Anaerobic Treatment of Wastewater with High Concentrations of Lipids or Sulfate

Promotoren: Dr.ir. G. Lettinga, bijzonder hoogleraar in de anaerobe waterzuivering
Dr.ir. J.A.M. de Bont, hoogleraar in de industriële microbiologie

Arjen Rinzema

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Proefschrift
ter verkrijging van de graad van
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Stellingen

1. Sam-Soon *et al* concluderen dat de vorming van anaeroob korrelslib met acetaat, propionaat, of hogere vetzuren als enig substraat, onwaarschijnlijk is. Deze conclusie is onjuist.

   P.A.L.N.S. Sam-Soon, et.al. (1987) Hypothesis for Pelletisation in the Upflow Anaerobic Sludge Bed Reactor. Water SA **13** (2), 69

2. Het is zinloos om richtlijnen voor de toelaatbare sulfideconcentratie in het influent van een anaerobe zuiveringsreactor op te geven, zonder vermelding van de concentratie van afbreekbaar organisch materiaal in het influent en van de pH in de reactor.

   G.F. Parkin, R.E. Speece (1983) Attached Versus Suspended Growth Anaerobic Reactors: Response to Toxic Substances. Wat. Sci. Technol. **15** (8/9), 261

   Z. Isa, S. Grusenmeyer, W. Verstraete (1986) Sulfate Reduction Relative to Methane Production in High-Rate Anaerobic Digestion: Technical Aspects. Appl. Environ. Microbiol. **51** (3), 572

3. Koster concludeert ten onrechte uit zijn experimentele resultaten dat de adaptatie van anaeroob korrelslib aan ammonia moet worden toegeschreven aan een verandering in het metabolisme van de reeds aanwezige methaanproducerende bacteriën.

   I.W. Koster (1986) Characteristics of the pH-influenced Adaptation of Methanogenic Sludge to Ammonium Toxicity. J. Chem. Tech. Biotechnol. **36**, 446

4. De conclusie van Schäfer dat metaaloxidehoudende chemische afvalstoffen niet kunnen worden uitgeloogd met *Thiobacillus* sp., is onvoldoende onderbouwd.

   W. Schäfer (1987) Leaching of Industrial Waste Products with Thiobacilli and Heterotrophic Microorganisms. In: Recent Progress in Biohydrometallurgy (G. Rossi and A.E. Torma, eds.), Associazione Mineraria Sarda, Iglesias, Italy, 427

5. Dietrich *et al* verwaarlozen bij de berekening van het afbraakrendement van vluchtige, slecht in water oplosbare gechloreerde koolwaterstoffen in het natte-oxidatieproces, ten onrechte de verdamping van deze verbindingen.

   M.J. Dietrich, T.L. Randall, P.J. Canney (1985) Wet Air Oxidation of Hazardous Organics in Wastewater. Environ. Progress **4** (3), 171

6. Bij onderzoek naar de invloed van remmende stoffen op de microorganismen in een anaerobe zuiveringsreactor moet terdege rekening worden gehouden met het maskerende effect van diffusielimitatie in biofilms of biomassa-aggregaten.

Dit proefschrift

7. Uit de resultaten van Kugelman en McCarty, Parkin *et al*, en De Baere *et al* mag niet de conclusie worden getrokken dat adaptatie van methanogeen slib aan natrium optreedt.

I.J. Kugelman, P.L. McCarty (1965) Cation Toxicity and Stimulation in Anaerobic Waste Treatment. II. Daily-feed Studies. In: Proc. 19th Ind. Waste Conf., Purdue Univ., 667

J. Yang, G.F. Parkin, R.E. Speece (1979) Recovery of Anaerobic Digestion After Exposure to Toxicants (Final Report). Drexel Univ., Philadelphia, PA, USA, US Dept. of Energy Contract No. EC-77-S-01-4391

L.A. De Baere, et.al. (1984) Influence of High NaCl and NH_4Cl Salt Levels on Methanogenic Associations. Water Res. 18 (5), 543

8. Het door Parkin *et al* geïntroduceerde begrip "acclimation potential", bedoeld als een maat voor de capaciteit van methaanproducerend slib om te wennen aan remmende stoffen, is misleidend.

G.F. Parkin, et.al. (1983) Response of Methane Fermentation Systems to Industrial Toxicants. Journal WPCF 55 (1), 44

9. Yoda *et al* hebben niet de juiste experimenten verricht om hun model voor de biomassaretentie in een gefluïdiseerd bed reactor te toetsen.

M. Yoda, et.al. (1987) Anaerobic Fluidized Bed Treatment with a Steady-State Biofilm. Wat. Sci. Technol. 19 (Rio), 287

10. De suggestie dat milieuhygiënisch aanvaardbare verwerking van chemisch afval in de derde wereld tegen lagere kosten is te realiseren dan in het geïndustrialiseerde Westen, moet worden beschouwd als een goedkope smoes.

11. Gelet op het aantal jaren dat is verstreken sinds de anaerobe zuivering van afvalwater voor het eerst werd onderzocht, moet eerder rekening worden gehouden met een mid-life crisis dan met onvolwassenheid van deze techniek.

12. Door de discriminerende belastingwetgeving met betrekking tot de aftrek van hypotheekrente, gaan ongehuwd samenwonende huiseigenaren de boot in.

Stellingen behorende bij het proefschrift "Anaerobic Treatment of Wastewater with High Concentrations of Lipids or Sulfate" van A. Rinzema.

Wageningen, 14 oktober 1988

Dankwoord

Dit proefschrift zou niet tot stand zijn gekomen zonder de steun van velen. Een aantal mensen wil ik in het bijzonder bedanken.

Gatze Lettinga heeft in 1983 toegegeven aan zijn verlangen om een Friese collega te hebben. Gatze, bedankt voor de steun en raad die je mij de afgelopen viereneenhalf jaar hebt gegeven. Het afgelopen half jaar heb je ondanks je verschrikkelijk drukke werk steeds de tijd gevonden om mijn manuscripten snel te becommentariëren. Je hebt je best gedaan om mij van een vrij theoretische procestechnoloog om te vormen tot een praktische waterzuiveraar.

Jan de Bont wil ik bedanken voor zijn bereidwilligheid om ondanks het zeer laat gedane verzoek als tweede promotor op te treden, waarmee hij de laatste formele barrière voor mijn promotie heeft geslecht.

Zonder de inzet van Arne Alphenaar, Kees Baas, Martin Boone, Wil de Bruin, Letitia Buth, Jos Keurentjes, Karin van Knippenberg, Jules van Lier, Albertien Paardekooper, Herbert van Veen, André de Vegt, René Wijffels, en Frank Zeegers was het nooit gelukt. In het kader van hun doctoraalstudie hebben zij ongelooflijk veel werk verricht, dat logischerwijze niet allemaal in dit proefschrift wordt beschreven. Toon Helmink heeft ruim een jaar een belangrijke bijdrage geleverd aan de technische en analytische uitvoering van verschillende experimenten. Bedankt voor jullie bijdrage aan het onderzoek en de prettige samenwerking.

Carl Schultz heeft het onderzoek op semi-technische schaal naar anaerobe zuivering van zuur water uitgevoerd. Dit onderzoek kon niet meer in dit proefschrift worden beschreven, maar dit doet geen afbreuk aan de kwaliteit en het belang ervan. Carl, hartelijk bedankt voor de leuke samenwerking.

Johannes van der Laan en Arjen van de Peppel hebben bijna een jaar lang een groot deel van hun tijd gestoken in het ontwikkelen van een snelle, eenvoudige analysemethode voor de bepaling van hogere vetzuren. Door externe omstandigheden is dit niet gelukt, aan hun inzet heeft het zeker niet gelegen. Daarnaast zorgden zij en de andere technische medewerkers en medewerksters van de vakgroep waterzuivering voor de broodnodige technische ondersteuning.

De medewerkers en medewerksters van de technische dienst, de magazijnen, de tekenkamer, de fotografische afdeling, en de administratie van de Dreijen wil ik bedanken voor hun snelle service. Vooral bij de service- en projectenafdeling van de werkplaats heb ik heel wat keren een "spoedklus" aangemeld, die ook steeds met spoed is uitgevoerd.

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De leden van de begeleidingscommissie, ing. M.M.J. Allessi, dr. J. van Andel, ir. T. Huizenga, ir. A.J. van der Hulst, de heer M.G.A. Langerak, drs. C.J.M. Meershoek, ing. G.W.J. Rijs, ir. H.R. Roelfsema, ir. R. Schutte, ir. W. van Starkenburg, ir. A.J. Van der Vlugt, en ir. C. Velzeboer wil ik bedanken voor hun belangstelling voor het onderzoek.

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Abstract

Rinzema, A. (1988) Anaerobic Treatment of Wastewater with High Concentrations of Lipids or Sulfate. Doctoral Thesis, Wageningen Agricultural University, Wageningen, The Netherlands.

This thesis describes research on the application of granular sludge bed upflow reactors for anaerobic treatment of wastewaters contaminated with lipids and sulfate, two contaminants that have so far seriously hampered the application of anaerobic treatment in several branches of industry. The Upflow Anaerobic Sludge Bed reactor is the most popular anaerobic treatment system at this moment. However, it is mainly applied to wastewaters with readily biodegradable dissolved contaminants, and hardly to more complex wastewaters.

Two problems can occur in anaerobic treatment of lipid containing wastewater, viz. (1) inhibition of anaerobic bacteria by long chain fatty acids, and (2) flotation of the biomass. Sulfate may cause direct and indirect inhibition of methanogenic and acetogenic bacteria.

The first part of the thesis deals with the inhibitory effect of long chain fatty acids (LCFA) in anaerobic digestion. Inhibition can occur after overloading, i.e. during the start-up period of the digester, or as a result of a shock load. LCFA affect especially the acetotrophic methanogens, above a critical threshold concentration they exert a bactericidal effect. The methanogens do not adapt to LCFA. The threshold concentration for capric acid - one of the most toxic saturated acids - is approximately 1 kg/m^3. The precise value of the threshold concentration depends upon the mass transfer characteristics of the anaerobic reactor, and upon the particle size and specific activity of the biomass aggregates. Furthermore, the presence of phospholipids may enhance the inhibitory effect of LCFA. Inhibition can be prevented by addition of soluble calcium salts to the wastewater. However, the addition of calcium cannot eliminate the second deleterious effect of a shock load of LCFA, viz. flotation and subsequent wash-out of biomass aggregates.

The second part of this thesis describes the anaerobic treatment of solutions of LCFA and emulsions of triglycerides in the Expanded Granular Sludge Bed reactor. With LCFA solutions this modified upflow reactor can achieve a mineralization efficiency of at least 85-90% at space loading rates of ca. 30 kg COD/m^3.day. Modification of the sludge separation system is required to reduce sludge wash-out during treatment of triglyceride emulsions. A novel sieve-drum separator was developed, which allows stable operation. Although the treatment capacity is significantly lower with triglyceride emulsions than with LCFA solutions, the EGSB reactor with sieve-drum separator can accommodate higher organic and hydraulic loading rates than previously described anaerobic filter reactors. Upscaling of the EGSB system and flotation of lipids require further research.

The third part of the thesis deals with the inhibitory effect of sulfide and sodium sulfate. From pH 6.4 to 7.2 approximately 250 mg H_2S per litre causes a 50% decrease of the maximum specific activity of acetotrophic methanogens. The inhibitory effect of a given H_2S concentration increases significantly when the pH approaches 8. Consequently, an increase of the pH level in the anaerobic digester above ca. 7.2 is not beneficial. Immobilization in biomass aggregates or films may provide protection against H_2S inhibition. Propionate degradation may be the rate limiting step during treatment of sulfate containing wastewater, because it is affected more severely by sulfide than acetotrophic methanogenesis. At extremely high sulfate concentrations, also inhibition by cations has to be considered. At neutral pH levels, sodium concentrations up to 5 g/l cause no inhibition of acetotrophic methanogens. A sodium concentration of 10 g/l causes a 50% decrease of the maximum specific acetotrophic methanogenic activity, 14 g/l causes complete inhibition. Acetotrophic methanogens do not adapt to high sodium concentrations.

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Background of the Study

Biological processes have found wide application in the treatment of municipal and industrial wastewater, because in many instances they are substantially cheaper than physical, or chemical methods. Especially aerobic processes are widely employed for the removal or conversion of oxygen demanding organic pollutants, sulfur and nitrogen compounds. Until recently, anaerobic wastewater treatment was not considered as a feasible or attractive alternative for aerobic methods, despite the fact that various scientists and engineers already investigated the applicability of anaerobic biological treatment methods since the beginning of this century. During the 1950s and 1960s reactor types with the possibility for biomass retention were developed - similar to the aerobic activated sludge and trickling filter systems. These systems allowed direct anaerobic treatment of less concentrated wastewaters at economically acceptable hydraulic residence times (Stander *et al* 1950, Schroepfer *et al* 1955, Coulter *et al* 1957, Young and McCarty 1969). The promulgation of stricter legislation during the 1970s, promoted the application of on-site treatment of industrial wastewater. The rising energy prices, and the relatively high costs of conventional aerobic treatment systems caused a strong increase in the research efforts on anaerobic treatment, which was certainly promoted by the increase of the funds made available by the authorities in some Western European countries.

In the Netherlands, small scale investigations of anaerobic wastewater treatment were initiated by Lettinga and coworkers in 1970 (Lettinga *et al* 1972), in cooperation with the potato starch and beet sugar refining industries. Results obtained with fully packed upflow reactors resulted in the development of an unpacked reactor system with an integrated settler, the socalled Upflow Anaerobic Sludge Blanket reactor (Lettinga *et al* 1974, 1975, 1976, 1980a, 1980b). The UASB system was rapidly developed to the full scale level. Within five years after the start of the first lab scale experiments, the first full scale reactor was implemented at a CSM beet sugar refinery (Lettinga *et al* 1977, De Vletter 1977, Pette *et al* 1980). At present, over 60 UASB reactors are in operation all over the world (Lettinga *et al* 1984). Most of these systems treat carbohydrate containing wastewaters, for example from the sugar industry, the starch industry, the confectionery industry, and the paper industry.

In 1982 the Dutch Ministry of Public Health and Environment[1] indicated anaerobic digestion as a promising development for treatment of protein and lipid containing wastewater from various branches of industry (Ministerie VOMIL 1982). This led to several investigations, both on laboratory and on pilot-plant scale, with protein and lipid containing wastewater (De Zeeuw 1982, Van Dorp 1982, Nieuwenhof 1983, Sayed *et al* 1984, Breure 1986).

The first studies on anaerobic treatment of lipid containing wastewater indicated, that the treatment capacity and stability of high rate anaerobic wastewater treatment systems may be seriously hampered by two phenomena:

- biomass is encapsulated in layers of floating suspended and emulsified lipids, which impairs the nett biomass production.
- long chain fatty acids may cause strong inhibition of the anaerobic biomass, particularly the acetogenic and methanogenic bacteria.

For this reason, in 1983 the Ministry of Housing, Physical Planning and Environment decided to support a research project at the Agricultural University, in order to improve the knowledge on the technological aspects of anaerobic treatment of lipid containing wastewater. This project also received financial support from the Dutch Seed Crushers and Oil Processors Association (Vernof), because this organization was searching for a cheaper alternative for conventional aerobic activated sludge treatment to solve the wastewater problems at several edible oil refineries.

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[1] Currently the Ministry of Housing, Physical Planning and Environment

In addition to the presence of lipids, in many cases a second potential problem has to be solved for wastewater from edible oil refineries, viz. they frequently contain excessively high concentrations of sulfate. The sulfate results from the use of large amounts of sulfuric acid in the refinery for recovery of free long chain fatty acids from the process water. The sulfate may cause several problems in the actual digestion process, as well as in ancillary equipment:

- sulfate is reduced to hydrogen sulfide, which is a powerful inhibitor of methanogenesis.
- sulfate reduction reduces the amount of methane produced from a given amount of organic waste, because sulfate reducing bacteria compete with the normal anaerobic flora for these substrates.
- part of the hydrogen sulfide is transferred to the biogas and may cause corrosion problems in boilers and internal combustion engines.
- the remaining part of the sulfide causes a high oxygen demand in the effluent, and potential malodour problems.
- neutralization of the acidic wastewater requires a large amount of alkali, which leads to high concentrations of either sodium or calcium in the influent to the anaerobic digester. The use of calcium hydroxide is not advisable, because it would cause excessive scaling on the reactor walls and in piping, and very likely also serious problems with biomass retention. High concentrations of sodium may cause strong inhibition.

The research described in this thesis deals with: (1) inhibition of anaerobic bacteria by long chain fatty acids, (2) the development of modified UASB-type reactor systems for high rate anaerobic treatment of lipid containing wastes, and (3) toxicity phenomena caused by hydrogen sulfide and sodium sulfate.

Table 1. Estimated emission of organic contaminants in lipid containing wastewater generated by several Dutch industries (situation 1980)

branch of industry	number of factories	emission in wastewater (PE's)[a]
slaughterhouses and meat products industry	220	600,000
dairy products industry	150	590,000
edible oil and fat refineries, and margarine industry	20	160,000
rendering industry	5	220,000
petfood industry	30	160,000
textiles industry (especially cotton)	37	250,000
total	462	1,980,000

[a] PE = Population Equivalent
references: Ministerie VOMIL 1981; Ministerie VOMIL 1982

Lipid Containing Industrial Wastewater in the Netherlands

Table 1 summarizes the estimated emission of organic pollutants in lipid containing wastewater generated by several Dutch industries. The total waste production generated by the branches of industry listed in Table 1 is approximately 2,000,000 population equivalents (PE), i.e. ca. 14% of the domestic wastewater production of the Dutch population. It is clear from Table 1, that the slaughtering and meat products industry and the dairy industry are the largest contributors. However, the average waste production per factory is rather low in these branches of industry. This means that on-site wastewater treatment is not economically attractive for these industries, compared to discharge to public wastewater facilities. On-site treatment can be profitable for the rendering industry, and the edible oil and fat refining and margarine industry, where the waste production of individual factories is in the order of magnitude of several tenthousands PE's.

Table 2 provides some information about the wastewater composition in some of the industries listed in Table 1. The figures in Table 2 merely indicate orders of magnitude. Considerable differences may exist between different branches of industry, as well as between different factories within one branch of industry. Even within one factory, large differences may result from variations in feedstocks or operating conditions.

Table 2. Some examples of the composition of industrial wastewater contaminated with lipids

branch of industry	COD ($kg.m^{-3}$)	TFM[a] ($kg.m^{-3}$)	ref.
slaughterhouses	2.0- 3.0	0.35-0.52	1
dairy products industry	0.1-95.0	0.02- 1.3	2
edible oil and fat refineries			
. superdegummed soy bean oil	2.2	0.55	3
. waterdegummed soy bean oil	4.3- 6.8	1.0	3
. mixed fish oils	0.8- 8.9	0.04- 1.0	4
edible fat refineries	0.9- 8.3	0.2 - 3.8	5
margarine industry	2.4- 4.0	0.6 - 2.0	6
rendering industry	2.7- 7.4	0.1 - 0.54	7
wool scouring industry	9.0-85.0	2.0 -15.0	8

[a] TFM = Total Fatty Matter, petroleum-ether, hexane or freon extractables
references: 1 Sayed 1987, 2 Brown and Pico 1979, 3 Segers 1982, 4 Rinzema unpublished, 5 Liemburg and Van der Wal 1982, 6 Ministerie VOMIL 1981, 7 De Zeeuw 1982, 8 Genon et al 1984

Lipids in wastewater from industries that use vegetable or animal oils and fats as raw material are mainly simple esters of straight-chain, even-numbered long chain fatty acids and linear polyols (triglycerides, phospholipids) and their hydrolysis products (Smits 1977, Seegers 1982, 1985, Sayed 1987). Although most of these simple lipids are highly biodegradable, their presence may cause serious problems in aerobic and anaerobic biological treatment systems (Hrudey 1981, Krause 1982, De Zeeuw 1982, Sayed 1987). Pipyn and Verstraete (1980) classified several waste streams contaminated with lipids as slowly biodegradable in anaerobic digestion. The work of De Zeeuw (1982), Liemburg and Van der Wal (1982) and Sayed (1987) shows that problems with sludge flotation and wash-out may limit the loading potentials of anaerobic treatment systems. Wastewater from the textiles industry is frequently considerably more resistant to biodegradation (Spies 1985), because it contains significant amounts of waxes and esters of cyclic alcohols and branched chain fatty acids.

Part of the lipids present in industrial wastewater can be removed easily by physical methods, for example by gravity separation, or flotation. It is advisable to employ these comparatively cheap techniques as a pre-treatment before a biological treatment system, because the relatively coarse suspended material removed in this way will only be degraded very slowly in an anaerobic treatment system (De Baere *et al* 1984, Gujer and Zehnder 1983), and will consequently require

a relatively long retention time. This material is difficult to retain, due to its strong tendency for flotation. As a result of this tendency for flotation, this material may seriously impair the stability of both anaerobic and aerobic biological treatment systems.

However, even after partial physical removal of the lipids, there may still be a considerable amount of emulsified or colloidal lipids left in the wastewater, especially in the edible oils and margarine industry. The investigations described in this thesis are particularly directed to the effect and biodegradation of this finely divided lipid material in high rate anaerobic treatment systems.

Wastewater from edible oil and fat refineries are classified as difficult for a second reason, viz. their very high sulfate concentration (Pipyn and Verstraete 1980, Segers 1982, Rinzema *et al* 1986). In edible oil refineries the sulfate concentration can be as high as 40 kg $SO_4^{2-}.m^{-3}$. The average ratio between COD and sulfate concentration in the wastewater generated by Dutch edible oil refineries is 0.5 kg $COD.kg^{-1}$ SO_4^{2-} (Ministerie VOMIL 1981).

Anaerobic Versus Aerobic Wastewater Treatment

The advantages and drawbacks of anaerobic wastewater treatment compared to aerobic wastewater treatment have been discussed extensively in recent publications (Lettinga *et al* 1980a, 1980b, 1984, Olthof and Oleszkiewicz 1982, Speece 1983). The most significant advantages of anaerobic treatment are:

- the low consumption of high grade energy, and the nett production of biogas with a high caloric value if the COD of the wastewater is sufficiently high.
- the low production of well stabilized sludge.
- the high space loading rate that can be applied in modern anaerobic reactor systems, consequently the compact installations.

The most significant drawbacks of anaerobic treatment are:

- the need for aerobic post-treatment (removal of remaining organic contaminants, oxidation of sulfide and ammonia).
- the lack of experience with anaerobic treatment of some types of wastewater.

Two other drawbacks that are often mentioned, are the long start-up period required in anaerobic treatment systems, and the sensitivity of anaerobic microorganisms towards inhibitory compounds. These drawbacks have largely been eliminated by technological advances and improved insight in the microbiology of anaerobic digestion.

In the case of lipid containing wastewater, the difference in loading capacity between anaerobic and aerobic treatment systems is of special importance. In conventional activated sludge plants, only very low sludge loading rates can be applied and high biomass concentrations cannot be achieved, viz. ca. 0.1 kg $BOD.kg^{-1}$ $TSS.day^{-1}$ and 5 kg $TSS.m^{-3}$ (Krause 1982, Roelfsema 1987). Therefore, conventional activated sludge plants cannot accommodate organic space loading rates exceeding ca. 0.5 kg $COD.m^{-3}.day^{-1}$.

