass serum bottles (136 ± 1 ml) with inactivated or active granular sludge (final total solids (TS) concentration, 2.5 g/l), supplemented with various concentrations of oleate (300, 600, 1000, 1400 and 2000 mg/l). The liquid volume (incl. sludge) in the bottle was ca. 50 ml. Subsequently, the headspace of the bottles was flushed with N_2/CO_2 gas (70/30, v/v) for 5 min. Bottles were then placed in a temperature controlled (40°C) reciprocating water-bath shaker (approx. 50 strokes per min). The isothermal adsorption tests were carried out as described by Fernadez *et al.* (1995). Upon finishing the experiments, the appearance of the granules was studied by an Olympus ZS40 zoom microscope. Experiments with $LCFA_m$ were done with approx. 50 ml liquid (incl. 2.4 g TS/l) supplemented with $LCFA_m$ (150, 300, 600, 1000 or 1500 mg/l) in a 125 ± 1 ml glass serum bottle, incubated in a temperature controlled (35°C) rotary water-bath shaker (approx. 120 revolutions per min).

Continuous UASB Reactor Runs

A Plexiglas cylindrical UASB reactor (height 850 mm, width 100 mm) was used to investigate the quantitative relation between LCFA biosorption and sludge flotation. The reactor had a working volume of 6.67 l and was temperature controlled at 35°C by a water jacket. The reactor was seeded with 250.1 g volatile suspended solids (VSS) of the same sludge used in $LCFA_m$ batch tests. During reactor start-up, glucose (2 g COD/l) was used as the sole carbon and energy source. When 80% COD removal efficiency was stably reached over 5 days, to the feed of the reactor the same medium was supplied as used in the $LCFA_m$ batch tests. Adjustment to various sludge loading rates (SLR), ranging from 0.086 to 0.250 g $LCFA_m$-COD/g VSS·d (in arbitrary order), was obtained by altering $LCFA_m$ concentrations or hydraulic retention times (HRT). The liquid superficial upflow velocity (V_{up}) was 2 m/h throughout the study.

The initial volume of the sludge bed in each test run was recorded. Sludge flotation was monitored by visual observation and the time was noted when sludge started to float from the sludge bed. A test run was terminated either when flotation became trivial or when the entire sludge bed floated. In the latter case, the time period from initial to entire floating was recorded. Between two test

loadings, buoying matter consisted of fat-like materials, sludge granules entrapped in and biogas bubbles adhered to these materials was collected and gently stirred to separate from each other. This procedure allowed sludge granules to re-settle down. After reintroducing the sludge into the UASB reactor, anoxic tap water was pumped through the reactor until no biogas production was detectable. This operation ensured that the non-separable LCFA residues on granules' surface, if any, were mineralized prior to the following run.

Analyses

In batch tests using oleate, samples from the supernatant were taken at certain time intervals and analyzed for their oleic acid content. If not analyzed immediately, samples were acidified below pH 2 and stored at –18°C. Samples (0.5 ml) were acidified with 2 drops of 6 N HCl and subsequently extracted with 5 ml petroleum ether (boiling point 40–60°C). A known concentration of sodium oleate was also analyzed in parallel as a positive control. After 1 h mixing, the ether phase was transferred to another tube using a Pasteur pipette. Ether was entirely evaporated by heating the tube in an 80°C water bath. Methylation reagent (1 ml) composed of fuming HCl and dry methanol (1:19, v/v) was added, well-mixed and then placed back in the 80°C water bath. After 30 min esterification, the tube containing methyl esters was cooled down to room temperature. Subsequently, 2 ml petroleum ether was added and vortexed for 3 min. One ml of this ether phase was Pasteur pipetted into a glass vial and immediately capped.

The oleic (or other LCFA, from capric to arachidic, $C_{20:0}$) concentration in the vial was determined by a gas chromatograph (HP 5890 II) equipped with an auto sampler and a flame ionization detector. The column (25 m x 0.25 mm) used was coated with CP-WAX-58 (film thickness: 0.2 mm). Temperature conditions were: injector, 275°C; detector, 250°C; oven, programmed 140–240°C at 5°C/min. Helium was used as carrier gas with a flow rate of 1.1 ml/min. In each analysis, a fatty acids methyl esters (FAME) standard was also analyzed, which was used to identify and quantify the LCFA in the samples. Over 90% of the recovery for the positive control was usually found. The errors of the FAME standard between each analysis were less than 3%.

COD was determined for samples from the experiments using $LCFA_m$. Otherwise stated, COD values were converted and expressed as mg $LCFA_m$/l based

on the calculation that each gram of $LCFA_m$ theoretically equaled 2.893 g COD. The methane content was measured by gas chromatography (Hwu *et al.*, 1996). TS, VSS and COD determinations followed the procedures described in the standard methods (APHA, 1992).

Chemicals

Sodium salts of LCFA and FAME standard (AOCS No. 5) were purchased from Sigma, USA. All chemicals were of analytical grade.

RESULTS AND DISCUSSION

Characterization and Isotherm of Oleate Biosorption

In the oleate biosorption tests, oleic acid methyl ester was the main constituent among all LCFA peaks shown in the GC-chromatograms. No other intermediates with the carbon chain-length longer than capric could be detected or, if any, in trifling concentrations. Fig. 4.2 represents the profiles of residual oleate concentrations (ROC) in the aqueous phase against methane production expressed as percentages of theoretical maximum values from oleate conversion. Active sludge granules showed a different sorption behaviour from inactivated sludge granules. In general, the inactivated granules had a slightly higher initial biosorption capacity for oleate as indicated by the lower ROC after 1 d of incubation. This might be due to the alteration of the surface properties of sludge granules by autoclaving, as reported by Tsezos and Bell (1989) that the surface properties of microbial cells change after their death. With respect to the overall biosorption, however, the variation may not be large enough to address significant differences between inactivated and active granules. This agrees with Ning *et al.* (1996), who postulated that anaerobic biosorption is mainly a physical-chemical process. The present work further confines the biosorption of LCFA merely to a physical process (see below).

It is clear that oleate became adsorbed prior to being biodegraded, because active granules removed 40–70% of oleate from the aqueous phase in the first day while less than 1% of methane (on COD basis) was produced (Fig. 4.2). This indicates that the primary mechanism for COD removal of LCFA is biosorption

rather than biodegradation, as was also found for synthetic milk substrate with anaerobic granules (Riffat and Dague, 1995).

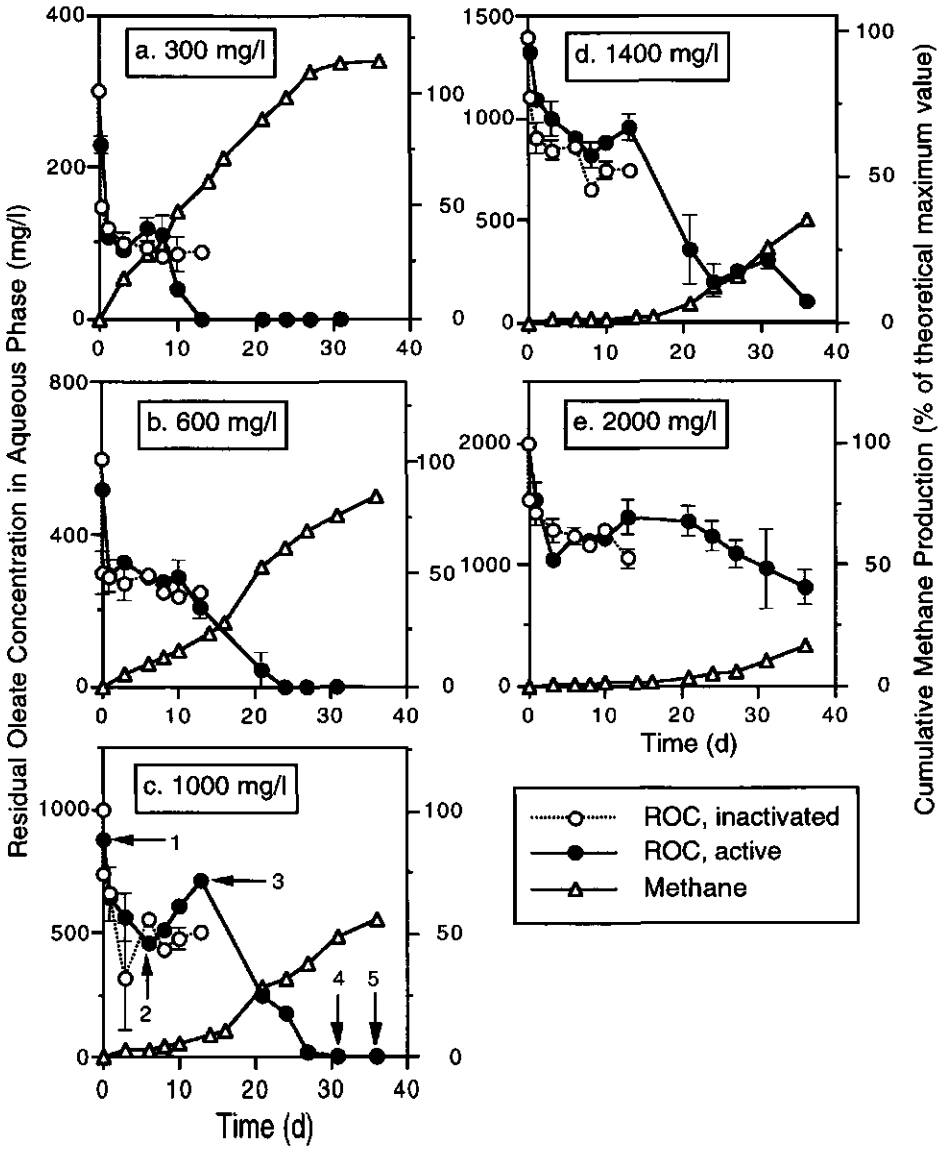


Fig. 4.2 Profiles of cumulative methane production (expressed as the volumetric percentage of the theoretical total conversion of oleate) by active sludge granules and residual oleate

on the calculation that each gram of $LCFA_m$ theoretically equaled 2.893 g COD. The methane content was measured by gas chromatography (Hwu *et al.*, 1996). TS, VSS and COD determinations followed the procedures described in the standard methods (APHA, 1992).

Chemicals

Sodium salts of LCFA and FAME standard (AOCS No. 5) were purchased from Sigma, USA. All chemicals were of analytical grade.

RESULTS AND DISCUSSION

Characterization and Isotherm of Oleate Biosorption

In the oleate biosorption tests, oleic acid methyl ester was the main constituent among all LCFA peaks shown in the GC-chromatograms. No other intermediates with the carbon chain-length longer than capric could be detected or, if any, in trifling concentrations. Fig. 4.2 represents the profiles of residual oleate concentrations (ROC) in the aqueous phase against methane production expressed as percentages of theoretical maximum values from oleate conversion. Active sludge granules showed a different sorption behaviour from inactivated sludge granules. In general, the inactivated granules had a slightly higher initial biosorption capacity for oleate as indicated by the lower ROC after 1 d of incubation. This might be due to the alteration of the surface properties of sludge granules by autoclaving, as reported by Tsezos and Bell (1989) that the surface properties of microbial cells change after their death. With respect to the overall biosorption, however, the variation may not be large enough to address significant differences between inactivated and active granules. This agrees with Ning *et al.* (1996), who postulated that anaerobic biosorption is mainly a physical-chemical process. The present work further confines the biosorption of LCFA merely to a physical process (see below).

It is clear that oleate became adsorbed prior to being biodegraded, because active granules removed 40–70% of oleate from the aqueous phase in the first day while less than 1% of methane (on COD basis) was produced (Fig. 4.2). This indicates that the primary mechanism for COD removal of LCFA is biosorption

rather than biodegradation, as was also found for synthetic milk substrate with anaerobic granules (Riffat and Dague, 1995).

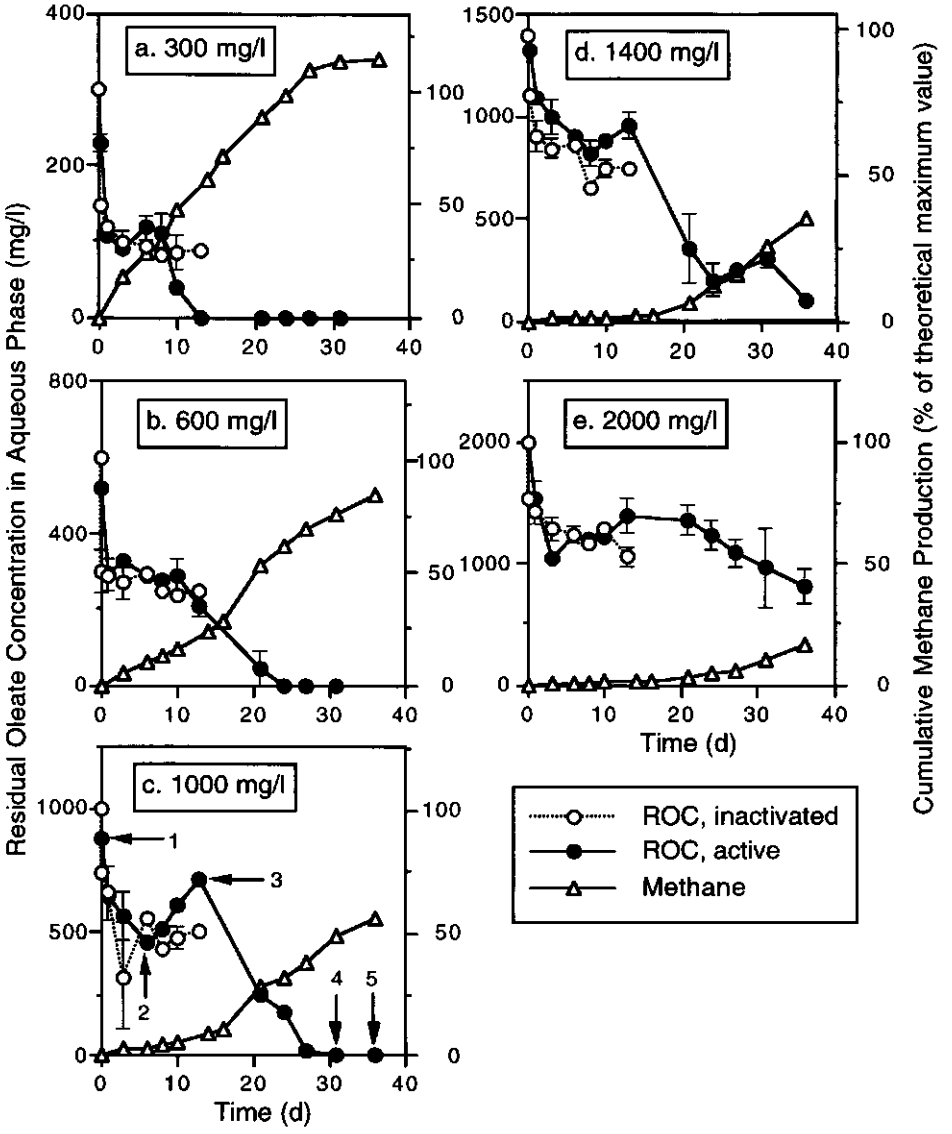


Fig. 4.2 Profiles of cumulative methane production (expressed as the volumetric percentage of the theoretical total conversion of oleate) by active sludge granules and residual oleate concentrations (ROC) in aqueous phase with active and inactivated sludge granules. Numbered arrows in graph c indicate data taken for mass balance calculation at their corresponding time points (Fig. 4.8a). Bars indicate standard deviations (n = 3).

Methane production started after a lag period at oleate concentrations exceeding 600 mg/l, which corresponds well with the results of the oleate toxicity test (IC_{50} = 612 mg/l) performed with the same granules at the same temperature (Hwu *et al.*, 1996). The initial sorption dynamics between aqueous and the sludge may be expected to detrimentally influence the rate of methane production, as a consequence of oleate toxicity. Indeed, Fig. 4.3 clearly shows that the more oleate adsorbed onto the biomass the lower becomes the methanogenic activity. The oleate adsorption apparently is highly concentration dependent, i.e., the higher the initial oleate concentration the more oleate becomes adsorbed, and this corresponds with a higher methanogenic inhibition (longer lag period). Since the cell wall of most methanogens resembles that of gram-positive bacteria (Zeikus, 1977), our results support the ideas of Galbraith *et al.* (1971) that LCFA adsorption on cell surface will lead to cytolysis of gram-positive bacteria.

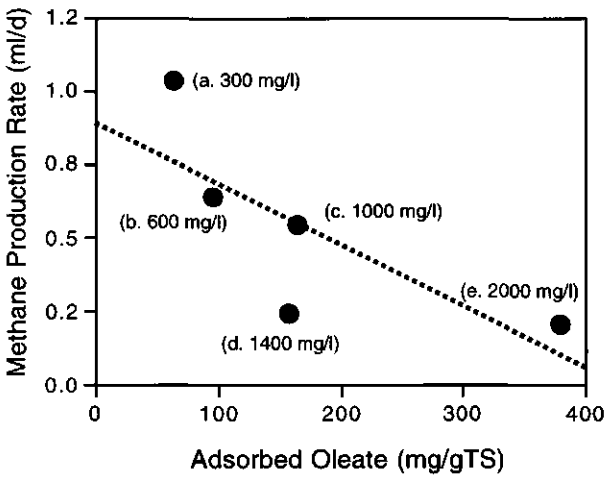


Fig. 4.3 Effect of initial oleate adsorption on initial methane production rate. Calculations are based on data obtained within the first 3 days, i.e., the first two data plots of each graph in Fig. 4.2. In parentheses indicate the initial oleate concentration.

Fig. 4.2 further shows that a certain desorption (increasing ROC) commonly occurred for the active sludge after the initial adsorption (decreasing ROC). Unlike the active granular sludge, no clear desorption was observed with the inactivated granular sludge, suggesting that the desorption was biologically mediated. This finding is in agreement with Tsezos and Bell (1989), who attributed the desorption of organic pollutants from live microbial biomass to the biodegradation of organic

molecules. Indeed, the desorption was accompanied by a significant increase in the methane production, simultaneous with (at oleate concentrations of 300 and 600 mg/l) or succeeding (1000 mg/l and higher) the oleate desorption. At an initial oleate concentration of 1000 mg/l, at least 67.4 mg oleate/g TS of the adsorbed oleate desorbed, corresponding to an average desorption rate of 16.9 mg oleate/g TS·d, before the start of smooth methane production on day 16. In contrast, at initial oleate concentrations of 1400 and 2000 mg/l, the smooth production did not start during the desorption process until the second adsorption took place (Fig. 4.2d and e). Therefore the occurrence of the desorption apparently is not a prerequisite for the increasing rate of methanogenesis. It is possible that the desorption of the preliminarily adsorbed oleate was due to the perturbation (expulsion) of oleate molecules by fine biogas bubbles rising from granule's surface through the adsorbed layer then to the aqueous phase. (Fig. 4.9 preferentially represents this concept.) However, no quantitative relation could be established between the amount of desorption and biogas production.

Following the desorption, the ROC decreased to concentrations lower than during the first adsorption (Fig. 4.2). This decrease can be attributed to oleate adsorption as well as to rapid methanogenesis, as indicated by a steeper slope in a methanation curve. The increasing rate of methanogenesis, however, did not induce oleate desorption but, to the contrary, oleate adsorption prevailed again. The adsorbed oleate on granules amounted to 46.8, 74.5, 239.8, 412.5 and 348.5 mg oleate/g TS, respectively, for the various imposed initial oleate concentrations. In some cases the amount was even higher than that during the first (initial) adsorption (cf. Fig. 4.3). It is very interesting to note that, unlike the strongly inhibitory effect occurring during the initial adsorption, little if any methanogenic inhibition prevailed during the second adsorption. Regarding these contradictory observations, one might speculate that there was formation of channeling which allowed biogas bubbles to pass through the adsorption layer with least perturbation (as conceptually illustrated in Phase C in Fig. 4.9), and that the oleate toxicity decreased due to the increased long-term recovery (see Chapter 3) or the increasing of biodegradability. Also very interesting is that the methane production continued after the ROC already dropped to values below the detection limit (20 mg/l) for 7 days (Fig. 4.2a–c). This clearly indicated that the adsorbed oleate present on the surface of sludge granules was biodegraded without any detectable desorption.

The oleate adsorption rate by anaerobic sludge granules was rather low, viz., it proceeded in terms of h^{-1} or d^{-1}, compared with the values found for oleate/LCFA soaps by textile fibers, where it ranged at a min^{-1} scale (Weatherburn *et al.*, 1950; Meader and Fries, 1952). This big difference very likely can be attributed to the significantly larger specific surface area of textile fibers compared to that of sludge granules. According to Jafvert and Heath (1991) and West and Harwell (1992), critical micelle concentrations (CMC) of soaps (surfactants) play a role on the predominant mechanism of the disappearance of the sorbates, viz., by adsorption or by precipitation. Precipitation becomes the dominant mechanism when soaps' concentrations approach their CMC levels while adsorption prevails when the concentrations exceed the CMC. The oleate concentrations (0.99–6.57 mM) used in the present work were much higher than the oleate CMC-level of 0.56 mM (calculated as described by Gerrens and Hirsch, 1974). Moreover, since the oleate concentrations in terms of equivalents (0.99–6.57 meq/l) were also much higher than those of the bivalent cations (maximum 0.75 meq/l, including Ca^{++} and Mg^{++}) in solution, the occurrence of oleate precipitation can be considered as minor importance or negligible in our experiments. Apparently oleate sorption by anaerobic sludge granules is a relatively slow process, as also was found by Keurentjes and Rinzema (1986) in their adsorption experiments with caprate by inactivated anaerobic sludge granules, where an equilibrium even did not establish after 8 days.

It has to be noted here that methane production with the inactivated granules commenced after 21 days of incubation (data not shown). Thus pseudo-equilibria of oleate adsorption by the inactivated granules were presumed on day 13 (Fig. 4.2). These data were elaborated in terms of adsorption isotherms according to the Freundlich and the Langmuir models. Of the two models considered, the Freundlich gave the highest correlation ($r = 0.992$), with a $K = 12$ mg/g and $1/n = 0.521$:

$$q = KC^{1/n} \quad \text{..... (1)}$$

Where q = equilibrium amount of sorbate on sorbent, mg oleate/g TS;
C = equilibrium concentration in aqueous phase, mg oleate/l; and
K, $1/n$ = Freundlich parameters.

The $1/n$ derived in the present study is in agreement with the values reported by Riffat and Dague (1995), indicating similar strength of adsorption using anaerobic

granular sludges. Notwithstanding, the obtained K value is significantly larger in comparison with those established in batch biosorption tests on nonfat milk (Riffat and Dague, 1995) and on 2,4-dichlorophenol (Ning *et al.*, 1996) using anaerobic granules. Apparently the sorption capacity for oleate is substantially higher. The relatively large sorption capacity may imply potentials for application of sorption as a pretreatment process (Riffat and Dague, 1995) for wastewaters containing high concentrations of LCFA. After completion of the biosorption process, the LCFA covered granules can be removed to another reactor, where sufficient time is prevailed to achieve biodegradation of the adsorbed LCFA. Then the "recuperated" granules can be recycled to the sorption reactor. Considering its relatively slow rate (in h^{-1} or d^{-1} scale) and its very reversible pattern (significant desorption), biosorption of oleate by anaerobic granules can be regarded as a physical multilayer adsorption, in contrast to sorption of soaps by textile fibers which according to Weatherburn *et al.* (1950) is of more chemical character.

Fig. 4.4 Normal appearance of active granules after biosorption tests with 2000 mg/l oleate. Biogas bubbles can be observed around granules' surface (bar = 1 mm).

The appearance of granules was examined visually and by microscopy upon termination of the experiments (day 36). A higher fraction of "white granules", probably due to the presence of a coating of adsorbed oleate, were observed at

higher oleate concentrations. Fig. 4.4 shows that oleate apparently was not evenly distributed (adsorbed) over the surface of the granules. Moreover, the examination also revealed that big differences existed between the separate granules, although this was less the case for inactivated granules. The observed differences between the individual granules can be attributed to differences in size, surface properties and metabolic activities (biodegradation of oleate by active granules). Description and discussion regarding "white granules" formed in continuous reactors has been provided elsewhere (Hwu *et al.*, 1997c; Chapter 5).

Characterization of $LCFA_m$ Biosorption

The results in Fig. 4.5 reveal that the trend of $LCFA_m$ biosorption is similar to that of oleate (Fig. 4.2). Assuming a linear relationship between time 0 and 2.28 h (the first two data points of the $LCFA_m$ curves, Fig. 4.5), the initial adsorption rates of $LCFA_m$ amounted to 0.023, 0.027, 0.032, 0.045 and 0.065 h^{-1} for concentrations of 150, 300, 600, 1000 and 1500 mg/l, respectively. Increasing LCFA concentrations clearly give rise to much higher initial adsorption rates. Like oleate biosorption, $LCFA_m$ biosorption apparently is also concentration dependent. As follows from the results in Fig. 4.6 the initial amount of the $LCFA_m$ adsorbed on granules also significantly affects the initial methanogenic activity. An increase of 45 mg $LCFA_m$/g TS in the initial adsorption amount caused a 10% loss in the initial methane production rate. The lag period of methane production becomes longer at increasing $LCFA_m$ concentrations and the methane production even drops to almost nil at the two highest concentrations investigated, suggesting a nearly complete inhibition of methanogenesis.

In contrast to oleate adsorption, $LCFA_m$ adsorption by anaerobic sludge granules proceeded much faster than that of oleate. More than 90% of the $LCFA_m$ was removed from the aqueous phase within the first 3 h (Fig. 4.5), while it took at least 24 h before 40–60% of the oleate was removed (Fig. 4.2). In an attempt to predict the $LCFA_m$ adsorption, the oleate adsorption isotherm (Eq. 1) was applied to estimate the equilibrium amount of adsorbed $LCFA_m$. It was assumed that an equilibrium was reached at 2.28 h when the methane production not yet commenced and that the differences in the experimental conditions between oleate and $LCFA_m$ biosorption tests were negligible. However, as follows from Fig. 4.7 the predicted isotherm does not fit the experimental data obtained for the $LCFA_m$ adsorption.