Modern anaerobic treatment systems can accommodate at least ten times higher organic space loading rates than conventional activated sludge plants (Lettinga *et al* 1983, Morper 1986, Backman *et al* 1986, Sayed *et al* 1987). Therefore, the required reactor volume for complete treatment can be reduced by at least 60% by applying a combined anaerobic-aerobic treatment system, provided the wastewater contains no more than 0.006-0.03 kg $nitrogen.kg^{-1}$ COD (Speece and McCarty 1964). The power consumption for aeration will be reduced by a comparable percentage.

Microbiology of Anaerobic Digestion at Low Sulfate Concentration

The current status of our knowledge of the microbiology of anaerobic digestion is visualized in Figure 1. Five steps can be distinguished in the anaerobic mineralization process, when the concentrations of electron acceptors like sulfate and nitrate are negligible (McCarty 1982, Gujer and Zehnder 1983):

(1) Hydrolysis of macro-molecules. In the case of carbohydrates and proteins this results in the formation of soluble monomers. In the case of lipids the long chain fatty acids represent the major portion of the reduction equivalents, and are poorly soluble. Hence, in the case of lipids hydrolysis is not equivalent to liquefaction (c.f. Eastman and Ferguson 1981).

(2) Fermentation of sugars, amino acids, and alcohols by acidogenic bacteria, yielding mainly volatile fatty acids, hydrogen and carbon dioxide, and smaller amounts of ethanol and lactic acid. These acidogenic bacteria cannot degrade long chain fatty acids, although some species are capable of hydrogenation of unsaturated acids (Dawson and Kemp 1970, Kemp *et al* 1975).

(3) Anaerobic oxidation of long chain fatty acids. Tracer studies have shown that the principal mechanism is ß-oxidation (Jeris and McCarty 1965). The bacteria catalyzing this reaction are obligate hydrogen producers (Roy *et al* 1985, 1986), their metabolism is inhibited by hydrogen (Novak and Carlson 1970). This implies that a syntrophic association with hydrogen oxidizing bacteria is required for anaerobic degradation of long chain fatty acids.

(4) Anaerobic oxidation of volatile fatty acids by obligate hydrogen producing bacteria, yielding acetate and hydrogen, and depending on the chain length of the fatty acids also carbon dioxide. As in step 3, these bacteria are obligate syntrophs, i.e. they require the company of hydrogen oxidizing bacteria (Bryant *et al* 1967, McInerney *et al* 1979, 1981, Boone and Bryant 1980).

(5) Production of methane from acetate, and from hydrogen plus carbon dioxide. These reactions finally lead to the desired reduction in the oxygen demand of the wastewater. Jeris and McCarty (1965) have shown that approximately 70% of the methane is produced by cleavage of acetate, against 30% by oxidation of hydrogen.

The historical development of our knowledge of the microbiology of anaerobic digestion has been extensively reviewed by McCarty (1982). Some relevant discoveries that have been published more recently, will be discussed briefly, with emphasis on anaerobic digestion of lipids.

Although there is a substantial body of literature about the anaerobic degradation of lipids in the rumen and other parts of the gastrointestinal tract (Garton *et al* 1958, Lough 1970, Bryant 1977, Prins 1977, Harfoot 1978, Hobson and Wallace 1982), very little is known about the microbiology of lipid degradation in anaerobic digesters. Several bacteria capable of hydrolyzing triglycerides and phospholipids have been isolated from the rumen, as well as from pig manure digesters (Dawson 1959, Hobson and Mann 1961, Henderson 1971, Hobson *et al* 1974, Prins *et al* 1975, Hazlewood and Dawson 1975, 1979). It should be emphasized that there is a large difference between the environmental conditions in the rumen and those in an anaerobic digester, e.g. with respect to retention time, hydrogen partial pressure, pH and product concentration.

It has been demonstrated that extensive hydrogenation of unsaturated long chain fatty acids occurs in the rumen (Dawson and Kemp 1970, Kemp *et al* 1975, Kemp and Lander 1983, 1984, Hobson and Wallace 1982). However, the importance of hydrogenation in anaerobic digesters has not been clarified. There is some circumstantial evidence for hydrogenation in anaerobic digesters (Novak and Carlson 1970), but recently isolated *Syntrophomonas* sp. are capable of direct oxidation of unsaturated long chain fatty acids (Roy *et al* 1986).

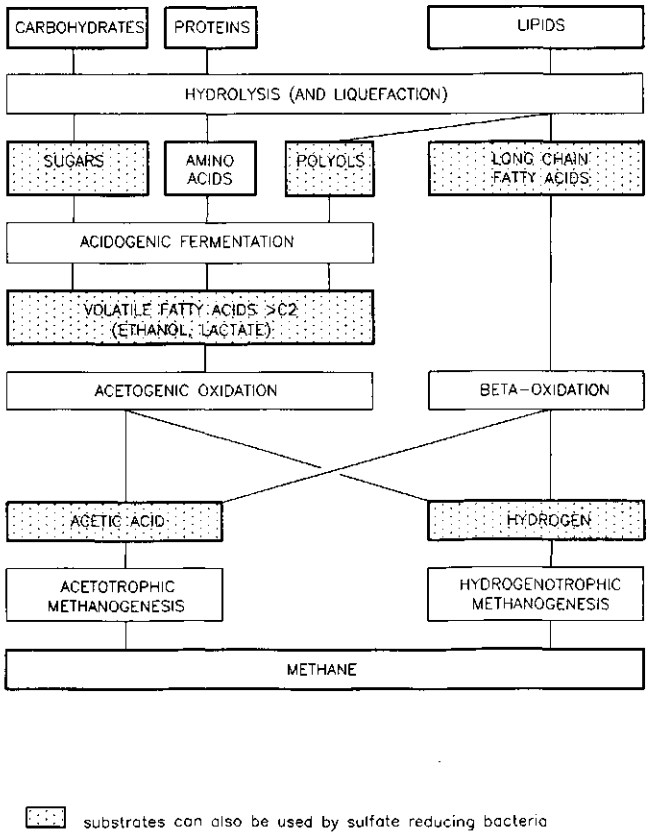

Figure 1. Degradation pathways in anaerobic digestion at low sulfate concentration. Compounds in shaded boxes can also be converted by sulfate reducing bacteria, if sufficient sulfate is available.

Table 3. Kinetic parameters for enrichment cultures degrading long chain fatty acids (Novak and Carlson 1970)

fatty acid	$\hat{\mu}$ (day^{-1})	K_s ($kg.m^{-3}$)
myristic acid ($C_{14:0}$)	0.10	105
palmitic acid ($C_{16:0}$)	0.11	143
stearic acid ($C_{18:0}$)	0.08	417
oleic acid ($C_{18:1}$)	0.44	3180
linoleic acid ($C_{18:2}$)	0.55	1816

CSTR experiments (37 °C, pH unknown), $\hat{\mu}$ values calculated from maximum specific activities and yield data presented by the authors, long chain fatty acids were added as sodium salts

Table 4. Maximum specific growth rates of *Syntrophomonas sapovorans* on various long chain fatty acids (Roy *et al* 1985, 1986)

fatty acid	$\hat{\mu}$ (day^{-1})
butyric acid ($C_{4:0}$)	0.62
lauric acid ($C_{12:0}$)	0.55
myristic acid ($C_{14:0}$)	0.55
stearic acid ($C_{18:0}$)	0.37
oleic acid ($C_{18:1}$)	0.42

batch experiments (35 °C, pH 7.1), initial concentration of fatty acids 4 $kg.m^{-3}$, long chain fatty acids were added as calcium salts

The early studies of Heukelekian and Mueller (1958) already indicated that ß-oxidation is the primary mechanism in anaerobic degradation of long chain fatty acids. This was proven conclusively by tracer studies conducted by Jeris and McCarty (1965). Novak and Carlson (1970) obtained the first enrichment cultures on long chain fatty acids, and studied the degradation kinetics of several of these acids. Only very recently, Roy *et al* (1985, 1986) isolated the first obligate hydrogen producing bacterium capable of oxidizing fatty acids with more than ten carbon atoms, *Syntrophomonas sapovorans*. Tables 3 and 4 summarize the kinetic parameters determined by Novak and Carlson (1970) and Roy *et al* (1985).

Bryant and coworkers (Bryant *et al* 1967, McInerney *et al* 1979, 1981, Boone and Bryant 1980) discovered that ethanol and fatty acids with more than two carbon atoms can only be degraded by syntrophic associations of acetogenic bacteria and hydrogenotrophic methanogens (or sulfate reducers). The discovery of Bryant and coworkers also led to a better appreciation of the crucial role of hydrogen in anaerobic digestion. Extremely low partial pressures of hydrogen are required to make the socalled acetogenic reactions - steps 3 and 4, c.f. Figure 1 - thermodynamically possible. Thus very close spatial associations of both partners in the symbiotic relationship are required, in order to maintain these low hydrogen concentrations (Gujer and Zehnder 1983). An important implication is that biomass aggregates (flocs, granules, or films) may offer significant advantages with respect to acetogenesis. This is the basis for the current microbiological theory that the mutual dependance of both organisms in these associations may be the driving force behind the formation of biomass aggregates (Dubourguier *et al* 1988).

The discovery of Bryant *et al* prompted the transition from the two-phase model of anaerobic digestion introduced by Buswell and Neave (1930), to the currently accepted four-phase or five-phase model illustrated in Figure 1. It established the current theory that methanogens fall into two trophic groups, with acetic acid and hydrogen as principal substrates. About a decade after Bryant's discovery of the S-organism, this theory was firmly established by the isolation of the first acetotrophic methanogens, belonging to the genera *Methanosarcina* (Mah *et al* 1978, Weimer and Zeikus 1978) and *Methanothrix* (Huser 1980, Zehnder *et al* 1980). Especially this latter genus is of great importance in modern high-rate anaerobic wastewater treatment systems with biomass retention. The 'rod-like' or 'filamentous' organisms that are frequently observed in biomass aggregates and biofilms (Van den Berg 1984, Hulshoff Pol *et al* 1983, Dubourguier *et al* 1985, Heijnen 1983, Heijnen *et al* 1986) in these reactor systems, belong to this genus. A comparison of the kinetic parameters of *Methanosarcina* and *Methanothrix* sp. indicates that the long biomass retention times realized through immobilization cause the selection of *Methanothrix*, because it has a much higher substrate affinity at low specific growth rates (Gujer and Zehnder 1983).

Microbiology of Anaerobic Digestion at High Sulfate Concentration

High concentrations of sulfate cause a change in the metabolic routes in an anaerobic digester. The obligate hydrogen producing acetogens and the methanogens indicated in Figure 1 (steps 3-5) will have to compete with sulfate reducing bacteria that are capable of utilizing the same substrates. The importance of the competition between sulfate reducing bacteria and methanogenic or acetogenic bacteria increases as the COD:SO_4-ratio of the waste decreases.

The developments in the field of microbiology were reviewed extensively by Rinzema and Lettinga (1988), therefore only a short description is presented here. The microbiological studies of Hoppe-Seijler (1886), Rubentschik (1928) and Baars (1930), already indicated that complete mineralization of organic electron donors by sulfate reducing bacteria is possible. Baars (1930) isolated a sulfate reducer, *Desulfovibrio rubentschikii*, that oxidized acetate and butyrate. This organism was also isolated from sewage sludge by Basu and Ghose (1961). Because most attempts to repeat the isolation of this bacterium failed, the existence of sulfate reducers capable of fatty acid oxidation, was not generally accepted until the end of the seventies (Postgate 1979). Sulfate reducers capable of utilizing hydrogen, lactate and ethanol as substrates were studied in pure culture since the 1950s. Therefore, until the end of the 1970s, sulfate reducers were regarded as a group of microorganisms with a rather limited scala of substrates. Three genera were recognized, viz. *Desulfovibrio* sp., *Desulfotomaculum* sp. and *Desulfomonas* sp. (Postgate 1979, Moore *et al* 1976).

However, sediment studies published during the 1970s (Jørgensen 1977, Winfrey and Zeikus 1977) indicated that sulfate reducing bacteria capable of mineralization of fatty acids do exist. Furthermore, Middleton and Lawrence (1977) succeeded in obtaining an enrichment culture of acetate oxidizing sulfate reducers from sewage sludge. Widdel provided a major break-through with his description of the first acetotrophic sulfate reducer isolated in pure culture, *Desulfotomaculum acetoxidans* (Widdel and Pfennig 1977), which was soon followed by the description of several sulfate reducers capable of fatty acid oxidation (Widdel 1980, Widdel and Pfennig 1981a, 1981b, 1982, 1983). At present, six genera are distinguished besides the three that were already known, viz. *Desulfobulbus* sp., *Desulfobacter* sp., *Desulfococcus* sp., *Desulfonema* sp., *Desulfosarcina* sp., and *Desulfobacterium* sp. Sulfate reducers are now regarded as a very versatile group of microorganism, capable of using a wide range of substrates, including all straight-chain fatty acids from formic to stearic acid (Widdel 1980), several aromatic acids (Widdel 1980, Imhoff-Stuckle and Pfennig 1983), methanol (Nanninga and Gottschal 1986, 1987), glycerol (Nanninga and Gottschal 1987), sugars (Cord-Ruwisch *et al* 1986, Joubert and Britz 1988), and several phenolic compounds (Bak and Widdel 1986a, 1986b, Szewzyk and Pfennig 1987).

Competition Between Sulfate Reducers and Methanogens or Acetogens

The versatility of sulfate reducing bacteria as a group has severe implications for anaerobic wastewater treatment. Thermodynamic and, more important, kinetic data indicate an advantage for sulfate reducers over their acetogenic and methanogenic competitors (Rinzema and Lettinga 1988). Consequently, sulfide instead of methane will theoretically be the end product of the anaerobic mineralization process until sulfate is completely exhausted. This prediction is confirmed by the available experimental data from ecological studies on marine and freshwater

sediments (Winfrey and Zeikus 1977, Zaiss 1981, Smith and Klug 1981, Banat 1981, Lovley *et al* 1982, Oremland and Polcin 1982, Schönheit *et al* 1982, Lovley and Klug 1983, King 1984, Lovley and Klug 1986).

The results obtained by Middleton and Lawrence (1977) and Olthof *et al* (1985) in completely mixed reactors (CSTR's) without biomass retention, also confirm that sulfate reducers have a kinetic advantage over methanogens or acetogens.

Our optimism with regard to the possibility to produce methane from sulfate loaded wastewater from edible oil refineries, was based on the work of Mulder (1982, 1984) and Hoeks *et al* (1983, 1984). Mulder treated yeast factory wastewater with excess sulfate in UASB reactors. He concluded that hydrogen produced by acidogenic and acetogenic bacteria is completely oxidized by sulfate reducers when sufficient sulfate is present, but all the acetate remains available for methanogenesis. Hoeks *et al* observed that anaerobic contact process reactors inoculated with granular sludge from an industrial UASB reactor continued to produce methane during treatment of wastewater from edible oil refineries (acid water) and glycerol solutions, both with an extremely unfavorable COD:SO_4-ratio. Hoeks' mass balance calculations supported the conclusion of Mulder that acetate remains available for methanogenesis, i.e. ca. 70% of the COD was converted to methane. Hoeks *et al* (1984) suggested several explanations for their results, viz. mixotrophic growth of methanogens on hydrogen and acetic acid, or an iron limitation of sulfate reducers. However, they were not able to prove any of these hypotheses.

Since then, several studies on anaerobic treatment of wastes with excess sulfate have been published (Tables 5 and 6). An examination of these results clearly shows that hydrogen produced as an intermediate during anaerobic digestion is captured virtually completely by sulfate reducers. The results with regard to acetate utilization are, however, rather unpredictable. Evidently, sludge retention is a key factor in the competition. However, comparable systems do not always give comparable results. Our lab scale studies indicated that UASB reactors with granular sludge can give the acetotrophic methanogens sufficient advantage (Rinzema *et al* 1986, Rinzema and Lettinga 1988, Rinzema unpublished, c.f. Table 6). However, the lab scale results could not be reproduced on a semi-technical scale (Rinzema and Schultz 1987, c.f. Table 6). Published results for contact process (CP) and upflow anaerobic filter (UAF) reactors show similar inconsistencies (Table 5). No general guidelines on the effect of operational variables and type of inoculum can be delineated from the available experimental data.

Two theories have been published, that claim to explain the successful competition of acetotrophic methanogens against sulfate reducers. Both theories will be discussed briefly.

Isa *et al* (1986a, 1986b) concluded that the successful competition of methanogens can be attributed to two factors: (1) their superior capability to colonize support materials, and (2) a high substrate concentration. Their superior colonization capabilities would enable the methanogens to outcompete the sulfate reducers, despite the kinetic superiority of the latter organisms (higher maximum specific activity and higher substrate affinity). A high substrate concentration appeared to favor the methanogens. Isa *et al* treated sulfate containing synthetic wastewater in anaerobic filter reactors with reticulated polyurethane foam sponges as support material. These reactors were fed semi-continuously (once daily). Acetic acid, ethanol, and even formic acid were converted largely to methane, in the presence of excess sulfate. Cell counts performed by Isa *et al* (1986b) indicated that the ratio between the number of organisms present in the porous packing material and the number of freely suspended organisms in the liquid was much higher for methanogenic bacteria than for sulfate reducing bacteria. Furthermore, activity tests also indicated that the methanogenic activity was mainly associated with the biomass present on the support material.

A rigorous evaluation of the work of Isa *et al* shows that in fact their results do not allow definite conclusions regarding the effect of substrate concentration and residence time, because conflicting results were obtained in different sets of experiments. The semi-continuous feeding strategy employed by these authors makes a straightforward investigation of the effect of residence time, influent concentration and space loading rate impossible. Very high substrate concentrations occur during part of the time. We conducted computer simulations of Isa's experiments, which demonstrate that this feeding strategy may lead to dominance of bacterial species, that would not dominate in a continuously fed reactor with biomass retention, operated at a comparable space loading rate (the substrate affinity is less important in the semi-continuous system). As a matter of fact the only direct evidence for the superior colonization capability of

Table 5. Overview of literature data on anaerobic treatment of wastewater with excess sulfate

reactor type[a] (volume)	inoculum	biomass type	HRT (days)	electron donor	r_{COD}/r_{H2S}	r_{CH4}/r_{H2S}	ref.
CSTR (6 dm^3)	digested sewage sludge	dispersed	1-20	acetic acid	1.0	0.0	1
CSTR (12 dm^3)	unknown	dispersed	15	sewage sludge	1-1.25	0.0	2
UASB (6 dm^3)	unknown	flocs	0.2	synthetic sewage sludge	1.8	0.4	2
UASB (2.5 dm^3)	flocculent sludge	flocs	0.6-0.74	yeast waste water	3	-	3
CP (3 dm^3)	granular sludge (sugar)	flocs	0.9-4.6	acid water,[b] glycerol	4.0	1.1	4
CP (0.5 m^3)	digested sewage sludge	flocs	2.0-9.3	acid water	1.55	-	5
UAF (0.5 m^3)	digested sewage sludge	flocs, biofilm	0.7-8.1	acid water	1.8	-	5
UAF (1.5 dm^3)	aerobic activated sludge	flocs, biofilm	0.5	molasse	3.5	-	6
UAF (1 dm^3)	flocculent sludge	flocs, biofilm	0.5	acetic acid, ethanol	ca. 10	ca. 9	7
FB (3.2 dm^3)	anaerobic biofilm	biofilm	0.3	acetic acid	1.6	0.5	8
FB (3.2 dm^3)	anaerobic biofilm	biofilm	0.02-0.09	acetic acid	-	$\geq$ 0.9	8

[a] CSTR completely mixed reactor without biomass retention, CP contact process reactor, UASB upflow anaerobic sludge bed/blanket reactor, UAF upflow anaerobic filter reactor, FB fluidized bed reactor

[b] acid water is wastewater from edible oil refineries

references: 1 Middleton and Lawrence 1977; 2 Olthof et al 1985; 3 Mulder 1982; 4 Hoeks et al 1984; 5 Donnelly et al 1986; 6 Maree and Strydom 1986; 7 Isa et al 1986a, 1986b; 8 Yoda et al 1988

Table 6. Authors own results on anaerobic treatment of wastewater with excess sulfate

reactor type[a] (volume)	inoculum	biomass type	HRT (days)	electron donor	$\frac{r_{COD}}{r_{H2S}}$	$\frac{r_{CH4}}{r_{H2S}}$	ref.
UASB (1.5 dm^3)	granular sludge (potato)	granules	0.2-2	acid water	4	3	9
UASB (1.5 dm^3)	granular sludge (potato)	granules	0.5-1.6	acetic acid	∞	∞	10
UASB (1.5 dm^3)	granular sludge (potato)	granules	0.6-2	propionic acid	3.5	2.5	11
UASB (2.5 dm^3)	granular sludge (sugar)	granules	0.12	glycerol, lipids	2	1	10
UASB (20 m^3)	granular sludge (sugar)	granules	0.25-0.5	acid water	1.5	0.5	12

[a] UASB upflow anaerobic sludge bed/blanket reactor

references: 9 Rinzema et al 1986; 10 Rinzema unpublished; 11 Rinzema and Lettinga 1988; 12 Rinzema and Schultz 1987

the methanogens provided by Isa *et al*, i.e. the cell counts, has a very limited accuracy. It is hardly possible to make reliable counts of immobilized bacteria, the choice of the medium may influence the result of plate counts, and the use of epifluorescence for a direct count of methanogens may be hampered by differences in fluorescence intensity between species. The indirect evidence for the superior colonization capability of the methanogens, provided by Isa's activity tests, is invalid. Incomplete conversion of the substrate mixture was achieved with the freely suspended biomass, and therefore preferential use of one substrate may have caused an incorrect estimate of the ratio between methanogenic and sulfidogenic activities.

Recently, Yoda *et al* (1988) concluded that acetotrophic methanogens growing in a biofilm can outcompete their sulfate reducing competitors, because the specific growth rate of the methanogens exceeds that of the sulfate reducers when the acetate concentration in the bulk of the liquid exceeds 8.8 $mg.l^{-1}$. Yoda *et al* (1988) evaluated their experimental results with a model for biomass retention in a fluidized bed reactor (Yoda *et al* 1987), which assumes that the attrition of methanogens and sulfate reducers from the biofilm can be described as a first order process, with equal rate constants for both species. They calculated apparent kinetic parameters for acetotrophic methanogens and sulfate reducers in the biofilm, which differ markedly from previously published values for suspended organisms (Table 7).

The starting-points of the model used by Yoda *et al* (1987, 1988), i.e. equal colonization capability for both species and first order attrition kinetics, have not been verified. In fact, the model predicts that the substrate concentration in the reactor and thus the dominating species will be independent of the hydraulic residence time. This prediction is contradicted by the experimental results (Yoda *et al* 1988). Furthermore, several assumptions made in the calculation

Table 7. Kinetic parameters for acetotrophic methanogens and sulfate reducers

organism	$\hat{\mu}$ (day^{-1})	K_s (mM)	k_d (day^{-1})	Y (kg VSS / kg COD)	ref.
acetotrophic methanogens					
enrichment culture	0.037	0.55	0.002	0.019	1
enrichment culture	0.21	0.333	0.037	0.051	2
Methanothrix söhngenii	0.11	0.46-0.7	-	0.012-0.023	3,4,5[a]
Methanothrix concilii	0.7	1.2	-	-	6
Methanosarcina barkeri	0.21-0.55	4.0 -5.0	-	0.018-0.07	7,8,9[a]
acetotrophic sulfate reducers					
enrichment culture	0.015	0.16	0.0016	0.015	1
enrichment culture	0.54	0.1	0.00	0.061	10
Desulfotomaculum acetoxidans	0.55-1.44	-	-	0.086-0.118	11,12[a]
Desulfobacter postgatei	0.84-1.03	0.08-0.23	-	0.040-0.075	13-17

[a] ca. 35 °C; others 30 °C

references: 1 Yoda et al 1988; 2 Lawrence and McCarty 1969; 4 Huser 1981; 5 Zehnder et al 1980; 6 Patel 1984; 7 Smith and Mah 1980; 8 Scherer and Sahm 1981; 9 Wandrey and Aivasidis 1983; 10 Middleton and Lawrence 1977; 11 Widdel and Pfennig 1977; 12 Widdel and Pfennig 1981; 13 Widdel 1980; 14 Brandis-Heep et al 1983; 15 Widdel and Pfennig 1981; 16 Schönheit et al 1982; 17 Ingvorsen et al 1984

of the maximum specific growth rates, specific decay rates, and yield coefficients have not been verified. Therefore, only the maximum specific activities and the substrate affinity constants (K_s), which have been derived from batch experiments in a straightforward manner, are reliable.

The conclusion must be drawn that so far no satisfactory explanation for the dominance of (acetotrophic) methanogens in reactors with biomass retention is available. Conflicting explanations have been suggested by Isa *et al* (1986a, 1986b) and Yoda *et al* (1988). Where the latter authors assume equal colonization capability for both species, the former authors indicate a difference in colonization capability as the main cause for the dominance of methanogens. A rigorous evaluation of the publications of both authors shows, that the experimental evidence for their theories is meager. Further experimental research is required to predict the performance of a given reactor type during treatment of a waste stream with excess sulfate.

Inhibition Phenomena in Anaerobic Digestion

A popular misconception about anaerobic wastewater treatment is, that the process is relatively unreliable, because it is extremely sensitive to inhibitory compounds. A large number of recent publications clearly demonstrates that properly designed and operated modern reactor systems can handle wastewater with toxic compounds (e.g. Parkin and Speece 1983, Parkin *et al* 1983).

Nevertheless, the information available in the literature does not allow the formulation of unambiguous guidelines for anaerobic treatment of wastewater with high concentrations of long chain fatty acids, sulfides, or cations accompanying sulfate. The available information will be discussed briefly, and the need for further investigations will be identified.

Inhibition by Long Chain Fatty Acids

Long chain fatty acids (LCFA) are well known inhibitors of various microorganisms (Glassman 1948, Camien and Dunn 1957, Prince 1959, Bell 1971, Kabara *et al* 1977). Gram-negative microorganisms are to some extent protected by the lipopolysaccharide layer in their cell wall (Sheu and Freese 1973), but gram-positive microorganisms are affected at low concentrations (Blaxter and Czerkawski 1966, Kodicek and Worden 1945, Nieman 1954, Kabara *et al* 1977). Since the cell wall of most methanogenic bacteria resembles that of gram-positive bacteria (Zeikus 1977, Wolfe 1979), it is reasonable to expect that they are sensitive to LCFA. Apart from the methanogens, many other gram-positive bacteria play a crucial role in anaerobic digestion, e.g. acetogenic bacteria capable of long chain fatty acid oxidation (Roy *et al* 1986) and sulfate reducers (Widdel 1980). Research on rumen microorganisms has revealed that LCFA may indeed be severely inhibitory to cellulolytic and methanogenic species (Henderson 1973, Prins *et al* 1972, Maczulac *et al* 1981).

Saturated fatty acids with 12-14 carbon atoms and unsaturated fatty acids with 18 carbon atoms are usually indicated as the most versatile inhibitors (Nieman 1954, Blaxter and Czerkawski 1966, Galbraith *et al* 1971, El Hag and Miller 1972). The inhibitory effect of unsaturated LCFA increases with the number of double bonds (Demeyer and Henderickx 1967, Prins *et al* 1972), and cis-isomers that are abundant in natural lipids are considerably more inhibitory than their counterparts with a trans-orientation (Demeyer and Henderickx 1967, Galbraith *et al* 1973). Triglycerides and several non-ionic fatty acid derivatives are not inhibitory (Kodicek and Worden 1945, Blaxter and Czerkawski 1966, Czerkawski *et al* 1966a, 1966b, Demeyer and Henderickx 1967, Van Nevel *et al* 1971). Sulfonates and monoglycerides, however, are reported to be inhibitory (Czerkawski *et al* 1966b, Kabara *et al* 1977).

Most authors ascribe the inhibitory effect of LCFA to adsorption of the surface active acids onto the cell wall, which may damage the transport function or the protective function of the cell wall (Kodicek and Worden 1945, Hotchkiss 1946, Franke *et al* 1949, Demeyer and Henderickx 1967, Galbraith *et al* 1973). Hotchkiss (1946) suggests that adsorption of surface active compounds onto the microbial cells destroys the cell wall, and causes the release of the cell contents. Kodicek and Worden (1945) suggest three possible mechanisms for the inhibitory effect of adsorbed LCFA: (1) the fatty acids change the membrane permeability, (2) the fatty acids affect the surface tension, which exerts a negative influence on cell division, and (3) a non-defined chemical influence. The second mechanism is contradicted by the results of Blaxter and Czerkawski (1966), who observed that lauric acid and oleic acid cause different degrees of inhibition, while giving a comparable change in surface tension. Lai *et al* (1976) observed that LCFA affect the transport properties of membrane vesicles from *Escherichia coli*.

The inhibitory effect of LCFA in anaerobic digestion was already observed by McCarty (1964), in research on anaerobic digestion of sewage sludge. McCarty concluded that anaerobic digestion of wastes containing LCFA is possible, provided a continuously fed digester is used, and sudden overloading is avoided. This implies that conventional sludge digesters operated at hydraulic retention times of 15-20 days will hardly ever suffer from LCFA inhibition. However, during anaerobic treatment of relatively dilute industrial wastewater in high-rate anaerobic digesters operated at a hydraulic retention time of only a few days or even a few hours, shock loads of LCFA may cause serious problems.

Hanaki *et al* (1981) conducted an extensive study of the inhibitory effect of LCFA in anaerobic digestion. They concluded that LCFA affect the obligate hydrogen producing acetogenic bacteria which are responsible for the ß-oxidation of LCFA, as well as the hydrogenotrophic and acetotrophic methanogenic bacteria which convert the intermediates from the ß-oxidation process. Even when the degradation of LCFA was severely retarded, the hydrogen partial pressure remained very low (50 Pa), indicating that the LCFA affect the acetogens more seriously than the hydrogenotrophic methanogens. Inhibition of acetogens and acetotrophic methanogens caused a pronounced lag phase in batch experiments, whereas inhibition of hydrogenotrophic methanogens merely caused a decrease of the hydrogen conversion rate. Hanaki *et al* also found that LCFA disappear from the solution and accumulate in the solid phase, within 24 hours. This seems to be in accordance with the theories attributing LCFA inhibition to physical interactions between the acids and the membrane of the microorganisms.

Koster and Cramer (1987) studied the influence of individual and mixed LCFA on the specific activity of acetotrophic methanogens in granular sludge from UASB reactors. Their results indicate that LCFA with ten carbon atoms or more are inhibitory. Lauric acid is the most versatile inhibitor among the saturated acids, and the effect of oleic acid is comparable to that of lauric acid. Mixtures of LCFA can exert a synergistic effect. The results of a very limited number of batch tests in which the degradation of lauric acid was studied, suggest that toxicity is not solely determined by adsorption equilibria.

In a second study, Koster (1987) has shown that LCFA affect the cells very rapidly: 50% of the acetotrophic methanogenic activity was lost, already 7.5 minutes after the introduction of lauric acid in an anaerobic digester.

Several authors have shown that soluble calcium salts can reduce or eliminate the inhibitory effect of LCFA (Kodicek and Worden 1945, Demeyer and Henderickx 1967, Galbraith *et al* 1971, El Hag and Miller 1972, Roy *et al* 1985, Koster 1987). Surface active neutral lipids, like cholesterol, calciferol and lecithin are also mentioned as antagonists to LCFA (Kodicek and Worden 1945, Demeyer and Henderickx 1967, Galbraith *et al* 1971). The results of Koster (1987) as well as those of Hanaki *et al* (1981) indicate that calcium salts have to be added within minutes after the introduction of the acids into the digester. Hotchkiss (1946) concluded that surface active compounds have to be added simultaneously with the LCFA, to obtain an antagonistic effect. The requirement for timely addition is in agreement with the bactericidal effect of LCFA observed by some authors (Hotchkiss 1946, Galbraith *et al* 1973).

No reliable guidelines concerning the allowable amount of LCFA in an anaerobic digester can be derived from the available literature. It is not clear, whether the fatty acid concentration, or the ratio between long chain fatty acid and biomass quantities governs inhibition. Furthermore, it is not clear whether anaerobic microorganisms can recover after inhibition by LCFA. So far, the results of batch inhibition studies have not been validated by shock load experiments in continuous digesters.

Inhibition by Sulfide

Sulfide is an essential nutrient for methanogenic bacteria (Zehnder and Wuhrman 1977, Bryant *et al* 1971, Rönnow and Gunnarsson 1981, De Zeeuw 1984), but only a low concentration is required for optimal growth; higher concentrations cause severe inhibition.

The stimulative and inhibitory effect of sulfide is supposed to be determined by the concentration of undissociated hydrogen sulfide, as only the neutral molecule can permeate the cell wall (Schlegel 1981). Therefore, chemical and physical equilibria have to be considered in a study of the effect of sulfide on anaerobic bacteria, as is schematically illustrated in Figure 2.

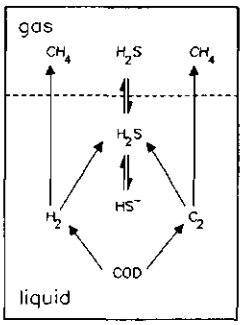

Figure 2. Schematic representation of chemical and physical equilibria relevant with respect to sulfide inhibition

The concentration of undissociated hydrogen sulfide in an anaerobic digester is affected by chemical dissociation:

$$H_2S\ (liq) \rightarrow HS^- + H^+,$$

as well as by the distribution of hydrogen sulfide between gas and liquid phase:

$$H_2S\ (liq) \rightarrow H_2S\ (gas)$$

The pK_a-value of the dissociation equilibrium of hydrogen sulfide is estimated at 6.9 at 30 °C (based on extrapolation of values from Weast (1978), using the Van 't Hoff equation). Consequently, small pH variations within the pH range (6.5-8) that is considered as optimal for anaerobic digestion can have a very significant effect on the inhibition. The gas-liquid distribution coefficient is 2.27 at 30 °C (Wilhelm *et al* 1977). This implies that the methane production from an amount of organic waste equivalent to 5 kg $COD.m^{-3}$ can already remove 30% of the sulfide from the liquid phase, in a digester operated at pH 6.9.

Unfortunately, insufficient attention was paid to these chemical and physical equilibria in the majority of the studies on sulfide inhibition (Bannink and Muller 1951, Rudolfs and Amberg 1952, Aulenbach and Heukelekian 1955, Butlin *et al* 1956, Parkin *et al* 1983, Isa *et al* 1986a).

Lawrence *et al* (1966) were the first to consider the influence of pH and gas production on sulfide toxicity. They determined the effect of sulfide in continuously fed, completely stirred tank reactors (CSTR's) without sludge retention, that were in a steady state situation. The actual sulfide concentration in the digester fluid, as well as the pH were determined. Lawrence *et al* concluded that the maximum allowable sulfide concentration in CSTR's operated at a retention time of 20 days, is 200 mg $S.l^{-1}$. This is equivalent to ca. 50 mg H_2S-$S.l^{-1}$ under the experimental conditions employed by the authors. However, these experiments provide no information on the effect of hydrogen sulfide at concentrations between 50 mg H_2S-$S.l^{-1}$ (allowing successful digestion) and 150 mg H_2S-$S.l^{-1}$ (causing process failure). Furthermore, the results are only valid for digesters operated at a sludge residence time of 20 days, and do not allow any clear conclusions regarding the influence of sulfide on the maximum specific activity of the methanogens. The results of Lawrence *et al* are still quoted rather often, but it is clear that the threshold level advised by these authors is very conservative, especially for modern reactor systems with biomass retention.

Consequently, from all results published prior to 1980 only a few qualitative conclusions with regard to the effect of sulfides on modern reactor systems can be drawn: (1) non-dissolved metal sulfides are not inhibitory (Aulenbach and Heukelekian 1955, Lawrence *et al* 1966), and (2) methanogenic bacteria are far more sensitive to sulfide than acidogenic bacteria and sulfate

reducing bacteria (Rudolfs and Amberg 1952).

Since 1980, three studies have been published that provide information on the effect of hydrogen sulfide on the maximum specific activity of methanogenic cultures. Kroiss and Plahl-Wabnegg (1983) found a very strong effect of hydrogen sulfide on the specific activity of acetotrophic methanogens in flocculent sludge from an industrial digester: ca. 50 mg H_2S-S.l^{-1} already caused 50% inhibition. Speece *et al* (1986) found stimulation of acetotrophic methanogens in digested sewage sludge, up to ca. 70 mg H_2S-S.l^{-1}, and a very sharp decrease in activity at higher concentrations. Karhadkar *et al* (1987) found a less severe effect of hydrogen sulfide on the methanogenic activity of a mixed anaerobic population cultivated on synthetic distillery waste.

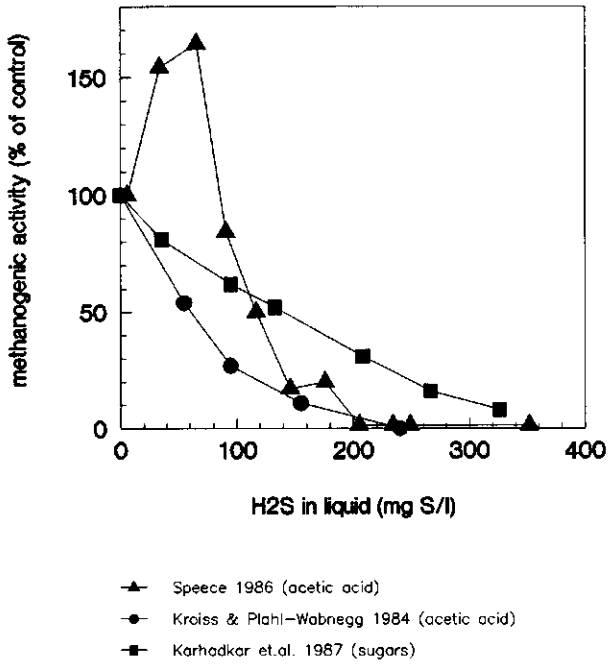

Figure 3. Inhibitory effect of sulfide on methanogenesis (substrates indicated in legend, Kroiss and Plahl-Wabnegg 1983, Speece *et al* 1986, Karhadkar *et al* 1987)

Figure 3 shows that there is a considerable difference in the results of these three studies. Furthermore, these studies share one shortcoming: although the authors correlated the activity to the concentration of undissociated hydrogen sulfide and paid sufficient attention to pH control and removal of hydrogen sulfide by the biogas, they failed to prove that the inhibitory effect of undissociated H_2S is independent of the pH-level. This precludes a proper evaluation of the possibilities of pH-regulation as a control strategy against H_2S toxicity.

Model calculations based on physico-chemical equilibria and mass balance equations, show that an increase of the pH in an anaerobic digester reduces the amount of sulfide that is stripped by the biogas. The dissociation equilibrium shifts sufficiently, however, to give a decrease of the H_2S concentration (Rinzema and Lettinga 1988). Consequently, operation at increased pH may provide a simple method to reduce the inhibitory effect of sulfide and improve the capacity of the

digester, provided that the inhibitory effect of hydrogen sulfide does not increase at high pH-levels. A verification of the supposed independence is required, to assess the merits of pH-regulation.

A second obvious limitation of almost all experimental studies conducted so far, is that they were focussed only on inhibition of the acetotrophic methanogens. These organisms are indeed more sensitive to hydrogen sulfide than the hydrogenotrophic methanogens, acidogens and sulfate reducers (Rudolfs and Amberg 1952, Gunnarsson and Rönnow 1982, Kroiss and Plahl-Wabnegg 1983). However, there is some evidence that obligate hydrogen producing acetogens are also very sensitive to hydrogen sulfide (Boone and Bryant 1980, Grotenhuis 1987). This may be important with respect to anaerobic treatment of wastewater from edible oil refineries, as glycerol is an important organic pollutant in these wastes (Smits 1977, Segers 1982, Hoeks *et al* 1983), and propionic acid is probably one of the most important intermediates in the anaerobic fermentation of glycerol (Johns 1953, Hobson and Mann 1961, Prins *et al* 1975). Consequently an investigation of the sensitivity of propionate degrading cultures is warranted.

Inhibition by Cations

Wastewater with a high sulfate concentration inevitably also contains a high concentration of cations, because neutralization or buffering will be required before anaerobic treatment. Although calcium bases may be substantially cheaper than sodium bases, the addition of high amounts of calcium hydroxide should be avoided, because it will cause insurmountable operational problems. It has been shown that calcium concentrations above ca. 400 $mg.l^{-1}$ may cause very serious problems. Calcium precipitates may cover biomass aggregates, which results in transport limitations (Lettinga *et al* 1987). Precipitates will build up in the digester and in piping, which results in a loss of biomass and plugging problems. Furthermore, precipitation may cause a phosphate deficiency (Callander and Barford 1983, Lettinga *et al* 1987).

Therefore, sodium hydroxide or sodium (bi)carbonate has to be preferred for neutralization and buffering purposes. There is a substantial body of literature on sodium toxicity in anaerobic digestion. Nevertheless, no unambiguous guidelines concerning the maximum admissable sodium concentration in high-rate anaerobic digesters with biomass retention are available. The available literature and the need for additional research are discussed extensively in Chapter 8.

Anaerobic Wastewater Treatment Technology

Table 8 gives an overview of the development of anaerobic wastewater treatment technology since the introduction of the first septic tanks at the end of the nineteenth century in France and the United Kingdom. The most important improvement in the anaerobic technology is the introduction of biomass retention systems. This allows a considerable shortening of hydraulic retention times, i.e. from several weeks - as applied in sewage sludge digesters - to only a few hours for liquid waste treatment systems. Wash-out of the slow-growing methanogens at these short hydraulic retention times is prevented by immobilization or recycling of the biomass.

Basically two concepts were introduced during the 1950s and 1960s. In the first concept, the socalled contact process, the retention of biomass is accomplished by means of settling and sludge recycling (Stander 1950, Stander and Snyders 1950, Schroepfer *et al* 1955). In the second concept, the anaerobic filter, the biomass is retained through the use of support materials (Coulter *et al* 1957, Young and McCarty 1969). Since then, these two concepts have been developed into several socalled 'high-rate' wastewater treatment systems (Speece 1983, Henze and Harremoes 1983, Lettinga *et al* 1984, Van den Berg 1984, Hulshoff Pol and Lettinga 1986), a term that should not be confused with the 'high-rate' sewage sludge digesters introduced in the 1950s (Table 8).

At present over 100 high-rate anaerobic digesters have been installed or commissioned for treatment of industrial wastewater (Lettinga *et al* 1984, Nyns 1985, Pohland and Harper 1985, De Zeeuw 1988). The majority of these reactors is of the Upflow Anaerobic Sludge Bed (UASB) reactor type (Lettinga *et al* 1984, De Zeeuw 1988). The principal reasons for the comparatively large success of the UASB system are its simple and inexpensive construction and its ability to retain very high amounts of high quality biomass, and thus to accommodate high organic space loads and provide ample safety against shock loads. The system becomes especially attractive when

biomass aggregates (granular sludge) develop. The excellent settling properties of these granules allow low hydraulic retention times, and result in a very high concentration of active biomass in the digester compartment of the UASB reactor.

The large number of full-scale UASB reactors that are operative in Western Europe and North and South America, allows the use of granular sludge as an inoculum for new-built reactors in these continents. This largely removes one of the most serious drawbacks of anaerobic digesters, i.e. the long start-up period (Lettinga *et al* 1984). It also implies, that UASB systems can now be considered for the treatment of wastes which do hardly or not allow the formation of granular sludge from low grade inocula, for example lipid containing wastewater from slaughterhouses and rendering plants (De Zeeuw 1982, Sayed 1984, Van Campen *et al* 1986). Lab-scale and pilot scale experiments with these wastewaters, as well as with cold domestic sewage indicate that granular sludge can be recommended for use as an inoculum. The granular sludge can be maintained and even augmented under proper conditions (De Zeeuw 1982, Lettinga *et al* 1983b, Sayed *et al* 1987).

Nevertheless, the application of the UASB process, and of anaerobic wastewater treatment in general, is still limited to the treatment of wastewater containing mainly rapidly degradable compounds, like volatile fatty acids and carbohydrates (Lettinga *et al* 1984, De Zeeuw 1988). The number of full scale applications to wastewater containing lipids or proteins is still very limited (Samson *et al* 1985, Van Campen *et al* 1986, De Man and Piscaer 1986, De Zeeuw 1988). The main reasons for this are:

- the lack of experience with the application of anaerobic treatment systems to these types of wastewater.
- reported problems with sludge retention encountered during lab scale and pilot scale studies and full-scale applications, viz. the occurrence of sludge flotation and wash-out (De Zeeuw 1982, Lieburg and Van der Wal 1982, Sayed *et al* 1984, Samson *et al* 1985, Van Campen *et al* 1986).
- the possibility of inhibition by long-chain fatty acids (Prins *et al* 1972, Chou *et al* 1978, Hanaki *et al* 1981, Koster and Cramer 1987), which is especially threatening in systems operated at a low hydraulic retention time.

A prerequisite for an increased application of anaerobic digestion for the treatment of lipid containing wastewater would be the control of sludge wash-out and long chain fatty acid inhibition. This requires the choice, or - if not available yet - the development of a proper type of reactor.

There is still some discussion regarding the benefits of support materials among scientists involved in research on anaerobic wastewater treatment. Nevertheless, the following partition of the 'high-rate' anaerobic wastewater treatment reactors listed in Table 8 is justified within the scope of this thesis:

- reactors with mobile biomass aggregates which may or may not include a carrier material.
- reactors with biofilms attached to stationary carrier material.

It is generally accepted that the biological conversion capacity of upflow anaerobic filter reactors (UAF) with random packing is mainly associated with suspended biomass aggregates in the lower part of the reactor, and that the attachment of biofilms to the packing is only of marginal importance (Young and Dahab 1983, Dubourguier *et al* 1988). Therefore, all systems except the Downflow Stationary Fixed Film reactor and the Anaerobic Rotating Biological Contactor can be classified in the first category (mobile aggregates).