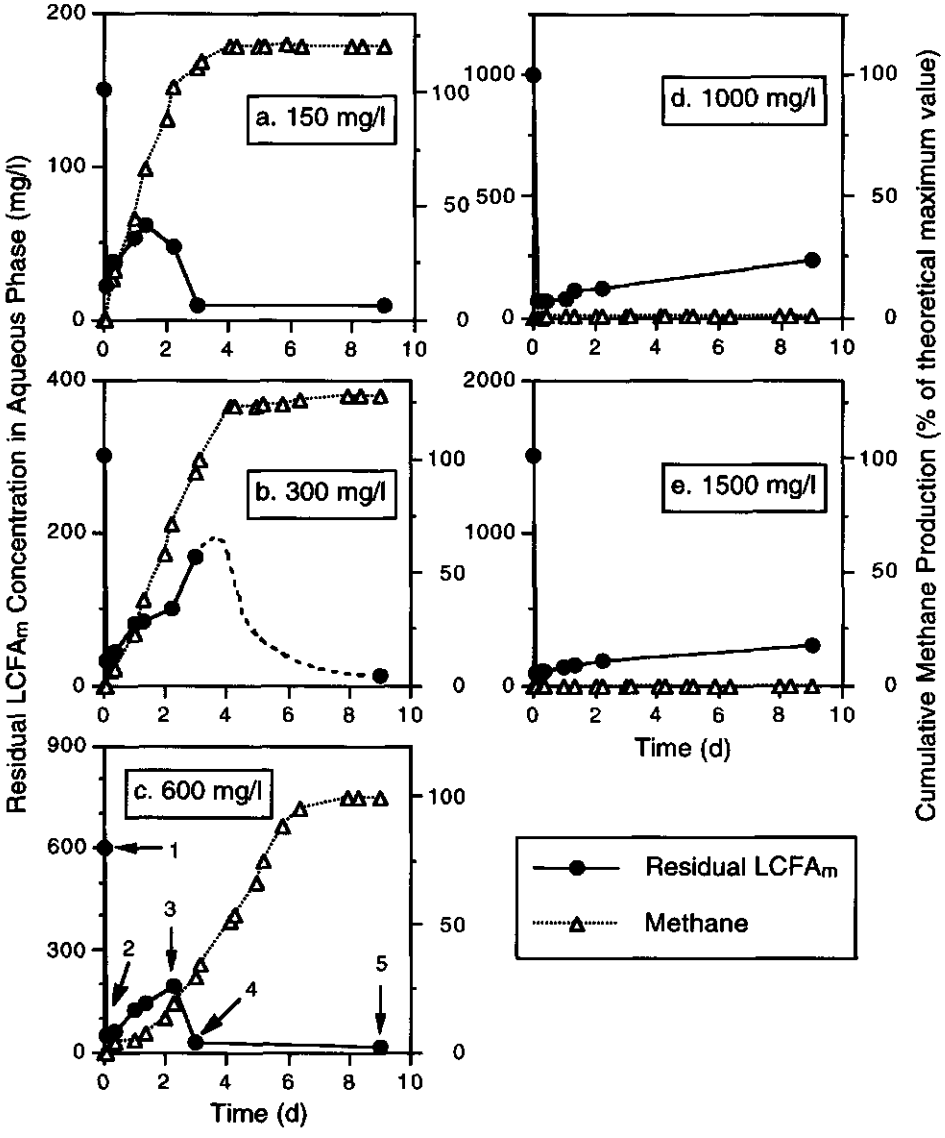


Fig. 4.5 Changes of cumulative methane production (expressed as the volumetric percentage of the theoretical total conversion of $LCFA_m$) and residual $LCFA_m$ concentrations. Numbered arrows in graph c indicate data taken for mass balance calculation at their corresponding time points (Fig. 4.8b).

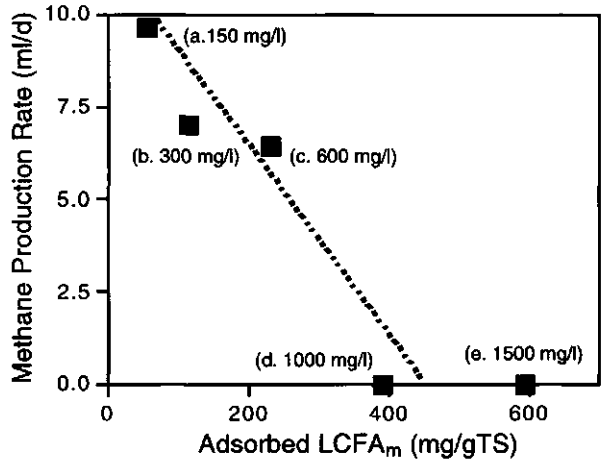


Fig. 4.6 Effect of initial $LCFA_m$ adsorption on initial methane *production rate. Data were derived based on the first two* measures of the methane production and residual $LCFA_m$ concentrations, respectively, in each graph in Fig. 4.5. In parentheses indicate the initial $LCFA_m$ concentrations.

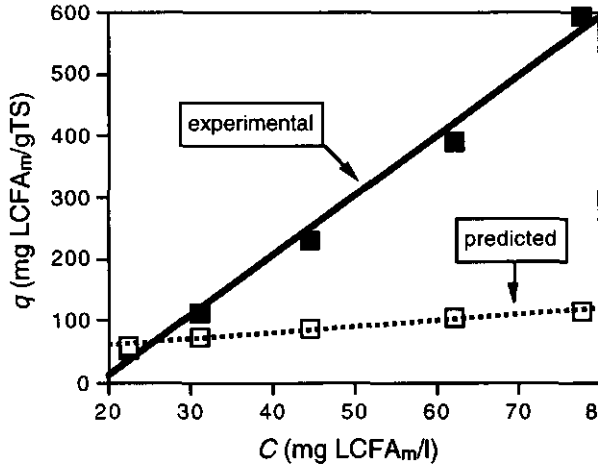


Fig. 4.7 Distinct difference of $LCFA_m$ biosorption between the experimental results and the predicted by using the adsorption *isotherm (Eq. 1) which describes oleate adsorption onto* inactivated anaerobic granular sludge.

The amount of $LCFA_m$ adsorbed onto granules exceeded significantly that estimated from the oleate isotherm. These big differences presumably can not be attributed to the 5°C difference in test temperature, because according to Weatherburn *et al.* (1950) sorption of LCFA soaps by textile fibers at 30 and 50°C does not differ significantly. Obviously, a mixture of LCFA is more "adsorptive", particularly at higher concentrations, than a single LCFA. The observed different behaviour between oleate and $LCFA_m$ biosorption on granular sludge might result from the differences in sludge origin (Ning *et al.*, 1996), consequently differences in granule size (Riffat and Dague, 1995) as well as other various characteristics. Despite of that, the observed considerably higher adsorption rate (Fig. 4.5) and larger adsorption capacity (Fig. 4.7) on an LCFA mixture might point to the prevalence of the synergistic toxic effects as suggested by Koster and Cramer (1987) and Cánovas-Diaz (1992). This means that a mixture exerts a distinctly higher bactericidal effect than the additive effect of the single constituents when acting independently. However, unfortunately such a synergism could not be verified in the present work due to the fact that sludges from different origins were used in the experiments with oleate and $LCFA_m$. The sludge used in the latter experiments was readily well acclimated to slaughterhouse wastewater.

The results in Fig. 4.5 show that generally following the initial adsorption, a distinct desorption occurred, which is accompanied with a distinct increase of methanation in the experiments with $LCFA_m$ concentrations of 150, 300 and 600 mg/l. In the experiments at 1000 and 1500 mg/l this was not the case. Here also no noticeable desorption occurred, in fact like this was found for oleate biosorption by inactivated sludge. It therefore can be concluded that the desorption of LCFA was mediated by biological activity!

In the experiment conducted at 600 mg $LCFA_m$/l, at first approximately 44.5 mg $LCFA_m$/g TS desorbed at a mean desorption rate of 49.5 mg $LCFA_m$/g TS·d, before the methane production started to proceed smoothly on day 2. Despite the fact that the amount of $LCFA_m$ desorbed is less than that found for oleate experiment at 1000 mg oleate/l, the desorption rate of the $LCFA_m$ was 2.6 times higher than that of oleate. Presumably the faster methanation rate found for $LCFA_m$ is clearly related to its higher desorption rate which results from the dynamic process mentioned earlier in this Chapter. In line with that, any clear desorption did not occur for $LCFA_m$ concentrations of 1000 and 1500 mg/l due to the almost complete absence of methanogenesis. Moreover, in contrast to oleate biosorption, a second adsorption on granules occurred merely at the initial

concentration of 600 mg $LCFA_m$/l (Fig. 4.5c). The second drops of $LCFA_m$ concentrations in aqueous phase at 150 and 300 mg $LCFA_m$/l (Fig. 4.5a and b) can not be attributed to the second adsorption on granules. According to mass balance calculations (data not shown), these two drops were due wholly to the complete conversion to methane and not to the adsorption at all.

Dynamic Process of LCFA Biosorption by Anaerobic Sludge Granules

Both big similarities and distinct differences were found between oleate and $LCFA_m$ biosorption. The LCFA biosorption therefore clearly can be regarded as a very complex phenomenon. To generalize the biosorption behaviour, data at some important time instants in Figs. 4.2c and 4.5c (indicated by arrows with Arabic numerals) were converted to their corresponding COD balance diagrams, shown in Fig. 4.8a and b. Based on the trends shown in Fig. 4.8a and b, a four-phase dynamic process is proposed explaining the course of the profiles of the cumulative methane production and the residual LCFA concentration (Fig. 4.8c). This dynamic process is derived from data obtained in the experiments with active granules only and it simulates the sequential interrelated steps proceeding in the anaerobic treatment of LCFA in bioreactors (see Table 4.1). The schematic diagrams presented in Fig. 4.9 may be useful in understanding the complexity of the biosorption and biodegradation processes.

In Fig. 4.8a and b, COD recoveries from the biomass yield are not shown because kinetic parameters available in the literature (Novak and Carlson, 1970) are insufficiently reliable and therefore not applicable. The proposed descriptive dynamic process agrees with the view of Sayed *et al.* (1988), who reported that adsorption is the primary mechanism involved in the COD removal of slaughterhouse wastewater. According to our above-mentioned dynamic process (Fig. 4.8c, Table 4.1) the rapid and large initial COD removal only partially can be attributed to biodegradation. Hence, when LCFA removal merely would be monitored by COD, TOC (total organic carbon) or DOC (dissolved organic carbon), LCFA bioconversion might be overestimated. An adequate and accurate description of the anaerobic removal and conversion of LCFA in an anaerobic reactor requires also reliable information on their metabolites, especially the end product, i.e., methane. Methane represents an elegant and easily detectable indicator for biological activity on LCFA and therefore differentiates the biological substrate removal (bioconversion) from physical removal (adsorption).

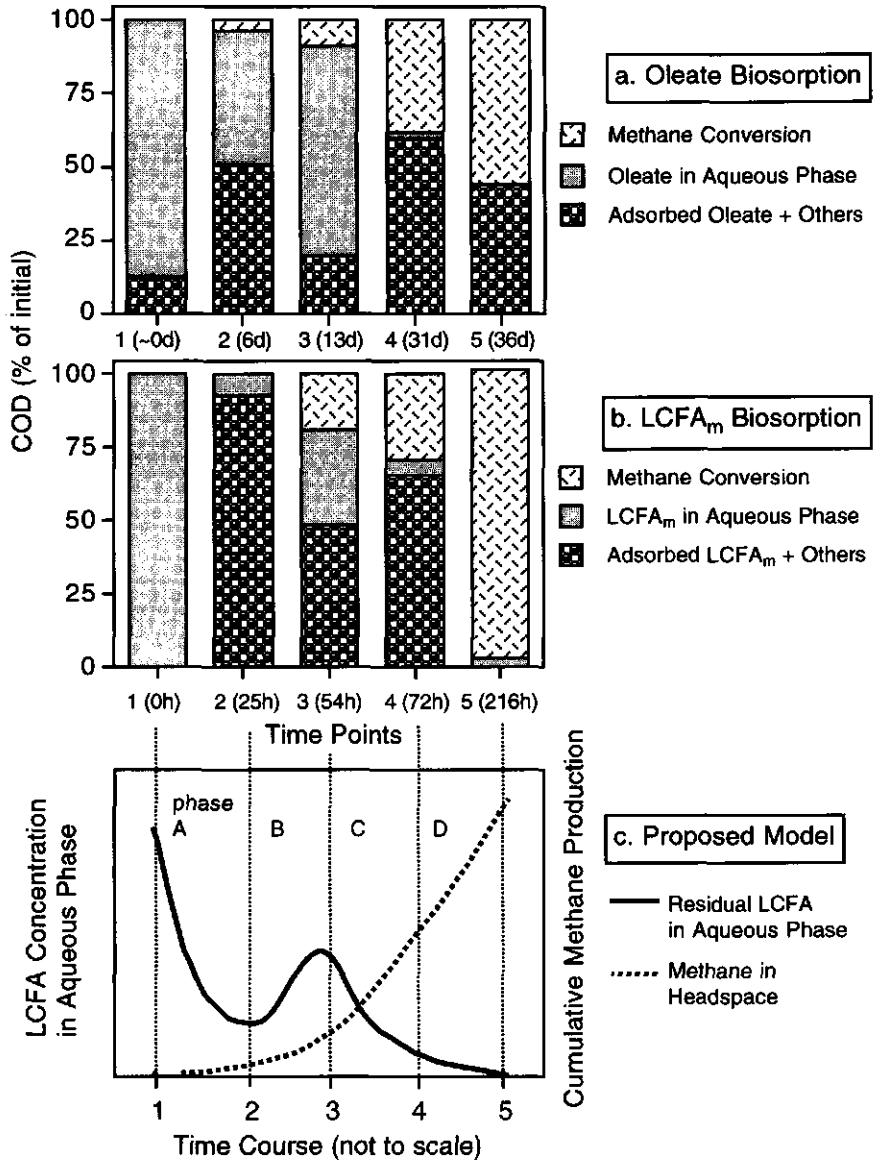
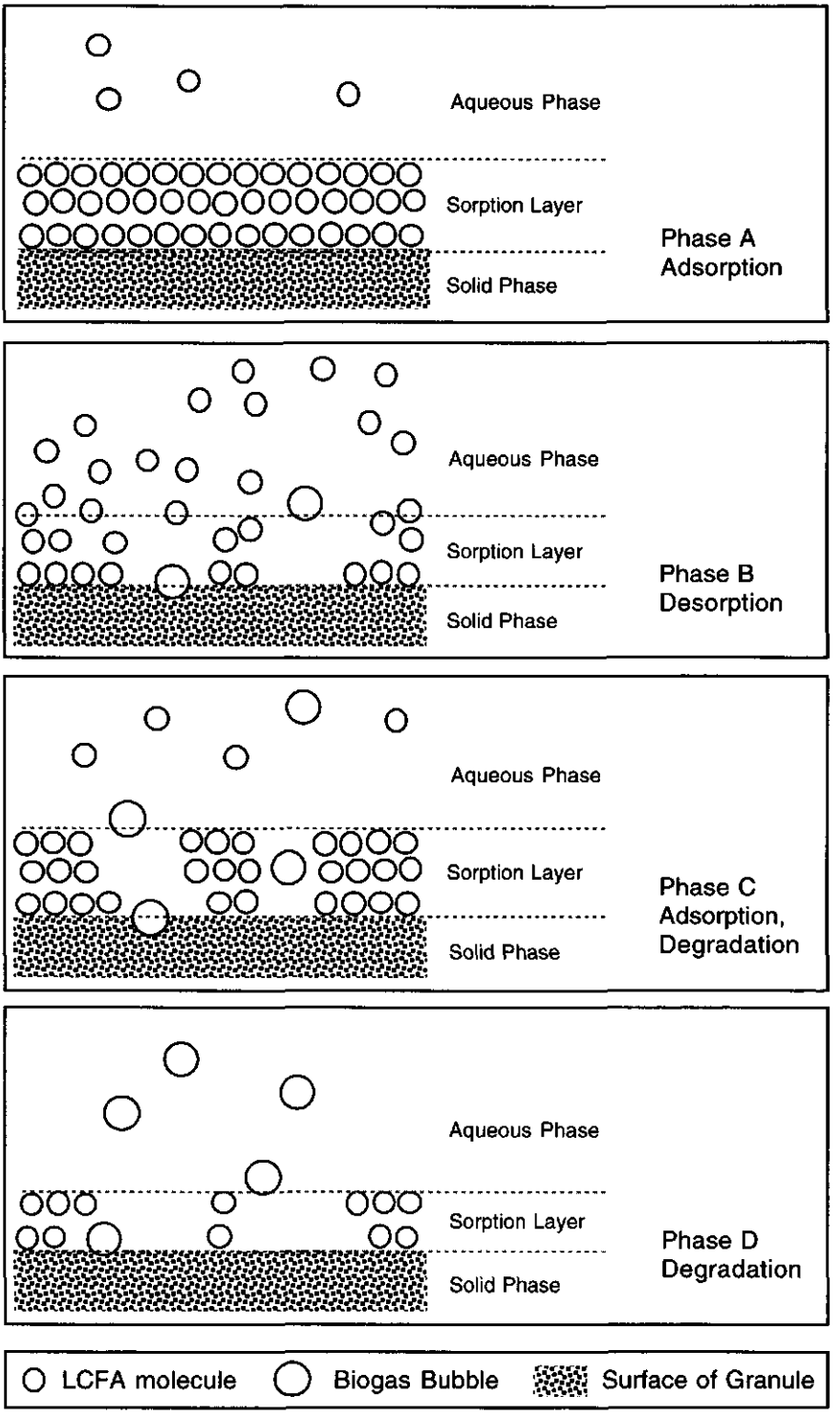


Fig. 4.8 Mass balance diagrams of (a) oleate and (b) $LCFA_m$ biosorption tests at 1000 and 600 mg/l initial concentrations, respectively. Time points, with the respective real time in parentheses, refer to Figs. 4.2c and 4.5c, respectively. The four-phase dynamic process (c) illustrating LCFA biosorption by anaerobic sludge granules (see Table 4.1 for further description).

Table 4.1 Description of the proposed four-phase dynamic process (Fig. 4.8c) illustrating LCFA biosorption by anaerobic sludge granules

Phase	Dominant reaction	Description
A	adsorption	• LCFA dramatically disappear from aqueous and accumulate in solid phase, presumably increasingly in a multilayer at higher LCFA concentrations • no significant methane production, presumably due to a lack of adaptation or a response to toxicity or both • concentration dependent; the higher concentration leads to the faster initial adsorption rate and larger adsorption amount; the more LCFA adsorbed the greater methanogenic inhibition occurs, resulting in a subsequent longer lag period in methane production
B	desorption	• increasing LCFA in aqueous phase, presumably due to perturbation of biogas fine bubbles that randomly "escape" from granule's surface through the multilayer to aqueous phase • increasing of methane production indicates biodegradation • biologically mediated reaction
C	adsorption and degradation	• second dramatic disappearance of LCFA from aqueous phase, presumably due to "re-adsorption" onto the cavities created by the bubbles • significant methane production • little or no perturbation of biogas possibly due to formation of "escaping channels"
D	degradation	• continuing conversion of adsorbed LCFA • no significant desorption phenomenon

Fig. 4.9 Schematic diagrams preferentially representing the dynamic process of the LCFA biosorption on surface of anaerobic granular sludge.


Aqueous Phase
Sorption Layer
Solid Phase
Phase A
Adsorption
Aqueous Phase
Sorption Layer
Solid Phase
Phase B
Desorption
Aqueous Phase
Sorption Layer
Solid Phase
Phase C
Adsorption,
Degradation
Aqueous Phase
Sorption Layer
Solid Phase
Phase D
Degradation
LCFA molecule
Biogas Bubble
Surface of Granule

Effect of $LCFA_m$ Biosorption on UASB Treatment Process

To simulate the LCFA biosorption in a practical treatment process, we conducted 8 test runs with a UASB reactor fed with the $LCFA_m$. Table 4.2 and Fig. 4.10 compare the experimental conditions and the results from the present study with a previous study on lauric acid adsorption (Rinzema *et al.*, 1989) . Based on (i) the observed different biosorption characteristics between oleate and $LCFA_m$ described earlier in this Chapter (viz., a faster initial adsorption rate and higher adsorption of the $LCFA_m$) and (ii) the 20 times higher V_{up} used in the present work (Table 4.2), which may lead to a higher longitudinal hydraulic shear; one might expect a more serious sludge flotation in the present work. The contrary is the case, Rinzema *et al.* (1989) observed a more severe sludge flotation, because in their experiments a complete sludge bed flotation occurred already at the SLR of 0.118 g laurate-COD/g VSS·d compared to only 43% sludge flotation at the SLR of 0.120 g $LCFA_m$-COD/g VSS·d in our experiments (Table 4.2). Apart from the often encountered "piston effect", which easily occurs in laboratory scale reactors with small diameters, the very short HRT applied in their study (1.6 h) might be another possible explanation for the observed differences. The positive effect of a longer HRT on minimizing sludge flotation has been further verified in later work (Hwu *et al.*, 1997a–c; Chapters 5 and 6). In the present work, the $LCFA_m$ clearly initiated serious sludge flotation, despite the good COD removal efficiencies (82–93%) achieved under all loadings tested. Such high efficiencies, however, can not be guaranteed for full scale reactors, as sophisticated devices to retain sludge as used in laboratory scale reactors are not available for industrial scale treatment plants.

The results of the continuous experiments revealed a heavier sludge flotation at higher sludge loading rates and also that less time is required for confronting a complete flotation (Fig. 4.10 and Table 4.2). In contrast to the batch-type biosorption experiments, where adsorption was found to be concentration dependent, the results of the continuous experiments further showed that sludge flotation in UASB reactors depended upon LCFA loading rates. Based on these important observations, we may deduce the antecedents and consequences in LCFA biosorption, as shown in Fig. 4.11. As HRT is one of the common parameters in continuous reactor operation, it is reasonable to expect that HRT will affect the fate of LCFA in (bio)sorption process. Unfortunately, we were unable to measure the methane production during this continuous experiment due to frequent clogging of the biogas collector. This hampered an accurate estimation of sorption rate (denoted by the question marks in the squares in Fig. 4.11).

Table 4.2 Sludge flotation in UASB reactors exposed to LCFA

Compound investigated	Reactor Vol. (l)	Reactor Temp. (°C)	Dimensions (mm) Height	Width	HRT (h)	V_{up} (m/h)	Influent concentration (mg LCFA/l)	Sludge loading rate (gCOD/gVSS·d)	Degree of sludge flotation %[a] (time[b])	Data source
$LCFA_m$	6.67	35	850	100	27.6	2.0	192	0.086	0	This study
					27.6		236	0.106	19	
					27.7		387	0.173	74	
					27.6		495	0.222	100 (97)	
					27.9		565	0.250	100 (93)	
					16.0		155	0.120	43	
					17.5		203	0.144	68	
					16.0		263	0.203	100 (107)	
Lauric acid	0.20	30	170	39	1.6	0.1	102	0.059	0	adapted from
							205	0.118	100 (7)	Rinzema *et al.*,
							307	0.177	100 (4)	1989
							409	0.235	100 (3)	
							511	0.294	100 (3)	

[a]degree of sludge flotation was defined as the ratio of total amount of floated sludge (ml) to the total amount of inoculated sludge (ml)
[b]in parentheses, time (hours) required for complete sludge flotation

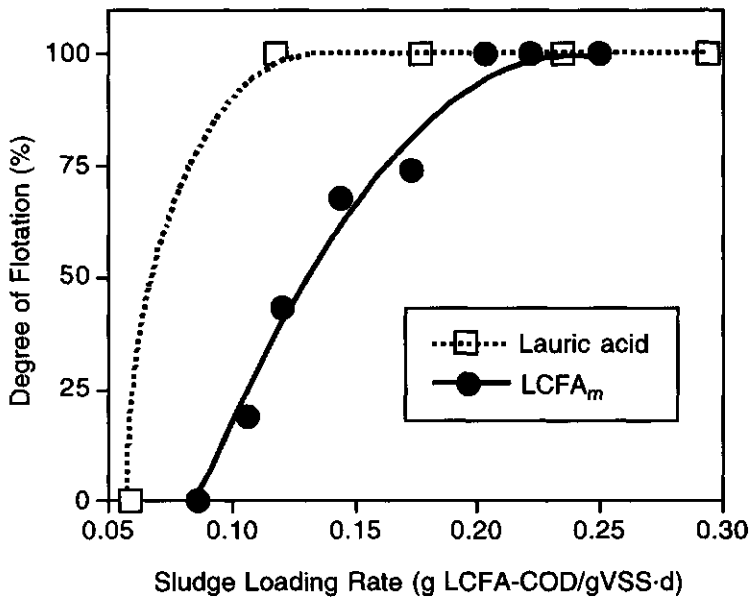


Fig. 4.10 *Relation between sludge loading rate and sludge flotation in UASB reactors treating* $LCFA_m$ (this study) and lauric acid (adapted from Rinzema *et al.*, 1989).

However, still it looks justified to draw the conclusion that the degree of inhibition is more influenced by the concentration while flotation is more affected by the HRT. More discussion on this matter in connection with the effects of other common operational parameters, e.g., reactor V_{up}, temperature and the presence (addition) of cosubstrate, is presented in Chapters 5 and 6. Fig. 4.11 implies an important operational strategy in the prevention of sludge from flotation in the continuous treatment of LCFA-containing wastewaters, viz., HRT can be shortened only if the influent LCFA concentration is relatively low.

In the present work, flotation in the UASB reactor started when the sludge loading rate exceeded 0.086 g $LCFA_m$-COD/g VSS·d while complete flotation occurred at loading rates above 0.203 g $LCFA_m$-COD/g VSS·d. Regarding the trends of the two curves in Fig. 4.10, we may expect a farther right-shifted curve, with a lower slope, for an industrial scale reactor. According to Samson *et al.* (1985), the treatment failure of an industrial scale UASB reactor treating milk fat (whose major hydrolysis product is oleic acid) was due to sludge flotation. The findings in the present study support the hypothesis of Lettinga and Hulshoff Pol (1992) that adsorption of fatty matter on sludge particles leads to sludge flotation.

The results of this study clearly reveal the existence of a distinct relation between $LCFA_m$ biosorption and sludge flotation (Fig. 4.6). This is, to the best of our knowledge, the first time such a clear relation presented in the anaerobic treatment of wastewaters containing fatty matter or their main ingredient—LCFA.

It has to be noted that even at 0.203 g COD/g VSS·d condition, the corresponding $LCFA_m$ concentration (263 mg/l) in the influent was far below the minimum inhibitory concentration of methanogenesis, i.e., 401 mg $LCFA_m$/l (Yuan, 1995). This might implicate that, under practical conditions, complete washout of sludge bed can occur prior to inhibition of methanogens. Hence, biosorption of LCFA by anaerobic sludge granules might affect more the sludge retention of the system than the sludge activity. Therefore complimentary research has been conducted towards bioreactor systems in which adsorption is minimized (e.g., by maximizing biodegradation) or which will not be affected by sludge flotation (e.g., by returning of washed out biomass), as is presented in the following two Chapters.