Table 8. Overview of anaerobic wastewater treatment technology

reactor type	introduction	comment	references
septic tanks	1880s	exclusion of air, combined solids settling and digestion (liquefaction)	McCarty 1982
heated sludge digester	1920s	increased reaction rates	McCarty 1982
'high-rate' sludge digester	1950s	improved contact between microorganisms and substrate increases reaction rates	Morgan 1954, Torpey 1955
clarigester, contact process (CP)	1950s	biomass retention or recycling allows shorter hydraulic retention times	Stander et al 1950, Schroepfer et al 1955, Steffen and Bedker 1961, Stander 1966
upflow anaerobic filter (UAF)	1960s	solves problems with sludge separation/settling in CP	Coulter et al 1957, Young and McCarty 1969
upflow anaerobic sludge bed (UASB)	1970s	solves problems with sludge settling in CP, and problems with plugging and channeling in UAF	Lettinga et al 1972, 1974, 1975, 1976, 1977, 1980b
anaerobic rotating biological contactor (AnRBC)	1976	improvement of UAF, plugging and channeling problems solved	Antonie 1976, Tait and Friedman 1980
anaerobic attached film expanded bed (AAFEB)	1978	improvement of UAF, plugging and channeling problems solved	Jewell et al 1980, Switzenbaum and Jewell 1980
downflow stationary fixed film (DSFF)	1979	improvement of UAF, plugging and channeling problems solved	Van den Berg and Lentz 1979
fluidized bed (FB)	1981	similar to AAFEB, but higher upflow velocity prevents accumulation of inert suspended solids	Hakulinen and Salkinoja-Salonen 1981, Binot et al 1981, Jenkins et al 1981, Hall 1981, Li et al 1982, Jeris 1983, Heijnen 1983
upflow sludge bed filter (USBF)	1982	hybrid design incorporating a sludge bed (UASB) and upflow filter (UAF), aimed at high capacity (UASB) and good solids retention (UAF)	Olthof and Oleszkiewicz 1982, Guiot and Van den Berg 1984, Oh and Yang 1985, Reynolds and Colleran 1986
anaerobic baffled reactor (ABR)	1982	modification of AnRBC, also considered as modification of UASB	McCarty 1982, Bachman et al 1985

Table 8. (Continued)

reactor type	introduction	comment	references
anaerobic gas-lift reactor (AGLR)	1983	attempt to solve gas-solids separation and solids retention problems in UASB and FB reactors treating non-acidified wastes	Beeftink and Staugaard 1983, Beeftink and Van den Heuvel 1987
internal circulation reactor (IC)	1986	comparable to AGL, liquid recirculation induced by biogas, no forced gas recirculation	Vellinga et al 1986, Hack et al 1988

A choice among the currently existing types essentially boils down to a choice between mobile biomass aggregates and stationary biofilms. The pro's and contra's of both systems can be summarized as follows:

mobile aggregates

- a high biomass concentration can be reached (30-50 kg VSS.m^{-3}), which implies high maximum conversion rates, and a high safety-factor against overloading with long chain fatty acids.
- the system is potentially unstable during treatment of lipids, i.e. there is a relatively high risk for sludge flotation and wash-out.

stationary biofilms

- the biomass concentration is considerably lower (15-25 kg VSS.m^{-3}) due to the limited specific surface area of the support material. Consequently the maximum conversion rate is lower, which implies either a lower design capacity, or a lower safety-factor against overloading with long chain fatty acids.
- the stationary packing material gives a high risk of clogging and channeling.
- the system provides an inherent safeguard against sludge wash-out.

Consequently, a choice has to be made between a higher degree of safety with regard to sludge wash-out and a higher design capacity. A fully rational choice among the existing reactor types is not possible, as there is very little experimental experience with treatment of lipid containing wastewater. However, the risk of channeling and the lack of industrial experience with the currently available systems with stationary biofilms makes these reactor types less attractive for treatment of lipid containing wastewater. Furthermore, it should be emphasized that the risk of inhibition by long chain fatty acids is directly related to the overcapacity and the mixing characteristics of the reactor system, as these inhibitors are biodegradable (McCarty 1964). This favors the systems with mobile biomass aggregates.

I decided to investigate a system with mobile biomass aggregates, and modify this system if necessary to minimize the risk of sludge wash-out. The granular sludge UASB reactor was chosen as a starting-point, because of its relatively easy start-up and the vast practical experience that has been obtained with this reactor system. Furthermore, reactor systems with biofilms on mobile carriers very likely will not provide more safety against sludge wash-out. Sludge flotation will also occur in these systems when the balance between upflow liquid velocity and settling velocity of the aggregates is disturbed by adhering lipids.

Scope and Organisation of this Thesis

The present study deals with a number of aspects of anaerobic treatment of wastewaters containing high concentrations of lipids or sodium sulfate. The thesis can be divided into two parts: Chapters 2-5 describe experiments related to anaerobic digestion of lipid containing wastewater, Chapters 6-8 describe problems arising from the presence of high concentrations of sodium sulfate in the wastewater.

In all experiments upflow reactors without carrier materials were applied, because of the advantages discussed in previous paragraphs. In all cases, granular sludge from existing full-scale UASB reactors was chosen as an inoculum, because of the superior characteristics of this type of sludge, particularly with respect to the start-up of the system.

Aspects of anaerobic treatment of lipid containing wastes covered by this thesis are: toxicity of long chain fatty acids (Chapter 2), inhibition and sludge flotation resulting from shock loads of long chain fatty acids (Chapter 3), the performance of conventional and modified granular sludge bed upflow reactors in the treatment of long chain fatty acid solutions (Chapter 4), and investigations concerning the further improvement of the upflow reactor with respect to the retention of granular sludge during treatment of triglyceride emulsions (Chapter 5).

The following aspects of anaerobic treatment of wastewater with a high sodium sulfate concentration are described: inhibition of acetotrophic methanogens by sodium sulfide (Chapter 6), the effect of sulfide on the anaerobic degradation of propionate (Chapter 7), and inhibition of acetotrophic methanogens by sodium salts (Chapter 8).

Chapter 9 summarizes the results of the investigations, and provides the general conclusions.

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CHAPTER 2

Bactericidal Effect of Long Chain Fatty Acids in Anaerobic Digestion

(submitted for publication)

CHAPTER 2

Bactericidal Effect of Long Chain Fatty Acids in Anaerobic Digestion

Arjen Rinzema, Martin Boone, Jos Keurentjes, Karin van Knippenberg and Gatze Lettinga
Dept. of Water Pollution Control, Wageningen Agricultural University, Bomenweg 2, 6703 HD Wageningen, The Netherlands

Summary

The effect of shock loads of long chain fatty acids (LCFA) on the activity of granular methanogenic sludge was studied in batch experiments, with capric acid as model substrate. The lag phase during the start-up of a continuous anaerobic treatment system for lipid containing wastes, is shortened by a low LCFA (lipid) concentration in the influent. Inhibition is primarily related to the LCFA concentration; the ratio between LCFA and biomass concentrations is less important. A lethal threshold LCFA concentration can clearly be distinguished. Capric acid has a lethal effect on acetotrophic methanogens at approximately 1 $kg.m^{-3}$. The difference in the threshold level found in different sets of experiments, can be explained by differences in the mass transfer characteristics, and the specific activity and particle size of the biomass granules. When the LCFA concentration in a methanogenic digester exceeds this threshold level, less than 0.1% of the acetotrophic methanogens survives. Acetotrophic methanogens do not adapt to LCFA, neither upon repeated exposure to toxic concentrations, nor after prolonged exposure to non-toxic concentrations. Lecithin acts as a synergist to capric acid. Furthermore, lecithin and capric acid mutually reduce each others degradation rate.

Introduction

The inhibitory effect of long chain fatty acids (LCFA) in anaerobic digestion was already described in the 1960s by McCarty,[1] in connection with anaerobic digestion of sewage sludge. McCarty concluded that anaerobic digestion of wastes containing LCFA is possible, provided a continuously fed digester is used, and sudden overloading is avoided. This implicates that conventional sludge digesters operated at hydraulic residence times of 15-20 days will hardly ever suffer from LCFA inhibition. However, during anaerobic treatment of relatively dilute industrial wastewater in high-rate anaerobic digesters operated at a hydraulic residence time of only a few days, shock loads of LCFA may cause serious problems. Hanaki *et al*[2] conducted an extensive study of the inhibitory effect of LCFA in anaerobic digestion. They concluded that LCFA affect the obligate hydrogen producing acetogenic bacteria, which are responsible for the ß-oxidation of LCFA, as well as the hydrogenotrophic and acetotrophic methanogenic bacteria, which convert the intermediates from the ß-oxidation process. Even when the degradation of LCFA was severely retarded, the hydrogen partial pressure remained very low (50 Pa), indicating that the LCFA affect the acetogens more seriously than the hydrogenotrophic methanogens. Inhibition of acetogens and acetotrophic methanogens caused a pronounced lag phase in batch experiments, whereas inhibition of hydrogenotrophic methanogens merely caused a decrease of the hydrogen conversion rate. Hanaki *et al* also found that LCFA disappear from the solution and accumulate in the solid phase, within 24 hours. This seems to be in accordance with theories attributing LCFA inhibition to physical interactions between the acids and the membrane of the microorganisms.[3-7]

These theories suggest that inhibition is a function of the LCFA:biomass ratio (the ratio between the LCFA and biomass concentrations), but this has not yet been proven. Taking the maximum allowable LCFA:biomass ratio derived from preliminary batch toxicity tests as a guideline, we made several attempts to start an Upflow Anaerobic Sludge Bed reactor (UASB) for continuous digestion of LCFA. In all cases the UASB reactors were started-up in batch mode, i.e. a slug dose of LCFA was added, feeding was interrupted and effluent recirculation was applied. The LCFA:biomass ratio was kept below the maximum allowable level indicated by the batch tests. None of the start-up attempts was successful. The crucial difference between the UASB start-up experiments and the batch toxicity tests, was the absolute concentration of biomass (granular sludge) and LCFA. The sludge concentration in the UASB reactors exceeded that in the

batch reactors by at least a factor five, and therefore a much higher influent concentration of LCFA was chosen. The failure of these start-up attempts necessitated further research into the relationship between inhibition and LCFA concentration.

In this paper we describe batch experiments, in which the same LCFA concentration was combined with different sludge concentrations. Capric acid was chosen as model substrate, because it is one of the most toxic saturated LCFA,[8] and the concentration of all its ß-oxidation intermediates can be determined very easily.

Furthermore, we also studied the toxicity of mixtures of capric acid and lecithin, because in practice LCFA usually are accompanied by neutral lipids. The presence of these lipids may change the physico-chemical behavior of the acids significantly, and consequently also their inhibitory effect. Kodicek and Worden[4] observed a distinct antagonistic effect of lecithin, cholesterol and other surface active compounds against linoleic acid, in toxicity studies with *Lactobacillus helveticus*. Galbraith *et al*[7] found an antagonistic effect of cholesterol and ergocalciferol with several Gram positive bacteria.

Theory

Assuming that the hydrogen produced during ß-oxidation is instantly oxidized by hydrogenotrophic methanogens,[2] the anaerobic degradation of capric acid can be described as a series of consecutive reactions:

$$f_8\,C_{10} \rightarrow f_8\,C_8 + f_8\,C_2 + 0.5\,f_8\,CH_4 \quad (1)$$

$$f_6\,C_8 \rightarrow f_6\,C_6 + f_6\,C_2 + 0.5\,f_6\,CH_4 \quad (2)$$

$$f_4\,C_6 \rightarrow f_4\,C_4 + f_4\,C_2 + 0.5\,f_4\,CH_4 \quad (3)$$

$$f_2\,C_4 \rightarrow 2\,f_2\,C_2 + 0.5\,f_2\,CH_4 \quad (4)$$

$$f_{ACH4}\,C_2 \rightarrow f_{ACH4}\,CH_4 \quad (5)$$

where the coefficients f_8, f_6, f_4, f_2, f_{ACH4} represent the number of moles converted according to reactions 1-5. For simplicity, CO_2 and H_2O production are not shown.

According to this description by five consecutive reactions, the coefficients f_8-f_{ACH4} can be calculated from matrix equation 6, if the concentrations of all intermediates and end products are known:

$$\begin{vmatrix} f_8 \\ f_6 \\ f_4 \\ f_2 \\ f_{ACH4} \end{vmatrix} = \begin{vmatrix} 0.786 & 0.571 & 0.357 & 0.143 & 0.143 \\ -0.214 & 0.571 & 0.357 & 0.143 & 0.143 \\ -0.214 & -0.429 & 0.357 & 0.143 & 0.143 \\ -0.214 & -0.429 & -0.643 & 0.143 & 0.143 \\ -0.071 & -0.143 & -0.214 & -0.286 & 0.714 \end{vmatrix} \begin{vmatrix} [C_8]/[C_0] \\ [C_6]/[C_0] \\ [C_4]/[C_0] \\ [C_2]/[C_0] \\ [CH_4]/[C_0] \end{vmatrix} \quad (6)$$

where C_0 is the initial capric acid concentration (all concentrations are expressed as moles.l^{-1}).

With the calculated value of f_{ACH4} (from equation 6), the contribution of hydrogenotrophic methanogens (reactions 1-4) and acetotrophic methanogens (reaction 5) to the measured total methane production can be distinguished.

Table 1. Summary of experimental conditions

experiment	purpose	reactor	biomass concentration (kg VSS.m^{-3})	C_{10}-concentrations added during subsequent feedings (kg C_{10}.m^{-3})
A	UASB start-up simulation	A1	27.97	1.940, 1.950, 2.360, 2.710, 2.720
B	effect of LCFA concentration and sludge load on toxicity	B1	2.52	0.410, 0.590, 0.770, 0.970, 1.180, 1.350
		B2	5.04	0.400, 0.590, 0.770, 1.000, 1.160, 1.350, 1.550
		B3	10.08	0.400, 0.600, 0.780, 0.970, 1.160, 1.360, 1.550, 1.760
C	c.f. B	C1	2.74	0.390, 0.390, 0.390, 0.440, 0.440, 0.830, 0.830, 0.710, 0.890, 0.890, 0.890, 0.890, 1.160
		C2	5.48	0.390, 0.390, 0.390, 0.440, 0.440, 0.830, 0.830, 0.710, 0.710, 0.710, 0.890, 1.160
		C3	10.96	0.390, 0.390, 0.390, 0.440, 0.440, 0.830, 0.830, 0.710, 0.710, 0.710, 0.710, 0.890, 1.160, 1.160
D	effect of lecithin on LCFA toxicity	D1	5.00	0 (0.760)[a], 0 (1.140), 0 (1.520), 0 (2.280)
		D2	5.00	0.340 (0), 0.510 (0), 0.680 (0)
		D3	5.00	0.340 (0.760), 0.510 (1.140), 0.680 (1.520)
		D4	5.00	0.340 (0.076), 0.510 (0.114), 0.680 (0.152)

[a] between brackets the lecithin concentrations (kg COD.m^{-3}). In reactor D3, the pursued ratio between the COD of capric acid and lecithin was 1:1; the pursued ratio in reactor D4 was 10:1.

Materials and Methods

Overview of Experiments

Four series of batch experiments were performed. In series A (1 reactor), the start-up of a UASB reactor was simulated in a stirred batch reactor. In series B and C (3 reactors each), the toxicity of capric acid as a function of the capric acid concentration and the LCFA:biomass ratio (ratio between capric acid and biomass concentrations) was investigated. In the latter two series of experiments, three stirred batch reactors were inoculated with different amounts of biomass, and subsequently fed with comparable quantities of capric acid. After complete degradation of capric acid, feeding was repeated with step-wise increasing quantities of capric acid, until inhibition was observed. In experiments A-C, capric acid was the sole substrate. In experiment D (4 reactors), the degradation of mixtures of lecithin and capric acid was studied. Table 1 summarizes the biomass and substrate concentrations that were investigated.

Reactors

Gas tight plexiglass batch reactors with 2.5 l liquid volume were used in experiments A and B (internal diameter 150 mm, height 150 mm, no baffles). PVC batch reactors with 5 l liquid volume were used in experiments C and D (internal diameter 150 mm, height 290 mm, four baffles). The liquid was stirred intermittently (two-blade impeller in all cases, impeller diameter 100 mm; experiments A and B 120 rpm, pulse 1 minute, pause 1 minute; experiments C and D 120 rpm, pulse 1 minute, pause 2 minutes). Methane production was measured with Mariotte flasks filled with 1% NaOH solution. All experiments were performed at 30 ± 1 °C, in a temperature controlled room.

Biomass

Elutriated granular sludge from a full scale UASB reactor treating potato processing wastewater was used as inoculum (Aviko, Steenderen, The Netherlands). The inoculum for experiments A and B was taken from the full scale reactor in September 1984, that for experiments C and D in June 1985. The predominant acetotrophic methanogens in the inoculum were of the genus *Methanothrix*.[8] The granular sludge was stored in a refrigerator at 4 °C, and re-activated with a VFA solution prior to the experiments.

Media

All experiments were performed with a basal medium containing ($mg.l^{-1}$): NH_4Cl (1044), KCl (270), $MgCl_2.6H_2O$ (150), KH_2PO_4 (170), $(NH_4)_2SO_4$ (170), yeast extract (20), and 1 $ml.l^{-1}$ trace element solution.[9] The medium was prepared in tap water containing approximately 35 mg $Ca^{2+}.l^{-1}$. The desired capric acid concentration was obtained by addition of the required amount of a concentrated sodium caprate stock solution containing (g): capric acid (20), NaOH (4.65), demineralized water (1000). Capric acid was melted *au bain-marie* and dissolved in the vigorously stirred, hot NaOH solution (ca. 50 °C). The pH of the stock solution was lowered to approximately 8 with CO_2, in order to avoid pH shocks in the batch reactors. In experiment D raw soy bean lecithin was used, containing (% w/w): phospholipids (57), neutral oil (39.5), water (2.5). Lecithin was added in the form of a stock emulsion.

Prior to the experiments with capric acid, the inoculum was re-activated by adding 20 $ml.l^{-1}$ of a VFA solution containing ($kg.m^{-3}$): acetic acid (31.24), propionic acid (22.02), and butyric acid (18.34), which was neutralized to pH 6.5 with concentrated NaOH solution.

All chemicals were of analytical grade (Merck AG, Darmstadt, FRG), except the yeast extract (Gist-brocades, Delft, The Netherlands) and the soy bean lecithin (Unimills, Zwijndrecht, The Netherlands).

Procedure

Known amounts of basal medium, granular sludge and VFA solution were brought into the batch reactors (VFA-COD 2 $kg.m^{-3}$). Liquid and gas phase were flushed with N_2 to remove oxygen. VFA addition was repeated three times, prior to the first addition of capric acid. Additions of VFA or sodium caprate solution were made after the previously added substrate had been completely mineralized. The liquid volume in the reactors was maintained constant, by removal of the required amount of liquid - after proper settling of the biomass - before the introduction of new substrate solution. After each addition, as well as during the batch digestion process, the pH was corrected to 7.0 ± 0.2 with HCl or NaOH. The supernatant was completely replaced with basal medium, once the calculated sodium concentration exceeded 5 $kg.m^{-3}$, in order to prevent sodium inhibition.[9]

Sampling and Analyses

Liquid samples were taken with a syringe, after settling of the biomass for at least 5 minutes. Samples were centrifuged for 10 minutes at 3500 rpm. Fatty acids with up to 8 carbon atoms were analyzed by gas chromatography, as described previously.[9] Capric acid was determined on the same column, after increasing the column temperature to 190 °C. COD, TSS and VSS were analyzed according to Dutch Standard Methods (NEN 3235-4.1 and NEN 3235-5.3). The pH was determined with a pH electrode, inside the reactors.

Regression Analysis

The maximum specific growth rate ($\hat{\mu}$) and the initial concentration (X_o) of the acetotrophic methanogens have been calculated by non-linear regression analysis (BFGS method),[10] utilizing the expression for exponential growth:

$$P = Y_{p/x} \cdot X_o \cdot (e^{\hat{\mu} \cdot t} - 1) \qquad (7)$$

where: P is the cumulative acetotrophic methane production calculated using equation 6 (expressed as a concentration based on the liquid volume, kg $COD.m^{-3}$), $Y_{p/x}$ is the methane yield coefficient

(kg CH_4-COD.kg^{-1} VSS), X_o is the initial concentration of acetotrophic methanogens (kg VSS.m^{-3}), $\hat{\mu}$ is their maximum specific growth rate (d^{-1}), and t is the time elapsed since the introduction of the capric acid (d). The use of equation 7 to calculate the maximum specific growth rate of the acetotrophic methanogens is allowed, despite the fact that acetic acid was not added as substrate, because acetate accumulated far above the K_s-value (half rate constant in Monod kinetics), in batch reactors inhibited by capric acid. A yield coefficient $Y_{p/x}$ = 25 kg CH_4-COD.kg^{-1} VSS was calculated from published $Y_{x/s}$ values[12,17,25] (assuming $Y_{p/x} = 1/Y_{x/s}$ - 1.4, with $Y_{p/x}$ the product over biomass yield coefficient expressed in kg CH_4-COD.kg^{-1} VSS, $Y_{x/s}$ the biomass over substrate yield coefficient expressed in kg VSS.kg^{-1} $COD_{reduced}$, and 1.4 the COD per kg VSS).

The correlation between both calculated values for the amount of methane produced by acetotrophic methanogens (from equations 6 and 7 respectively), is expressed as the Percent Goodness of Fit (PGF), which is defined as:

$$PGF = 100 \cdot (1 - \Sigma(P_i - P_{i1})^2 / \Sigma(P_i - P_{i2})^2)^{0.5} \quad (8)$$

where P_i and P_{i1} are the values for the acetotrophic methane production calculated from equations 6 and 7 respectively, and P_{i2} is the average of the values calculated from equation 6.

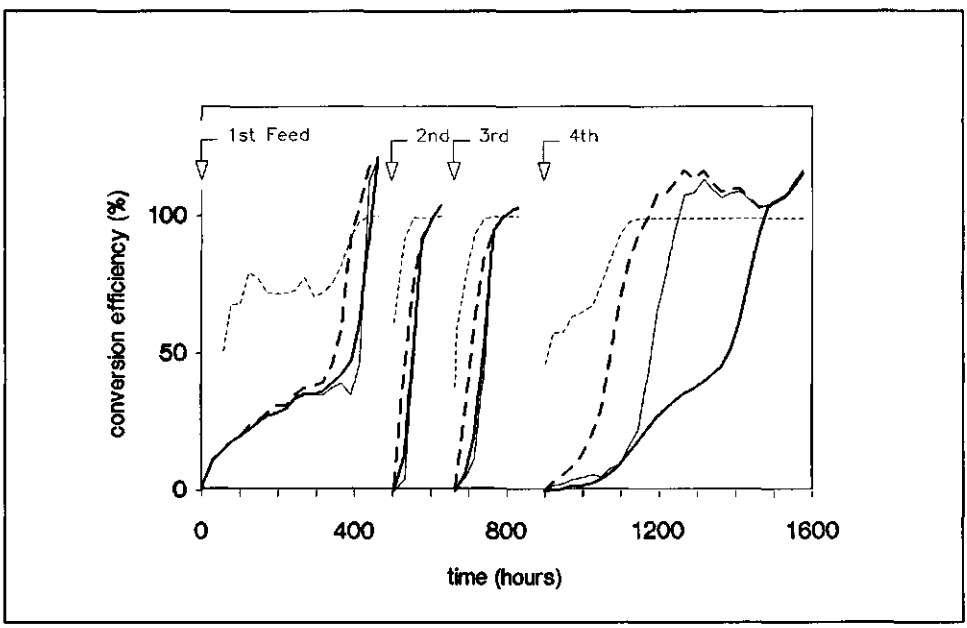

Figure 1. Simulation of UASB reactor start-up (experiment A; -- capric acid removal; -- capric acid degradation (f_8); — acetate formation (f_2); — methanogenesis; c.f. equations 1-6 and Table 1)

Results and Discussion

Simulation of UASB Reactor Start-up

Figure 1 shows the degradation of four consecutive additions of capric acid, in an intermittently stirred batch reactor (A1), with a biomass concentration comparable to that in a UASB reactor (28 kg VSS.m^{-3}). The fraction of the capric acid disappeared from solution, and the calculated conversions through reaction 1 (f_8) and reactions 1-4 (f_2), and the calculated conversion to methane (through reactions 1-4 plus reaction 5) are presented. After the first addition, only 35% of the added capric acid had been converted to methane after 350 hours. Reaction 1 was clearly rate limiting, as no accumulation of intermediates of the ß-oxidation occurred. COD recovery from the first feed was higher than 100%, probably as a result of biomass decay. The second and third capric acid addition were completely converted to methane within 100 hours, also without any accumulation of intermediates. Problems occurred after the fourth addition of capric acid: the acetogens responsible for ß-oxidation, and especially the methanogens were inhibited.

The results of experiment A clearly show that immediately after the first introduction of LCFA, ß-oxidation is the rate limiting step. Adaptation of the inoculum, i.e. growth of acetogenic bacteria, is required to alleviate this limitation. The results of experiments B-D (presented below) reveal that the lag phase can be shortened significantly by applying capric acid concentrations lower than those applied in experiment A. Usually only 24-48 hours were required to achieve rapid degradation of capric acid in experiments B-D. With respect to the start-up of UASB reactors for treatment of lipid containing wastes, this implies that a low LCFA concentration - preferably below 0.4 kg.m^{-3} - should be applied. Experiments with LCFA esters show that lipolysis is not the rate limiting step in lipid degradation[2] (Rinzema, A., unpublished). Therefore we can conclude that a low influent concentration during the start-up period is advisable for all

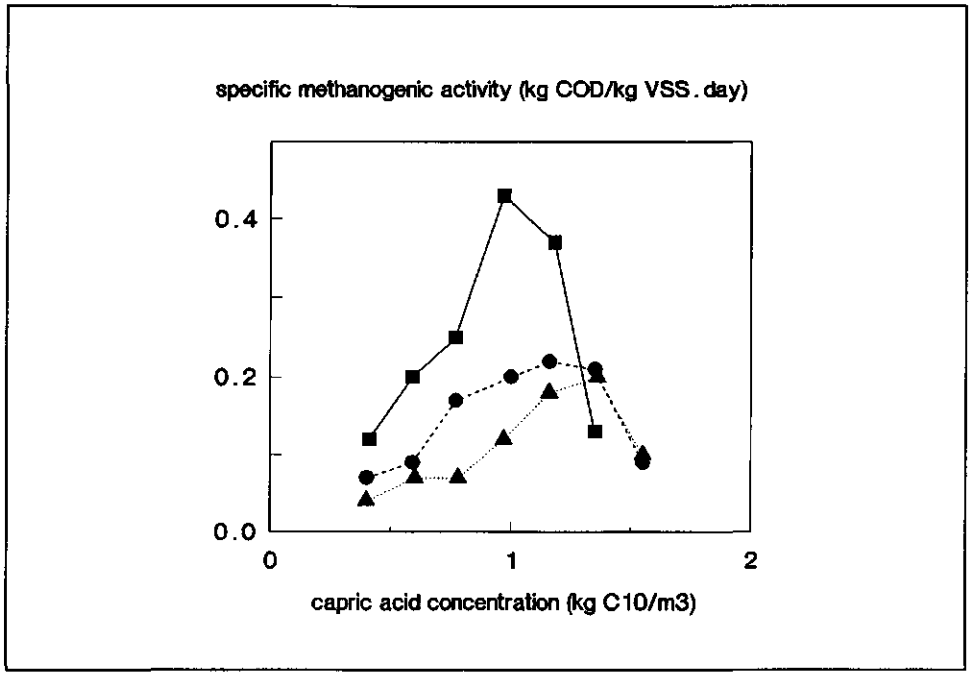

Figure 2. Maximum specific methanogenic activity as a function of the initial capric acid concentration in three batch reactors with different sludge concentrations (experiment B; ■— B1 2.52; ● -- B2; ▲... B3)

lipid containing wastes.

Toxicity Studies

The course of the capric acid degradation process after the fourth feeding in experiment A (Figure 1), is characteristic for all reactors in series B and C. Once adaptation had been achieved, capric acid was always rapidly degraded, without significant accumulation of ß-oxidation intermediates. However, above a certain capric acid concentration, again a lag phase in the capric acid degradation (reaction 1) was observed. The length of this lag phase ranged from 150 to more than 800 hours. This indicates substrate inhibition of the acetogenic bacteria, as already described previously by Hanaki *et al.*[2] Therefore, overloading will cause serious malfunctioning of an anaerobic treatment system, for a period of time that is far too long for practical purposes. Recovery of the methanogens requires even more time.

Effect of LCFA Concentration and LCFA:biomass ratio

Figure 2 shows the maximum specific methanogenic activity found in experiment B, as a function of the capric acid concentration added to the batch digesters. Clear inhibition occurred above ca. 1.25 kg $C_{10:0}.m^{-3}$, irrespective of the biomass concentration. Apparently, the inhibition is primarily governed by the LCFA concentration, and the LCFA:biomass ratio is only of minor importance in the range tested. The increase of the activity at capric acid concentrations below the toxicity threshold, reflects either a steady increase in the number of acetogenic bacteria upon repeated feeding, or an increase in the mass transfer rate. Below the toxic concentration ß-oxidation was rate limiting in all cases. The inverse relationship between the activity and the biomass concentration must probably be attributed to physical rate limitations; similar observations have been made with VFA (Lettinga, G., unpublished).

In experiment C, also a very clear toxicity threshold concentration was found, which was once again independent of the LCFA:biomass ratio. However, the threshold level in experiment C was slightly lower than in experiment B, viz. ca. 1.0 kg $C_{10:0}.m^{-3}$. Experiments A and D do not allow any conclusions regarding the relative importance of capric acid concentration and LCFA:biomass ratio. In both experiments, however, the toxic concentrations differed significantly from those found in experiments B and C (c.f. Table 2).

Experiments B and C provide clear evidence for the existence of a toxic threshold concentration, i.e. inhibition occurs above a critical capric acid concentration. This is in contradiction with previous publications which suggest that inhibition is determined by adsorption equilibria.[3-7] The differences between the threshold concentrations found in various experiments, can probably be attributed to mass transfer across the boundary layer of liquid surrounding the granules, and to intra-particle diffusion limitations. As a result of both physical limitations, the actual capric acid concentration at the granule-water interface and inside the granules may differ significantly from that in the bulk of the liquid (Figure 3), as will be demonstrated below.

External Mass Transfer

In a steady state situation, the concentration gradient across the boundary layer surrounding the biomass granules can be calculated from the mass balance:

$$\phi_m = r \tag{9}$$

$$\phi_m = k_L \cdot A_g \cdot (C_L - C_S) \tag{10}$$

$$r = k_r \cdot X \tag{11}$$

and hence:

$$C_L - C_S = \frac{k_r}{k_L} \cdot \frac{X}{A_g} \tag{12}$$

where ϕ_m is the mass transport rate across the boundary layer ($kg.m^{-3}.s^{-1}$), r is the average conversion rate in the biomass granules ($kg.m^{-3}.s^{-1}$), k_L is the mass transfer coefficient ($m.s^{-1}$), A_g is the interfacial area between biomass granules and liquid ($m^2.m^{-3}$ liquid volume), C_L and C_S

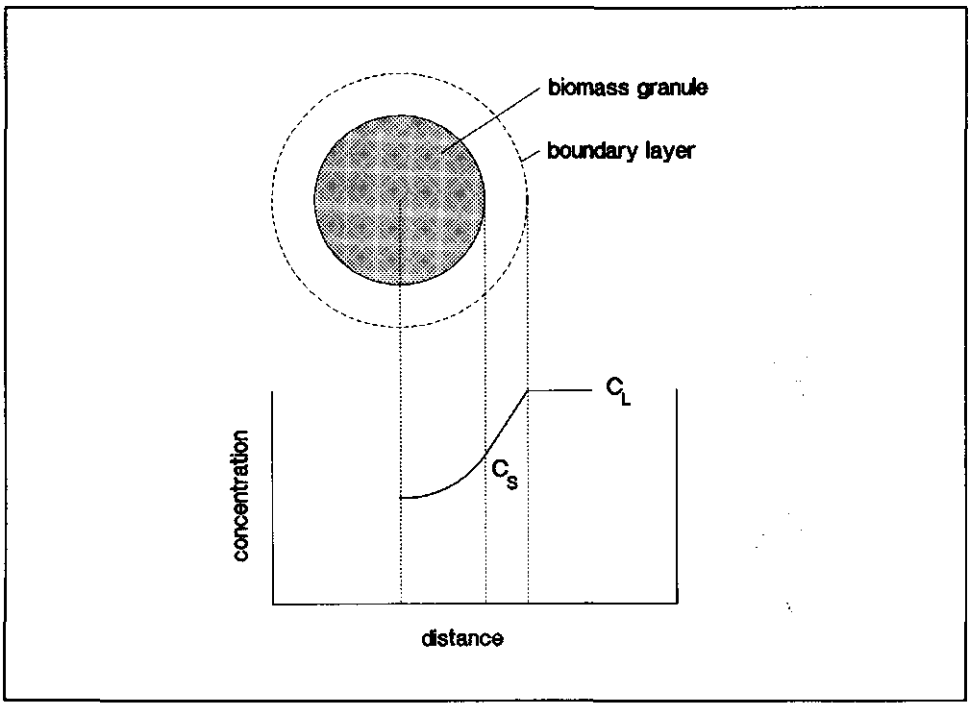

Figure 3. Schematic representation of the model describing transport of capric acid from the solution to the biomass granules, and inside the granules

are the concentrations of dissolved capric acid salts in the bulk of the liquid and at the biomass-liquid interface respectively ($kg.m^{-3}$), k_r is the average specific activity of the biomass ($kg\ C_{10}.kg^{-1}\ VSS.s^{-1}$), and X is the biomass concentration ($kg\ VSS.m^{-3}$).

The bulk concentration of dissolved capric acid salts (C_L) required to achieve a concentration at the biomass-liquid interface (C_S) above the toxicity threshold, depends on the (maximum) specific activity of the biomass (k_r), the mixing characteristics (k_L), and the specific surface area of the granules (A_g/X). This may explain the differences observed in different series of experiments, provided that C_L is proportional to the total capric acid concentration (dissolved plus undissolved). Figure 4 shows that this was indeed the case in our experiments, at the critical moment with respect to inhibition: the concentration of dissolved capric acid salts determined 15-30 minutes after the introduction of a new feed, was directly proportional to the amount of capric acid added. The concentrations shown in Figure 4 are well below the solubility indicated by the literature, viz. ca. 4 $kg\ C_{10}.m^{-3}$ for sodium caprate at pH 7.[26] The difference between the added amount of sodium caprate and recovery of soluble caprate, may be attributable to precipitation of calcium caprate[27].

Assuming realistic figures for all parameters in equation 12, it can be shown that the combined effect of differences in the mass transfer coefficient (mixing intensity), biomass particle size and specific activity between the four series of experiments, may have caused variations up to 0.7 $kg.m^{-3}$ in the required driving force across the boundary layer (c.f. Appendix A). Therefore, comparable differences in the bulk concentration of capric acid that is required to achieve a value of C_S above the toxicity threshold can be expected. The observed difference between experiments B and C may have been caused by differences in mixing frequency, reactor construction and biomass properties. The high threshold level found in experiment A may be the result of poor mixing caused by the extremely high biomass concentration, and the low threshold

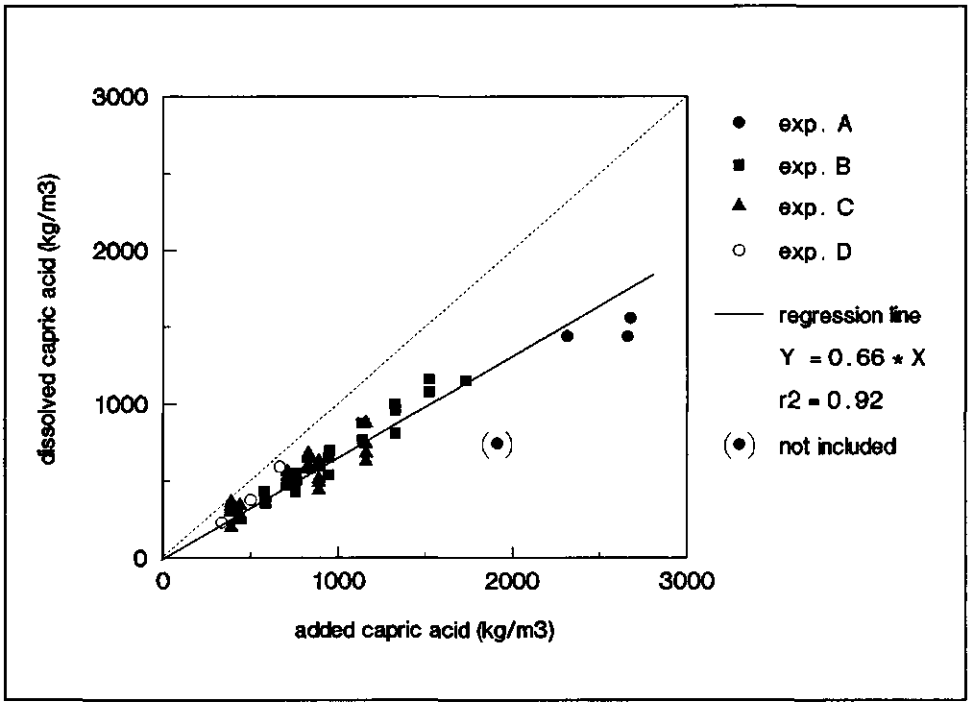

Figure 4. Relationship between the concentration of dissolved capric acid and the total capric acid concentration (quotient of added quantity and liquid volume), determined 15-30 minutes after addition of capric acid to the batch digesters

level observed in experiment D by the low specific activity of the biomass.

Intra-particle Limitations

McKay *et al*[29] have shown that adsorption of sodium dodecyl sulfate on activated carbon is significantly retarded by intra-particle diffusion limitation. The probability of a similar phenomenon in the case of granular sludge and capric acid is indicated by a comparison of the time constants of the biological reaction and the intra-particle diffusion process (Appendix B).

It is therefore conceivable that only the outer layer of the granule is supplied with substrate, as long as the capric acid concentration at the granule-water interface (C_S) remains below a certain level. An increase of the interfacial concentration allows deeper penetration of the substrate into the granule, which may mask the inhibition of the bacteria in the outer layers of the granule. We observed similar masking during toxicity studies with sodium salts.[9] Differences in granule structure and specific activity may thus result in differences in the observed toxicity threshold.

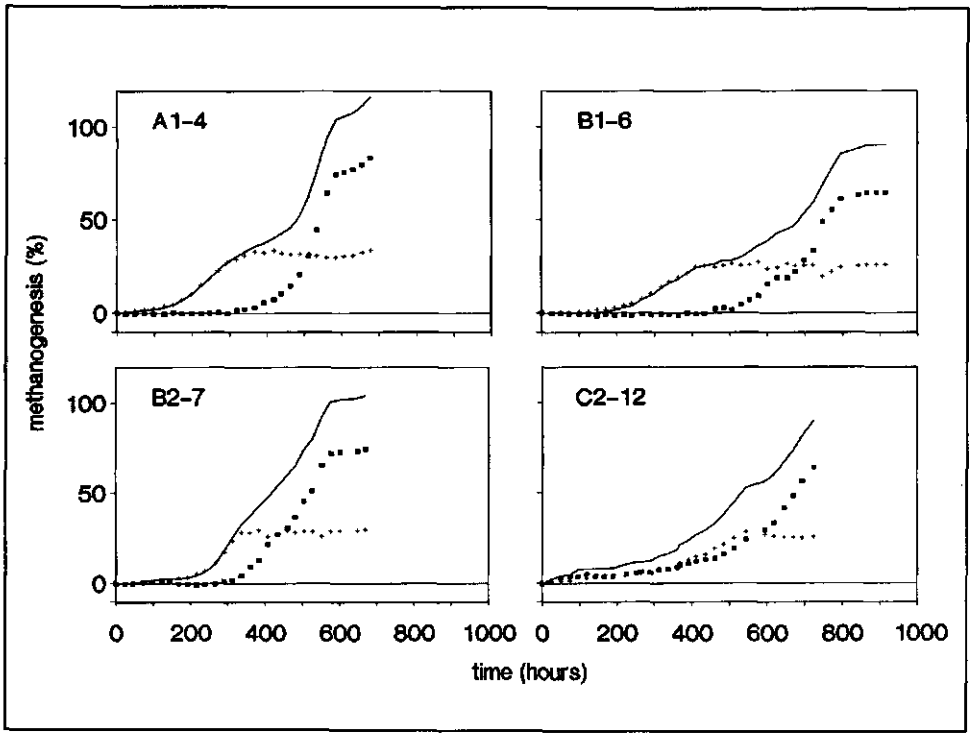

Figure 5. Typical examples of the measured total methane production (—), and the hydrogenotrophic (+) and acetotrophic (■) methane production calculated from equation 6, for severely inhibited batch reactors

Verification of the explanations postulated above, should be obtained from experiments where variations in specific activity and mixing intensity are carefully monitored. This will require the use of a reactor system which allows accurate prediction and control of mass transfer rates, and proper quantification of the relevant granule properties. The main practical problem will be to measure the maximum specific activity independently from the toxicity threshold level, because the conversion rate may be limited by physical processes below the threshold level. This problem can perhaps be overcome by measuring the specific activity with caprylic acid (C_8), as this acid has a relatively high solubility[26] and low toxicity.[8]

The fact that the actual inhibitory LCFA concentration depends upon hydrodynamics and biomass properties, may have important practical implications. It means that the performance of continuous flow reactors can hardly be predicted from batch toxicity studies. Furthermore, the capability of different continuous flow reactor systems to deal with toxic shock loads of LCFA may vary substantially.

Recovery of Methanogenesis after Inhibition

In all inhibited reactors, methane production clearly lagged behind the ß-oxidation. The course of methanogenesis observed during the fourth feeding in experiment A (Figure 1) is characteristic: initially, methanogenesis closely follows the acetogenesis (f_2), but after some time it stagnates and acetate accumulates; however, finally methanogenesis recovers rather rapidly. Figure 5 shows the contribution of hydrogenotrophic and acetotrophic methanogens to the observed methane production, as calculated from mass balances (equation 6). These calculations

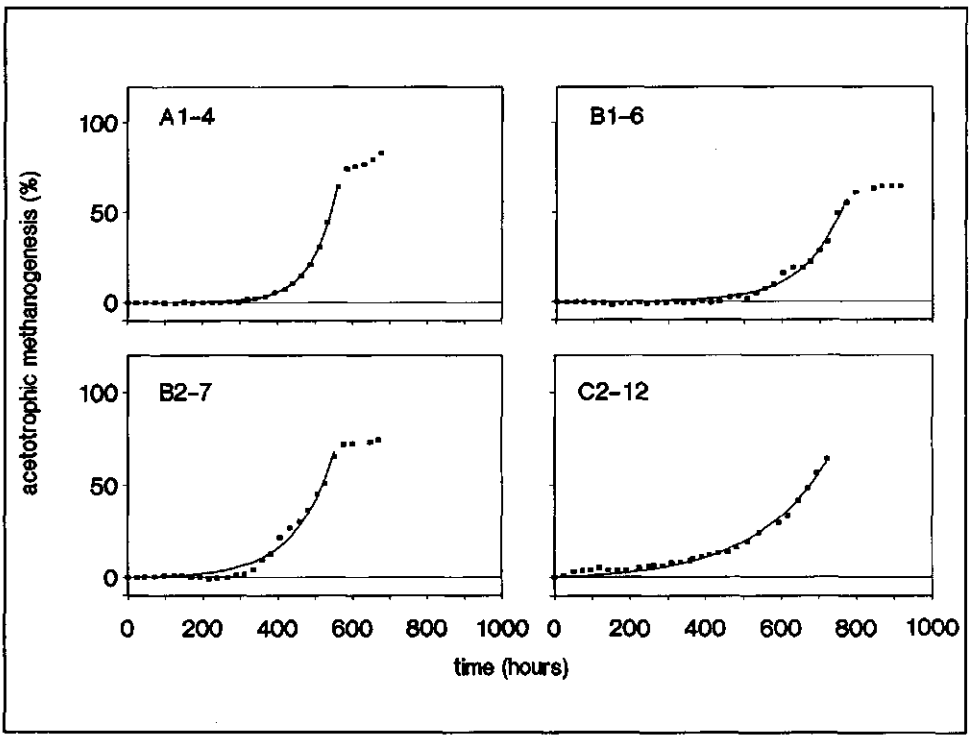

Figure 6. Comparison of acetotrophic methane production calculated from equations 6 (—) and 7 (■), in severely inhibited batch reactors

show that prior to the stagnation of the methane production, virtually no methane was produced from acetate. The acetotrophic methanogens recovered a long time after completion of reaction 1, i.e. after all capric acid had been removed from the system. The observed long 'lag phase' certainly cannot be attributed to substrate limitation, as the acetate concentrations were always far above reported K_s-values.[11]

We found that the course of the acetotrophic methanogenesis can be described with acceptable accuracy by an exponential function. Figure 6 shows a comparison between the course of acetotrophic methanogenesis calculated from equations 6 and 7. Table 2 gives a resume of the kinetic parameters obtained by non-linear regression analysis, for all inhibited reactors receiving capric acid as sole substrate. Four out of eight experiments gave a $\hat{\mu}$ value that corresponds closely to the values published for *Methanosarcina* spp.[11,13-15] In two experiments, the $\hat{\mu}$ value obtained by regression analysis closely corresponded to published values for *Methanothrix* spp.[11,16,17] The remaining two experiments were interrupted before sufficient methane was produced from acetate to allow a regression analysis. These results indicate that the observed recovery of methanogenesis must be attributed to growth of a few acetotrophic methanogens that survived the addition of a lethal dose of LCFA, for reasons that remain to be clarified. No 'adaptation' or 'recovery' of inhibited bacteria takes place.

Hanaki *et al*[2] observed a methane production pattern that is very similar to the typical pattern found in our experiments. In our opinion it is reasonable to conclude that the 'recovery' after a 'lag phase' observed by Hanaki *et al* also must be attributed to growth of a few surviving acetotrophic methanogens.

Table 2. Lethal capric acid concentrations and parameter values for exponential growth of acetotrophic methanogens, determined by non-linear regression analysis (experiments with capric acid as sole substrate)

experiment[a]	concentrations		parameter values		
	biomass	C_{10}	$\hat{\mu}$	X_0[b]	PGF
	(kg VSS.m^{-3})	(kg.m^{-3})	(d^{-1})	(g VSS.m^{-3})	(%)
A1-4	27.97	2.71	0.36	0.04	99.91
B1-6	2.52	1.35	0.23	0.05	99.24
B2-7	5.04	1.35	0.23	0.60	98.90
B3-7	10.08	1.55	0.40	0.42	98.98
C1-13	2.74	1.16	n.d.[c]	n.d.	n.d.
C2-12	5.48	1.16	0.12	4.08	98.60
C3-13	10.96	1.16	n.d.	n.d.	n.d.
D2-3	5.0	0.68	0.09	1.28	96.64

[a] codes indicate reactor and feed number (c.f. Table 1)
[b] calculated assuming $Y_{p/x}$ = 25 kg CH_4-COD.kg^{-1} VSS (c.f. equation 7)
[c] n.d. = not determined, experiment interrupted too early

The number of acetotrophic methanogens that survived the capric acid addition, can be estimated from equation 7, by assuming a constant yield value (Table 2). These calculations show that less than 0.1% of the acetotrophic methanogens survived, when the capric acid concentration in the digester exceeded the threshold level. Consequently, poisoning of a methane digester with LCFA is virtually irreversible. Recovery of the anaerobic treatment system through growth of the very few survivors, will require approximately 70 days for *Methanothrix* sp. This is far too long for practical purposes. Consequently re-inoculation of the reactor will be inevitable. This is an expensive operation, unless sufficient excess sludge is stored on site. Obviously, shock loads of LCFA to the sensitive methane digester should be prevented, for instance (a) by installing sufficient buffering capacity and a physical lipid removal system (flotation) in front of the anaerobic reactor, (b) by constant liming of the wastewater, or (c) by installing a separate acidification reactor in front of the methanogenic reactor, as proposed by Hanaki *et al.*[18]

Possibilities for Adaptation

Neither repeated exposure of granular sludge to capric acid above the threshold level, nor prolonged exposure to concentrations below the threshold level resulted in increased tolerance (adaptation) of the acetotrophic methanogens towards capric acid. Figure 7 shows the results of two consecutive additions of ca. 2.7 kg C_{10}.m^{-3} in experiment A. The close correspondence between the product formation pattern in both cases, indicates that the methanogens that had survived the first addition did not posses a higher tolerance level.