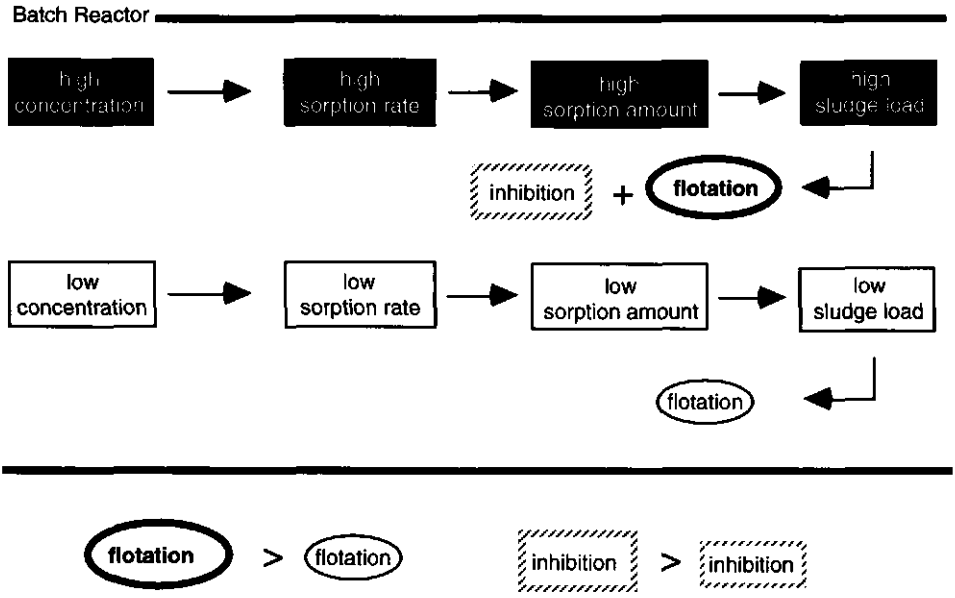


Fig. 4.11 Flow chart representation for the causal relation between methanogenic inhibition, sludge flotation and LCFA biosorption.

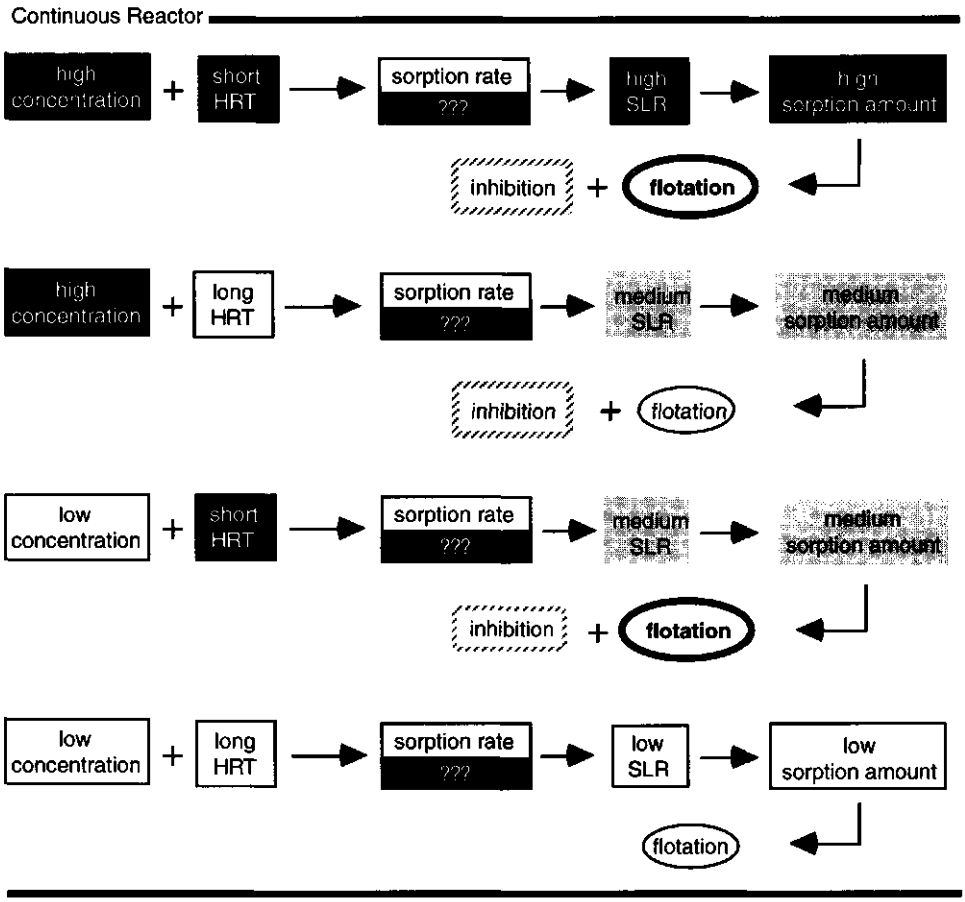


Fig. 4.11 (continued).

CONCLUSIONS

Adsorption of LCFA by anaerobic sludge granules can be described by the Freundlich model. The initial adsorption proceeds fast (within 1 day) but establishment of the adsorption equilibrium is a relatively slow process (weeks). Significant desorption prevails only with active methanogenic granules, suggesting that the desorption phenomenon is due to a biologically mediated reaction, presumably perturbation induced by biogas production.

A hypothesized four-phase dynamic process describing the behaviour of LCFA biosorption by anaerobic sludge granules is proposed to simulate the practical treatment process. Both LCFA adsorption and toxicity to anaerobic granular sludge are concentration dependent.

Sludge flotation in a continuous reactor is caused by adsorption and depends more on LCFA loading rates rather than on LCFA concentrations. LCFA biosorption can impede the success of a high-rate anaerobic wastewater treatment system such as a UASB reactor. Deterioration of the UASB treatment process by biosorption of LCFA is mainly due to sludge flotation rather than to the intoxication of the methanogenic consortia.

ACKNOWLEDGMENTS

I am indebted to Dr. Piet N. L. Lens for his invaluable views and suggestions on these two manuscripts published. I am grateful to Johannes van der Laan and Ilse Bennehey for their technical assistance with the LCFA analyses. Suggestions for granule inactivation by Dr. Jim A. Field are highly appreciated. This work was financially supported by the Ministry of Education and the Council for Agricultural Planning and Development (contract no., 83-AST-2-9-03(12)), Taiwan, R.O.C.

REFERENCES

APHA. (1992). *Standard Methods for the Examination of Water and Wastewater*, 18th edn., Am. Publ. Health Assoc., Washington, DC., pp. 2-54–2.57, 5-9–5-10.

Cánovas-Diaz, M., Sánchez-Roig, M.J. and Iborra, J.L. (1992). Myristic and oleic acid degradation by an acclimated anaerobic consortia: synergistic behaviour. In: *Proc. 6th EC Conf. Biomass for Energy, Industry and Environment,* Grassi, G., Collina, A. and Zibetta, H. (Eds.), Athens, Greece, Apr. 1991, Elsevier, UK, pp. 580–584.

Demeyer, D.I. and Henderickx, H.K. (1967). The effects of C_{18} unsaturated fatty acids on methane production in vitro by mixed rumen bacteria. *Biochim. Biophys. Acta*, **137**, 484–497.

Fernadez, N.A., Chacin, E., Gutierrez, E., Alastre, N., Llamoza, B. and Forster, C.F. (1995). Adsorption of lauryl benzyl sulphonate on algae. *Bioresource. Technol.*, **54**, 111–115.

Galbraith, H., Miller, T.B., Paton, A.M. and Thomson J.K. (1971). Antibacterial activity of long chain fatty acids and the reversal with calcium, magnesium, ergocalciferol and cholesterol. *J. Appl. Bacteriol.*, **34**, 803-813.

Gerrens, H. and Hirsch, G. (1974). Critical micelle concentration. In: *Polymer Handbook*, Brandrup, J. and Immergut, E.H. (Eds.), Wiley-Interscience Publ., London, pp. II-483–II-485.

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

Hrudey, S.E. (1982). Factors limiting emulsified lipid treatment capacity of activated sludge. *J. Water Pollut. Control Fed.*, **54**, 1207–1214.

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

Hwu, C.-S., Molenaar, G., Garthoff, J., van Lier, J.B. and Lettinga, G. (1997a). Thermophilic high-rate anaerobic treatment of wastewater containing long-chain fatty acids: impact of reactor hydrodynamics. *Biotechnol. Lett.*, **19**, 447–451.

Hwu, C.-S., van Beek, B., van Lier, J.B. and Lettinga, G. (1997b). Thermophilic high-rate anaerobic treatment of wastewater containing long-chain fatty acids: effect of washed out biomass recirculation. *Biotechnol. Lett.*, **19**, 453–456.

Hwu, C.-S., van Lier, J.B. and Lettinga, G. (1997c). Physicochemical and biological performance of expanded granular sludge bed reactors treating long-chain fatty acids. *Process Biochem.*, **32**, in press.

Jafvert, C.T. and Heath, J.K. (1991). Sediment- and saturated-soil-associated reactions involving an anionic surfactant (dodecylsulfate). 1. precipitation and micelle formation. *Environ. Sci. Technol.*, **25**, 1031–1038.

Keurentjes, J. and Rinzema, A. (1986). Adsorption of capric acid on granular methanogenic sludge. In: *Proc. EWPCA Conf. Anaerobic Treatment, A Grown-Up Technology*, Sep. 1986, Amsterdam, pp. 645–648.

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

Koster, I.W. (1987). Abatement of long-chain fatty acid inhibition of methanogenesis by calcium addition. *Biol. Wastes*, **22**, 295–301.

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.

Lettinga, G. and Hulshoff Pol, L.W. (1992). UASB process design for various types of wastewaters. In: *Design of Anaerobic Processes for the Treatment of Industrial and Municipal Wastes*, Malina, Jr., J.F. and Pohland F.G. (Eds.), Technomic Publ., Basel, Ch. 3, pp. 119–146.

Meader, Jr., A.L., Fries, B.A. (1952). Adsorption in the detergent process. *Ind. Eng. Chem.*, **44**, 1636–1648.

Ning, Z., Kennedy, K.J. and Fernandes, L. (1996). Biosorption of 2,4-dichlorophenol by live and chemically inactivated anaerobic granules. *Water Res.*, **30**, 2039–2044.

Novak, J.T. and Carlson, D.A. (1970). The kinetics of anaerobic long chain fatty acid degradation. *J. Water Pollut. Control Fed.*, **42**, 1932–1943.

Prins, R.A., van Nevel, C.J. and Demeyer, D.I. (1972). Pure culture studies of inhibitors for methanogenic bacteria. *Antonie van Leeuwenhoek*, **38**, 281–287.

Quéméneur, M. and Marty, Y. (1994). Fatty acids and sterols in domestic wastewaters. *Water Res.*, **28**, 1217–1226.

Riffat, R. and Dague, R.R. (1995). Laboratory studies on the anaerobic biosorption process. *Water Environ. Res.*, **67**, 1104–1110.

Rinzema, A., Alphenaar, A. and Lettinga, G. (1989). The effect of lauric acid shock loads on the biological and physical performance of granular sludge in UASB reactors digesting acetate. *J. Chem. Technol. Biotechnol.*, **46**, 257–266.

Rinzema, A., Boone, M., van Knippenberg, K. and Lettinga, G. (1994). Bactericidal effect of long chain fatty acids in anaerobic digestion. *Water Environ. Res.*, **66**, 40–49.

Samson, R., van den Berg, B., Peters, R. and Hade, C. (1985). Dairy waste treatment using industrial-scale fixed-film and upflow sludge bed anaerobic digesters: design and start-up experience. In: *Proc. 39th Purdue Industrial Waste Conference*, Bell, J.M. (Ed.), Butterworth, Boston, pp. 235–241.

Sayed, S.K.I., van der Zanden, J., Wijffels, R. and Lettinga, G. (1988). Anaerobic degradation of the various fractions of slaughterhouse wastewater. *Biol. Wastes*, **23**, 117–142.

Taylor, R.J. (1965). *The Chemistry of Glycerides*, Unilever Ltd., England.

Tsezos, M. and Bell, J.P. (1989). Comparison of the biosorption and desorption of hazardous organic pollutants by live and dead biomass. *Water Res.*, **23**, 561–568.

Viswanathan, C.V., Meera Bai, B. and Pillai, S.C. (1962). Fatty matter in aerobic and anaerobic sewage sludges. *J. Water. Pollut. Control Fed.*, **34**, 189–194.

Weatherburn, A.S., Rose, G.R.F. and Bayley, C.H. (1950). The sorption of soap by textile fibers. *Can. J. Res.* **28F**, 51–61.

West, C.C. and Harwell, J.H. (1992). Surfactants and subsurface remediation. *Environ. Sci. Technol.*, **26**, 2324–2330.

Yuan, C.-Y. (1995). *Treatment of Lipid-Ingredients in Slaughterhouse Wastewater by Upflow Anaerobic Sludge Bed Reactor*, M.Sc. thesis, Grad. Inst. of Environ. Eng., Natl. Taiwan Univ., Taipei, Taiwan, R.O.C.

Zeikus, J.G. (1977). The biology of methanogenic bacteria. *Bacteriol. Rev.*, **42**, 514–541.

5

Performance of Expanded Granular Sludge Bed Reactors Treating Oleic Acid

This Chapter contains two papers published/accepted as:

Hwu, C.-S., van Lier, J.B., Kulik, Z., Mishra, P.K. and Lettinga, G. (1997). Feasibility of expanded granular sludge bed (EGSB) system for the treatment of wastewaters containing long-chain fatty acids. In: *Proc. the 8th Intl. Conf. on Anaerobic Digestion*. 25–29 May 1997, Sendai, Japan, Vol. 3, pp. 103–108.

Hwu, C.-S., van Lier, J.B. and Lettinga, G. (1997). Physicochemical and biological performance of expanded granular sludge bed reactors treating long-chain fatty acids. *Process Biochem.*, **32**, in press.

5

Performance of Expanded Granular Sludge Bed Reactors Treating Oleic Acid

ABSTRACT

The effects of reactor hydrodynamics, temperature and cosubstrate on the performance of expanded granular sludge bed (EGSB) reactors treating oleic synthetic wastewaters were investigated. When operating at liquid superficial upflow velocities (V_{up}) at about 3.4–4 m/h and without addition of cosubstrate, COD removal efficiencies of 66% and 73% were attained, respectively, at hydraulic retention times (HRT) of 3 h and 6 h in thermophilic (55°C) runs. The corresponding mesophilic (30°C) removal efficiencies were 44% and 69%, respectively. The achieved highest methane conversion was only 15% in the thermophilic reactor and 9% in the mesophilic reactor, both operated at the HRT of 6 h. In both systems, floating layer which consisted of sludge granules and non-degraded oleate clusters frequently clogged the gas collector. At an HRT of 0.6 h, both reactors failed due to severe washout of sludge granules.

When operating at a constant HRT of 24 h, in the presence of glucose and acetate, and at V_{up} of 4, 7 and 1 m/h; COD removal efficiencies of 82–89% were attained at both temperatures while no significant washout or flotation of granules or fatty matter was observed in all runs. Yet, the higher V_{up} resulted in the lower methane conversion. Methane conversion ratios of 49% (4 m/h) and 39% (7 m/h) in thermophilic and 59% (4 m/h) and 53% (7 m/h) in mesophilic runs were attained. The highest methane conversion ratio, 70%, was achieved at the V_{up} of 1 m/h at

both temperatures. Many white granules prevailed in sludge bed, probably due to cumulative oleate adsorption onto granule surface.

INTRODUCTION

In anaerobic digestion, lipids are hydrolyzed to long-chain fatty acids (LCFA) and glycerol, where LCFA are further degraded to acetate and hydrogen via β-oxidation (Weng and Jeris, 1976; Nuck and Federle, 1995). While hydrolysis of lipids proceeds rapidly (Hanaki *et al.*, 1981), β-oxidation of LCFA is generally considered as the rate-limiting step (Novak and Carlson, 1970). Successful treatment of LCFA-containing wastes is possible under conventional conditions (McCarty, 1964), e.g., of hydraulic retention times of 10–40 days.

In the past two decades, high-rate anaerobic treatment systems have been developed and are nowadays considered as a grown-up technology. Among these systems, the upflow anaerobic sludge bed (UASB) reactor system is the most widely applied for the treatment of industrial and increasingly also domestic wastewaters. To date, industrial-scale anaerobic reactors have been installed and operated worldwide, of which some 930 are UASB reactors (Habets, 1997). When treating more complex (insoluble or inhibitory) wastewaters, e.g., those containing lipids and/or LCFA, however, treatment failure of UASB reactors has been encountered (Samson *et al.*, 1985; Rinzema *et al.*, 1989; Hawkes *et al.*, 1995). The failure is mainly due to two problems: (i) occurrence of flotation of sludge granules and fatty matter at low loadings (Rinzema *et al.*, 1989; Hawkes *et al.*, 1995; Hwu *et al.*, 1996b) and (ii) LCFA inhibition of anaerobic microorganisms at millimolar concentrations (Koster and Cramer, 1987; Hwu *et al.*, 1996a, 1997a). It has been demonstrated that the former problem is more significant since sludge flotation already commenced in a UASB reactor operated at an influent LCFA concentration far below the minimum inhibition concentration (Chapter 4; Hwu *et al.*, 1996b).

A modified-UASB reactor system characterized by upflow velocities (V_{up}) higher than 4 m/h (Lettinga, 1996) and hydraulic retention times (HRT) shorter than 10 h (reviewed by Van Haandel and Lettinga, 1994), the so-called expanded granular sludge bed (EGSB) reactor, was found to be capable of providing better mixing of the reactor contents and more efficient contact between substrate and

biomass (Lettinga, 1995). According to Rinzema *et al.* (1993), this reactor concept significantly improves the treatment performance of lauric acid ($C_{12:0}$), viz., flotation of granular sludge was not found and approximate 83% of the added laurate COD was converted to methane at considerably high organic loading rates (OLR) up to 31.4 g COD/l·d. Considering the genuine compositions of lipids/LCFA-containing wastewaters, however, lauric acid is rarely present but oleic acid ($C_{18:1}$) is the most abundant constituent (Viswanathan *et al.*, 1962; Quéméneur and Marty, 1994). A 65% of COD removal efficiency of oleate was achieved in a UASB reactor (Sam-Soon *et al.*, 1991), yet, methane production was not quantified. The latter is of interest since particularly with LCFA, a large discrepancy between COD removal and methane production rate can be encountered (Cánovas-Diaz *et al.*, 1992) due to physical entrapment of LCFA onto biomass surface (Chapter 4; Birch *et al.*, 1989; Hwu *et al.*, 1996b).

In this Chapter, the performance of the EGSB reactor system treating oleic acid was evaluated by comparing the methane conversion capacity and COD removal efficiency under thermophilic (55°C) and mesophilic (30°C) conditions. The effects of cosubstrates, HRT and V_{up} on methanogenesis of the selected LCFA and on physicochemical characteristics of sludge granules were investigated under both temperature conditions.

MATERIALS AND METHODS

Inoculum of Reactors

Both reactors used in the present work were inoculated with granular sludge originated from a 110 m^3 anaerobic internal circulation (IC) reactor treating potato-processing wastewater (Agrico, Wezep, The Netherlands) under mesophilic conditions. Prior to the inoculation, the sludge was elutriated by tap water to remove fines and grovel. The amount of inoculum in the thermophilic reactor (R-T) was 92 g volatile suspended solids (VSS) while that in the mesophilic reactor (R-M) was 106 g VSS.

Compositions of Media

The composition of the basal medium has been described previously (Chapter 2; Hwu *et al.*, 1996a). Table 5.1 presents the compositions of the substrates which served as carbon and energy source in the present study. During the reactor start-up period, a medium with a concentration of 1000 mg COD/l, comprising glucose, sodium acetate and sodium caprylate ($C_{8:0}$), was supplemented to the influent. In test runs I–III the influent was completely replaced by a technical grade sodium oleate (Riedel de Haën, Germany), analyzed with a composition of about 82% (w/w) oleate ($C_{18:1}$) and 17% palmitate ($C_{16:0}$). The medium used during restart-up period contained the same constituents as used for the reactor start-up but with a COD ratio of 1.5:1.5:1 (glucose:acetate:caprylate) and with three concentrations (1000, 2000 and 4000 mg COD/l). In test runs IV–VI caprylate was substituted with sodium oleate, thus making a cosubstrates (glucose plus acetate) to substrate (oleate) ratio of 3:1. The pH of the influent in all runs was adjusted to 7.2. Except for oleate, all chemicals were of analytical grade.

Table 5.1 Compositions of influents used in each stage

Run	Constituents	COD ratio	Total concentration (mg COD/l)
start-up	glucose, acetate, caprylate	1:1:1	1000
I–II	oleate		1000
III	oleate		100
restart-up	glucose, acetate, caprylate	1.5:1.5:1	1000, 2000, 4000
IV–VI	glucose, acetate, oleate	1.5:1.5:1	4000

Expanded Granular Sludge Bed Reactors

Two identical glass EGSB reactors, each with a working volume of 4.4 l (inner diameter: main body = 56 mm, gas-liquid-solid separator = 100 mm; Fig. 5.1) were operated in parallel under the same conditions except for the temperatures, of which one (R-M) was controlled at 30°C and the other (R-T) at 55°C by a thermostat-bath-circulator connected to the double wall of each reactor. Biogas was led through a 10% (w/v) NaOH solution and a column packed with soda lime pellets with indicator (Merck, Darmstadt, Germany) before entering a wet gas meter (Meterfabriek Dordrecht, Dordrecht, The Netherlands) where methane production was quantified. A Plexiglas settler with a working volume of 1.0 l (70 mm diameter) was installed to collect washed out fatty matter and biomass. Thus

total volume of each EGSB system was 5.4 l, which was used for volume-based calculations.

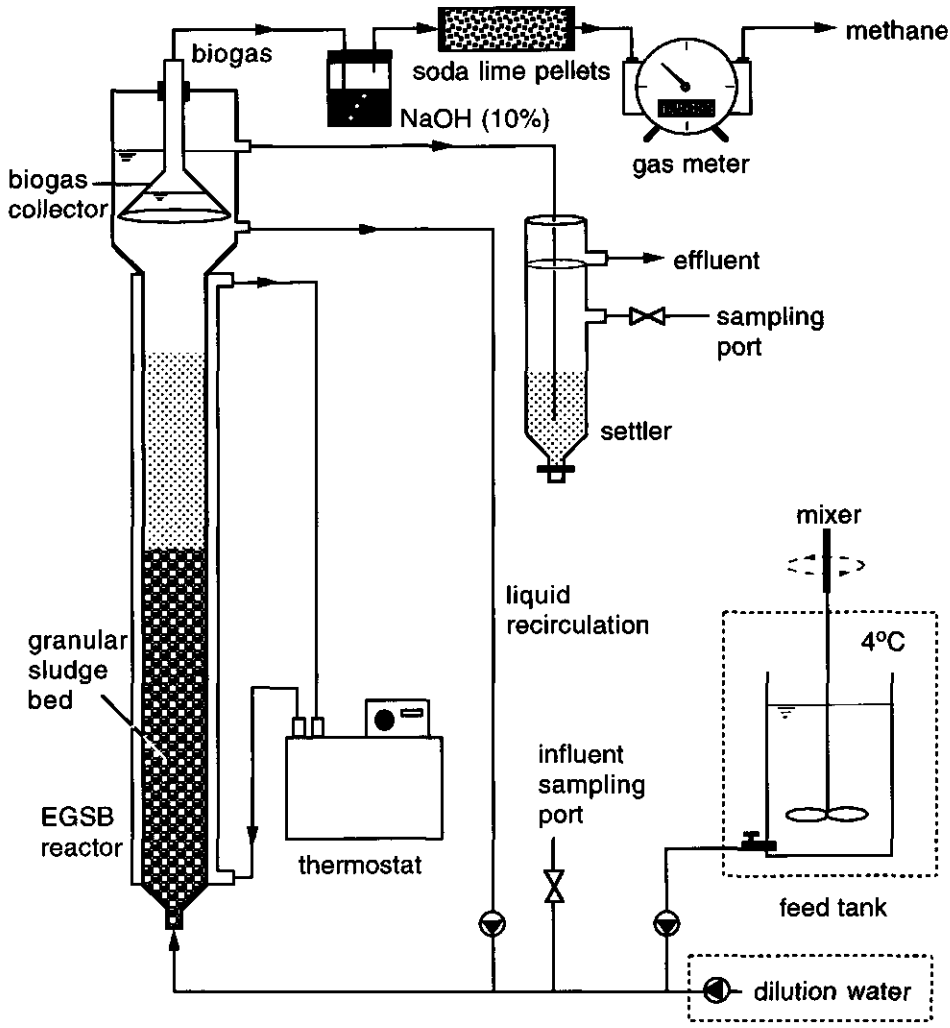


Fig. 5.1 Schematic diagram representing the EGSB reactor set-up (not to scale). The dilution water and 4°C condition was omitted during runs IV–VI.

Table 5.2 presents the operational parameters of the EGSB reactors. Feeding to both reactors was started directly after inoculation. After a 24-hour operation at 30°C, reactor temperature of R-T was one-step shifted to 55°C. OLR were started at 2 and 8 g COD/l·d for reactors R-T and R-M, respectively, and were

terminated at 16 g COD/l·d for both reactors after 53 days (R-T) and 15 days (R-M) of the start-up periods.

Table 5.2 Operational parameters of the EGSB reactors operated at 55°C (R-T) and 30°C (R-M)

	Operational Parameter					
Run	OLR (g COD/l·d)	COD_{oleic} (mg/l)	COD_{total} (mg/l)	HRT (h)	V_{up} (m/h)	Duration (HRTs)
start-up	2–16	0	1000	12–1.5	3.3–4.0	53 days (R-T)
	8–16	0	1000	3–1.5	4.0	15 days (R-M)
I	8	1000	1000	3	3.6	104
II	4	1000	1000	6	3.4	44
III	4	100	100	0.6	4.0	120
restart-up	4	0	1000	6	4.0	17 days (0–17)
	8	0	2000	6	4.0	7 days (18–24)
	16	0	4000	6	4.0	13 days (25–37)
IV	4	1000	4000	24	4.0	15
V	4	1000	4000	24	7.2	10
VI	4	1000	4000	24	1.0	13

In runs I–III, oleate was used as the sole carbon and energy source. To compare the effect of HRT on the treatment at the two temperatures, the V_{up} was similarly kept at about 3.4–4 m/h. The hydrodynamic parameters were selected as such (see Table 5.2) because previously an OLR of 31.4 g COD/l·d with an 83% methane yield was achieved in an EGSB reactor treating sodium laurate as sole substrate at an HRT as short as 2 hours (Rinzema *et al.*, 1993). Concentrated feed stock of 10 g COD/l was prepared twice per week during runs I–III and was housed at 4°C. The feed tank was equipped with continuously mechanical mixing. The speed of the mixer was set as such that the feed stock had an emulsion-like appearance. The feed stock was then diluted with hot tap water to the designated concentrations. The final influent temperature was about 40°C.

Both reactors were failed in run III (see Results), thus requiring a restart-up procedure to revive sludge activity for the remaining experiments. During this restart-up period, the HRT was constantly kept at 6 h, which consequently resulted in an OLR of 4 g COD/l·d during day 0–17, 8 g COD/l·d during day 18–24 and 16 g COD/l·d during day 25–37 (Table 5.2). This restart-up was accomplished for both on day 37. Subsequently, the effects of cosubstrate and V_{up} were to be verified in runs IV–VI. During runs IV–VI a readily soluble feed (4 g COD/l),

including the substrate (oleate) and the cosubstrate (glucose plus acetate), was freshly prepared for daily use. The HRT was constantly kept at 24 h while the V_{up} varied at 4, 7.2 and 1 m/h.

The start-up and restart-up processes were terminated when methane production maintained nearly constant for a week in both reactors, indicating the reactor system had reached a pseudo-steady state. In each stage the different HRTs were imposed by adjusting the flow rate of the dilution water or the feed or both. The various V_{up} were set by co-adjusting the flow rate of the liquid recirculation. At least a time period of 10 HRTs was implemented during each run.