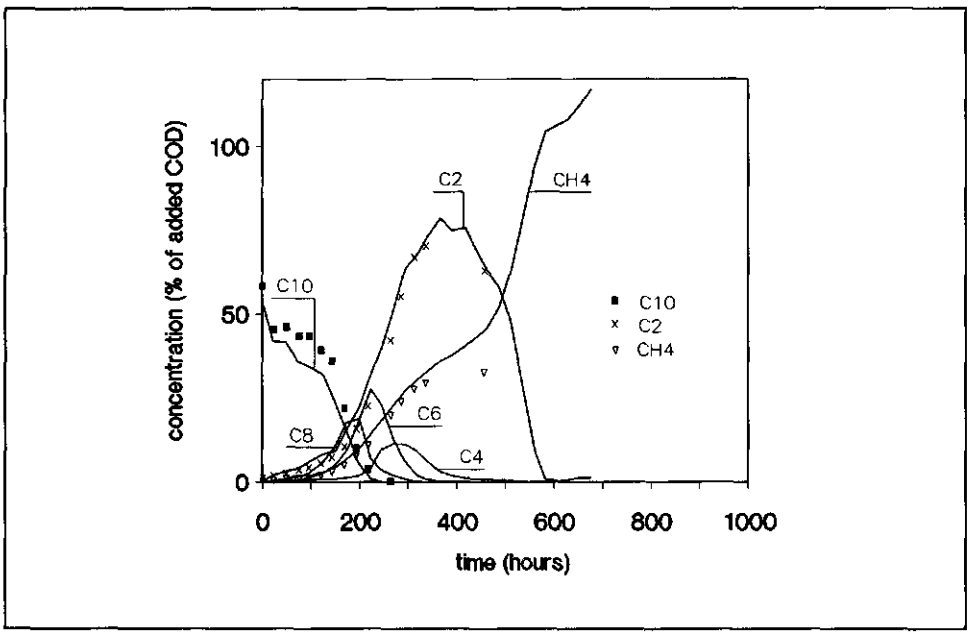

Figure 7. Degradation of capric acid upon repeated exposure to a lethal concentration (experiment A; drawn lines 4th feeding; symbols 5th feeding, interrupted after 450 hours)

Accumulation of ß-oxidation Intermediates

A successive accumulation of straight chain fatty acids with an even number of carbon atoms was observed, especially when capric acid additions exceeded the threshold level (Figure 7). This confirms the ß-oxidation mechanism for anaerobic LCFA degradation indicated by previous studies.[19-21]

The release of fatty acids with a shorter chain, coinciding with the rapid uptake of higher fatty acids, as shown in Figure 7, also indicates that the binding of ß-oxidation intermediates to the enzymes is reversible, and that the enzymes have a higher affinity for acids with a longer carbon chain. Novak and Carlson[22] observed no significant accumulation of intermediates other than acetate during anaerobic digestion of saturated long chain fatty acids. They concluded that the activation step is rate limiting in the ß-oxidation process. Our results indicate that - at least in an overloaded digester - the actual ß-oxidation reaction is rate limiting. Furthermore, our results are in conflict with those of Novak and Carlson[22] and Green[23], who found that the affinity of the enzymes decreases as the chain length of the LCFA increases.

Effect of Lecithin

Figure 8 shows the results of experiment D. The percentage indicated on the Y axis relates to the lecithin addition in the upper graph, and to the capric acid addition in the other three. Feeding was repeated immediately after complete digestion of the previous feed, and not necessarily after 1000 hours as indicated. Reactor D2 (capric acid only) showed the characteristic behavior of an inhibited digester - i.e. strong acetate accumulation - after addition of 0.68 kg $C_{10:0}.m^{-3}$, whereas reactor D3 (capric acid plus lecithin) was already inhibited at 0.51 kg $C_{10:0}.m^{-3}$. The relatively high lecithin concentration employed in reactor D3 clearly enhanced the inhibitory effect of capric acid. This is in contradiction with the results obtained by Kodicek and Worden[4] and Galbraith *et al*,[7] who found that lecithin and other surface active compounds act as antagonists to LCFA. A possible explanation for these conflicting observations may be the release of LCFA

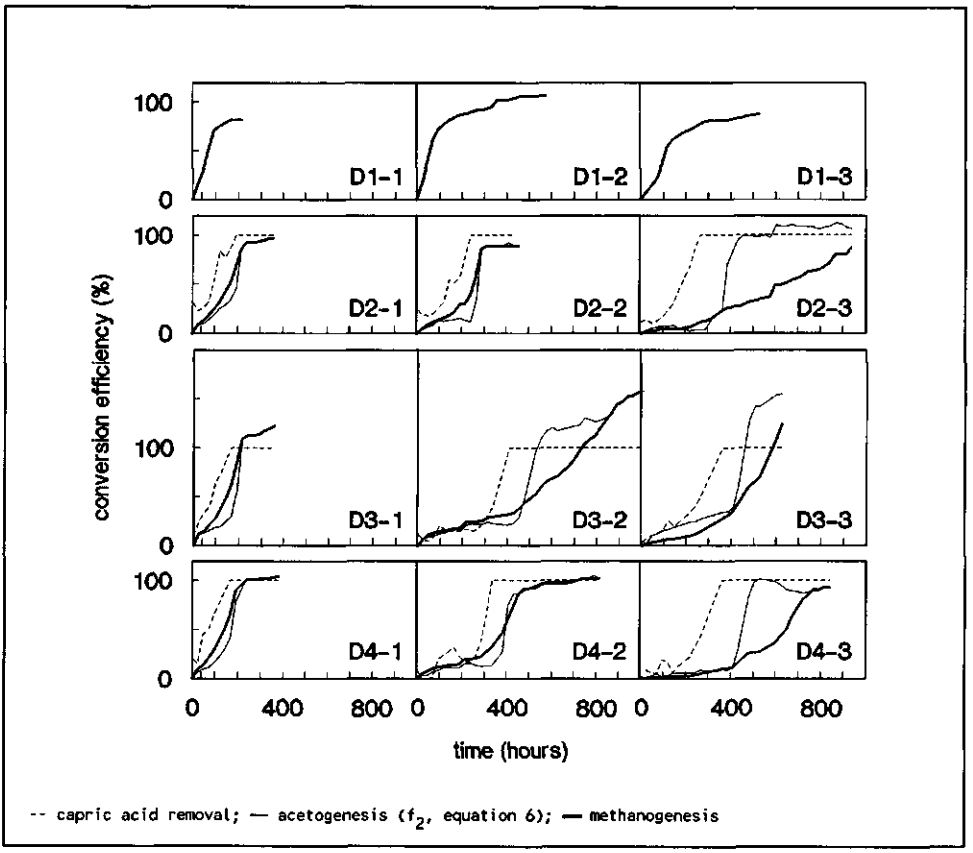

Figure 8. Degradation of lecithin (top graph), of capric acid (second graph), and of mixtures of capric acid and lecithin (third and fourth) (experiment D; Y axis shows % of added lecithin in top graph, % of added capric acid in others)

during lecithin degradation, which can act as synergists to capric acid.[8] Another explanation may be a change in the physical characteristics of the dispersed capric acid in the presence of lecithin. In experiments that will be described elsewhere, we observed that oleic acid is an extremely powerful inhibitor when added in an emulsion with triglycerides (Rinzema, A., unpublished).

Besides the synergistic effect of lecithin on capric acid toxicity, a second interaction was noticed: a comparison between the results of reactor D1 that received only lecithin, and those of the reactors receiving a mixture, indicates that capric acid reduced the degradation rate of lecithin. This can probably be attributed to its influence on the surface properties of the dispersed lecithin. Studies of lipid degradation in the digestive tract show that surface active compounds can depress the rate of lipolysis.[24] Our own studies indicate that emulsions of triglycerides stabilized with sodium oleate are hydrolyzed at a much lower rate than emulsions of the same triglycerides stabilized with gum arabic (Rinzema, A., unpublished). Capric acid might exert a similar effect on lecithin hydrolysis. On the other hand, experiment D indicates that a relatively low lecithin concentration (reactor D4) already retards the disappearance of capric acid from solution, as well as its ß-oxidation. Further research is needed to clarify the complex phenomena observed in mixtures of neutral lipids and LCFA.

Conclusions

During the start-up of a continuous anaerobic treatment system for lipid containing wastes, a lag phase in the degradation of long chain fatty acids (LCFA) can be expected. 'Adaptation' will occur as the result of growth of acetogenic bacteria capable of LCFA degradation. A low LCFA (lipid) concentration in the influent promotes faster adaptation.

Inhibition by LCFA is primarily related to the LCFA concentration; the LCFA:biomass ratio is less important. A lethal threshold LCFA concentration can clearly be distinguished. Our study indicates that capric acid has a lethal effect on acetotrophic methanogens at concentrations in the order of magnitude of 1 $kg.m^{-3}$. Some variation of the threshold level was observed, however, between different series of experiments. This variation can be explained with a mathematical model describing the relationship between LCFA concentrations in the bulk of the liquid and at the interface between biomass granules and liquid, the mass transfer characteristics, and the specific activity and particle size of the biomass granules.

Less than 0.1% of the acetotrophic methanogens survives, when the LCFA concentration in a methanogenic digester exceeds the lethal threshold level. Recovery of the methane production can be described by exponential growth of a small number of survivors. Consequently, restoration of the treatment capacity requires either several months to allow for growth, or re-inoculation. Acetotrophic methanogens do not adapt to LCFA, neither upon repeated exposure to toxic concentrations, nor after prolonged exposure to non-toxic concentrations.

Experiments with mixtures of lecithin and capric acid indicate that guidelines obtained from experiments with LCFA solutions cannot be used to predict the effect of mixtures of LCFA and other (neutral) lipids. Lecithin acts as a synergist to capric acid. Furthermore, lecithin and capric acid mutually reduce each others degradation rate.

The results concerning acetotrophic methanogenesis presented in this paper, primarily reflect the sensitivity of *Methanothrix* sp. towards capric acid, as this was the predominant acetotrophic methanogenic species in our inoculum.

Acknowledgements

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Appendix A. External Mass Transfer

Assuming spherical granules, equation 12 can be rewritten as:

$$C_L - C_S = \frac{k_r}{k_L} \cdot \frac{d_g \cdot X_g}{6} \qquad (13)$$

where C_L and C_S are the concentrations of dissolved capric acid salts in the bulk of the liquid and at the biomass-liquid interface respectively (kg.m^{-3}), k_r is the average specific activity of the biomass (kg C_{10}.kg^{-1} VSS.s^{-1}), k_L is the mass transfer coefficient (m.s^{-1}), d_g is the diameter of the biomass granules (m), and X_g is the local biomass concentration in the granules (kg VSS.m^{-3}).

The value for the mass transfer coefficient k_L is calculated from:[28]

$$k_L = 0.13 \cdot \left(\frac{P \cdot \eta}{V \cdot \rho^2} \right)^{0.25} \cdot Sc^{-0.67} \qquad (14)$$

$$P = N_p \cdot \rho \cdot N^3 \cdot D_i^5 \qquad (15)$$

where P is the energy dissipated by the impeller (J.s^{-1}), V is the liquid volume (m^3), η is the liquid viscosity (kg.m^{-1}.s^{-1}), ρ is the liquid density (kg.m^{-3}), Sc is the Schmidt number (-), N_p is the power number (derived from standard graphs[28]), and D_i is the diameter of the impeller (m).

The value of N_p for a six-blade turbine impeller would be ca. 5 at the rotational velocity and impeller diameter used in our study,[28] therefore a value of 5/3 was used for our two-blade impeller. The k_L-value calculated from equation 14 is 10^{-5} m.s^{-1}. Taking into consideration that intermittent mixing was applied in all experiments, and that experiments A and B were performed in reactors without baffles, we estimate that the actual k_L-value will be in the range 5 10^{-7}-10^{-5} m.s^{-1}.

Using values of 1 10^{-3}-2.5 10^{-3} m for d_g, 0.5 kg C_{10}.kg^{-1} VSS.day^{-1} for k_r, and 150 kg VSS.m^{-3} for X_g, the minimum and maximum value for the driving force can be estimated at:

$$(C_L - C_S)_{min} = 0.029 \text{ kg.m}^{-3}$$
$$(C_L - C_S)_{max} = 0.723 \text{ kg.m}^{-3}$$

Appendix B. Internal Diffusion Limitation

The time constants of the biological reaction and the intra-particle diffusion process are defined as:

$$t_r = C_L / k_r \cdot X_g \quad (16)$$

$$t_{dg} = d_g^2 / ID_g \quad (17)$$

where t_r and t_{dg} are the time constants for the biological reaction and the diffusion process (s), C_L is the concentrations of dissolved capric acid salts in the bulk of the liquid ($kg.m^{-3}$), k_r is the maximum specific activity of the biomass ($kg\ C_{10}.kg^{-1}\ VSS.s^{-1}$), X_g is the local biomass concentration in the granules ($kg\ VSS.m^{-3}$), d_g is the granule diameter (m), and ID_g is the effective diffusion coefficient of caprate in the granule ($m^2.s^{-1}$).

Assuming that C_L is 1.0 $kg.m^{-3}$, k_r is 0.2-1.0 $kg\ C_{10}.kg^{-1}\ VSS.s^{-1}$, X_g is 100-150 $kg\ VSS.m^{-3}$, d_g is 1-2 10^{-3} m, and ID_g is 5 $10^{-10}\ m^2.s^{-1}$, the time constants are estimated at:

t_r = 20-200 s
t_{dg} = 1,000-40,000 s

CHAPTER 3

The Effect of Lauric Acid Shock Loads on the Biological and Physical Performance of Granular Sludge Bed UASB Reactors Digesting Acetate

(submitted for publication)

CHAPTER 3

The Effect of Lauric Acid Shock Loads on the Biological and Physical Performance of Granular Sludge Bed UASB Reactors Digesting Acetate

Arjen Rinzema, Arne Alphenaar and Gatze Lettinga
Dept. of Water Pollution Control, Wageningen Agricultural University, Bomenweg 2, 6703 HD Wageningen, The Netherlands

Abstract

The specific activity of acetotrophic methanogens and the physical behavior of granular sludge in lab scale Upflow Anaerobic Sludge Bed reactors subjected to shock loads of lauric acid was studied, both in the absence and presence of calcium. In the absence of calcium, lauric acid completely inhibits acetotrophic methanogens above a threshold level of 100 mg $C_{12:0}$ dm^{-3}, whereas no inhibition occurs below this threshold concentration. Addition of an equivalent amount of calcium to wastewater containing lauric acid, prevents inhibition of acetotrophic methanogens at least up to 1500 mg $C_{12:0}$ dm^{-3}. Addition of less than an equivalent amount of calcium apparently removes more than a stoichiometric amount of lauric acid: 50% inhibition occurs at approximately 700 mg 'free' or excess $C_{12:0}$ dm^{-3}. Complete sludge wash-out from conventional UASB reactors will occur within 2-8 hours, if the system is overloaded with an influent containing more than 100 mg $C_{12:0}$ dm^{-3}. Calcium does not prevent wash-out.

1. Introduction

Long chain fatty acids are well known as versatile inhibitors of methanogenic bacteria.[1-3] The early work of McCarty,[4] nevertheless already indicated that anaerobic treatment of wastes with high concentrations of lipids can be successful in well mixed, continuously fed digesters. Inhibition will only occur in case of (sudden) overloading. Until now, the inhibitory effect of long chain fatty acids in anaerobic digestion has only been studied in batch reactors.[3,5-7] Hanaki *et al*[3] studied the mechanism of long chain fatty acid inhibition. They concluded that long chain fatty acids induce a lag phase in the activity of acetotrophic methanogens as well as obligate hydrogen producing acetogens. We found that the initial retardation of methanogenesis (lag phase) can be explained by the death of the majority of the methanogens and subsequent exponential growth of the survivors (Rinzema, A., unpublished). This implicates that a shock load of long chain fatty acids can render an anaerobic digester inactive for several months, unless it is re-inoculated. The results published by Koster[7] indicate, that long chain fatty acids affect the cells very rapidly: already after 7.5 minutes, 50% of the acetotrophic methanogenic activity was lost. Several authors have shown that soluble calcium salts can reduce the inhibitory effect of long chain fatty acids. The results of Koster[7] as well as those of Hanaki *et al*[3] indicate that calcium salts have to be added within minutes after the introduction of the acids into the digester. So far, however, the results of the above mentioned batch inhibition studies have not been validated by shock load experiments in continuous digesters.

Apart from their inhibition potential, long chain fatty acids may have a second negative effect. Samson *et al*[8] reported strong sludge wash-out after the accidental introduction of a shock load of milk fat into a full scale Upflow Anaerobic Sludge Bed reactor (UASB). This emphasizes the importance of continuous treatment studies. Therefore, we studied the effect of shock loading with lauric acid on the behavior of granular sludge in lab scale UASB reactors. The specific activity of acetotrophic methanogens and the physical behavior of the granules were monitored.

Table 1. Summary of experimental conditions

experiment	sludge concentration (kg VSS m^{-3})	lauric acid concentrations applied to parallel UASB reactors (mM C_{12})	cation concentrations Ca^{2+} (mM)	Mg^{2+} (mM)
A	16.9	0.000, 0.460, 0.925, 1.845, 2.770	0.0	0.0
B	12.8	0.460, 0.925, 1.385, 1.845, 2.305	0.0	0.0
C	15.8	0.445, 0.590, 0.885, 1.105, 1.325	0.0	0.0
D	18.5	0.000, 0.460, 1.385, 2.305, 3.690	2.0	0.75
E	15.8	3.690, 3.690, 3.690, 3.690, 7.380	0.7-3.7	0.0

2. Materials and methods

2.1. Summary of experiments

Granular sludge fed with acetate solutions was exposed to lauric acid, in lab scale UASB reactors. The sludge sample used in these experiments had not been exposed to lauric acid before. Earlier work indicated that this type of sludge does not degrade lauric acid within a period of at least 24 hours. Consequently the activity of the acetotrophic methanogens could be monitored in this case, simply by measuring the methane production.

The granular sludge was maintained on an acetate feed for a period of 4-5 days, until the increase in activity was insignificant (<10% per day). At that moment, the feed was switched to a mixture of sodium acetate and laurate. Table 1 summarizes the principal influent characteristics, during five series of experiments with five UASB reactors operated in parallel. During the start-up period on acetate, the cation composition of the media was identical to that listed in Table 1. After the first introduction of lauric acid, the methane production and the acetate concentration in the effluent were monitored for 24-30 hours.

In series D and E, the 'free' lauric acid concentration is defined as (all concentrations in moles dm^{-3}):

$$['free'\ C_{12:0}] = [total\ C_{12:0}] - 2\ .\ [Ca^{2+}]$$

No attempt was made to calculate the concentration of undissociated lauric acid in solution, because experimental data available in the literature on the dissociation constant and the solubility are conflicting.[9,10] Complexation by magnesium ions was not taken into account, because their antagonistic influence can be neglected.[11]

2.2. Reactors

Five glass UASB reactors with a liquid volume of 200 ml were used (internal diameter 39 mm, height ca. 170 mm). The UASB reactors were equipped with a heavy glass marble placed on top of a ring above the sludge bed (Figure 1). This construction guaranteed virtually complete retention of floating sludge. No mechanical mixing or effluent recirculation was applied in the reactors. The influent stock solution was continuously stirred, in order to prevent settling or flotation of undissolved laurate. Methane production was measured with Mariotte flasks filled with 1% (w/v) NaOH solution. The reactors were situated in a temperature controlled room (30 ± 1 °C).

2.3. Biomass

Elutriated granular sludge from a full scale UASB reactor treating potato processing wastewater was used as an inoculum (Aviko, Steenderen, The Netherlands). The average maximum specific acetotrophic methanogenic activity was 1.1 kg $COD.kg^{-1}$ $VSS.day^{-1}$ (30 °C). The activity was calculated from the methane production and acetate degradation rates measured during the start-up period on acetate. The predominant acetotrophic methanogens in the inoculum were of the genus *Methanothrix*.[6,12]

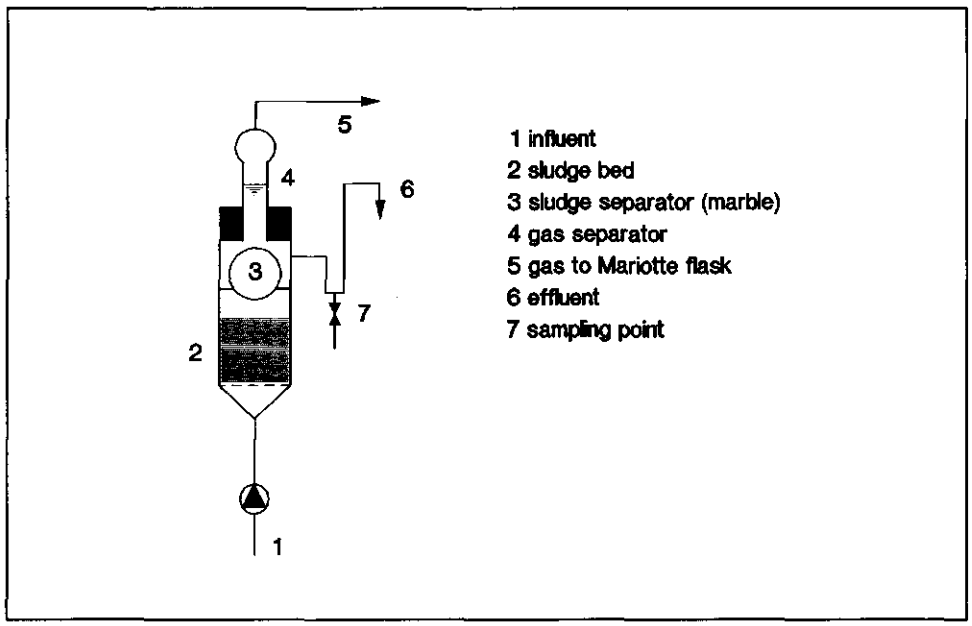

Figure 1. Schematic representation of the UASB reactors used in this study.

2.4. Media

All UASB reactors received a basal acetate, containing (mg dm^{-3}): acetic acid (2340), $CaCl_2.2H_2O$ (see Table 1), $MgCl_2.6H_2O$ (see Table 1), NH_4Cl (1044), KCl (270), $MgCl_2.6H_2O$ (150), KH_2PO_4 (170), $(NH_4)_2SO_4$ (170), and trace element solution (0.001 dm^3 dm^{-3}, c.f. Rinzema *et al*[13]). The basal medium was prepared in demineralized water; the pH was increased to 7.0 $\pm$ 0.2 with NaOH.

The desired lauric acid concentration was obtained by adding the required amount of a concentrated sodium laurate stock solution. This stock solution contained (g): lauric acid (10.0), NaOH (2.0), and demineralized water (1000). Lauric acid was melted *au bain-marie* and dissolved in the vigorously stirred, hot NaOH solution (ca. 50 °C). The pH of the stock solution was 9.8. The solution became solid at room temperature, and had to be reheated before use.