Floating layer present in the gas collector and in the settler, consisting of sludge granules and fat-like materials, was removed whenever necessary. The oleate-adsorbed granules were gently stirred by using a glass bar to separate them from the adsorption layer. Then, the "recuperated" granules were reintroduced to the reactor. If not used for analyses, the fat-like materials were discarded.

Analyses and Calculations

Appearance of sludge granules was examined by a stereo zoom microscope (Olympus ZS40, Japan). Analytical procedures for volatile fatty acids (from acetate to caprylate) and LCFA (from caprate, $C_{10:0}$, to arachidate, $C_{20:0}$) have been described, respectively, in Chapter 2 and Chapter 4 (Hwu *et al.*, 1996b). For COD determination, well-mixed influent samples were analyzed (as COD_{total}) while effluent samples were membrane-filtered (as COD_{mf}; pore size of membrane = 0.45 μm, Schleicher and Schuell BA85, Germany) in runs I–III and paper-filtered (as COD_{pf}; pore size = 4.4 μm, same supplier 595$^1/_2$) in runs IV–VI. If not being analyzed immediately, both influent and effluent samples were acidified below pH 2 with concentrated H_2SO_4 and stored at 4°C. All other analyses were determined according to the standard methods (APHA, 1992).

The COD removal efficiencies described in this Chapter were calculated as the percentages of $(COD_{total} - COD_{mf})/(COD_{total})$ for runs I–II and $(COD_{total} - COD_{pf})/(COD_{total})$ for runs IV–VI. The amount of methane production was converted to its COD equivalent, based on the calculation that 1 g COD equaled to 388 ml methane. The methane conversion values are expressed as the ratios of methane-COD produced to influent COD_{total} added.

RESULTS AND DISCUSSION

Table 5.3 summarizes the experimental results in this study. No data were available in run III due to serious washout of the reactor contents after a 2-day run. In general, thermophilic runs (R-T) achieved slightly better COD removal efficiencies. It was also indicated that longer HRT resulted in better COD removal efficiencies: during run II (HRT = 6 h) reactors R-T and R-M obtained 73% and 69%, respectively, compared to 66% in R-T and 44% in R-M during run I (HRT = 3 h). Moreover, the COD removal efficiencies were considerably higher in runs with addition of cosubstrates (IV–VI) compared to those in runs with solely oleate. It should be noted that, however, in the treatment of adsorptive compounds (e.g., LCFA) in bioreactors, COD removal does not actually imply biodegradation because a huge portion of these compounds may have been adsorbed to biomass (Birch *et al.*, 1989). For instance, Cánovas-Diaz *et al.* (1992) demonstrated that 84% of oleate was removed while no methane was produced. Our previous batch biosorption tests have shown that sludge granules became surrounded with "white coats" due to oleate adsorption (Chapter 4; Hwu *et al.*, 1997c).

Table 5.3 Summary of treatment performance[a] in the thermophilic (R-T) and mesophilic (R-M) reactors under various test conditions (cf. Table 5.2)

Run	COD removal (%)		Methane conversion[b] (%)	
	R-T	R-M	R-T	R-M
I	66.2 ± 13.7	43.5 ± 4.1	11.8 ± 0.3	6.7[c]
II	72.5 ± 7.1	68.8 ± 4.5	14.7 ± 2.8	9.1 ± 4.8
III	NA	NA	NA	NA
IV	89.1 ± 1.7	86.5 ± 2.0	48.8 ± 3.4	58.7 ± 7.8
V	89.1 ± 1.6	86.9 ± 1.0	38.7 ± 2.5	52.6 ± 7.8
VI	83.7 ± 3.6	81.9 ± 5.5	69.6 ± 2.8	69.9 ± 3.8

[a]data expressed as mean ± standard deviation (n > 9)
[b]based on influent total COD
[c]one measurement
NA: not available

Indeed, many white granules were observed both in the floating layer during runs I and II and in the sludge bed during all runs. Fig. 5.2a shows a typical example of the white granules. It is surprising that the white coat was a relatively thick layer, with the thickness of ca. 0.2–0.3 mm, compared to the diameter of a granule (ca. 2–3 mm). This is in a good agreement with the above batch tests that LCFA adsorption onto anaerobic granules is a physical multilayer adsorption. Besides, it is clear that to some extent the added oleate-COD was "removed" from

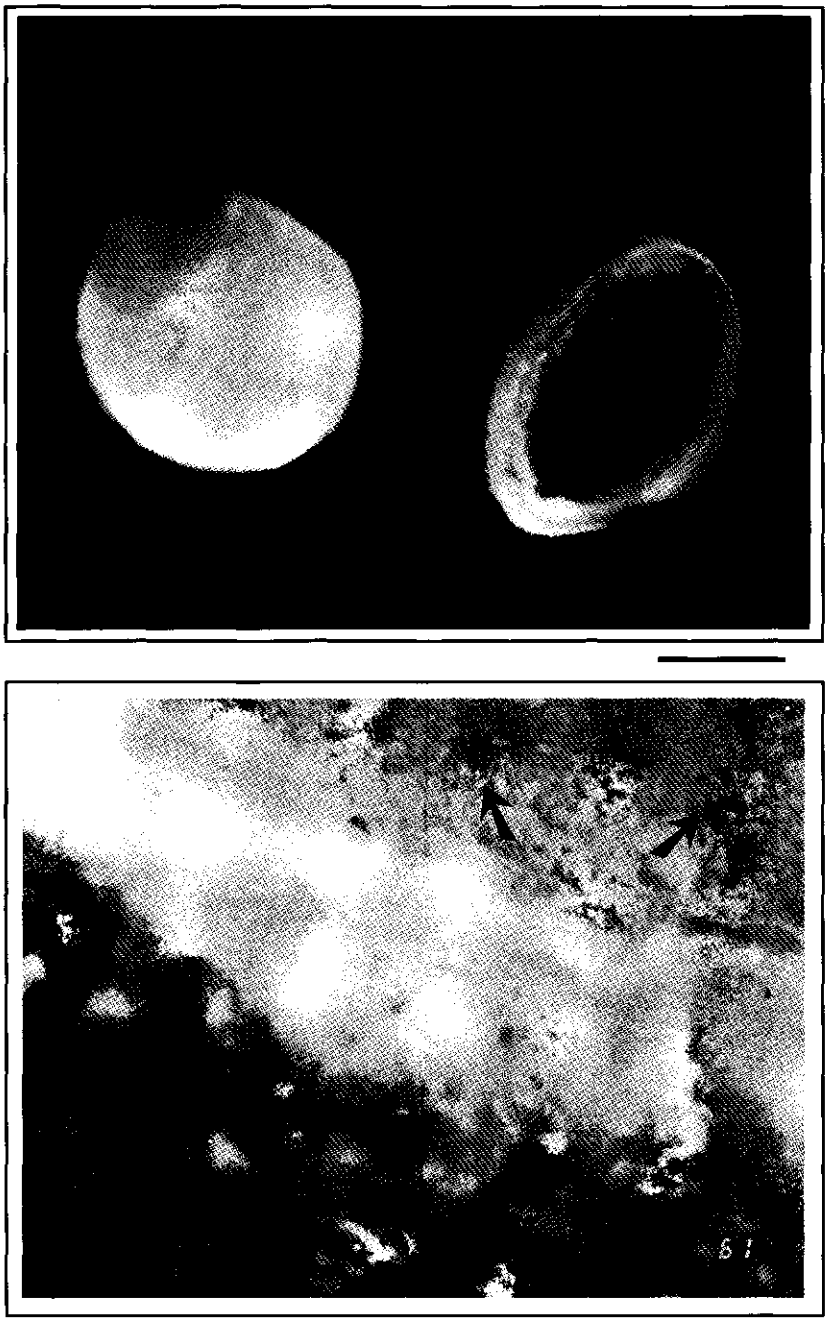


Fig. 5.2 Micrographs representing (a) the appearance of an intact white granule (left side) and a sectioned granule (right side) showing the adsorbed LCFA white coat (bar = 1 mm) and (b) a close-up look of the above sectioned granule, showing crystal-like matter (arrow) in the adsorbed layer (bar = 50 μm).

the aqueous phase to the solid phase. This physicochemical removal of oleate-COD consequently contributed to the overall removal efficiencies. The adsorbed matter showed a crystal-like appearance (Fig. 5.2b) under microscopic examination at higher magnification. Its composition should be identified because optimum strategies for minimizing the negative adsorption, eventually leading to flotation, can be dependent on its physicochemical properties.

Fig. 5.3 represents a possible development of the formation of white granules. It is likely that the adsorption was not homogeneously but locally initiated on the surface and, subsequently, stretched over a granule. On the other hand, it is also likely that the granules with incomplete coating were due to locally active microbial degradation. However, more and more granules changed their appearance from black to white in the later runs. This indicates that the change of granule color in the EGSB reactors was a long-term, cumulative effect of LCFA adsorption; compared to the batch tests (Chapter 4) where the change was rather fast due to the much higher oleate concentration applied (5800 mg COD/l) and the much less amount of biomass present (ca. 2.5 g total solids per litre). Moreover, floating fatty matter on top of the reactors during runs I and II required daily clearing to prevent from clogging in biogas collector. Analytical results showed that oleate and palmitate largely prevailed with a concentration of ca. 4 g COD per litre of the floating matter (mesophilic runs). Obviously, in addition to the adsorptive removal, a relatively large fraction of oleate-COD was removed through the build-up of this floating fatty matter. These observations are rather important in practice for two reasons: (i) initial good COD removal efficiencies cannot guarantee a successful treatment and (ii) periodic replacement of sludge is obligate if a reactor is mainly served as an adsorption tank. In the latter case, characterization of reactor breakthrough has to be carried out in pilot-scale prior to treatment in full-scale reactors.

Therefore the methane production is a better parameter to evaluate the biological conversion process than the overall COD removal efficiency, particularly in the bioreactors where, to certain extent, physicochemical interactions are prevailing (see also Chapter 4; Birch *et al.*, 1989; Hwu *et al.*, 1996b). Methane conversion ratios ranging 79–85% were achieved in both reactors during the start-up period. When oleate, as the sole substrate, was introduced to both reactors during runs I and II, however, the conversion ratios dramatically decreased to 7–15% (Table 5.3). This can probably be attributed to the occurrence of oleate inhibition or the prevalence of a poor substrate degradation. The highest methane

conversion ratio of 15% was attained in reactor R-T operated at the HRT of 6 h. No significant differences in methane conversion between reactors R-T and R-M can be seen. This observation is contrary to the results of the batch experiments described in Chapter 3 (and Hwu *et al.*, 1997f), where the thermophilic sludge achieved much higher methane conversion than the mesophilic sludge (discussed later). Yet, though small, these ratios clearly indicate that the added oleate not only was adsorbed or floated but also was partially degraded. During runs IV–VI, no significant floating matter was formed at the HRT of 24 h. In Chapter 4 (and Hwu *et al.*, 1997c) we have speculated that operating a reactor at a longer HRT might minimize excessive LCFA adsorption and, consequently, little flotation will occur. This speculation is further supported by the present results. Compared to runs I–II, runs IV–VI achieved significantly higher methane conversion ratios, viz., 7–15% for the former and 39–70% for the latter (Table 5.3). These results agree with Elefsiniotis and Oldham (1994), who demonstrated that an increase in HRT results in better methanogenesis of lipids. Regarding runs IV–VI, it must be noted that the exact degree of methane conversion from oleate alone is not clear. Very likely, the anaerobic digestion of oleate requires a longer contact time between oleate and oleate-oxidizers.

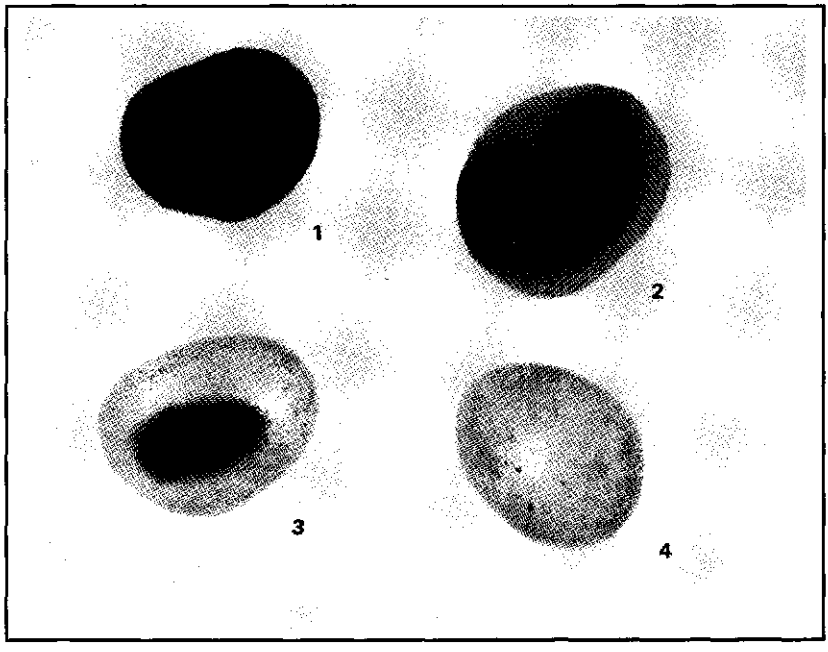


Fig. 5.3 Photograph showing a possible evolution of the formation of LCFA adsorption layer (the white coat), in the order of Arabic numerals. Bar = 1 mm.

Fig. 5.4 compares the effect of the three V_{up} on the degree of methane conversion during runs IV–VI. Regardless of reactor temperature, a higher V_{up} resulted in a significantly lower degree of methane conversion. Besides, at higher V_{up} more sludge debris appeared above the sludge bed and in the settler as well (Fig. 5.1). These results suggest that a more vigorous longitudinal mixing in the liquid phase, induced by a higher V_{up}, could abrade or disintegrate the granules and thus very likely deteriorate the syntrophic degradation of oleate between β-oxidizers and methanogens. On the other hand, the washed out debris might be essential to the β-oxidation. Recently, Omil *et al.* (1996) also found that high V_{up} (4–6 m/h) negatively influenced the performance of a sulphidogenic reactor by washout of biomass in particle form. Apparently, whether or not biomethanation is enhanced by a high V_{up} depends on the substrate to be treated and the sludge which is inoculated. Also, the high temperature in reactor R-T did not enhance methanogenesis. To the contrary, methane conversion under thermophilic conditions was even more susceptible to higher V_{up} as indicated by a steeper slope of the curve (Fig. 5.4). These results are in contradiction to the results of a parallel batch study. The batch study was performed by using serum bottles inoculated with the sludge taken from reactors R-T and R-M at the end of the restart-up period, which showed a 4–5 times higher oleate conversion rates under thermophilic than under mesophilic conditions (Chapter 3; Hwu *et al.*, 1997f). The most apparent difference between the batch and the EGSB reactors is that the latter are uniquely affected by hydrodynamics or, more likely, liquid superficial upflow velocity. Quarmby and Forster (1995) found that thermophilic granular sludge fed with palm oil mill effluent, an LCFA-rich wastewater, had a lower strength as compared to its mesophilic counterpart. Consequently, more serious abrasion or breaking of thermophilic granules can occur when the system is operated at higher V_{up} accompanied with high shear forces.

During the start-up period, an 85% methane conversion was achieved and both caprylate ($C_{8:0}$) and caproate ($C_{6:0}$) were not detectable in the effluents of both reactors (< 5 mg/l); indicating the occurrence of good β-oxidation and methanation of caprylate. The good conversion, however, did not sustain when oleate was present in the reactors. Therefore, it is questionable that caprylate-oxidizers are able to β-oxidize oleate. Also, the treatment performance of oleate was much lower than that of laurate conducted by Rinzema *et al.* (1993), although both LCFA were treated in EGSB reactors. Similarly, one might speculate that the oleate-oxidizers and laurate-oxidizers are not the same bacteria. Besides, the different performance can also be attributed to the differences in molecular size of

the hydrophobic aliphatic moieties, viz., oleate is more hydrophobic and has higher surface activity than laurate. Thus the liquid surface tension in reactors fed with oleate is lower than that in reactors fed with laurate. Recently, it was reported that surface thermodynamics in anaerobic reactors has selective force on different trophic groups of anaerobes (Daffonchio *et al.*, 1995; Thaveesri *et al.*, 1995), viz., with addition of surfactants, the surface tension is lowered, thus favors the aggregation of rather hydrophilic bacteria and disfavors that of rather hydrophobic bacteria. As most of the acetogens are considered rather hydrophobic (Daffonchio *et al.*, 1995), it therefore is likely that the oleate-degraders are more susceptible to high V_{up} than the laurate-degraders (see also Chapter 6; Hwu *et al.*, 1997b,d). Concerning anaerobic treatment in practice, cultivation of oleate-degraders is rather important in reactors inoculated with non-adapted sludge, since oleate is the most abundant constituent in LCFA-contaminated wastewaters. Based on our current insights, a fed-batch manner is preferred for the cultivation. To shorten the adaptation (start-up) period of an anaerobic reactor treating oleate-containing wastewater, we recommend to inoculate the reactor with sludge from reactors treating similar types of wastewater.

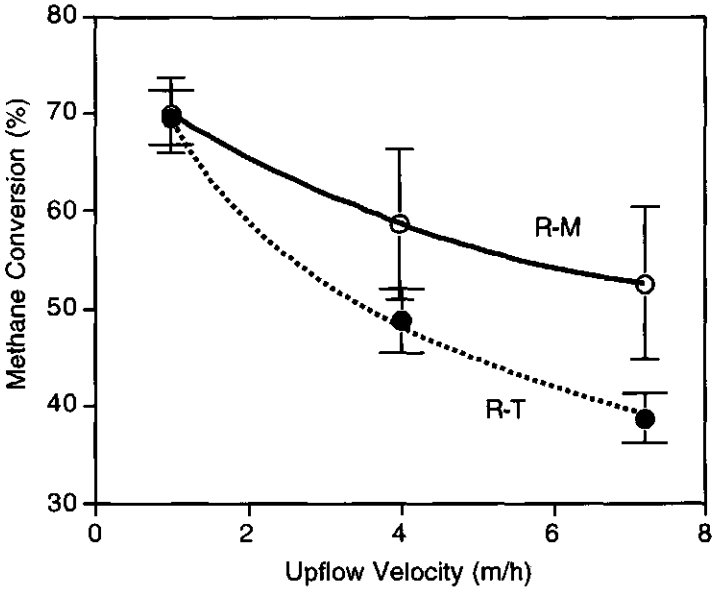


Fig. 5.4 Influence of upflow velocity on methane conversion in EGSB reactors operating at thermophilic (R-T) and mesophilic (R-M) temperatures.

Although in runs IV–VI the fractions of methane conversion were not differentiated between the cosubstrates and oleate, it is reasonable to expect that the added oleate was degraded, according to the conversion ratios obtained in runs I-II. Thus the methane production in runs IV–VI is regarded as an overall result. Compared to runs I–III (Table 5.3), the better biological (higher methane conversion) and physicochemical (no flotation of fatty matter or granules) performance was obtained in runs IV–VI. This indicates that the addition of the cosubstrates can be a prerequisite in increasing the overall performance. The effect of cosubstrate was in fact evidenced by Beccari *et al.* (1996) who found that oleic acid was not degraded at all within a 66-day incubation period, without addition of an easily biodegradable substrate, i.e., glucose. Our recent work on the enhancement of oleate mineralization by addition of acetate, caprylate or glucose found that glucose exerted the best cosubstrate effect (Hwu *et al.*, 1997e). The addition of exergonic-rich substrate(s) likely stimulates the endergonic β-oxidation of oleate (refer to the LCFA degradation pathway described in Chapter 1).

Apart from the cosubstrate effect, the experimental results imply that reactor hydrodynamics plays an important role in anaerobic digestion of oleate. The highest reactor performance was achieved at the V_{up} of 1 m/h and the HRT of 24 h, suggesting that the "low-rate" operational parameters are rather preferred than the "higher-rate" ones. A maximum growth rate of 0.3 d^{-1} for thermophilic β-oxidizers was established by Angelidaki and Ahring (1995). Obviously, each HRT applied in the present work was very likely to create a dilution rate exceeding the increase in the growth rate. This consequently might hamper β-oxidation capacity, known as the rate-limiting step in the anaerobic digestion of oleate. Under such circumstances, abruptly applying higher V_{up} can merely aggravate the adverse situation due to the accompanying higher longitudinal shear forces, and subsequent washout of a fraction of the present oleate-oxidizers.

CONCLUSIONS

Performance of the EGSB reactors treating oleic acid was investigated under various conditions. The experimental results in this Chapter demonstrate that a good anaerobic digestion of oleate-containing wastewaters needs addition of cosubstrate, operating at lower V_{up} or at longer HRT or both. The typical EGSB reactor hydrodynamic parameters, being a high V_{up} and a short HRT, are

apparently unfavorable in the anaerobic treatment of oleate. The reasons of the unfeasibility of the EGSB reactor concept for the digestion of oleate are described in the next Chapter which discusses the interactions between reactor hydrodynamics, surface thermodynamics and the oleate-oxidizing biomass.

ACKNOWLEDGMENTS

I am grateful to Zoltán Kulik and Pradeep K. Mishra for their technical assistance on part of the experimental work.

REFERENCES

Angelidaki, I. and Ahring, B.K. (1995). Establishment and characterization of anaerobic thermophilic (55°C) enrichment culture degrading long-chain fatty acids. *Appl. Environ. Microbiol.*, **61**, 2442–2445.

APHA. (1992). *Standard Methods for the Examination of Water and Wastewater.* 18th edn., American Public Health Association, Washington, D.C., pp. 2-54–2-57, 5-9–5-10.

Beccari, M., Bonemazzi, F., Majone, M. and Riccardi, C. (1996). Interaction between acidogenesis and methanogenesis in the anaerobic treatment of olive oil mill effluents. *Water Res.*, **30**, 183–189.

Birch, R.R., Biver, C., Campagna, R., Gledhill, W.E., Pagga, U., Steber, J., Reust, H. and Bontinck, W.J. (1989). Screening of chemicals for anaerobic biodegradability. *Chemosphere*, **19**, 1527–1550.

Cánovas-Diaz, M., Sánchez-Roig, M.J. and Iborra, J.L. (1992). Myristic and oleic acid degradation by an acclimated anaerobic consortia: synergistic behaviour. In: *Proc. 6th EC Conf. Biomass for Energy, Industry and Environment,* Grassi, G., Collina, A. and Zibetta, H. (Eds.), Athens, Greece, Apr. 1991, Elsevier, UK, pp. 580–584.

Daffonchio, D., Thaveesri, J. and Verstraete, W. (1995). Contact angle measurement and cell hydrophobicity of granular sludge from upflow anaerobic sludge bed reactors. *Appl. Environ. Microbiol.*, **61**, 3676–3680.

Elefsiniotis, P. and Oldham, W.K. (1994). Effect of HRT on acidogenic digestion of primary sludge. *J. Environ. Eng. ASCE*, **120**, 645–660.

Habets, L.H.A. (1997). Personal communication. Paques B.V., PO Box 52, 8560 AB Balk, The Netherlands.

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

Hawkes, F.R., Donnelly, T. and Anderson, G.K. (1995). Comparative performance of anaerobic digesters operating on ice-cream wastewater. *Water Res.*, **29**, 525–533.

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

Hwu, C.-S., Tseng, S.-K., Yuan, C.-Y., Kulik, Z. and Lettinga, G. (1996b). Adsorption of long-chain fatty acids onto granular sludges and its effect on upflow anaerobic sludge bed reactor operation. In: *Proc. 1st IAWQ Specialized Conf. on Adsorption in Water Environment and Treatment Processes*, Nov. 1996, Wakayama, Japan, pp. 91–98.

Hwu, C.-S. and Lettinga, G. (1997a). Acute toxicity of oleate to acetate-utilizing methanogens in mesophilic and thermophilic anaerobic sludges. *Enzyme Microb. Technol.*, **21**, in press.

Hwu, C.-S., Molenaar, G., Garthoff, J., van Lier, J.B. and Lettinga, G. (1997b). Thermophilic high-rate anaerobic treatment of wastewater containing long-chain fatty acids: impact of reactor hydrodynamics. *Biotechnol. Lett.*, **19**, 447–451.

Hwu, C.-S., Tseng, S.-K., Yuan, C.-Y., Kulik, Z. and Lettinga, G. (1997c). Biosorption of long-chain fatty acids in UASB treatment process. *Water Res.*, in press.

Hwu, C.-S., van Beek, B., van Lier, J.B. and Lettinga, G. (1997d). Thermophilic high-rate anaerobic treatment of wastewater containing long-chain fatty acids: effect of washed out biomass recirculation. *Biotechnol. Lett.*, **19**, 453–456.

Hwu, C.-S., van Beek, B., van Lier, J.B. and Lettinga, G. (1997e). Effects of cosubstrates and sludge acclimation on anaerobic degradation of oleic acid by granular sludge. in preparation.

Hwu, C.-S., van Lier, J.B. and Lettinga, G. (1997f). Anaerobic toxicity and degradability of oleic acid under mesophilic and thermophilic conditions. In: *Proc. Forum for Appl. Biotechnol.*, 25–26 Sep. 1997, Gent, Belgium, in press.

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.

Lettinga, G. (1995). Anaerobic digestion and wastewater treatment systems. *Antonie van Leeuwenhoek*, **67**, 3–28.

Lettinga, G. (1996). Sustainable integrated biological wastewater treatment. *Water Sci. Technol.*, **33**(3), 85–98.

McCarty, P.L. (1964). Anaerobic waste treatment fundamentals. Part III: Toxic materials and their control. *Public Works*, **95**(Nov.), 91–94.

Novak, J.T. and Carlson, D.A. (1970). The kinetics of anaerobic long chain fatty acid degradation. *J. Water Pollut. Control Fed.*, **42**, 1932–1943.

Nuck, B.A. and Federle, T.W. (1995). Batch test for assessing the mineralization of ^{14}C-radiolabeled compounds under realistic anaerobic conditions. *Environ. Sci. Technol.*, **30**, 3597–3603.

Omil, F., Lens, P., Hulshoff Pol, L. and Lettinga, G. (1996). Effect of upward velocity and sulphide concentration on volatile fatty acid degradation in a sulphidogenic granular sludge reactor. *Process Biochem.*, **31**, 699–710.

Quarmby, J. and Forster, C.F. (1995). A comparative study of the structure of thermophilic and mesophilic anaerobic granules. *Enzyme Microb. Technol.*, **17**, 493–498.

Quéméneur, M. and Marty, Y. (1994). Fatty acids and sterols in domestic wastewaters. *Water Res.*, **28**, 1217–1226.

Rinzema, A., Alphenaar, A. and Lettinga, G. (1989). The effect of lauric acid shock loads on the biological and physical performance of granular sludge in UASB reactors digesting acetate. *J. Chem. Technol. Biotechnol.*, **46**, 257–266.

Rinzema, A., Alphenaar, A. and Lettinga, G. (1993). Anaerobic digestion of long-chain fatty acids in UASB and expanded granular sludge bed reactors. *Process Biochem.*, **28**, 527–537.