All chemicals were of analytical grade (Merck AG, Darmstadt, FRG).

2.5. Sampling and analyses

Samples for VFA analyses were filtered (Schleicher & Schull filter no. 589-1). VFA up to valeric acid were analyzed by gas chromatography.[13] TSS and VSS were analyzed according to Dutch Standard Method NEN 3235-4.1.

3. Results and discussion

In all experiments discussed in this paper, methane production from lauric acid was negligible, as indicated by COD balances.

3.1. Experiments with calcium-free media

Figure 2 shows the specific acetotrophic methanogenic activity of the sludge exposed to various lauric acid concentrations, determined 5 and 30 hours after lauric acid was first introduced into the UASB reactors. After 30 hours exposure, a threshold lauric acid concentration at approximately 100 mg $C_{12:0}$ dm^{-3} is clearly visible. Lower concentrations caused no inhibition, whereas above 100 mg dm^{-3}, inhibition was virtually complete. Comparable observations have

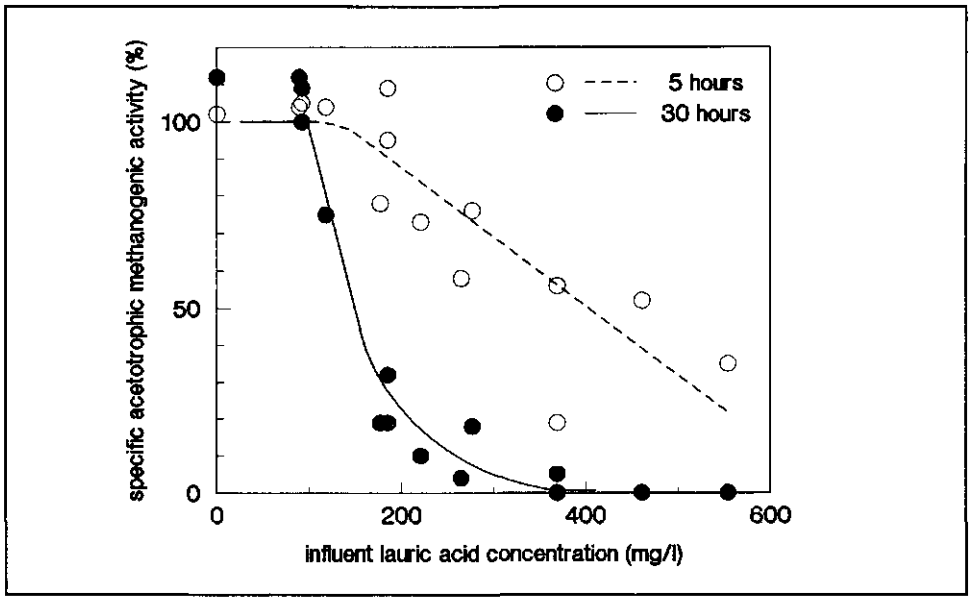

Figure 2. The maximum specific methanogenic activity of granular sludge subjected to various lauric acid concentrations, in the absence of calcium, after 5 (○ --) and 30 (● —) hours exposure.

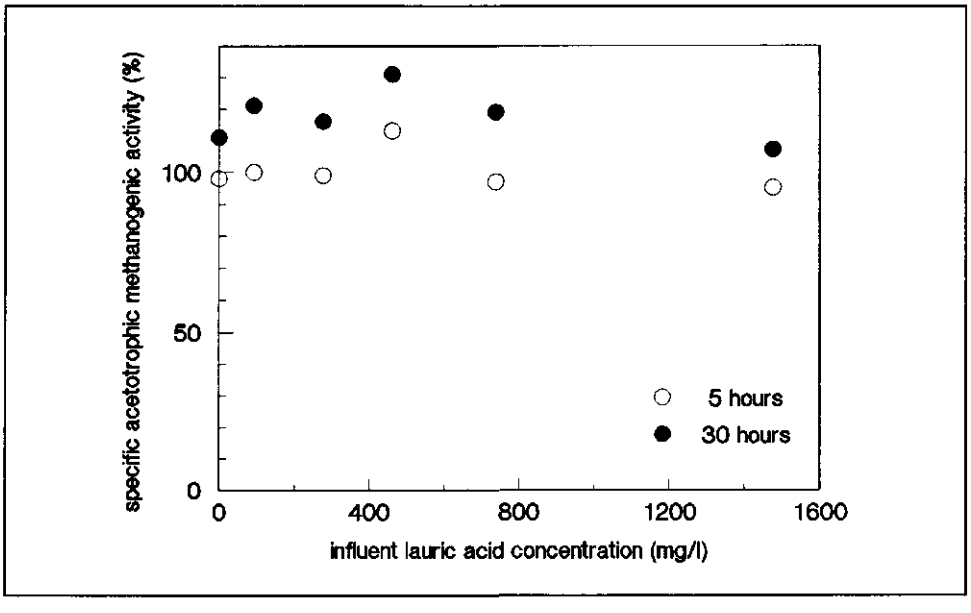

Figure 3. The maximum specific acetotrophic methanogenic activity of granular sludge subjected to various lauric acid concentrations, in the presence of an equivalent amount of calcium, after 5 (○) and 30 (●) hours exposure.

been described by other authors.[14-16] Galbraith *et al*[15] found a bactericidal effect of long chain fatty acids towards Gram-positive bacteria, above a threshold level. The mechanism behind the threshold level remains to be clarified.

The inhibitory effect of lauric acid was clearly time dependant. This dependency cannot be attributed to residence time distribution in the reactors. Even if we assume ideal mixing of the liquid in the reactors, the lauric acid concentration should have approached the influent level very closely after 5 hours (the hydraulic residence time was 1.6-1.9 hours).

Koster[7] observed a very rapid decrease of the acetotrophic methanogenic activity when granular sludge from the same origin as our inoculum was exposed to 7.5 mM lauric acid (ca 1 mM Ca^{2+}), in batch experiments: the addition of an equivalent amount of calcium chloride only 7.5 minutes after the introduction of the lauric acid, already resulted in 50% loss of activity. Our results are clearly in conflict with these findings. A possible explanation for the difference might be either the difference in lauric acid concentration, or a difference in mass transfer rate (hydrodynamics).

The results presented in this paper do not yet allow a proper evaluation of the consequences of the observed delay in the response of the UASB reactors to shock loads of long chain fatty acids. Corrective actions applied within the first five hours may preserve part of the methanogenic activity. On the other hand, the methane production observed after several hours exposure to lauric acid in the absence of an equivalent amount of lauric acid, does not necessarily indicate that the methanogens are still vital. The delayed response may even prevent that the shock load is noticed in time. Further research is clearly needed to clarify the mechanism underlying the time dependance of the response of the methanogens to shock loads of long chain fatty acids.

3.2. Experiments with calcium-containing media

Figure 3 shows that addition of an equivalent amount of calcium chloride to the influent prevented inhibition up to 1500 mg $C_{12:0}$ dm^{-3}; higher concentrations were not investigated. The increase of the specific activity after 30 hours exposure, indicates that growth of acetotrophic methanogens occurred. From the average increase over 25 hours (17 %, s.d. 4%, n 6), the nett growth rate was calculated at 0.15 d^{-1}, a value that corresponds reasonably to published values for *Methanothrix* sp.[17]

Figures 4 and 5 illustrate that a higher reduction of toxicity was found in the experiments with a calcium:laurate ratio < 1 eq eq^{-1}, than expected on the basis of the 'free' laurate concentration (correction for calcium laurate precipitation, c.f. materials and methods section). This may indicate that calcium binds more than an equivalent amount of laurate, most probably as a result of co-precipitation of lauric acid crystals.

The activity remaining after 30 hours exposure corresponded very well to the results obtained in batch experiments by Koster and Cramer[6] (Figure 5). Hence it can be concluded that batch experiments predict the long term effect of fatty acids in continuous feed systems satisfactorily.

3.3. Sludge flotation

Very severe sludge flotation was observed at lauric acid concentrations exceeding 100 mg dm^{-3}, in the presence, as well as in the absence of calcium. Table 2 summarizes the length of the periods between the start of the shock load and the moment when complete sludge flotation was observed. The "marble-on-ring" construction employed in these experiments to ensure retention of floating sludge, cannot be employed on a larger scale. This means that a shock load of LCFA will cause very serious problems in a conventional UASB reactor system; complete sludge wash-out will most likely occur, within several hours after the start of the shock load. Even if sufficient calcium is added to the wastewater to prevent inhibition, complete failure of a conventional UASB system is likely to occur. The occurrence of this type of process failure in a full scale UASB installation treating cheese factory wastewater, has been described by Samson *et al.*[8] Other high rate reactor systems like the Fluidized Bed and Expanded Bed systems very likely will suffer the same problems. Therefore, it is justified to conclude that there is a need for the development of advanced reactor types, capable of retention of large amount of floating sludge.

Calcium retarded the sludge flotation only slightly (Table 2). In the absence of calcium, flotation primarily resulted from the poor release of gas bubbles by the granules (Figure 6). In the presence of calcium, attachment of precipitated fatty acid salts to the granules was clearly visible, causing similar problems with the release of biogas. Furthermore, precipitated LCFA salts accumulated at the bottom of the reactor, thus expelling the sludge from the reactor (Figure 6).

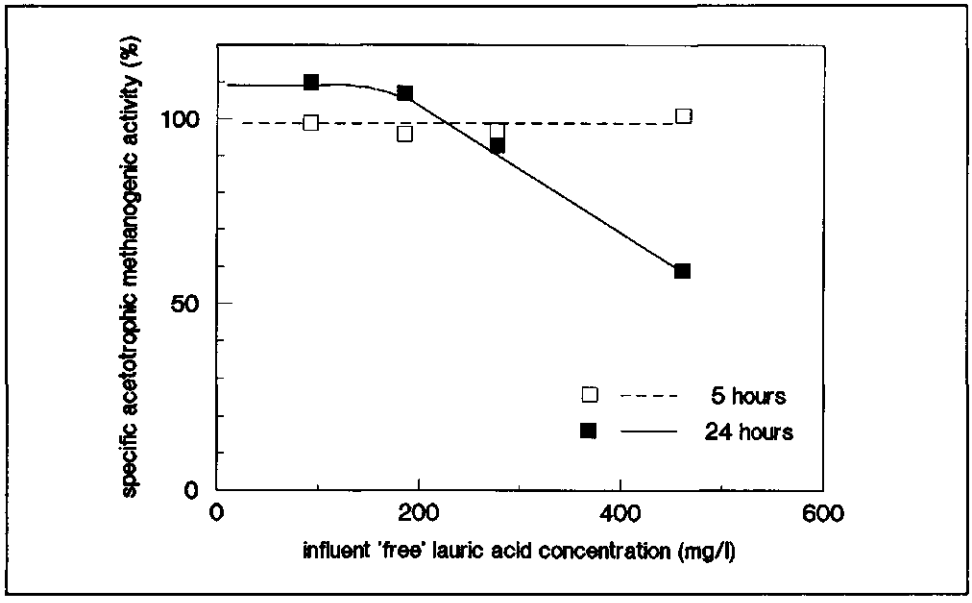

Figure 4. The maximum specific acetotrophic methanogenic activity of granular sludge subjected to various lauric acid concentrations, in the presence of less than an equivalent amount of calcium, after 5 (□--) and 24 (■—) hours exposure.

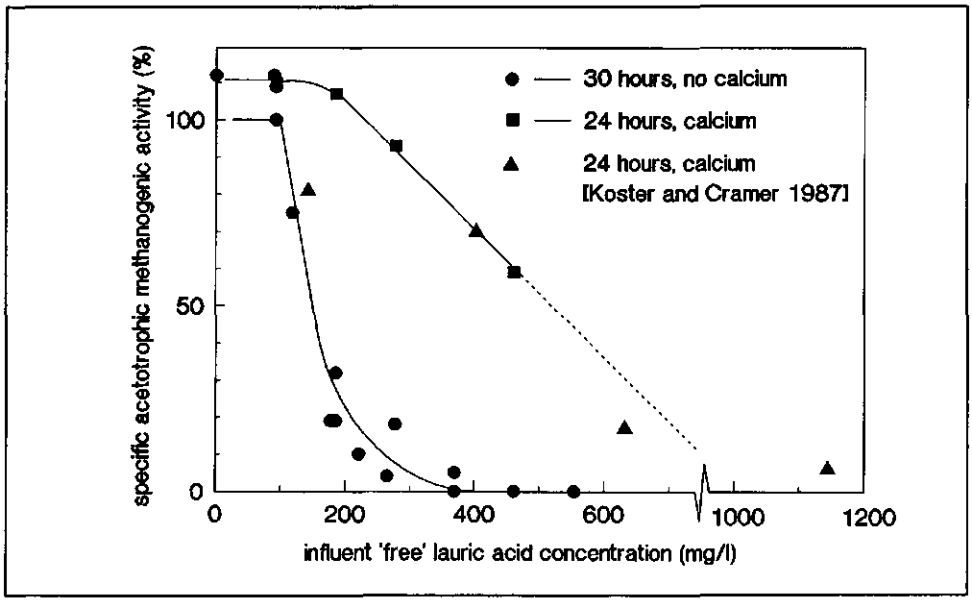

Figure 5. A comparison of the inhibitory effect of lauric acid, as observed in the absence of calcium (•—), and in the presence of less than an equivalent amount of calcium (■— this study, ▲Koster and Cramer 1987)

Table 2. Time required for complete sludge flotation after lauric acid shock loads

influent concentration (mM $C_{12:0}$)	required time (hours)[a]	
	no Ca^{2+}	2.0 mM Ca^{2+}
0.460	no flotation	no flotation
0.925	> 6.4	n.d.[b]
1.385	4.0	8.0
1.845	3.0	n.d.
2.305	3.0	5.0
3.690	n.d.	1.9

[a] hydraulic retention time 1.6 hours

Acknowledgements

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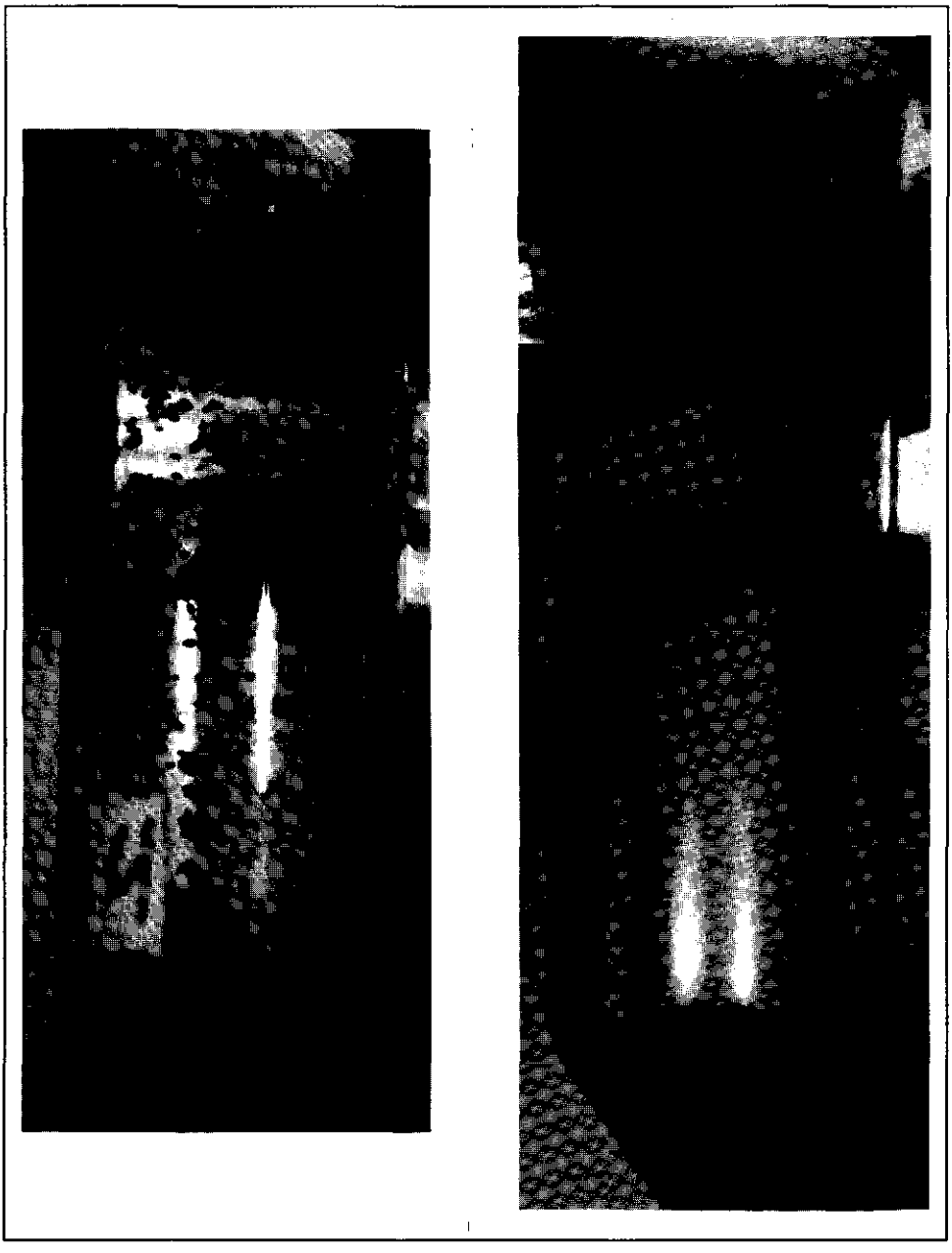

Figure 6a. Formation of clusters of granules shortly after the start of a shock load of lauric acid. **Figure 6b.** Complete flotation of the granular sludge bed after several hours exposure to lauric acid (both cases in the absence of calcium).

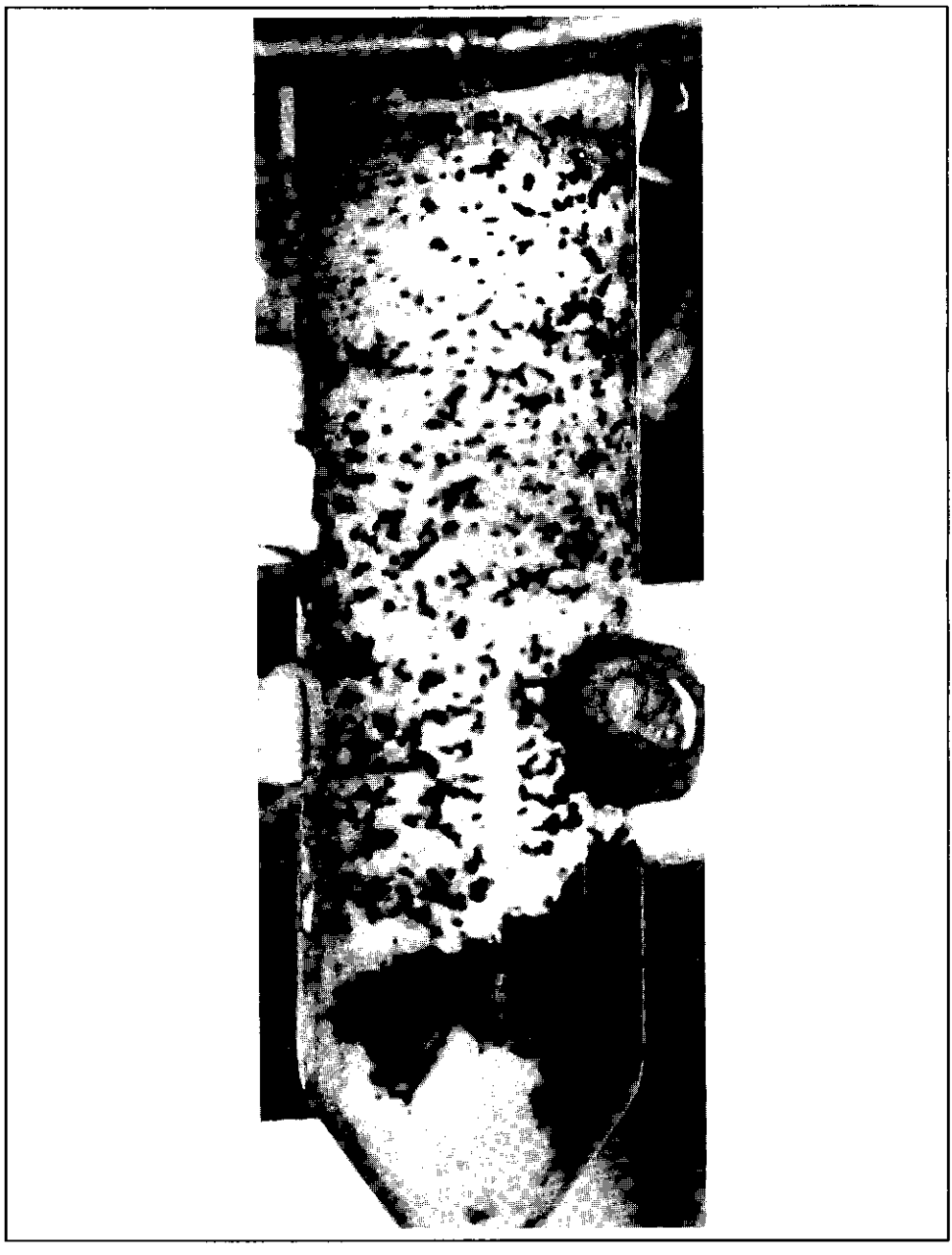

Figure 6c. Flotation of granular sludge and accumulation of fatty acid precipitates after a shock load of lauric acid, in the presence of an equivalent amount of calcium

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CHAPTER 4

Anaerobic Digestion of Long Chain Fatty Acids in Conventional and Expanded Granular Sludge Bed Reactors

(submitted for publication)

Mueller 1958, O'Rourke 1968, Hanaki *et al* 1986). The overall conversion rate is limited either by the biological conversion of the LCFA, or by the physical processes of dissolution and mass transfer of these acids. The work of Loehr and Roth (1968) on aerobic degradation of LCFA salts, indicates that mass transfer limitations can be very significant, especially with calcium salts of LCFA. Novak and Carlson (1970) investigated the anaerobic digestion of several LCFA in continuously fed completely stirred tank reactors. In contrast to Loehr and Roth, they concluded that conversion rates were limited by the biological reactions, not by physical processes. The apparent discrepancy between the results obtained for aerobic and anaerobic degradation of LCFA salts, must probably be attributed to the difference in maximum specific activity between aerobic and anaerobic microorganisms.