Samson, R., Van den Berg, B., Peters, R. and Hade, C. (1985). Dairy waste treatment using industrial-scale fixed-film and upflow sludge bed anaerobic digesters: design and start-up experience. In: *Proc. 39th Purdue Industrial Waste Conference*, Bell, J.M. (Ed.), Butterworth Publ., Boston, pp. 235–241.

Sam-Soon, P.A.L.N.S., Loewenthal, R.E., Wentzel, M.C. and Marais, Gv.R. (1991). A long-chain fatty acid, oleate, as sole substrate in upflow anaerobic sludge bed (UASB) reactor systems. *Water SA*, **17**, 31–36.

Thaveesri, J., Daffonchio, D., Liessens, B., Vandermeren, P. and Verstraete, W. (1995). Granulation and sludge bed stability in upflow anaerobic sludge bed

reactors in relation to surface thermodynamics. *Appl. Environ. Microbiol.*, **61**, 3681–3686.

Van Haandel, A.C. and Lettinga, G. (1994). *Anaerobic Sewage Treatment: A Practical Guide for Regions with a Hot Climate*. John Wiley and Sons, Chichester, Ch. 2, pp. 54–55.

Viswanathan, C.V., Meera Bai, B. and Pillai, S.C. (1962). Fatty matter in aerobic and anaerobic sewage sludges. *J. Water Pollut. Control Fed.*, **34**, 189–194.

Weng, C.-N. and Jeris, J.S. (1976). Biochemical mechanisms in the methane fermentation of glutamic and oleic acids. *Water Res.*, **10**, 9–18.

6

Thermophilic High-Rate Anaerobic Treatment of Wastewaters Containing Oleic Acid: Effects of Reactor Hydrodynamics and Recirculation of Washed Out Biomass

This Chapter contains two papers published as:

Hwu, C.-S., Molenaar, G., Garthoff, J., van Lier, J.B. and Lettinga, G. (1997). Thermophilic high-rate anaerobic treatment of wastewater containing long-chain fatty acids: impact of reactor hydrodynamics. *Biotechnol. Lett.*, **19**, 447–451.

Hwu, C.-S., van Beek, B., van Lier, J.B. and Lettinga, G. (1997). Thermophilic high-rate anaerobic treatment of wastewater containing long-chain fatty acids: effect of washed out biomass recirculation. *Biotechnol. Lett.*, **19**, 453–456.

6

Thermophilic High-Rate Anaerobic Treatment of Wastewaters Containing Oleic Acid: Effects of Reactor Hydrodynamics and Recirculation of Washed Out Biomass

ABSTRACT

Regarding the research in this thesis, till now oleic acid ($C_{18:1}$) can be successfully mineralized in batch rather than in continuous reactors. In a previous study (Chapter 5) we found that the typical expanded granular sludge bed (EGSB) hydrodynamic parameters, viz., liquid superficial upflow velocity (V_{up}) > 4 m/h and hydraulic retention time (HRT) < 10 h, negatively affect oleate treatment and consequently the methane conversion. Therefore in the present study we systematically investigated the effects of V_{up} and HRT on the thermophilic treatment of oleate using four EGSB reactors operated under twelve conditions. In comparison with the operation at HRT = 24 h, operation at HRT = 6 h was found to lead to a less favorable treatment performance, mainly due to biomass washout in particulate form. When the reactor system was modified to a configuration with complete biomass retention, no differences could be found in methane yield between V_{up} of 1 and 8 m/h.

Biomass particulates washed out from one of the EGSB reactors were tested for their oleate conversion capacity. The specific activity on oleate conversion of this biomass (129 mg CH_4-COD/g VS·d) was significantly higher than that of the granular sludge left in the sludge bed of the EGSB reactor. Recirculation of washed out biomass to the reactor remarkably improved the treatment performance. The highest methane production rate of 600 mg CH_4-COD/g VS·d was achieved while

treating oleate at the concentration as high as 4000 mg oleate-COD/l (4.5 mM), corresponding to a sludge loading rate of 0.58 g COD/g VS·d (0.29 g oleate-COD/g VS·d). The results obtained are better than those previously reported regarding anaerobic treatment of wastewaters containing oleic acid.

INTRODUCTION

Since its introduction in the seventies, the upflow anaerobic sludge bed (UASB) reactor has become the most widely applied high-rate anaerobic wastewater treatment process. The success of the UASB reactor can be attributed to its capability of biomass retention in the reactor systems, which is attained by mutual attachment of bacteria, resulting in the formation of granular sludge with excellent settling velocities as high as 90 m/h (Lettinga *et al.*, 1983). More than nine-hundred industrial scale UASB reactors are currently under operation (Habets, 1997), successfully treating a wide variety of easily biodegradable wastewaters from, e.g., potato processing and brewery industries (Lettinga and Hulshoff Pol, 1991). However, success is still not certain when treating some complex (difficult) wastewaters. For instance, treatment failure of UASB reactors has been reported both at laboratory and industrial scale, following shock loads of long-chain fatty acids (Rinzema *et al.*, 1989) or milk fats (Samson *et al.*, 1985).

Long-chain fatty acids (LCFA) are products of lipid hydrolysis. They are well known inhibitors of anaerobic micro-organisms at millimolar concentrations (Koster and Cramer, 1987; Hwu *et al.*, 1996a, 1997a). Apart from their inhibitory effect, LCFA can cause severe flotation/washout of granular sludge due to their adsorption onto the sludge granules (Rinzema *et al.*, 1989; Hwu *et al.*, 1996b). When comparing the anaerobic digestion of sodium laurate ($C_{12:0}$) in UASB and expanded granular sludge bed (EGSB) reactors, Rinzema *et al.* (1993) recommended the use of EGSB reactors under thermophilic conditions for minimizing flotation of granular sludge and maximizing degradation of LCFA. The EGSB process is a modified-UASB, high-rate process, conceptually operated at relatively higher liquid superficial upflow velocities, i.e., V_{up} > 4 m/h (Lettinga, 1996) and shorter hydraulic retention times, i.e., HRT < 10 h (Van Haandel and Lettinga, 1994). The better biomass–substrate contact in EGSB reactors, compared to conventional UASB reactors, allows anaerobic treatment of

wastewaters containing lipids and/or higher fatty acids (Lettinga, 1995) and even recalcitrant or toxic substrates (Lettinga, 1996).

Since it is the most abundant constituent in LCFA-containing wastewaters (Viswanathan *et al.*, 1962; Quéméneur and Marty, 1994), sodium oleate was used as a model LCFA in a previous study (Chapter 5; Hwu *et al.*, 1997b). In contrast to Rinzema *et al.* (1993), EGSB hydrodynamic parameters, i.e., V_{up} = 4–7 m/h or HRT = 0.6–6 h, gave a less favorable reactor performance compared to the typical UASB operational parameters, i.e., V_{up} = 1 m/h or HRT = 24 h. The reasons for the contrariety between the two reactor concepts were not well understood. In addition, although flotation of sludge granules did not occur, much sludge debris appeared in the blanket zone at higher V_{up}. It was speculated that a too vigorous mixing in liquid phase, induced by higher V_{up}, could disintegrate the granules and thus very likely deteriorate the syntrophic degradation of oleate between β-oxidizers and methanogens (Chapter 5; Hwu *et al.*, 1997b).

To date the washout of biomass in the form of finely suspended sludge or debris from high-rate reactors has not drawn much serious attention compared to problems with flotation/washout of big biomass, e.g., granular sludge. This lack of attention can be attributed to the fact that washout of particulates is supposed not to impede the reactor performance (Shieh and Hsu, 1996). This, however, is only valid if the growth-in rate of a specific substrate-utilizer exceeds the washout rate. In anaerobic wastewater treatment systems, lipids are rapidly hydrolyzed to long-chain fatty acids (LCFA) and glycerol but the degradation (β-oxidation) of LCFA to acetate proceeds slowly (Hanaki *et al.*, 1981). The β-oxidation is the rate-limiting step in anaerobic degradation of capric acid ($C_{10:0}$) (Rinzema *et al.*, 1994) and oleic acid ($C_{18:1}$) (Novak and Carlson, 1970). These LCFA β-oxidizers (proton-reducing, acetogenic bacteria), according to Angelidaki and Ahring (1995), have a rather slow growth rate (0.3 d^{-1}) compared to short-chain fatty acid, β-oxidizers (0.48–0.77 d^{-1}; Ahring and Westermann, 1987) and to methanogens (0.48–2.04 d^{-1}; Van Lier *et al.*, 1993). Consequently, application of a high-rate reactor at hydrodynamic stress conditions very likely aggravates the washout of specific biomass composed of the key microorganisms, due to the increased liquid and particle shear forces (Gjaltema *et al.*, 1995) and the high dilution rates (Yang and Okos, 1987).

Hence, we systematically studied the effects of the EGSB reactor hydrodynamics on oleate removal efficiencies, methane yield and biomass

characteristics using four thermophilic (55°C) EGSB reactors operated under twelve conditions. In the second stage of experiment, the washed out biomass sampled from one of the EGSB reactors was characterized for its oleate degradation capacity and compared to the granules retained in the reactor. Also the effect of recirculating the biomass particulates to the reactor on the treatment performance was investigated under three conditions. Based on the observations made, it is attempted to explain the poor treatment performance under EGSB hydrodynamic parameters.

MATERIALS AND METHODS

Biomass

The granular sludge used for the experiments with continuous flow was harvested from a 4.4 l thermophilic (55°C) EGSB reactor treating oleate (Hwu *et al.*, 1997b). Operational conditions of this EGSB reactor have been described in Chapter 5. Prior to inoculation, the sludge had been batch-wise exposed to a solution of sodium oleate (95% purity, Sigma, USA), with a chemical oxygen demand (COD) of 2000 mg/l for a period of 16 days. The sludge was elutriated with tap water to remove fines and floating matter. The sludge had an initial specific methanogenic activity (SMA) on acetate of 0.23 g COD per g volatile solids (VS) per day at 55°C.

For the batch experiment, three types of sludge originating from an EGSB reactor were used as inocula. The EGSB reactor was used in the present investigation dealing with the effect of HRT on oleate conversion and denoted by R3 below. Upon finishing the HRT investigation, washed out sludge in the settler and granules in the sludge bed were sampled.

Chemicals and Media

The composition of the basal medium used was described previously (Hwu *et al.*, 1996a). A solution of sodium butyrate (analytical grade, Merck, FRG) with a concentration of 4 g COD/l was continuously supplied to the reactors in the experiments for assessing the effects of V_{up} and HRT. To avoid cumulative inhibition of sodium ions in the reactors during the test runs in V_{up} study, butyrate

was replaced by butyric acid (analytical grade, Merck, FRG) with the same concentration. Instead of butyrate, glucose of 4 g COD/l was served in the experiment with recirculation of washed out biomass.

Analytical grade sodium oleate (Sigma, USA), comprising 95% (w/w) oleate and 5% palmitate ($C_{16:0}$), was used for the batch experiment. Technical grade sodium oleate (Riedel de Haën, FRG), consisting of 82% (w/w) oleate and 17% palmitate was supplied by pulse injection in V_{up} study or continuously in other studies. Influent pH of all experiments was adjusted to 7.0 ± 0.1, except for the runs using butyric acid, where the pH eventually ranged 6.2 ± 0.1 in the mixing flasks (see below). All media were buffered by $NaHCO_3$ with 5 g/l in batch experiment and various concentrations from 0.5 to 8 g/l, according to influent COD and effluent pH, in continuous flow experiments.

Batch Experiments

Batch tests were carried out to compare the anaerobic conversion of oleate by three types of sludge, i.e., granular, dispersed, and washed out, which all originated from an EGSB reactor (Fig. 6.2). The washed out sludge was collected from the settler. This sludge was placed in a separation funnel and repeatedly rinsed and settled with anaerobic basal medium under anaerobic conditions to remove floating matter. The granular sludge was homogeneously sampled from the sludge bed of the EGSB reactor and elutriated using the same basal medium. The diameters of the elutriated granules ranged from 1 to 3 mm. A known amount of the granules was placed in a pressured anaerobic serum bottle and, subsequently, granules were dispersed by succeedingly passing them through a series of sharp-pointed needles with a syringe. The finest needles used were coded 25G5/8.

The oleate degradation capacity was assayed by measuring the methane production using the "headspace" method (Hwu *et al.*, 1996a). Biomass (1 g VS/l) and oleate (0.2 g COD/l) were added to a serum bottle (136 ± 1 ml) containing 100 ml basal medium. The initial pH was adjusted to ca. 7.0 by adding drops of HCl. The headspace of the bottles was flushed with N_2/CO_2 (70/30, v/v) for 5 min and incubated in a reciprocal water-bath shaker at 50 strokes/min. The experiment was terminated 48 h after cessation of the methane production, because this indicates that oleate had been sufficiently mineralized.

Continuous Flow Experiments

I. Effect of V_{up}

Reactor Start-Up

The rather low sludge SMA of the inoculum, probably due to inhibition by oleate (2000 mg COD/l), required a start-up procedure to revive the activity. During the start-up period, the pseudo-steady state methane recovery from the cosubstrate, butyrate, could be estimated. Two identical glass EGSB reactors (Fig. 6.1), each with an inner diameter of 40 mm, a total height of 965 mm, and a working volume of 1.4 l, were initially inoculated with 19.2 g VS, respectively. After inoculation, both reactors, R1 and R2, were immediately fed with butyrate and operated at an HRT of 24 h and a V_{up} of 1.3 m/h. During this period LCFA was not supplied to the feed of the reactors and they were operated at an organic loading rate (OLR) of 4 g COD/l·d. The reactor temperature (55°C) was controlled by a thermostat-bath (Haake D8, FRG) with water recirculation through the reactor jacket. The mixing flask shown in Fig. 6.1 had not yet been installed during the start-up period. Influent was pumped using peristaltic pumps (Watson Marlow 202 S/1, UK) from a cooled (5°C), stirred feed tank and effluent was discharged after the siphon. The biogas was led through a 10% NaOH solution and a column packed with soda lime pellets with indicator (Merck, FRG) and finally to a wet gas meter (Meterfabriek Dordrecht, The Netherlands), where methane production was quantified. Ambient temperature was recorded for calibration in the calculation of methane production. From day 44 onwards, the V_{up} of reactor R2 was increased to 7.9 m/h solely by increasing the flow rate of recirculation. The start-up was terminated at day 92 because butyrate removal then exceeded 99% and methane recovery was about 60% over a 10-day period. An equal amount of sludge was withdrawn from each reactor, resulting in an initial total biomass content of 16.1 g VS per reactor for the remaining experiments, covering ca. 20% of reactor volume.

Test Runs

After the start-up, the reactor set-up was modified to a virtually closed system (Fig. 6.1) in order to monitor the impact of the V_{up} while minimizing the influence of the HRT. Effluent was collected in a 1.3 l flask, mixed with the cosubstrate and then recirculated. Highly concentrated butyric acid of 250 g COD/l

was pumped constantly at a flow rate of 22 ml/d, so that the OLR of 4 g COD/l·d and the total liquid volume of the system was nearly unaltered. Occasionally, anaerobic tap water was added to maintain the liquid level in the mixing flasks. An N_2/CO_2 (70/30, v/v) gas bag was connected to each flask to balance the gas pressure and liquid bufferity.

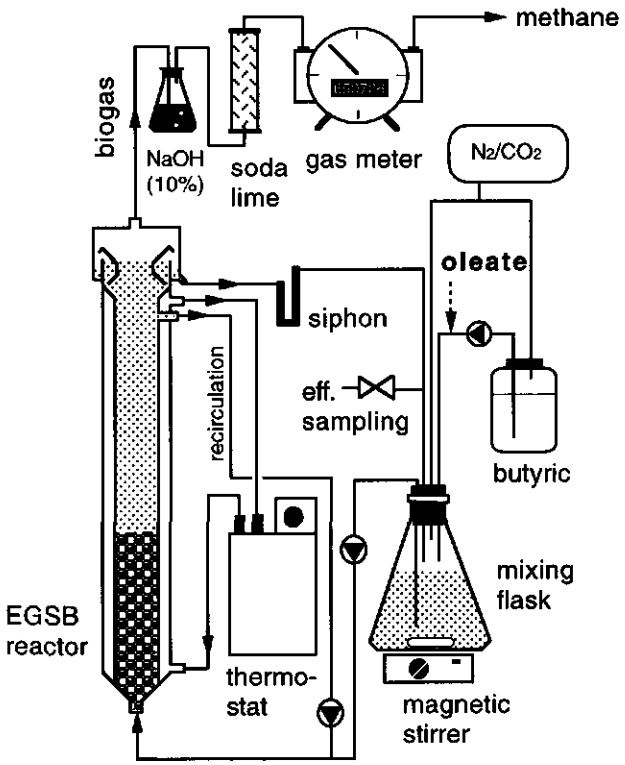


Fig. 6.1 Schematic diagram representing reactor set-up in V_{up} study. Not to scale.

The concentrated, well soluble oleate solution was fed by pulse injection into the butyric feed line (the dotted arrow in Fig. 6.1). Two injections with each of the three designated final concentrations were performed: day 0 & 6 with 500 mg COD/l, day 12 & 19 with 1000 mg COD/l and day 40 & 47 with 2000 mg COD/l. The time interval between two injections was large enough to reach a pseudo-steady state. The total time period of the three test runs (I, II and III) was 53 days. In each test run the impact of the V_{up} was evaluated, based on the difference (R1-R2) between the change of COD recovery ratio as methane (due to oleate

injection) in reactor R1 (V_{up} = 1.3 m/h) and the change in reactor R2 (V_{up} = 7.9 m/h). The change in each reactor was calculated by subtracting the ratio with butyrate (before oleate injection, mean value) from the ratio with butyrate plus oleate (maximum value).

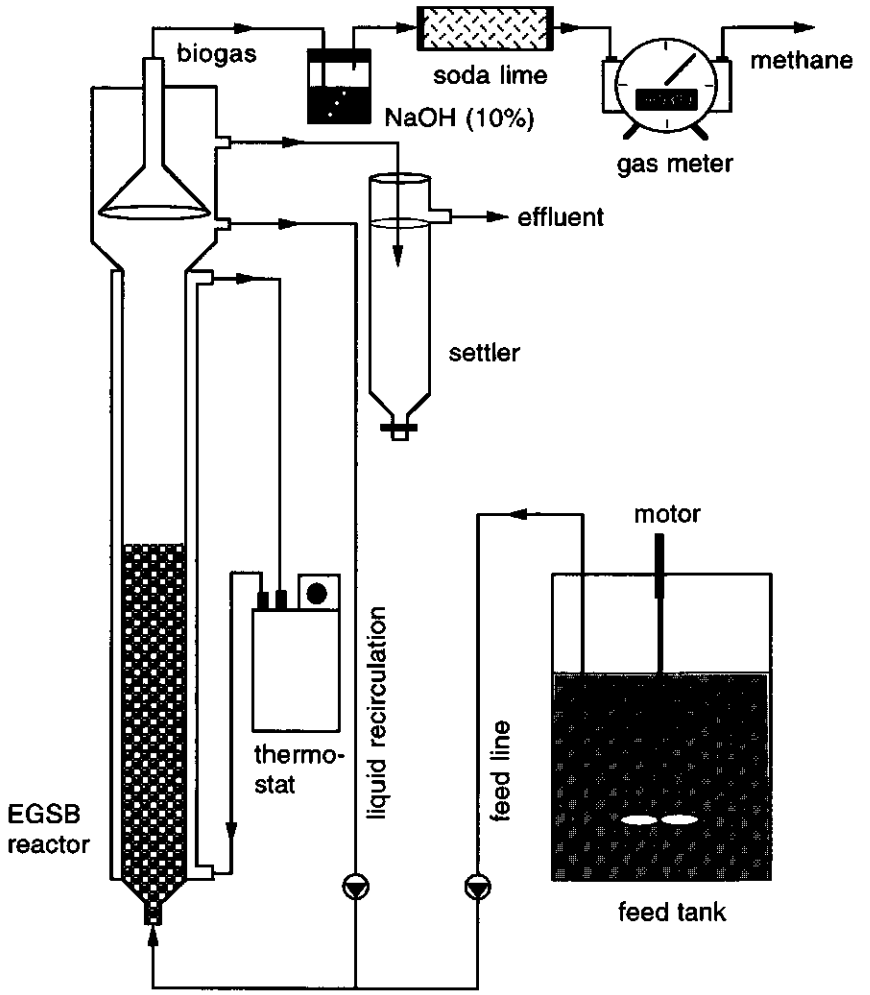


Fig. 6.2 Reactor set-up of the HRT experiment. Not to scale.

II. Effect of HRT

Reactor Start-Up

Two identical glass EGSB reactors, reactor R3 inoculated with 30.3 g VS and R4 with 35.6 g VS, were used to study the effect of the HRT (Fig. 6.2). The inner diameters of the reactor was 56 mm in the main body and 100 mm in the gas-liquid-solid separator. They were 1700 mm tall and the working volume was 4.4 l. In the beginning of the start-up period, both reactors were operated at HRT = 24 h and a V_{up} of 1 m/h. From day 52 onwards, the HRT of R4 was reduced to 6 h while the OLR (4 g butyrate-COD/l·d) remained constant by equivalent dilution. Once over 99% of the butyrate was removed and ca. 80% of methane recovery was obtained over 7 d in both reactors, the start-up was terminated (day 91).

Test Runs

Three OLR of oleate (OLR_{ole}) in series, i.e., 0.5, 1 and 2 g COD/l·d) were imposed to the system and denoted by test run IV, V and VI, respectively. Correspondingly, the total OLR (OLR_{tot}) in each run was 4.5, 5.0 and 6.0 g COD/l·d for both reactors. Each run was operated for 21 days.

III. Effect of Washed Out Recirculation

Upon completion of the experiment on the effect of the HRT and after sampling for the batch tests, sludge in reactor R3 and R4 was combined and mixed thoroughly. The mixed sludge was elutriated to remove fines, floating or fat-like matter and then 60.4 g (VS) was inoculated into one of the reactors. Fig. 6.3 shows the reactor set-up in order to study the effect of returning the washed out sludge to the system used in the present experiment. This reactor system is denoted by R5 throughout this Chapter, which differs from the set-up of reactors R3 and R4 (Fig. 6.2) with respect to the modified (1st) settler used and the recirculation line for returning the washed out biomass. A Plexiglas hollow cylinder was installed in the centre of the first settler, as to prevent effluent from direct discharge to the side-arm and to improve the settling of the washed out particulates. With combination of recirculation and feed, the settled biomass was continuously recirculated into the reactor at various rates adjusted to maintain a minimum volume of settled sludge.

The reactor hydrodynamic parameters applied in R5 were: V_{up} = 1 m/h and HRT = 24 h. R5 was started-up by feeding 4 g glucose-COD/l synthetic wastewater for 2 months. Three test runs (VII, VIII and IX), each with a time course of 11 HRTs, were performed with 500, 2000 and 4000 mg COD/l of oleate, respectively. Glucose (4 g COD/l) was used as cosubstrate and its concentration was kept constant throughout this work. The corresponding OLR_{ole} were 0.5, 2.0 and 4.0 g COD/l·d and the OLR_{tot} were 4.5, 6.0 and 8.0 g COD/l·d for reactor run VII, VIII and IX, respectively.

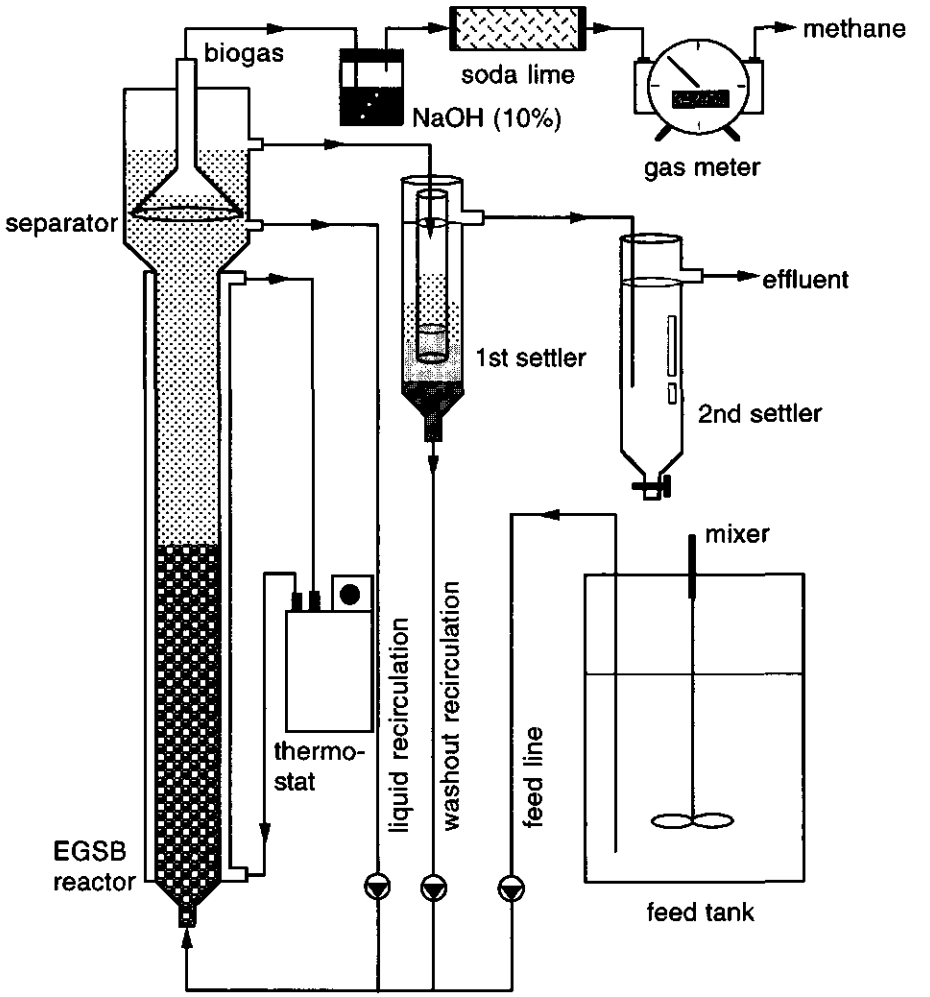


Fig. 6.3 Configuration of reactor set-up with washed out biomass recirculation (not to scale).

Analyses

Sludge size and appearance was examined using a stereo microscope (Olympus ZS40, Japan) equipped with a camera body for photography. For COD determinations, well mixed influent samples were used. Effluent samples were membrane-filtered (pore size 0.45 μm, Schleicher & Schuell, FRG) in the experiments concerning the V_{up} effect and paper-filtered (pore size 4.4 μm, same supplier) in the experiments with reactors R3, R4 and R5. In the V_{up} test runs, the reactor biomass was not quantified. In the HRT runs, VS were determined upon start and termination of a run, and averaged to calculate the mean conversion rate per unit biomass.

COD and VS determinations followed the procedures described in the standard methods (APHA, 1992). The volatile fatty acid (VFA) concentration and methane content in the headspace was analyzed by gas chromatography as described in Chapter 2 and elsewhere (Hwu *et al.*, 1996a), respectively. Procedures of LCFA analyses have been described in Chapter 4 (or Hwu *et al.*, 1996b).

RESULTS AND DISCUSSION

Effect of the V_{up} on Oleate Conversion

In each of the test runs, an oleate injection induced 10–20% increase in methane production in the next 1–3 days. Subsequently, methane production returned to its background level from butyrate, indicating that the added oleate was biodegraded without detectable inhibition. Otherwise, the methane production should not be able to return to the background level (discussed later with Run VI in Fig. 6.5) since LCFA toxicity eventually leads to cytolysis (Asther and Corrieu, 1987; Thies *et al.*, 1994; Hwu and Lettinga, 1997a) which necessitate a long-term recovery of methanogenic activity (Rinzema *et al.*, 1994). Table 6.1 compares the reactor performance at the two imposed different V_{up}. It is not surprising that excellent COD removal efficiencies (94–98%) were achieved in the thermophilic digestion of butyrate. The methane conversion rates from butyrate can be regarded as the pseudo-steady state, background levels during each test run in each reactor. Though fluctuating, the conversion rates in reactor R2 increased

with time course. This trend was not found in reactor R1, suggesting that the higher V_{up} (7.9 m/h) led to the increase in butyrate conversion.

Table 6.1 Comparison of the treatment performance of reactors R1 and R2 at a V_{up} of 1.3 m/h and 7.9 m/h, respectively.

Test run	Oleate[a] / Butyrate conc. (mg COD/l)	COD removal[b] (%)		Methane conversion rate[b,c] (mg CH_4-COD/g VS·d)		Difference R1 - R2 (%)[d]
		R1	R2	R1	R2	
I	500 / 4000	95 ± 6	94 ± 4	195 ± 21	143 ± 20	4.7 ± 0.8
II	1000 / 4000	96 ± 9	95 ± 5	210 ± 26	184 ± 18	1.9 ± 0.2
III	2000 / 4000	98 ± 9	98 ± 7	188 ± 23	226 ± 38	5.8 ± 0.6

[a]pulse feed
[b]based on butyrate only
[c]mean value of methane production from butyrate divided by the initial amount of biomass
[d]absolute value of the difference between the change of methane recovery in R1 and that in R2

Interestingly, no significant difference was found between the change of methane recovery following an oleate injection in R1 and that in R2. The difference never exceeded 6% (Table 6.1), indicating that the two reactors made a similar response in the degradation of oleate, regardless of the applied V_{up}. These results differ with a previous study on oleate conversion (Hwu *et al.*, 1997b), where methane recovery ratio in an EGSB reactor operating at a V_{up} of 1 m/h was 1.8 times higher compared to that at 7.2 m/h. However, it should be emphasized here that virtually closed systems were used, consequently a complete biomass retention prevailed in both reactor systems. This probably explains for the similarity in the reactor performance. It therefore is obvious that retention of oleate-degraders which are susceptible to higher upflow velocities is a factor of crucial importance.

Flotation of granular sludge did not occur under conditions of V_{up} as high as 7.9 m/h. We previously speculated that a longer HRT could minimize LCFA adsorption and consequently result in less granular sludge flotation (Chapter 4). The results of the present study confirm this speculation, considering the very long HRT (123 d, cosubstrate flow basis) applied in the present system. However, more small particulates were washed out at the higher V_{up}, as indicated by the higher turbidity in the mixing flask of reactor R2 system. Compared with mesophilic granules, the strength of thermophilic granules is lower (Quarmby and Forster, 1995) and perhaps they have a more loosely open structure (Macario *et al.*, 1991). An increased V_{up} enhances the liquid and particle shear forces, leading to abrasion

or breaking of granules; which results in washout of particulates and/or formation of smaller granules.

Indeed, microscopic examination (Fig. 6.4) upon finishing test run III showed that the average granular size in the reactor operating at 7.9 m/h (R2, average diameter = 1 mm) was half of that of the granules present in R1, operated at 1.3 m/h (average diameter = 2 mm). This might explain the increase in methane conversion rate in reactor R2 (Table 6.1), because as found by Van Lier *et al.* (1996) biomass with smaller particle size exerts a higher butyrate conversion rate, simply because of the better mass transfer.

Contrary to our observations in the present experiments, Arcand *et al.* (1994) found an increase of granule size at higher V_{up} in a fluidized bed reactor fed with glucose. Similar observations to those of Arcand *et al.* were also made by Kato *et al.* (1994) with an EGSB reactor treating ethanol. This discrepancies led us to speculate that the dynamic growth of granules, a dynamic process resulting from the attachment and detachment of micro-organisms, very likely is influenced by the type of substrate fed to a reactor. It indeed is reasonable to expect that the physico-chemical properties of a substrate such as effective diffusivity, molecular size and surface charge, as well as the specific substrate-utilizing bacteria, e.g., acidogens or acetogens, play a key role on the ultimate size of granules. Some of these factors are to be discussed later in this Chapter. However, our results clearly indicate that, in a realistic, "open" reactor system treating oleate-containing wastewaters and operated at high V_{up}, a distinct and possibly serious disintegration of granules may prevail, which ultimately even may lead to a complete loss of biomass.

Effect of the HRT on Oleate Conversion

Fig. 6.5 presents the methane conversion rates in reactor R3 and R4, operated at the HRT of 24 h and 6 h, respectively. An increase in methane production was generally found on the day following the increase of oleate load. During run IV conducted at an OLR_{ole} of 0.5 g COD/l·d and run V at 1.0 g COD/l·d, the levels of methane production rates remained more or less steadily. Apparently both reactors adapted well to oleate at both conditions. However, during run VI at an imposed OLR_{ole} of 2.0 g COD/l·d, after the expected initial increase of the methane production, viz., for a period of 3 days in reactor R3 and 7 days in R4 (Fig.

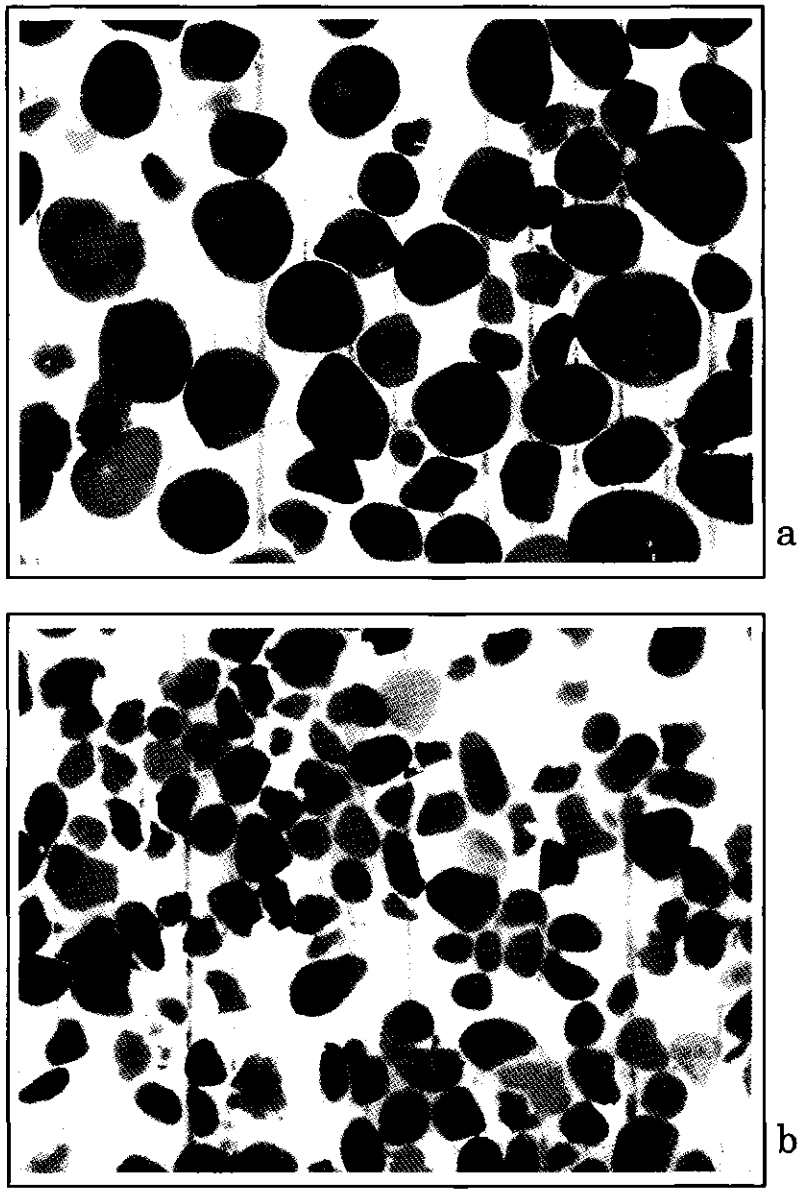


Fig. 6.4 Comparison of the size of granular sludge in (a) reactor R1 at the V_{up} of 1.3 m/h and (b) reactor R2 at the V_{up} of 7.9 m/h. Bar = 2 mm.

6.5), the methane production declined seriously throughout the rest of the test period. This clearly points to the occurrence of oleate inhibition. The slope of declining methane production curve is steeper for reactor R3 (HRT = 24 h) than R4 (HRT = 6 h), because four-fold of the oleate concentration was present in R3. These results are in accordance with earlier results (Rinzema *et al.*, 1994; Hwu *et al.*, 1996b) which also led to the conclusion that the LCFA toxicity is concentration dependent.

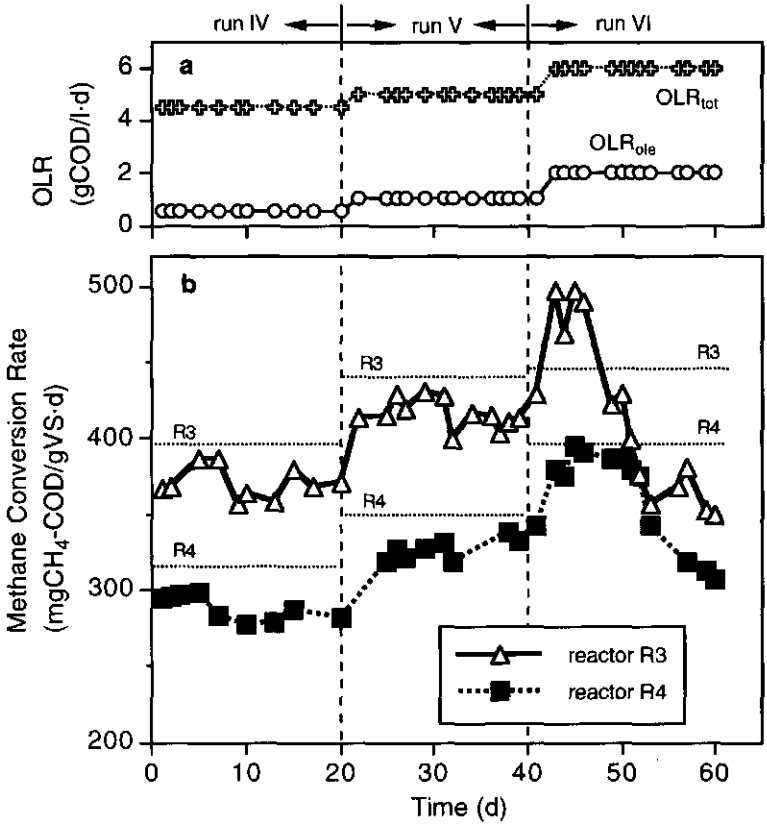


Fig. 6.5 (a) Changes of applied organic loading rates and (b) comparison of methane conversion rates, in reactor R3 and R4 operated at 24 h and 6 h HRT, respectively. Straight dotted lines (·····) indicate the 90% conversion from each OLR_{tot}.

It is worth mentioning that the highest methane conversion rates found in reactor R4 on days 43–46 (Fig. 6.5) significantly exceeded the maximum possible conversion value of 445.5 mg CH_4-COD/g VS·d based on the imposed oleate and butyrate load (assuming a biomass yield of 0.1 mg VS/mg COD). This clearly

indicates that another carbon source (lysed biomass) in addition to butyrate and oleate was involved in the conversion to methane. Moreover, although it seemed that the methane production in R3 ceased declining on day 54, there was no clear sign of recovery at the termination of this experiment on day 60. These observations further postulate that the decrease of methane production was characteristic of oleate toxicity—cytolysis (Hwu and Lettinga, 1997a). Under such circumstances, we can not expect a rapid recovery, e.g., not within one week (see also Chapter 3), because the recovery results from the growth of the micro-organisms who survive the exposure to the toxic compound (Rinzema *et al.*, 1994).

Table 6.2 summarizes the main results of the HRT test runs. Very high COD removal efficiencies were achieved in all cases. The observed high removal efficiencies (96–97%) and low methane recoveries during run VI once again indicate that an adsorptive removal prevailed along with biological conversion. Reactor R3 gave better results both in methane recovery and conversion rates during runs IV and V, suggesting that a longer HRT favors the anaerobic conversion of a feed containing oleate. The highest achievable sludge loading rates found for reactors R3 and R4 under the applied conditions were 0.49 g COD/g VS·d (corresponding to 0.1 g oleate-COD/g VS·d) and 0.39 g COD/g VS·d (0.08 g oleate-COD/g VS·d) in run V, respectively. Assuming that the pseudo-steady state methane recovery established during the start-up period remained unchanged during all test runs, the methane conversion rates originating solely from oleate in runs IV and V amounted to 74 ± 10 and 93 ± 8 in reactor R3, and 50 ± 8 and 83 ± 5 mg CH_4-COD/g VS·d in R4, respectively. Apparently in reactor R3 the oleate degradation rates are higher, which is in accordance with observations of Elefsiniotis and Oldham (1994), who also found that a better methanogenesis of lipids present in primary sludge is obtained in a UASB reactor operated at longer HRT.

Table 6.2 Summary of the treatment performance[a] of reactors R3 and R4 at an HRT of 24 h and 6 h, respectively

Test run	OLR_{ole} / OLR_{tot} (g COD/l·d)	COD removal (%)		Methane conversion rate (mg CH_4-COD/g VS·d)		Methane recovery (%)	
		R3	R4	R3	R4	R3	R4
IV	0.5 / 4.5	94 ± 3	95 ± 2	370 ± 10	289 ± 8	94 ± 4	88 ± 4
V	1.0 / 5.0	95 ± 2	96 ± 0	415 ± 10	327 ± 7	87 ± 4	83 ± 5
VI	2.0 / 6.0	96 ± 2	97 ± 0	415 ± 51	361 ± 30	71 ± 10	76 ± 7

[a]calculations of results based on influent total COD

Flotation of granular sludge did not prevail in any of the reactors. On the other hand, washout of particulates with diameters ranging between 50–100 μm occurred in both reactors already at the V_{up} as low as 1 m/h. Fig. 6.6 presents the typical appearance of the washed out sludge particulates collected from the settler (Fig. 6.2) of reactor R3. These particulates were found to have a higher oleate conversion capacity than the granules present in the reactor (see the next Section). The mean washout rate during test runs amounted to 0.03 and 0.08 g VS/d, respectively, in reactor R3 and R4. These different rates reflect the effect of the HRT on the sludge retention, as the dilution factor in R4 was four-fold of that in R3. These washout rates would be much higher if the reactors were operated by using typical EGSB flow regime, i.e., V_{up} > 4 m/h. In contrast to our results, Arcand *et al.* (1994) pointed out that V_{up} has little effect on the specific washout rate of biomass particulates. Despite the installation of a filter on top of their reactor, which probably contributed to biomass hold-up, very likely the use of glucose as a feed might give rise to the different results. Hence, in addition to operational parameters such as HRT, V_{up} or OLR, also the hydrophobicity of the specific substrate-utilizing biomass (Daffonchio *et al.*, 1995) and the liquid surface tension in reactors may play a role in biomass washout.

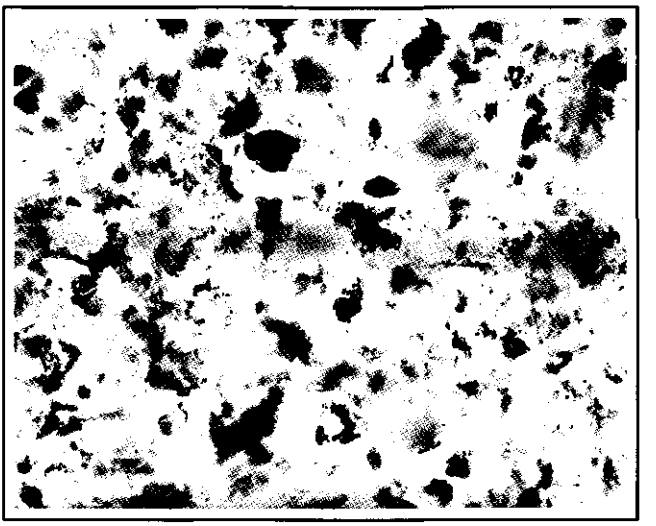


Fig. 6.6 Appearance of washed out sludge of reactor R3 operated at 24 h HRT and 1 m/h V_{up}. Bar = 500 μm.

One of the conceptual EGSB reactor operational parameters, a short HRT (< 10 h), was found to be less favorable for efficient oleate treatment. It should also

be noted that the highest oleate conversion rate found in R3 (93 mg CH_4-COD/g VS·d) is far below that (124 mg CH_4-COD/g VS·d) of specific oleate conversion obtained in batch tests (Chapter 3). The optimization of a high-rate reactor treatment system of oleate towards (and exceeding) these high conversion rates is presented in "Effect of Recirculation..." in this Chapter.

Oleate Conversion Capacity of Washed Out Biomass

The average diameter of the dispersed sludge was 50 μm. Thus the order of size of the sludges was: granular >> washed out > dispersed. Fig. 6.7 compares the methane production rates from oleate (95% purity) found for the three types of sludges. All sludges were able to convert oleate into methane and the same amount of methane was produced upon termination of the digestion. Granular sludge, however, reached the maximum methane production only after 110 h, whilst the dispersed and washed out sludge reached this plateau within a period of 60 h.

Moreover, the time required for each sludge to produce the same amount of methane was significantly different, e.g., 65 h for granular, 49 h for dispersed and 37 h for washed out to produce 4 ml methane. These different time periods imply the order of the oleate conversion rates: granular < dispersed < washed out. It is unquestionable that the granular sludge attained the lowest rate because sludge with bigger particle size has slower internal mass transfer, as was found by Van Lier (1996) that granular sludge achieves lower butyrate and acetate conversion rates than does its dispersed homologues. Very interesting is that the order of the conversion rates of the dispersed and the washed out is contrary to the effect of the size on mass transfer. These results suggest that more oleate-oxidizers were present in the washed out biomass, thus virtually attributed to the faster methane production rate since β-oxidation of oleate is the rate-limiting step (Novak and Carlson, 1970). Indeed, the specific oleate conversion rate of the washed out biomass, 129 mg CH_4-COD/g VS·d, exceeds that of the sludge granules, 84 mg CH_4-COD/g VS·d, and that of the dispersed, 98 mg CH_4-COD/g VS·d. Obviously, washout of the highly active biomass, leaving the reactor with the effluent, will diminish the reactor performance. Thus, besides washout of granules, also washout of small particulates should be considered during process evaluation.

In addition to reactor hydrodynamics and specific biomass growth rate, also surface thermodynamics triggers washout of biomass in suspended/free form.

Daffonchio *et al.* (1995) reported that most acetogens can be considered rather hydrophobic. LCFA act as surfactants at the pH ranges prevailing in the bioreactors (e.g., pH 6–8), viz., they lower the liquid surface tension; which disfavors aggregation of rather hydrophobic bacteria. Sam-Soon *et al.* (1991) found that no pelletisation occurred during the treatment of oleate in a UASB reactor. As a result, the presence of LCFA can hamper suspended LCFA-oxidizers (acetogens) to attach onto granules or prevent particulates to form granules, thus making them susceptible to wash out from reactors. Hence, abruptly applying high-rate parameters, which could be accompanied by high shear forces, to a reactor in which LCFA-oxidizing acetogens are required might aggravate their washout and thus deteriorate the reactor performance.

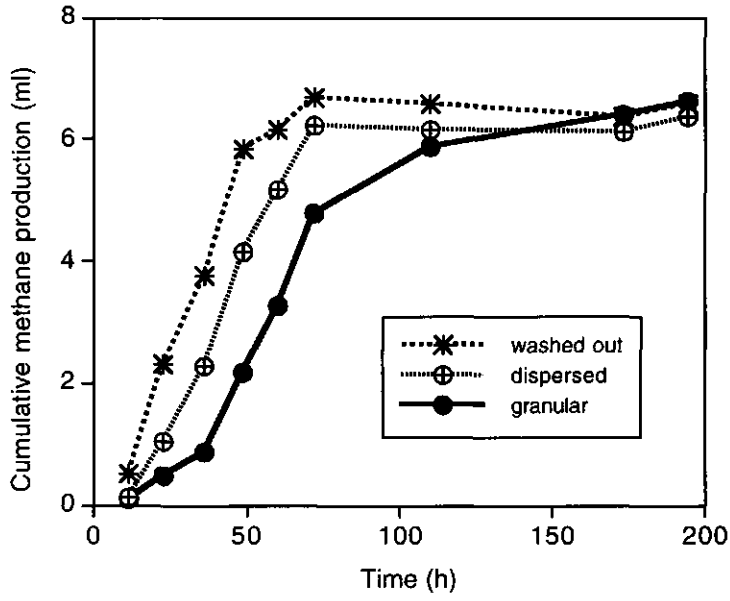


Fig. 6.7 Cumulative methane production from oleate (0.2 g COD/l) found in batch assays for the 3 different types of sludge originated in the EGSB reactor—R3.

This explanation is congruent with the data of the reactor performance above-described. The washed out biomass tested was collected from a reactor operated at an HRT of 24 h (R3). It can be expected that even more β-oxidizers were washed out from the reactor operated at a lower HRT (6 h, R4), which then would explain its lower treatment performance. Moreover, as found for the reactors R1 and R2 after they were modified to virtually closed systems, a complete

biomass retention did not result in an inferior treatment capacity, even when operating at an elevated V_{up} as high as 7.9 m/h.

Effect of Recirculation of Washed Out Biomass

The settling efficiency of the first settler (Fig. 6.3) was very satisfactory as any visible accumulation of washed out biomass did not occur in the second settler. Therefore we assumed that washout of free β-oxidizers had little or no effect on performance of the system investigated. Table 6.3 summarizes the experimental results of the various reactor runs with the recirculation of washed out biomass. The relatively poor methane recovery found in run VII (indicated in Figs. 6.8 and 6.9 as well) probably can be due to decay of LCFA-oxidizers during the two-month's start-up period, when the system was fed merely with glucose. In the last 3 days of run VII, methane recovery reached 80%, indicating a good stabilization in reactor R5. The methane recoveries obtained in runs VIII (95%) and IX (92%) are to date the highest achieved under the applied conditions. Let us assume an overestimated, 100% methane recovery from the easier substrate, glucose, during the test runs; the minimum specific methane conversion rates from the added oleate could be estimated as 156 ± 17 and 304 ± 48 mg CH_4-COD/g VS·d in runs VIII and IX, respectively. These values are much higher than those found in reactor R3 (93 mg CH_4-COD/g VS·d, run V) and even in batch tests (124 mg CH_4-COD/g VS·d, Chapter 3). It therefore is obvious that the novel reactor system design indeed allowed the development and retention of highly concentrated LCFA-oxidizers per unit VS.

Table 6.3 Treatment performance[a] of reactor R5 with washed out biomass recirculation

Test run	OLR_{ole} / OLR_{tot} (g COD/l·d)	COD removal (%)	Methane conversion rate (mg CH_4-COD/g VS·d)	Methane recovery (%)
VII	0.5 / 4.5	97 ± 3	254 ± 17	76 ± 5
VIII	2.0 / 6.0	95 ± 1	448 ± 17	95 ± 2
IX	4.0 / 8.0	97 ± 0	597 ± 48	92 ± 7

[a]calculation based on influent total COD

Fig. 6.8 contrasts the methane conversion rates established in reactors R3, R4 and R5. Compared with R3 and R4, which failed at an OLR_{ole} of 2.0 g COD/l·d (corresponding to an oleate concentration of 2 g COD/l) due to oleate toxicity (run VI in Fig. 6.5), reactor R5 mineralized oleate to methane without intoxication, even at OLR_{ole} as high as 4.0 g COD/l·(corresponding to an oleate concentration of 4 mg

COD/l and an OLR_{tot} of 8 g COD/l·d). The contrast between these different situations becomes even bigger when the conversion rates are plotted against their corresponding imposed sludge loading rates (Fig. 6.9). Reactor R5 reached the highest sludge loading rate at 0.58 g COD/g VS·d (corresponding to 0.29 g oleate-COD/g VS·d) with a methane conversion rate of 597 ± 48 mg CH_4-COD/g VS·d. Moreover, in spite of the poor performance in run VII, R5 attained a 18% higher conversion rate than R4 based on the same sludge loading rate. These better performance results can be attributed to the positive effect of recirculation of washed out biomass which allowed the retention of highly concentrated oleate-oxidizers in the reactor.

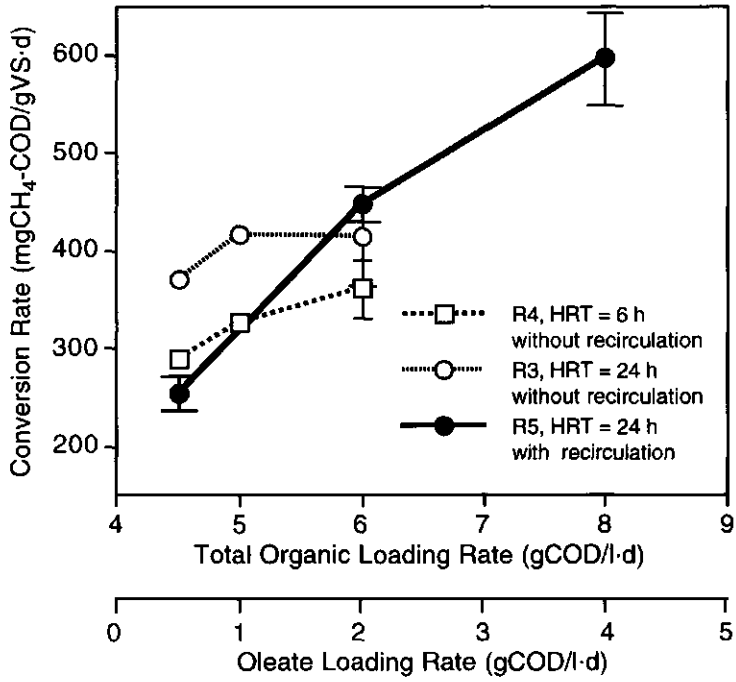


Fig. 6.8 Comparison of methane conversion rates under various organic loading rates in continuous flow reactors R3 (HRT = 24 h) and R4 (HRT = 6 h) without recirculation of washed out biomass, and R5 (HRT = 24 h) with recirculation of washed out biomass). Each reactor was operated at a V_{up} of 1 m/h.

In contrast to LCFA toxicity, which is toxicant-concentration dependent (Rinzema *et al.*, 1994; Hwu *et al.*, 1996b) and non-adaptable (Angelidaki and Ahring, 1992; Rinzema *et al.*, 1994), the results of the present experiment shown in Fig. 6.9 clearly indicate that oleate/LCFA biodegradation in high-rate reactors depends on the substrate to specific biomass (oleate-/β-oxidizers) ratio, and thus is

adaptable to higher oleate (LCFA) concentrations and/or loading rates if an appropriate reactor concept, e.g., the reactor design with washout recirculation, is applied.

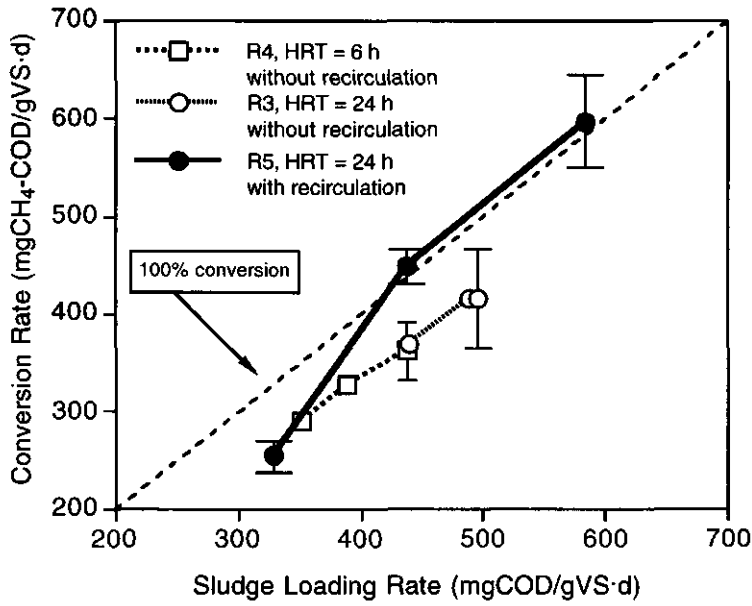


Fig. 6.9 Comparison of methane conversion rates under various sludge loading rates in reactors R3, R4 and R5. The initial amount of VSS is used in the calculation for R5. See Fig. 6.8 for more details.

To our knowledge, both the methane conversion rate (600 mg CH_4-COD/g VS·d) and COD removal efficiency (97%) achieved in run IX are to date the highest reported for the anaerobic treatment of typical oleate-containing wastewaters (Table 6.4). It has to be noted that fatty matter is considerably adsorptive, thus the COD removal presented in Table 6.4 does not necessarily imply biodegradation (Chapter 4; Hwu *et al.*, 1996b). Consequently comparison of the performance with other investigations should, preferably, be made on the basis of unit of biomass. However, in many of the references either data about the biogas production or the amount of inoculum was not present, thus hampering adequate comparison.

Totally fifteen conditions by means of EGSB reactors were investigated on thermophilic treatment of oleate. Under none of these conditions any flotation of sludge granules, reported as one of the most serious problems in anaerobic treatment of LCFA-containing wastewaters (Samson *et al.*, 1985; Rinzema *et al.*, 1989; Hawkes *et al.*, 1995; Hwu *et al.*, 1996b), was found. Moreover, it was found

Table 6.4 Comparison of anaerobic treatment of wastewaters containing oleic acid

Wastewater type[a]	Reactor type[b]	Reactor volume	Temp. (°C)	V_{up} (m/h)	HRT (d)	OLR (gCOD/l d)	COD$_r$[d] (%)	Methane conv. rate[g]	Ref.
slaughter-house	FB	1 l	35	—	0.3	27	94	—	(1)
slaughter-house	UASB	33.5 l	30	0.5	0.09	15	82	150*[h]	(2)
wool scouring	UAF + UFM	4.5 m^3	37	1	—	10	75[e]	—	(3)
			53	1	—	20	70[e]	—	
wool scouring	SCSTR	2 l	35	—	2.8	9.9	59	—	(4)
dairy	UASB	400 m^3	30	0.2*	1.2*	3	82	35*[i]	(5)
	DSFF	400 m^3	30	—	1.6*	5	66	43*[i]	
ice-cream	UASB	5 m^3	amb[c]	2–3*	1.6*	2.2	49	—	(6)
	FB	0.5 m^3	amb[c]	16-25*	1.5*	4.2	56	—	
edible oil	UASB	49 l	35	—	2	3.6	39	—	(7)
	AF	49 l	35	—	2	1.8	56	—	
OME	fed-batch	3 l	37	—	8	1.3	75	120*	(8)
OME	UASB	2 m^3	35	0.02	10.7	11	83	—	(9)
OME	CSTR	1.12 l	35	—	15	3.5*	85	330*	(10)
	CSTR	1.12 l	55	—	15	3.5*	90	410*	
POME	hUASB	4.2 l	35	0.04*	3.5	16.2	92	120*	(11)
oleate	batch	—	35	—	40	—	84[f]	—	(12)
oleate	UASB	9 l	30	0.08*	0.6*	4.2	65	—	(13)
oleate	EGSB	4.4 l	55	1	1	8	97	600[j]	(14)

—not reported or not applicable

*values transformed from the original data present in respective reference

[a]abbreviations: OME, olive oil mill effluent; POME, palm oil mill effluent, best performance in POME treatments is said in comparison with eight others

[b]*abbreviations:* FB, *fluidized bed;* UASB, *upflow anaerobic sludge bed;* UAF + UFM, *upflow anaerobic* filter with an additional ultrafiltration membrane in series; SCSTR, semi-continuous stirred tank reactor; DSFF, downflow stationary fixed film; AF, anaerobic filter; CSTR, continuous stirred tank reactor; hUASB, hybrid UASB, with a filter on the top; EGSB, expanded granular sludge bed

[c]ambient temperature

[d]COD removal efficiency; otherwise stated, on COD basis

[e]based on total oxygen demand (TOD)

[f]on LCFA basis

[g]methane conversion rate, otherwise stated, in terms of mg CH_4-COD/g VSS·d

[h]biomass unit: total suspended solids (TSS)

[i]biomass unit: suspended solids (SS)

[j]biomass unit: VS

Ref.: (1) Borja *et al.*, 1995a; (2) Sayed *et al.*, 1987; (3) Hogetsu *et al.*, 1992; (4) Cail *et al.*, 1986; (5) Samson *et al.*, 1985; (6) Hawkes *et al.*, 1995; (7) Eroglu *et al.*, 1990; (8) Tsonis and Grigoropoulos, 1993; (9) Dalis *et al.*, 1996; (10) Borja *et al.*, 1995b; (11) Borja *et al.*, 1996; (12) Cánovas-Diaz *et al.*, 1992; (13) Sam-Soon *et al.*, 1991; (14) this study

that by applying recirculation of washed out biomass, oleate concentrations up to 4000 mg COD/l (4.5 mM) did not impede reactor performance although oleate IC_{50} was found as low as 0.7 mM (Hwu and Lettinga, 1997a).

However, we also observed that any generation of sludge granules did not occur under all test conditions. This agrees well with the finding of Sam-Soon *et al.* (1991), who concluded from their research that pelletisation will not occur in the anaerobic digestion of oleate. They observed much debris prevailing in sludge bed but not discharging to blanket zone. By contrast, due to a higher V_{up} (cf. Table 6.4), we found a great deal of black debris floating above the sludge bed. Besides, we witnessed a decrease in granules diameters. The granular sludge bed height gradually dropped while the blanket zone became more and more darker. The last phenomenon was less significant during the HRT experiment, which can be attributed to washout of (small) debris in succession. We previously considered the disintegration of granules as one of the causes for the treatment deterioration (Chapter 5; Hwu *et al.*, 1997b). To our present view, washout of the small debris (particulates) rather than the disintegration of granules is the main cause behind. This also explains well the less satisfactory performance of a thermophilic reactor in that previous study: due to the weaker strength of thermophilic granules (Quarmby and Forster, 1995), there were more debris formed and washed out in the long run.

Both the tendency of washout (this study) and the rather slow growth rates (0.3 d^{-1}) of thermophilic LCFA-oxidizers (Angelidaki and Ahring, 1995) impose the need to develop a proper system which enables a highly efficient in-reactor accumulation of specific biomass (in relation to the rate-limiting step) so that high loading rates can be applied. The recirculation system used in this study allowed a greater accumulation and longer retention of LCFA-degraders in the EGSB/high-rate reactor system. This greatly enhanced the treatability of oleate. The system allows the application of high-rate hydrodynamics for this type of wastewaters, i.e., a short HRT, thus reducing reactor volume and space requirement. Practical application of the recirculation principle warrants further research towards the development of efficient particulate retention systems, e.g., an external settler or membrane filtration unit.

CONCLUSIONS

The impact of reactor hydrodynamics on anaerobic treatment of oleate was systematically investigated. The shorter HRT and the higher V_{up} was found to negatively affect the treatment to lower methane production as well as to more washout of biomass particulates. The later was identified to be the main cause of the low treatment efficiencies, as highly concentrated oleate-degraders were prevailed in the washed out biomass.

Recirculation of the biomass bearing the high oleate-degrading capability to the reactor dramatically enhanced the treatment efficiencies. In this study the up-to-date highest performance among reactor systems treating LCFA-containing wastewaters was achieved at the HRT of 24 h and the V_{up} of 1 m/h. Nonetheless, the room for further improvement by application of high-rate hydrodynamic parameters is remained, provided that the fine biomass can be retained in reactor systems.

ACKNOWLEDGMENT

I would like to express my appreciation for the views and comments of Dr. Piet N. L. Lens on the two papers published in series which are the backbone of this Chapter.

REFERENCES

Ahring, B.K. and Westermann, P. (1987). Thermophilic anaerobic degradation of butyrate by a butyrate-utilizing bacterium in coculture and triculture with methanogenic bacteria. *Appl. Environ. Microbiol.*, **53**, 429–433.

Angelidaki, I. and Ahring, B.K. (1992). Effects of free long-chain fatty acids on thermophilic anaerobic digestion. *Appl. Microbiol. Biotechnol.*, **37**, 808–812.

Angelidaki, I. and Ahring, B.K. (1995). Establishment and characterization of anaerobic thermophilic (55°C) enrichment culture degrading long-chain fatty acids. *Appl. Environ. Microbiol.*, **61**, 2442–2445.

APHA, AWWA and WEF. (1992). *Standard Methods for the Examination of Water and Wastewater.* 18th edn., American Public Health Association, Washington, DC, pp. 2-54–2-57, 5-9–5-10.

Arcand, Y., Guiot, S.R., Desrochers, M. and Chavarie, C. (1994). Impact of the reactor hydrodynamics and organic loading on the size and activity of anaerobic granules. *Chem. Eng. J.*, **56**, B23–B35.

Asther, M. and Corrieu, G. (1987). Effect of Tween 80 and oleic acid on liginase production by *Phanerochaete chrysosporium* INA-12. *Enzyme Microb. Technol.*, **9**, 245–249.

Borja, R., Banks, C.J. and Wang, Z. (1995a). Effect of organic loading rate on anaerobic treatment of slaughterhouse wastewater in a fluidised bed reactor. *Bioresource Technol.*, **52**, 157–162.

Borja, R., Martín, A., Banks, C.J., Alonso, V. and Chica, A. (1995b). A kinetic study of anaerobic digestion of olive mill wastewater at mesophilic and thermophilic temperatures. *Environ. Pollut.*, **88**, 13–18.

Borja, R., Banks, C.J., Khalfaoui, B. and Martín, A. (1996). Performance evaluation of an anaerobic hybrid digester treating palm oil mill effluent. *J. Environ. Sci. Health*, **A31**, 1379–1393.

Cail, R.G., Barford, J.P. and Lichacz, R. (1986). Anaerobic digestion of wool scouring wastewater in a digester operated semi-continuously for biomass retention. *Agric. Wastes*, **18**, 27–38.

Cánovas-Diaz, M., Sánchez-Roig, M.J. and Iborra, J.L. (1992). Myristic and oleic acid degradation by an acclimated anaerobic consortia: synergistic behaviour. In: *Proc. 6th EC Conf. Biomass for Energy, Industry and Environment,* Grassi, G., Collina, A. and Zibetta, H. (Eds.), Athens, Greece, Apr. 1991, Elsevier, UK, pp. 580–584.

Daffonchio, D., Thaveesri, J. and Verstraete, W. (1995). Contact angle measurement and cell hydrophobicity of granular sludge from upflow anaerobic sludge bed reactors. *Appl. Environ. Microbiol.*, **61**, 3676–3680.

Dalis, D., Anagnostidis, K., Lopez, A., Letsiou, I. and Hartmann, L. (1996). Anaerobic digestion of total raw olive-oil wastewater in a two-stage pilot-plant (up-flow and fixed bed bioreactors). *Bioresource Technol.*, **57**, 237–243.

Elefsiniotis, P. and Oldham, W.K. (1994). Effect of HRT on acidogenic digestion of primary sludge. *J. Environ. Eng. ASCE*, **120**, 645–660.

Eroglu, V., Ozturk, I., San, H.A. and Demir, I. (1990). Comparative evaluation of treatment alternatives for wastewaters from an edible oil refining industry. *Water Sci. Technol.*, **22**(9), 225–234.

Gjaltema, A., Tijhuis, L., van Loosdrecht, M.C.M. and Heijnen, J.J. (1995). Detachment of biomass from nongrowing spherical biofilms in airlift reactors. *Biotechnol. Bioeng.*, **46**, 258–269.

Habets, L.H.A. (1997). Personal communication. Paques B.V., PO Box 52, 8560 AB Balk, The Netherlands.

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

Hawkes, F.R., Donnelly, T. and Anderson, G.K. (1995). Comparative performance of anaerobic digesters operating on ice-cream wastewater. *Water Res.*, **29**, 525–533.

Hogetsu, A., Ishikawa, T., Yoshikawa, M., Tanabe, T., Yudate, S. and Sawada, J. (1992). High rate anaerobic digestion of wool scouring wastewater in a digester combined with membrane filter. *Water Sci. Technol.*, **25**(7), 341–350.

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

Hwu, C.-S., Tseng, S.-K., Yuan, C.-Y., Kulik, Z. and Lettinga, G. (1996b). Adsorption of long-chain fatty acids onto granular sludges and its effect on upflow anaerobic sludge bed reactor operation. In: *Proc. the First IAWQ Specialized Conf. on Adsorption in Water Environment and Treatment Processes*, Nov. 1996, Shirahama, Wakayama, Japan, pp. 91–98.

Hwu, C.-S. and Lettinga, G. (1997a). Acute toxicity of oleate to acetate-utilizing methanogens in mesophilic and thermophilic anaerobic sludges. *Enzyme Microb. Technol.*, **21**, in press.

Hwu, C.-S., van Lier, J.B. and Lettinga, G. (1997b). Physicochemical and biological performance of expanded granular sludge bed reactors treating long-chain fatty acids. *Process Biochem.*, **32**, in press.

Kato. M.T., Field, J.A., Versteeg, P. and Lettinga, G. (1994). Feasibility of expanded granular sludge bed reactors for the anaerobic treatment of low strength soluble wastewaters. *Biotechnol. Bioeng.*, **44**, 469–479.

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.

Lettinga, G., Hulshoff Pol, L.W., Hobma, S.W., Grin, P., de Jong, P., Roersma, R. and Ijspert, P. (1983). The use of a floating settling granular sludge bed reactor in anaerobic treatment. In: *Proc. Symp. on Anaerobic Waste Water Treatment*, Noordwijkerhout, The Netherlands, pp. 411–429.

Lettinga, G. and Hulshoff Pol, L.W. (1991). UASB-process design for various types of wastewaters. *Water Sci. Technol.*, **24**(8), 87–107.

Lettinga, G. (1995). Anaerobic digestion and wastewater treatment systems. *Antonie van Leeuwenhoek*, **67**, 3–28.

Lettinga, G. (1996). Sustainable integrated biological wastewater treatment. *Water Sci. Technol.*, **33**(3), 85–98.

Macario, A.J.L., Visser, F.A., van Lier, J.B. and Conway de Macario, E. (1991). Topography of methanogenic subpopulations in a microbial consortium adapting to thermophilic conditions. *J. Gen. Microbiol.*, **137**, 2179–2189.

Novak, J.T. and Carlson, D.A. (1970). The kinetics of anaerobic long chain fatty acid degradation. *J. Water Pollut. Control Fed.*, **42**, 1932–1943.

Quarmby, J. and Forster, C.F. (1995). A comparative study of the structure of thermophilic and mesophilic anaerobic granules. *Enzyme Microb. Technol.*, **17**, 493–498.

Quéméneur, M. and Marty, Y. (1994). Fatty acids and sterols in domestic wastewaters. *Water Res.*, **28**, 1217–1226.

Rinzema, A., Alphenaar, A. and Lettinga, G. (1989). The effect of lauric acid shock loads on the biological and physical performance of granular sludge in UASB reactors digesting acetate. *J. Chem. Technol. Biotechnol.*, **46**, 257–266.

Rinzema, A., Alphenaar, A. and Lettinga, G. (1993). Anaerobic digestion of long-chain fatty acids in UASB and expanded granular sludge bed reactors. *Process Biochem.*, **28**, 527–537.

Rinzema, A., Boone, M., van Knippenberg, K. and Lettinga, G. (1994). Bactericidal effect of long chain fatty acids in anaerobic digestion. *Water Environ. Res.*, **66**, 40–49.

Samson, R., van den Berg, B., Peters, R. and Hade, C. (1985). Dairy waste treatment using industrial-scale fixed-film and upflow sludge bed anaerobic digesters: design and start-up experience. In: *Proc. 39th Purdue Industrial Waste Conference*, Bell, J.M. (Ed.), Butterworth Publ., Boston, pp. 235–241.

Sam-Soon, P.A.L.N.S., Loewenthal, R.E., Wentzel, M.C. and Marais, G.vR. (1991). A long-chain fatty acid, oleate, as sole substrate in upflow anaerobic sludge bed (UASB) reactor systems. *Water SA*, **17**, 31–36.

Sayed, S.K.I., van Campen, L. and Lettinga, G. (1987). Anaerobic treatment of slaughterhouse waste using a granular sludge UASB reactor. *Biol. Wastes*, **21**, 11–28.

Shieh, W.K. and Hsu, Y. (1996). Biomass loss from an anaerobic fluidized bed reactor. *Water Res.*, **30**, 1253–1257.

Thies, E., Jenkins, T. and Stutzenberger, F. (1994). Effects of the detergent Tween 80 on *Thermomonospora curvata*. *World J. Microbiol. Biotechnol.*, **10**, 657–663.

Tsonis, S.P. and Grigoropoulos, S.G. (1993). Anaerobic treatment of olive oil mill wastewater. *Water Sci. Technol.*, **28**(2), 35–44.

Van Haandel, A.C. and Lettinga, G. (1994). *Anaerobic Sewage Treatment: A Practical Guide for Regions with a Hot Climate*. John Wiley and Sons, Chichester, Ch. 2, pp. 33–62.

Van Lier, J.B., Hulsbeek, J., Stams, A.J.M. and Lettinga, G. (1993). Temperature susceptibility of thermophilic methanogenic sludge: implications for reactor start-up and operation. *Bioresource Technol.*, **43**, 227–235.

Van Lier, J.B., Sanz Martin, J.L. and Lettinga, G. (1996). Effect of temperature on the anaerobic thermophilic conversion of volatile fatty acids by dispersed and granular sludge. *Water Res.*, **30**, 199–207.

Viswanathan, C.V., Meera Bai, B. and Pillai, S.C. (1962). Fatty matter in aerobic and anaerobic sewage sludges. *J. Water Pollut. Control Fed.*, **34**, 189–194.

Yang, S.T. and Okos, M.R. (1987). Kinetic study and mathematical modeling of methanogenesis of acetate using pure cultures of methanogens. *Biotechnol. Bioeng.*, **30**, 661–667.

7

State-of-the-Art Anaerobic Treatment of Wastewaters Containing Long-Chain Fatty Acids: Summary

7

State-of-the-Art Anaerobic Treatment of Wastewaters Containing Long-Chain Fatty Acids: Summary

INTRODUCTION

Lipids are one of the major organic pollutants in municipal and industrial wastewaters. Although domestic sewage typically contains about 40–100 mg/l lipids (Forster, 1992; Quéméneur and Marty, 1994), it is industrial wastewaters that are of greater concern when considering the higher lipid concentrations in the discharged effluents. Typical industries that generate lipids-containing wastewaters are dairy, edible oil and fat refinery, slaughterhouse and meat-processing, rendering and wool scouring.

In anaerobic wastewater treatment, lipids are readily biodegradable. However, the practical problems arisen in anaerobic treatment of lipids mainly are due to (i) inhibition of the methanogens and acetogens by long-chain fatty acids (LCFA), and (ii) washout/flotation of the biomass. These two problems manifest themselves particularly in the high-rate treatment systems. Among these systems, unsatisfactory treatment results in full-scale upflow anaerobic sludge bed (UASB) reactors and in lab-scale expanded granular sludge bed (EGSB) reactors are frequently encountered.

This thesis is directed to find solutions for the problems and, consequently, to guarantee the efficiency and reliability of the two above-mentioned high-rate reactor systems because the UASB presently are and the EGSB potentially will

become the most widely applied anaerobic wastewater treatment processes. In this respect, the research in this thesis was focused on the cytotoxicity, biosorption and biodegradation of LCFA. In addition to the achievement of the up-to-date highest loading rate, several novel findings from our investigations relevant to the insight of the complicated relationships between toxicity, sorption and degradation may facilitate the future treatment of wastewaters contaminated with LCFA.

Based on the experimental results obtained in this study, we recommend five methods as solutions for the practical problems and as the state-of-the-art techniques for the ultimate treatment. The five methods that are to be described in this Chapter are listed as follows:

Use of granular sludge as inoculum;

Acclimation of sludge to long-chain fatty acids;

Application of thermophilic conditions;

Prevention of excessive sorption; and

Recirculation of washed out biomass.

USE OF GRANULAR SLUDGE AS INOCULUM

Comparative toxicity of oleate to seven anaerobic sludges from various origins was studied (Chapter 2). The oleate toxicity on methanogenic activity was found to be rather closely correlated to the specific surface area of sludge than to the sludge origin and methanogenic activity, even though acclimated sludge (pre-exposed to LCFA) was the more active and the less susceptible. The suspended and flocculent sludges are much more susceptible to the toxicity than are the granular sludges. The oleate IC_{50} levels derived for the granular sludges were 3–13 times higher than those for the suspended sludges at 40°C (Chapter 2) and 2 times higher than that for the flocculent sludge at 55°C (Chapter 3). Since the recovery of methanogenic activity for granular sludge after the occurrence of inhibition will take time periods from one week to more than one month (Chapter 3), it is reasonable to expect that the time required for suspended/flocculent sludge will even be longer.

Apart from the lower susceptibility to oleate found for granular sludge in the present research, the high biomass densities in the granules minimize the distances between bacteria and maximize interspecies transfer of acetate and hydrogen between syntrophic fatty acid degraders and methanogens (Pauss *et al.*, 1990; Thiele, *et al.*, 1990). This of course favors the syntrophic degradation of LCFA.

Regarding the full-scale application, the availability of sufficient amount of granular sludge has to be taken into consideration. To date, over 930 full-scale UASB reactors have been built (Habets, 1997) and more are under construction. This means that granular sludge will become available in near future in increasing amount. The use of granular sludges as inocula for start-up of reactors treating LCFA-wastewaters is, therefore, an appropriate strategy.

ACCLIMATION OF SLUDGE TO LONG-CHAIN FATTY ACIDS

The expected synergistic toxic effect that should be exerted by the LCFA-mixture (50% oleate, 35% palmitate and 15% stearate) was not observed for the granular sludge well acclimated to slaughterhouse wastewater. On the contrary a longer lag period of methane production in the non-acclimated granular sludge occurred due to oleate inhibition (Chapter 4). Moreover, in a batch test the sludge pre-exposed to LCFA (82% oleate) showed 2 times higher oleate degradation rate than did the non-exposed sludge. In continuous flow experiments, the reactor inoculated with the non-exposed sludge fails already at the influent concentration of 500 mg LCFA- (82% oleate-) COD/l while the reactor with pre-exposed sludge can successfully treat 4000 mg LCFA- (82% oleate-) COD/l (Hwu *et al.*, 1997).

The requirement of acclimation may turn into a bottleneck of the LCFA-wastewater treatment because the sources of LCFA-/lipids-adapted granular sludge are currently limited (Hulshoff Pol, 1997) and the growth rate of LCFA-degraders is as slow as 0.3 d^{-1} (Angelidaki and Ahring, 1995). Hence, for full-scale treatment the introduction of LCFA-wastewaters to reactors should start at low concentrations and allow acclimation and retention (see below) of the bacteria capable of LCFA degradation.

APPLICATION OF THERMOPHILIC CONDITIONS

The oleate degradation rate and IC_{50} level of an LCFA-exposed granular sludge were compared at thermophilic (55°C) and mesophilic (30°C) conditions (Chapter 3). Although oleate IC_{50} levels obtained at mesophilic (2.8 mM) was 4-fold higher than that at thermophilic conditions (0.7 mM), the oleate degradation rate at thermophilic (124 mg oleate-COD/g VS·d) was also 4-fold higher than that at mesophilic conditions (33 mg oleate-COD/g VS·d). This means the thermophiles are more susceptible to oleate toxicity but, on the other hand, their growth rate is much higher and thus would rapidly recover the lost activity due to LCFA-inhibition by a rapid increase of bacterial population.

When exposed to a relatively high sludge loading, i.e., 1.2 g oleate-COD/g VS, the thermophilic sludge took a 5-day time period to revive the methanogenic activity similar to the control while the mesophilic sludge took 28 days to achieve the same activity (Chapter 3). These results provide an important implication for the LCFA-wastewater treatment in practices, viz., thermophilic reactors can sooner be re-operated after shutdown by shock loadings.

PREVENTION OF EXCESSIVE SORPTION

Sorption of LCFA onto the surface of sludge granules leads to sludge flotation and inhibition (Chapter 4). The higher LCFA concentrations result in the higher sorption rates as well as larger sorption amounts, therefore induces the more serious flotation and inhibition. In batch tests, we estimated that an increase of 45 mg LCFA/g TS in adsorption amount can cause a 10% decrease in methane production rate. In a UASB reactor, the flotation of granular sludge started at the sludge loading rates exceeding 0.09 g LCFA-COD/g VSS·d, while the complete flotation occurred at the loading rates exceeding 0.2 g LCFA-COD/g VSS·d.

Flotation of both sludge granules and LCFA clusters occurred when LCFA were treated as the sole substrate in EGSB reactors operated at hydraulic retention times (HRT) < 6 h and liquid superficial upflow velocities (V_{up}) > 3 m/h (Chapter 5). Although a COD removal efficiency of 73% could be attained, the highest methane recovery achieved was below 15% (corresponding to a low conversion rate of 1.6 mg LCFA-COD/g VS·d). When the HRT was prolonged to

24 h (V_{up} = 1, 4 or 7 m/h) and glucose or butyrate was supplied as cosubstrate (Chapters 5 and 6), the flotation became insignificant and LCFA degradation increased significantly. Herein, the most satisfactory results obtained have been 95% COD removal and 87% methane recovery (corresponding to the conversion rate of 93 mg LCFA-COD/g VS·d) in a reactor operated at HRT = 24 h and V_{up} = 1 m/h (Chapter 6).

It therefore is clear that the prevention of excessive (negative) LCFA adsorption onto granules' surface requires enhancement of LCFA biodegradation. This can be achieved by applying a longer substrate-biomass contact time, i.e., a prolonged HRT; provided that the present reactor system is in common use.

RECIRCULATION OF WASHED OUT BIOMASS

Although the conversion rate in continuous-flow reactors reached up to 93 mg LCFA-COD/g VS·d (Chapter 6), this rate was still lower than that obtained in batch experiments, i.e., 124 mg oleate-COD/g VS·d (Chapter 3). The difference between these two rates would be even more significant if the LCFA-acclimated sludge inoculated in the continuous-flow reactors (Chapter 6) also would have been used in the batch experiments (Chapter 3). Since almost all granular sludge was retained in the continuous reactor, the observed difference draws a question: should any other factors influenced by reactor hydrodynamic parameters contribute to the difference?

Based on the observations in Chapter 5 that the higher V_{up} results in the lower LCFA conversion, we first speculated that the breakage of granules found in a higher V_{up} deteriorates the syntrophic degradation of LCFA. We therefore conducted experiments using two reactors operating at the V_{up} of 1 and 8 m/h (Chapter 6). When the reactors were changed to closed systems, despite the diameter of granules became two times smaller at 8 m/h, any significant differences of oleate conversion were not found between the two distinct V_{up}. These results indicate that this speculation is inconclusive.

The answer for the question remained unclear until batch tests were conducted by use of the granular (diameters = 1–3 mm) and washed out (diameters = 50–100 μm) sludges originating from the same reactor operated at

HRT = 24 h and V_{up} = 1 m/h (Chapter 6). The LCFA conversion rate of the washed out sludge was 129 mg LCFA-COD/g VS·d while that of the granular sludge was 84 mg LCFA-COD/g VS·d. It therefore is clear that the loss of the fine biomass bearing highly active LCFA degradability will impede the reactor performance.

Subsequently, we verified the importance of in-reactor retention of the washed out biomass (Chapter 6). By recycling the washed out biomass to the reactor also operated at HRT = 24 h and V_{up} = 1 m/h, we eventually obtained a COD removal efficiency of 97% and an LCFA conversion rate of 304 mg LCFA-COD/g VS·d. Moreover, in comparison with the reactor operated at the same hydrodynamic parameters but without returning the washed out biomass, the recycled reactor system achieved an 18% higher conversion rate based on the same sludge loading rates imposed in both reactor systems. The increased conversion rate can be attributed to the increase of LCFA-degraders concentration that is due mainly to the recycling. The best treatment performance achieved in this thesis is the up-to-date highest among those reported in anaerobic bioreactor systems treating LCFA-containing wastewaters.

REFERENCES

Angelidaki, I. and Ahring, B.K. (1995). Establishment and characterization of anaerobic thermophilic (55°C) enrichment culture degrading long-chain fatty acids. *Appl. Environ. Microbiol.*, **61**, 2442–2445.

Forster, C.F. (1992). Oils, fats and greases in wastewater treatment. *J. Chem. Technol. Biotechnol.*, **55**, 402–404.

Habets, L.H.A. (1997). Personal communication. Paques BV, PO Box 52, 8560 AB Balk, The Netherlands.

Hulshoff Pol, L.W. (1997). Personal communication. Department of Environmental Technology, Wageningen Agricultural University, PO Box 8129, 6700 EV Wageningen, The Netherlands.

Hwu, C.-S., van Beek, B., van Lier, J.B. and Lettinga, G. (1997). Effects of cosubstrates and sludge acclimation on anaerobic degradation of oleic acid by granular sludge. in preparation.

Pauss, A., Samson, R. and Guiot, S. (1990). Thermodynamic evidence of trophic microniches in methanogenic granular sludge bed reactors. *Appl. Microbiol. Biotechnol.*, **33**, 88–92.

Quéméneur, M. and Marty, Y. (1994). Fatty acids and sterols in domestic wastewaters. *Water Res.*, **28**, 1217–1226.

Thiele, J.H., Wu, W.-M. and Jain, M.K. (1990). Ecoengineering high rate anaerobic digestion systems: analysis of improved syntrophic biomethanation catalysts. *Biotechnol. Bioeng.*, **35**, 990–999.

Stand van Zaken Betreffende de Anaërobe Zuivering van Hogere Vetzuren Bevattende Afvalstromen: Samenvatting

Stand van Zaken Betreffende de Anaërobe Zuivering van Hogere Vetzuren Bevattende Afvalstromen: Samenvatting

INLEIDING

Vetten behoren tot de belangrijkste organische verontreinigingen in huishoudelijk en industrieel afvalwater. Ondanks dat huishoudelijk afvalwater ongeveer 40-100 mg/l vetten bevat (Forster 1992; Quéméneur and Marty, 1994), zijn met name industriële afvalwaters van belang als gevolg van de hogere concentraties vetten. Industrieën die vethoudend afvalwater genereren zijn de zuivelindustrie, eetbare olieën en vetten verwerkende industrie, slachthuizen en vleesverwerkende industrie, en wol verwerkende industrie.

Gedurende anaërobe afvalwaterzuivering worden vetten snel afgebroken. Praktische problemen die gedurende anaërobe zuivering van vethoudend afvalwater echter kunnen optreden zijn het gevolg van (i) inhibitie van methanogenen en acetogenen door hogere vetzuren (HVZ), en (ii) uitspoeling/flotatie van de biomassa. Deze twee problemen komen met name tot uitdrukking in hoog-belaste zuiveringssystemen. Onbevredigende resultaten zijn geboekt met praktijkschaal opstroom anaërob slib-bed (upflow anaerobic sludge bed, UASB) reaktoren en in labschaal geëxpandeerde korrelslib-bed (expanded granular sludge bed, EGSB) reaktoren.

Het in dit proefschrift beschreven onderzoek is gericht op de oplossingen voor de eerder genoemde problemen. Specifieke doelstellingen zijn het verbeteren van het zuiveringsrendement en betrouwbaarheid van UASB en EGSB reaktoren voor de anaërobe

behandeling van HVZ-houdend afvalwater, omdat deze reaktorsystemen respectievelijk de meest toegepaste en meest veelbelovende anaërobe zuiveringstechnologieën zijn. Hiervan uitgaande is het in dit proefschrift beschreven onderzoek gericht op de toxiciteit, biosorptie en afbreekbaarheid van HVZ. Naast het succes van de, tot op heden beschreven, hoogst toepasbare belastbaarheid, kunnen diverse nieuwe resultaten met betrekking tot de mechanismen die een rol spelen in de complexe verhouding tussen toxiciteit, sorptie en afbreekbaarheid, bijdragen aan succesvolle anaërobe zuivering van afvalwaters verontreinigd met HVZ.

Op basis van de experimentele resultaten verkregen gedurende dit onderzoek, worden vijf methoden beschreven, ter bestrijding van praktische problemen, teneinde tot een uiteindelijk bevredigend anaërob zuiveringssysteem te kunnen komen. De vijf methoden die in dit hoofdstuk zullen worden beschreven zijn:

Gebruik van korrelslib als entmateriaal;
Adaptatie van slib aan hogere vetzuren;
Toepassing van thermofiele omstandigheden;
Het voorkomen van overmatige sorptie, en;
Recirculatie van uitgespoelde biomassa.

GEBRUIK VAN KORRELSLIB ALS ENTMATERIAAL

De toxiciteit van oleaat voor zeven anaërobe slibsoorten van verschillende oorsprong is onderzocht (hoofdstuk 2). De oleaat toxiciteit voor de specifieke methanogene activiteit van de verschillende slibsoorten, bleek sterker gecorreleerd aan het specifieke oppervlakte van de slibkorrels, dan aan de oorsprong van het slib of de specifieke methanogene activiteit. Slib dat voor uitvoering van het experiment was blootgesteld aan HVZ, bleek minder gevoelig voor HVZ en een hogere methanogene activiteit te bezitten. Gesuspendeerde en vlokkige slibsoorten zijn veel gevoeliger voor toxiciteit van oleaat dan methanogeen korrelslib. De IC_{50} concentraties bepaald met korrelslib waren 3-13 keer hoger in vergelijking met gesuspendeerd slib bij 40°C (hoofdstuk 2) en 2 keer hoger bij 55°C (hoofdstuk 3). Aangezien het herstel van de methanogene activiteit van korrelslib na het optreden van toxiciteit, een periode van een week tot een maand beslaat (hoofdstuk 3), mag worden aangenomen dat de tijd benodigd voor herstel van gesuspendeerd slib zelfs langer zal zijn.

ADAPTATIE VAN SLIB AAN HOGERE VETZUREN

Het verwachte synergistische effect in de toxiciteit van een mengsel van HVZ (50% oleaat, 35% palmilaat en 15% stearaat) werd niet waargenomen voor korrelslib dat was geadapteerd aan slachthuis afvalwater. In tegenstelling tot deze waarneming, werd bij gebruik van niet-geadapteerd korrelslib een langere lag-fase waargenomen in de methaanproduktie als gevolg van oleaat inhibitie (hoofdstuk 4). Slib dat voorheen was blootgesteld aan HVZ (82% oleaat), bleek in staat om oleaat 2 keer sneller af te breken dan niet-blootgesteld slib. Gedurende continu-experimenten bleek de reaktor geënt met niet-blootgesteld slib bij een concentratie van 500 mgHVZ-(82% oleaat)-CZV/l niet meer te werken, terwijl de reaktor geënt met blootgesteld slib in staat is om 4000 mg HVZ-(82% oleaat)-CZV/l succesvol te behandelen (Hwu *et al.*, 1997).

De voorwaarde van adaptatie zou het belangrijkste knelpunt kunnen blijken te zijn voor toepassing van anaërobe zuivering voor HVZ-houdend afvalwater, omdat de beschikbaarheid van slib dat aan HVZ en/of vet is geadapteerd beperkt is (Hulshoff Pol, 1997), en omdat de groeisnelheid van HVZ-afbrekende organismen slechts 0.3 d^{-1} bedraagt (Angelidaki and Ahring, 1995). Hiervan uitgaande dient de opstart van anaërobe reaktoren voor de zuivering van HVZ-houdend afvalwater te worden uitgevoerd bij lage concentraties HVZ, waardoor adaptatie en biomassaretentie kunnen worden gewaarborgd.

TOEPASSING VAN THERMOFIELE OMSTANDIGHEDEN

De afbraaksnelheid en toxiciteit (IC_{50}-waarde) van oleaat voor geadapteerd slib onder mesofiele (30°C) en thermofiele (55°C) omstandigheden is onderzocht. Ondanks dat IC_{50}-waarden voor oleaat onder mesofiele omstandigheden (2.8 mM) 4 keer hoger waren dan onder thermofiele omstandigheden (0.7 mM), bleek dat de specifieke afbraaksnelheid onder mesofiele omstandigheden (33 mg oleaat-CZV/g VS.d) 4 keer lager dan onder thermofiele omstandigheden (124 mg oleaat-CZV/g VS.d). Dit betekent dat thermofiele organismen gevoeliger zijn voor oleaat toxiciteit, maar dat anderzijds de groeisnelheid onder thermofiele omstandigheden aanzienlijk hoger is. Hierdoor zal in geval van HVZ-toxiciteit de tijd benodigd voor herstel van de bacteriële populatie veel korter zijn onder thermofiele omstandigheden.

Indien een mesofiele en een thermofiele, geadapteerde populatie werden blootgesteld aan een hoge slib-belading van 1.2 g oleaat-CZV/g VS, bedroeg de tijdsduur nodig voor herstel van de methanogene capaciteit in de thermofiele populatie ca. 5 dagen, terwijl onder mesofiele omstandigheden een periode van 28 dagen benodigd was (hoofdstuk 3). Een belangrijke praktische implicatie van deze resultaten is dat een herstart van een thermofiele anaërobe reaktor voor de behandeling van HVZ-houdend afvalwater veel sneller zal verlopen, dan de herstart van een mesofiele reaktor.

VOORKOMEN VAN OVERMATIGE SORPTIE

Sorptie van HVZ aan de oppervlakte van korrelslib leidt tot slib-flotatie en -inhibitie (hoofdstuk 4). Hogere HVZ-concentraties leiden tot zowel hogere sorptie-snelheden als meer sorptie. Hierdoor worden sterkere flotatie en inhibitie van de biomassa geïnduceerd. Aan de hand van resultaten verkregen met batchexperimenten, is geschat dat een sorptie toename van 45 mg HVZ/g TS kan leiden tot een daling van 10% in de methaanproduktiesnelheid. In een UASB-reaktor begon de flotatie van korrelslib bij slibbelastingen hoger dan 0.09 g HVZ-CZV/g VS.d, terwijl volledige flotatie van de biomassa werd waargenomen bij slibbelastingen hoger dan 0.2 g HVZ-CZV/g VS.d.

Flotatie van zowel korrelslib als clusters van HVZ, werd waargenomen in EGSB-reaktoren gevoed met HVZ als enig substraat, bij hydraulische verblijftijden (HVT) kleiner dan 6 uur en opstroomsnelheden (V_{up}) hoger dan 3 m/uur. (hoofdstuk 5). Ondanks dat op CZV-basis een efficiëntie van 73% kon worden bereikt, bleef de maximale omzetting tot methaan beperkt tot minder dan 15% (hetgeen overeenkomt met een lage specifieke afbraaksnelheid van 1.6 mg HVZ-CZV/g VS.d). Indien de HVT werd verlengd to 24 uur (V_{up} = 1, 4 of 7 m/uur), en glucose of butyraat werd gedoseerd als co-substraat, trad nauwelijks flotatie op en nam de HVZ-afbraak aanzienlijk toe (hoofdstuk 5 en 6). De beste resultaten die met deze methode zijn bereikt zijn een CZV-verwijdering van 95%, waarbij 87% werd omgezet in methaan (hetgeen overeenkomt met een afbraaksnelheid van 93 mg HVZ-CZV/g VS.d), bij HVT = 24 uur en V_{up} = 1 m/uur (hoofdstuk 6).

Hiermee is aangetoond dat overmatige sorptie van HVZ aan de oppervlakte van korrelslib kan worden voorkomen door verhoogde omzetting van HVZ. Dit kan worden bereikt door verlenging van de substraat-biomassa contacttijd; door bijvoorbeeld de HVT te verhogen.

RECIRCULATIE VAN UITGESPOELDE BIOMASSA

Ondanks dat de specifieke afbraaksnelheid in continu-experimenten maximaal 93 mg HVZ-CZV/g VS.d bedroeg, werden in batchexperimenten omzettingssnelheden waargenomen van bijvoorbeeld 124 mg oleaat-CZV/g VS.d (hoofdstuk 3). Het verschil tussen de snelheden waargenomen in continu- en batchexperimenten zou zelfs aanzienlijk groter zijn indien slib uit de continu-experimenten (hoofdstuk 6) zou zijn gebruikt in batchexperimenten (hoofdstuk 3). Aangezien in de continu bedreven reaktoren nagenoeg volledige biomassaretentie kon worden bewerkstelligd, blijft de volgende vraag onbeantwoord: wordt de specifieke afbraaksnelheid beïnvloedt door de hydrodynamische parameters?

De waarneming beschreven in hoofdstuk 5, dat een hogere V_{up} leidt tot een lagere omzetting van HVZ, heeft geleid tot speculaties omtrent de mogelijke verstoring van syntrofe relaties in het slib bij hogere V_{up}, als gevolg van desintegratie van de slibkorrels. Teneinde de invloed van de V_{up} op de omzetting van HVZ te onderzoeken zijn twee reaktoren bedreven bij opstroomsnelheden van 1 en 8 m/uur (hoofdstuk 6). Als de reaktoren werden bedreven als gerecirculeerde batchreaktoren werden geen verschillen in de afbraaksnelheden van oleaat waargenomen, terwijl de gemiddelde diameter van de slibkorrels bij 8 m/uur twee keer kleiner was dan bij 1 m/uur. Hiervan uitgaande konden geen definitieve conclusies worden getrokken aangaande de relatie tussen de korrelgrootte/opstroomsnelheid en de specifieke afbraaksnelheid.

Een antwoord op de eerder gestelde vraag werd gevonden door vergelijking van de specifieke afbraaksnelheid van HVZ door slib uit een reaktor (diamater = 1-3 mm) en slib dat uit dezelfde reaktor was gespoeld (diameter = 50-100 mm). De reaktor werd bedreven bij HVT = 24 uur en V_{up} = 1 m/uur (hoofdstuk 6). De specifieke afbraaksnelheid van HVZ door het slib uit de reaktor bedroeg 84 mg HVZ-CZV/g VS.d, en van het uitgespoelde slib 129 mg HVZ-CZV/g VS.d. Hiermee wordt duidelijk dat uitspoeling van biomassa met een hoge specifieke activiteit ten opzichte van HVZ de capaciteit van de reaktor beperkt.

Vervolgens is het belang van biomassa-retentie in de reaktoren onderzocht (hoofdstuk 6). Door recirculatie van de uitgespoelde biomassa bij HVT = 24 uur en V_{up} = 1 m/uur, werd uiteindelijk een rendement op CZV-basis van 97% gehaald, bij een specifieke afbraaksnelheid van 304 mg HVZ-CZV/g VS.d. In vergelijking met een reaktor die werd

bedreven zonder recirculatie van uitgespoelde biomassa, werd een 18% hogere afbraaksnelheid waargenomen in de reaktor waarin uitgespoelde biomassa werd gerecirculeerd. Deze hogere specifieke afbraaksnelheid kan worden toegeschreven aan de hogere concentratie HVZ-afbrekende biomassa in de reaktor waarin biomassa wordt gerecirculeerd. De maximale capaciteit verkregen gedurende dit onderzoek, is op dit moment de hoogst waargenomen capaciteit voor anaërobe bioreaktoren voor de behandeling van HVZ-houdend afvalwater.

Bibliography

Hwu, C.-S., Tseng, S.-K., Yuan, C.-Y., Kulik, Z. and Lettinga, G. (1997). Biosorption of long-chain fatty acids in UASB treatment process. *Water Res.*, in press.

Hwu, C.-S., van Lier, J.B. and Lettinga, G. (1997). Physicochemical and biological performance of expanded granular sludge bed reactors treating long-chain fatty acids. *Process Biochem.*, **32**, in press.

Hwu, C.-S. and Lettinga, G. (1997). Acute toxicity of oleate to acetate-utilizing methanogens in mesophilic and thermophilic anaerobic sludges. *Enzyme Microb. Technol.*, **21**, in press.

Hwu, C.-S., van Lier, J.B. and Lettinga, G. (1997). Anaerobic toxicity and degradability of oleic acid under mesophilic and thermophilic conditions. In: *Proc. Forum for Appl. Biotechnol.*, 25–26 Sep. 1997, Gent, Belgium, in press.

Lettinga, G. and Hwu, C.-S. (1997). Anaerobic treatment under more complex situations: Treatment of wastewaters containing long-chain fatty acids. *Lecture Notes, Intl. Course on Anaerobic Waste Water Treatment*, Art. 6, 1–11 Jul. 1997, Delft and Wageningen, The Netherlands.

Hwu, C.-S., Molenaar, G., Garthoff, J., van Lier, J.B. and Lettinga, G. (1997). Thermophilic high-rate anaerobic treatment of wastewater containing long-chain fatty acids: impact of reactor hydrodynamics. *Biotechnol. Lett.*, **19**, 447–451.

Hwu, C.-S., van Beek, B., van Lier, J.B., and Lettinga, G. (1997). Thermophilic high-rate anaerobic treatment of wastewater containing long-chain fatty acids: effect of washed out biomass recirculation. *Biotechnol. Lett.*, **19**, 453–456.

Hwu, C.-S., van Lier, J.B., Kulik, Z., Mishra, P.K. and Lettinga, G. (1997). Feasibility of expanded granular sludge bed (EGSB) system for the treatment of wastewater containing long-chain fatty acids. In: *Proc. the 8th Intl. Conf. on Anaerobic Digestion.* 25–29 May, 1997, Sendai, Japan, Vol. 3, pp. 103–108.

Hwu, C.-S., Tseng, S.-K., Yuan, C.-Y., Kulik, Z. and Lettinga, G. (1996). Adsorption of long-chain fatty acids onto granular sludges and its effect on upflow anaerobic sludge bed reactor operation. In: *Proc. the First IAWQ Specialized Conf. on Adsorption in Water Environment and Treatment Processes.* 5–8 Nov. 1996, Shirahama, Wakayama, Japan, pp. 91–98.

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

Hwu, C.-S., van Lier, J.B. and Lettinga, G. (1996). Comment on "Interaction between acidogenesis and methanogenesis in the anaerobic treatment of olive mill effluents,". *Water Res.*, **30**, 2229.

Hwu, C.-S. and Tseng, S.-K. (1996). Screening of immobilization materials for anaerobic wastewater treatment. In: *Progress in Biotechnology. Vol. 11, Immobilized Cells: Basics and Applications*. Wijffels, R.H., Buitelaar, R.M., Bucke, C. and Tramper, J. (Eds.), Elsevier Science, Amsterdam, pp. 98–105.

Tseng, S.-K., Hwu, C.-S. and Chang, W.-Z. (1996). Anaerobic degradation of ortho- and meta-nitrophenols in plug flow biofilters. *Environ. Technol.*, **17**, 1225–1233.

Donlon, B., Razo-Flores, E., Hwu, C.-S., Field, J. and Lettinga, G. (1995). Toxicity and biodegradability of selected N-substituted phenols under anaerobic conditions. In: *Microbial Processes for Bioremediation*. Hinchee, R.E., Brockman, F.J. and Vogel, C.M. (Eds.), Battelle Press, Columbus, pp. 251–257.

Hwu, C.-S., Tseng, S.-K. and Lin, C.-H. (1994). Effects of entrapment materials on anaerobic packed bed reactor performance. Poster Presentation in *the IAWQ 17th Biennial Intl. Conf. on Water Quality*. 24–29 Jul. 1994, Budapest, Hungary.

Hwu, C.-S., Chong, N.-M. and Tseng, S.-K. (1994). Effects of methanogens-rich sludge augmentation on UASB reactor start-up after shock load. Poster Presentation in *the IAWQ 17th Biennial Intl. Conf. on Water Quality*. 24–29 Jul. 1994, Budapest, Hungary.

Hwu, C.-S. and Sheu, C.-S. (1993). Comparison of treatment performance between UASB and anaerobic packed bed reactors. *Technical Report*, Development Center for Biotechnology. Taipei, Taiwan, ROC. in Chinese.

Hwu, C.-S. and Sheu, C.-S. (1993). Feasibility and economical evaluation of UASB process treating low strength wastewaters. *Technical Report* DCB-099-S302(82), Development Center for Biotechnology. Taipei, Taiwan, ROC. in Chinese.

Hwu, C.-S. and Sheu, C.-S. (1993). Survey of applicability of UASB reactor system treating various types of wastewater. *Survey Report* DCB-099-S301(82), Development Center for Biotechnology. Taipei, Taiwan, ROC. in Chinese.

Hwu, C.-S. and Chong, N.-M. (1992). Effects of methanogens-rich anaerobic sludge augmentation on UASB reactor start-up. In: *Proc. 17th Conf. on Wastewater Treatment Technology*. Dec. 1992, Chungli, Taiwan, ROC, pp. 129–140. in Chinese with abstract in English.

Hwu, C.-S. and Shiau, L.-D. (1992). Enrichment of *Methanosarcina* spp. in anaerobic sludge. *J. Chinese Inst. Environ. Engng.*, **2**, 101–110. in Chinese with abstract in English.

Chen, C.-Y. and Hwu, C.-S. (1992). Effect of storage on the performance of anaerobic sludge. *Technical Report* DCB-099-E201(81), Development Center for Biotechnology. Taipei, Taiwan, ROC. in Chinese.

Hwu, C.-S. and Shiau, L.-D. (1991). Examination of methanogens in anaerobic sludge with dark field microscopy—a novel and economical method. *Technical Innovation Report* DCB-099-Chung101(80), Development Center for Biotechnology. Taipei, Taiwan, ROC. in Chinese.

Hwu, C.-S. (1991). *Fast Start-Up on an Upflow Anaerobic Sludge Blanket Reactor.* MSc. thesis, Graduate Institute of Biotechnology, Chinese Culture University. Taipei, Taiwan, ROC. in Chinese with abstract in English.

Hwu, C.-S. and Shiau, L.-D. (1991). High enrichment of methanogens in an anaerobic reactor. *Technical Report* DCB-099-E102(80), Development Center for Biotechnology. Taipei, Taiwan, ROC. in Chinese.

Hwu, C.-S. and Lin, C.R. (1990). Screening and evaluation of microbial cultures on odor control of piggery waste. In: *Proc. 15th Conf. on Wastewater Treatment Technology.* Nov. 1990, Taichung, Taiwan, ROC, pp. 271–282. in Chinese with abstract in English.

Hwu, C.-S., Lin, C.R., Lu, C.-J. and Mou, D.-G. (1990). Odor control of livestock waste. *Technical Report*, Development Center for Biotechnology. Taipei, Taiwan, ROC. in Chinese.

Wang, S.D. and Hwu, C.-S. (1989). Mechanism of solid state treatment of piggery waste. *Technical Report*, Development Center for Biotechnology. Taipei, Taiwan, ROC. in Chinese.

Hwu, C.-S. and Lin, C.R. (1989). Screening of odor control cultures. In: *Proc. 1st Conf. on Specific Microbial Screening, Improvement, and Industrial Application.* May 1989, Taipei, Taiwan, ROC, pp. 56–61. in Chinese with abstract in English.

Chung, H.R. and Hwu, C.-S. (1988). Immunotype biosensor: an example of bioelectronics. *J. Science*, **19,** 173–175. in Chinese.

Curriculum Vitae

The author of this thesis, Ching-Shyung Hwu, was born in Tainan, Taiwan. He received his B.Sc. degree in Microbiology from Soochow University, Taipei, Taiwan in 1986. He then spent the next six years working in the Development Center for Biotechnology in Taipei. During these years, he was admitted to go to the Graduate Institute of Biotechnology, Chinese Culture University, Taipei, for his "in-service advanced study." In 1991 he carried out his M.Sc. work on "Fast Start-Up of UASB Reactors" and passed the entrance examination for Ph.D. study at the Graduate Institute of Environmental Engineering in National Taiwan University. He then conducted his Ph.D. research under the supervision of Professor Szu-Kung Tseng on "Cell Immobilization in Anaerobic Wastewater Treatment." In 1992 he was granted the "governmental scholarship for studying overseas" from the Ministry of Education. In 1993 he quitted the Ph.D. research in Taiwan and started a new research topic in the Netherlands. Since Nov. 1993 he has been working as a "gastmedewerker" at the Department of Environmental Technology, Wagenin-gen Agricultural University, where he finished the Ph.D. study as is described in this thesis.