The maximum specific growth rates that can be calculated from mixed culture studies published by O'Rourke (1968) and Novak and Carlson (1970), as well as the maximum specific growth rates of *Syntrophomonas sapovorans* published by Roy *et al* (1985, 1986), indicate the necessity for biomass retention, if short hydraulic residence times are to be achieved in anaerobic treatment of lipid containing wastes. Almost all results with high-rate digesters published in the literature so far, are based on poorly defined synthetic substrates or real wastes containing several other substrates besides lipids, for example suspended dog food (Norrman and Frostell 1978), slaughterhouse wastewater (Denmead 1973, Sayed *et al* 1984, 1987), wool scouring wastewater (Wilson and King 1984), meat extracts (Rudd *et al* 1985), and diluted whole milk (Backman *et al* 1985). Although these studies may be very useful to establish the feasibility of anaerobic treatment for specific wastes, they do not allow the assessment of limiting factors, or provide general guidelines for treatment of fat containing wastewater. Kunst *et al* (1985) investigated the anaerobic digestion of soy bean oil emulsions in upflow anaerobic filter reactors. In a one-stage reactor for combined lipolysis, acetogenesis and methanogenesis, they achieved COD reduction efficiencies of ca. 82% at space loading rates up to 5 kg $COD.m^{-3}.day^{-1}$ (hydraulic residence time ca. 1 day). In a two-stage system, acetogenesis in the first stage stagnated when the space loading rate exceeded 7.5 kg $COD.m^{-3}.day^{-1}$. The loading rates reported by Kunst *et al* are rather moderate for high-rate mesophilic digesters. In our opinion this must be attributed to the rather poor biomass retention capacity of fully packed upflow filters. Much higher biomass concentrations can be achieved in unpacked upflow reactors with granulated biomass. The experience obtained in our laboratory, with wastewater from slaughterhouses (Sayed *et al* 1987) and rendering plants (De Zeeuw 1982), as well as with domestic sewage (Lettinga *et al* 1983), indicates that granular sludge can be used very beneficially as an inoculum for upflow reactors, and that it can be maintained or augmented on wastes that would not allow granulation with low-grade inocula like digested sewage sludge. Therefore we decided to evaluate the suitability of upflow reactors inoculated with granular sludge for treatment of fat containing wastewater. Solutions of sodium caprate and sodium laurate were digested in conventional upflow anaerobic sludge bed (UASB) reactors inoculated with granular sludge, and in modified upflow reactors with a fully expanded or fluidized granular sludge bed, so-called expanded granular sludge bed (EGSB) reactors.

Materials and Methods

Reactors. Data on the experimental equipment are summarized in Table 1. Figure 1 illustrates the experimental arrangement, and details of the reactors. The UASB reactor used for experiment 1 with capric acid had four inlet pipes. The UASB reactor used for experiment 3 with lauric acid had only one inlet pipe. The reactors used in experiments 3 and 4 with lauric acid had a modified gas-liquid-sludge separator, which allows retention of floating material and prevents plugging of gas lines by this material (Figure 1c). Both reactors used for digestion of lauric acid had removable gas-tight covers, allowing manual removal of floating material. In non of the reactors mechanical mixing was applied. Effluent recirculation was applied in the EGSB reactors only. Gas tight butyl rubber tubing was used for the effluent recirculation lines, to prevent losses of methane and poisoning by oxygen. All experiment were conducted in a temperature controlled room maintained at 30 ± 1 °C.

Space loading rates were calculated on the basis of the total liquid volume (reactor + settler).

Biomass. Elutriated, and well settled granular sludge from a full scale UASB reactor treating potato processing wastewater (Aviko, Steenderen, The Netherlands) was used as an inoculum in the experiments with capric acid. Elutriated, and well settled granular sludge from a full scale UASB reactor treating sugar beet refinery wastewater (Suiker Unie, Roosendaal, The

Table 1. Description of reactors and inocula

experiment no.	1	2	3	4
reactor type[a]	UASB perspex	EGSB PVC	UASB glass	EGSB glass
reactor dimensions				
internal diameter (mm)	115	53	92	47
height (mm)	140	985	365	1000
settler type[b]	A	A	B	B
settler dimensions				
internal diameter (mm)	115	80	92	92
height (mm)	85	100	185	185
total liquid volume reactor + settler (dm^3)	2.5	3.25	3.95	3.25
no. of inlet points	4	1	1	1
recycle flow rate ($dm^3.hr^{-1}$)	0	30	0	10
recycle ratio[c]	0	14-96	0	5-11
substrate[d]	$C_{10:0}$	$C_{10:0}$	$C_{12:0}$ ($+C_2$)	$C_{12:0}$
inoculum[e]	A	A	SU	SU
quantity (g VSS)	104	83	53	53
specific acetotrophic methanogenic activity ($kg\ COD.kg^{-1}\ VSS.day^{-1}$)	0.7	1.4	1.3	1.3

[a] UASB = Upflow Anaerobic Sludge Bed reactor, EGSB = Expanded Granular Sludge Bed reactor
[b] A = conventional separator (inverted funnel, Figure 1a), B = modified separator (Figure 1c)
[c] quotient of recycle and influent flow rates
[d] $C_{10:0}$ = capric acid, $C_{12:0}$ = lauric acid
[e] A = Aviko granular sludge, SU = Suiker Unie granular sludge

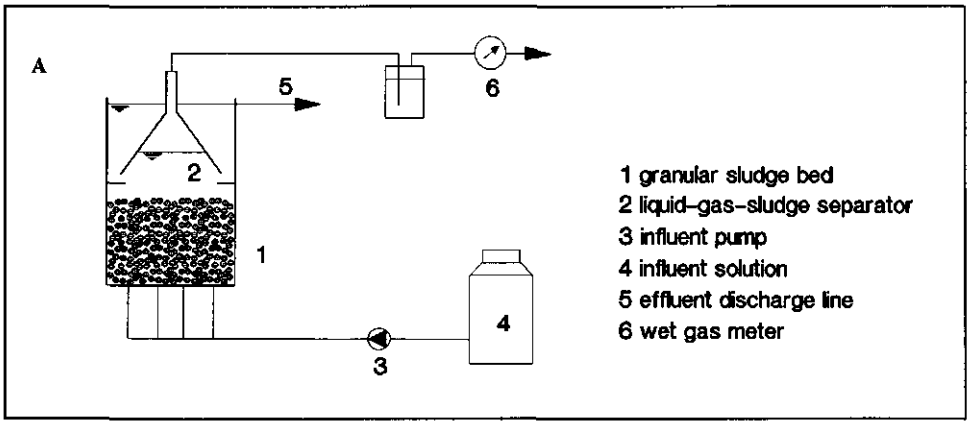

Figure 1a. Schematic representation of the UASB reactor with four inlet points used in experiment 1 (digestion of capric acid)

Netherlands) was used for the experiments with lauric acid. Table 1 provides more details on the inocula. The maximum specific acetotrophic methanogenic activity of the inocula for experiments 1 and 2 was determined in standard batch activity tests (>600 mg acetate.l^{-1} as sole substrate, 30 ± 1 °C), that of the inoculum for experiments 3 and 4 was determined in continuous feed tests with acetate as sole substrate (effluent concentration >600 mg.l^{-1}, 30 ± 1 °C).

Media. Concentrated stock solutions of capric ($C_{10:0}$) and lauric ($C_{12:0}$) acid were used. The capric acid stock solution contained (g): capric acid (20), NaOH (4.65), and demineralized water (1000). The lauric acid stock solution contained (g): lauric acid (10), NaOH (2.0), and demineralized water (1000). LCFA were melted *au bain-marie* and dissolved in the vigorously stirred, hot NaOH solution (ca. 50 °C). The pH of the capric acid stock solution was lowered to approximately 8.5 with HCl, the pH of the lauric acid stock solution was 9.8. The latter solution became solid at room temperature, and had to be reheated before use.

The required amounts of combined nutrients/trace elements stock solution and tap water were added to the LCFA stock solution *in situ* in all experiments (Figure 1b), except in the first UASB experiment with capric acid. In this experiment, influent with the required composition was prepared batch wise, every day. The combined nutrients/trace elements stock solution was a diluted mixture of the standard nutrients and trace elements solutions (volumetric ratio 6:1), described earlier (Rinzema *et al* 1988). This combined solution was mixed with the influent at a flow rate corresponding to the addition of 6 ml.l^{-1} standard nutrients solution, and 1 ml.l^{-1} standard trace elements solution.

In the UASB experiment with lauric acid, an acetic acid stock solution was used, containing 100 mg C_2.l^{-1}, neutralized to pH 6.5 with NaOH. The required amount of acetic acid stock solution was added to the combined nutrients/trace elements solution.

All chemicals were of analytical grade (Merck AG, Darmstadt, FRG), except the yeast extract used in the nutrients solution (Gist-brocades, Delft, The Netherlands).

Sampling and analyses. Effluent samples were taken from the settler of the UASB reactors, and from the recirculation lines of the EGSB reactors (through a septum). Samples for COD and fatty acid analyses were filtered (Schleicher & Schull paper filter no. 589-1, black). Fatty acids up to valeric acid were analyzed by gas chromatography (Rinzema *et al* 1987). Capric acid was determined on the same column, after increasing the column temperature to 190 °C. COD, TSS and VSS were analyzed according to Dutch Standard Methods (NEN 3235-4.1 and NEN 3235-5.3). The pH was determined with a pH electrode, immediately after exposure of the sample to the air.

Results and Discussion

Experiments with Capric Acid

Experiment 1: UASB reactor - capric acid

The results of batch experiments that will be published elsewhere indicated that a low influent concentration (< 400 mg $C_{10:0}$.l^{-1}) is favorable during the start-up period of a continuous digester treating LCFA (Rinzema, A., submitted). Therefore the UASB reactor was started up under the following conditions: influent concentration 384 mg $C_{10:0}$.l^{-1}, hydraulic residence time 40 hours, average sludge loading rate 0.014 kg COD.kg^{-1} VSS.day^{-1}, space loading rate 0.6 kg COD.m^{-3}.day^{-1}. As expected, virtually complete conversion of capric acid to methane was achieved within 5 days. Then the loading rate was gradually increased to 4.2 kg COD.m^{-3}.day^{-1} over a 70 days period (influent concentration 570 mg $C_{10:0}$.l^{-1}), without deterioration of the performance. No fatty acids were detected in the effluent during the entire period.

After approximately 80 days, the operation of the UASB reactor was seriously disturbed: plugging occurred in three of the four influent pipes (Figure 1a), and all influent entered the reactor through one pipe. This resulted in the formation of a white lump of precipitated capric acid (salts) in the sludge bed, within 24 hours (Figure 2). Still no fatty acids could be detected in the effluent, but the methane production rate dropped to zero within 24 hours. Feeding was stopped, and the precipitates were distributed over the sludge bed by manual mixing. Thereafter, the precipitates were degraded within three days. However, when feeding through one inlet pipe was resumed, the precipitates re-appeared immediately.

The most probable explanation for the precipitate formation is insufficient mixing of the liquid in the sludge bed. Superficial gas and liquid velocities were extremely low (ca. 0.01 and 0.03 m.hr^{-1} respectively). As a result, only the sludge in the vicinity of the inlet points

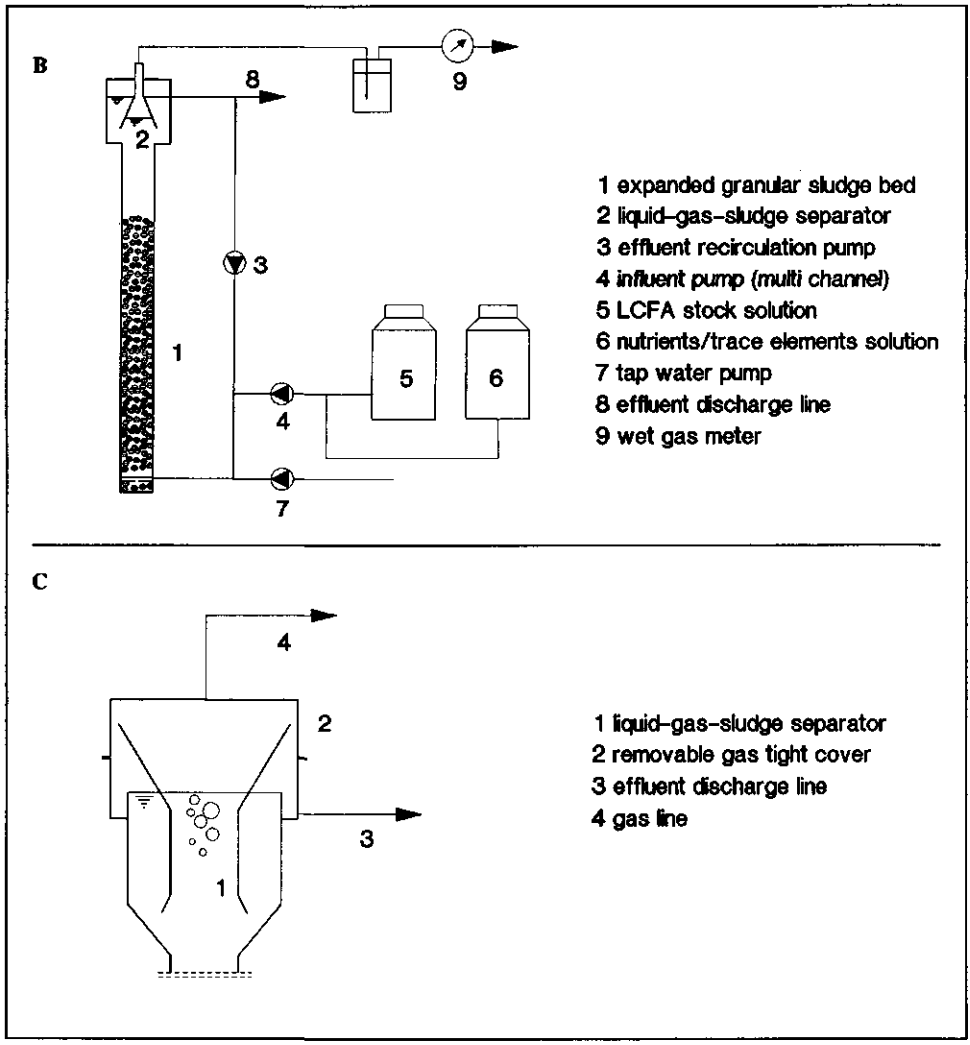

Figure 1b. *Schematic representation of the set-up used for digestion of capric and lauric acid* (experiments 2-4). The EGSB reactor used in experiment 2 is shown. **Figure 1c.** The modified gas-liquid-solids separator used in experiments 3 and 4.

participated in the digestion of capric acid, and plugging of the three inlet pipes caused local overloading of the biomass. The accumulation of capric acid resulted in the formation of precipitates, which were retained by the sludge bed (filtering action) and degraded very slowly as a result of mass transfer limitations.

Figure 2. Lump of capric acid (salt) precipitates (whitish) in the granular sludge bed (black) of the UASB reactor (experiment 1)

Experiment 2: EGSB reactor - capric acid

Considering the fact that the large number of inlet points per unit surface area, applied in the lab scale UASB reactor was completely unrealistic for full scale application, we decided to use a modified upflow reactor (higher H/D) with strong effluent recycling (Figure 1). At the applied superficial liquid velocity (7.2-7.7 $m.hr^{-1}$), the complete sludge bed was expanded or fluidized and thus any dead space was eliminated. As a result of the high recirculation factor (96-14) the liquid phase can be considered as a completely stirred tank reactor (CSTR).

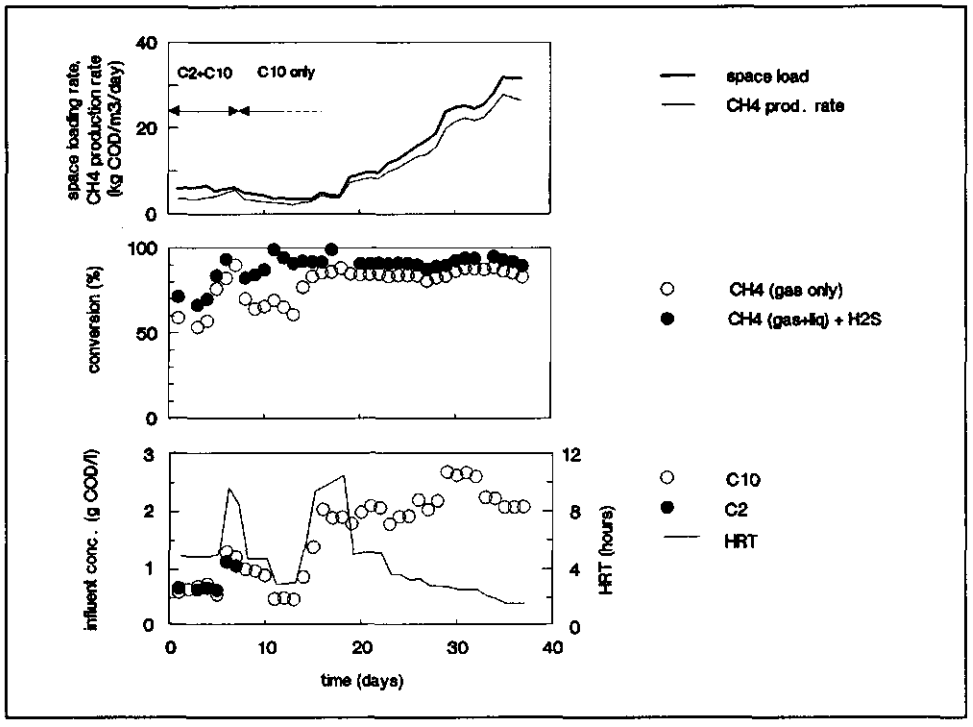

Figure 3. Results obtained with anaerobic digestion of capric acid in an EGSB reactor (experiment 2)

Figure 3 shows the treatment results achieved during digestion of a capric acid solution in the EGSB reactor. Initially, the reactor was fed with a mixture of capric and acetic acid (COD 1:1), in order to guarantee anaerobic conditions during the adaptation of the inoculum to capric acid. After 7 days, no capric, caprylic or caproic acid could be detected in the effluent, and the methane production indicated complete degradation of capric acid. Therefore acetate was omitted from the medium from day 7 onwards.

As shown in Figure 3, the space loading rate could be increased to 31.5 kg $COD.m^{-3}.day^{-1}$ within 35 days (sludge loading rate ca. 1 kg $COD.kg^{-1}$ $VSS.day^{-1}$). Between days 7 and 37, on average 91% of the COD added was recovered as methane and sulfide (σ 4%, n 27, including methane dissolved in the effluent and organic matter mineralized by sulfate reducing bacteria). The effluent COD remained below 50 $mg.l^{-1}$; except for acetic acid, fatty acids with up to ten carbon atoms remained below the detection limit. During the entire experiment, neither any foaming, nor the formation of a layer of floating lipids or sludge was observed.

The experiment clearly indicates that complete degradation of LCFA can be achieved at very high loading rates, in an anaerobic digester with completely mixed liquid phase and adequate contact between substrate and biomass. The capacity of the EGSB reactor used in this experiment was limited by the efficiency of the sludge separator, not by the biological capacity. At methane production rates above ca. 28 kg $COD.m^{-3}.day^{-1}$ (superficial gas velocity 0.95 $m.hr^{-1}$), the turbulence in the settling compartment immediately under the gas separator caused wash-out of granular sludge.

At methane production rates below 28 kg $COD.m^{-3}.day^{-1}$, no sludge wash-out occurred. From the measured increase in the quantity of granular sludge in the reactor after 37 days of continuous operation (from 83 g to 100 g VSS), an average net yield factor of ca. 0.01 kg $VSS.kg^{-1}$ COD-mineralized can be calculated. This value is lower than the yield coefficient of

approximately 0.04 kg VSS.kg^{-1} COD-mineralized determined by Novak and Carlson (1970). Taking the poor reliability of biomass determinations into account, however, the difference is probably not significant.

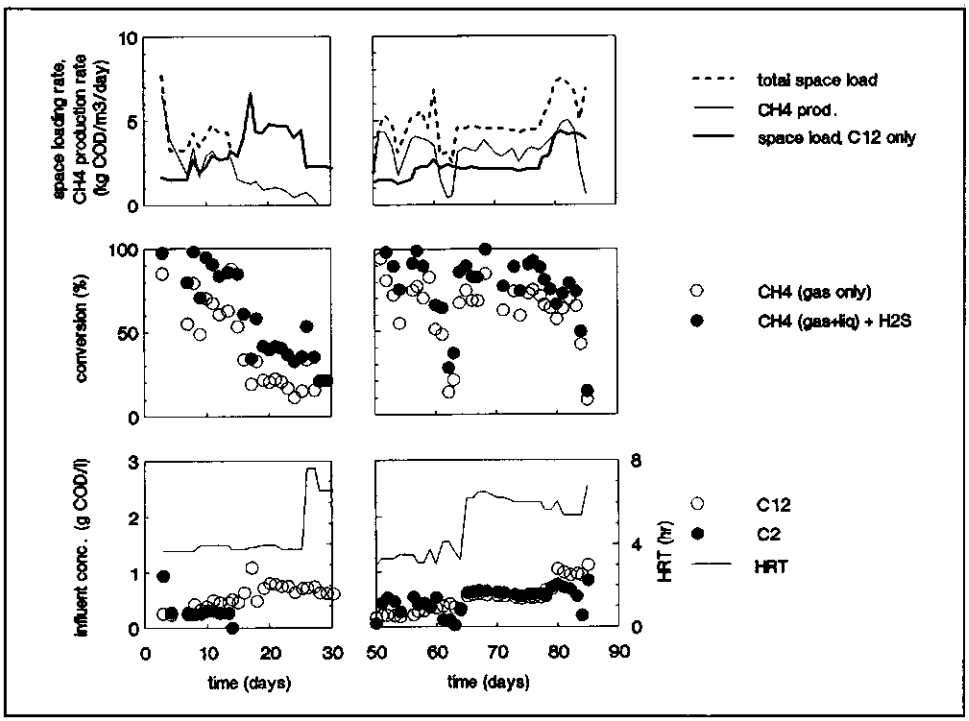

Figure 4. Results obtained with anaerobic digestion of mixtures of capric and acetic acid in a UASB reactor (experiment 3; days 1-15: C_{12}:C_2 0.65; days 50-86 C_{12}:C_2 1.12; ratios on COD basis)

Experiments with Lauric Acid

Experiment 3: UASB reactor - mixtures of lauric and acetic acid

In practice, a waste stream will seldom contain lipids as the sole organic contaminant. Usually, more easily degradable contaminants are also present. Anaerobic degradation of the latter compounds will cause gas production, and hence a certain degree of mixing of the digester contents. This may prevent the difficulties caused by insufficient mixing, that occurred during anaerobic digestion of capric acid in a UASB reactor. Therefore, the digestion of mixtures of lauric and acetic acid in a UASB reactor was investigated.

Figure 4 shows the results obtained with a mixture of lauric and acetic acid, with an average concentration ratio of 0.65 kg C_2-COD.kg^{-1} C_{12}-COD (σ 0.09, n 6, COD basis). Despite the complete degradation of acetic acid, the first lauric acid precipitates were already observed in the bottom of the sludge bed on day 7. On day 14 the addition of acetic acid was stopped, which resulted in a gradual decrease of the methane production rate to zero, accompanied by an increase of the amount of precipitates. This led to the formation of a floating layer containing precipitates and flocculent sludge. On day 20, the granular sludge suddenly agglomerated and part of these agglomerates started to float. However, the modified separator construction (Figure 1c) prevented wash-out of this floating sludge.

Between days 28 and 50, the UASB reactor was fed with acetate as sole substrate, which resulted in the complete disappearance of all accumulated lauric acid precipitates (results not shown).

From day 50 onwards, a second attempt was made to digest a mixture of lauric and acetic acid, this time with a higher C_2:C_{12}-ratio (average 1.12 kg C_2-COD.kg^{-1} C_{12}-COD, σ 0.57, n 32) and a higher acetic acid loading rate. The methane production efficiency was rather poor and varied sharply as a result of periodic sludge flotation (Figure 4). A doubling of the lauric acid concentration in the influent on day 80, resulted in very strong sludge wash-out. Between days 80 and 86 approximately 70% of the granular sludge was washed out of the reactor, and therefore the experiment was stopped.

From the results obtained with anaerobic digestion of capric and lauric acid in UASB reactors, we can conclude that a UASB system treating wastes with a high lipid content (ca. 0.5-1.5 kg COD.m^{-3}) can only accommodate space loads up to ca. 4-5 kg COD.m^{-3}.day^{-1}, even when operated on mixtures of lipids and easily degradable material. The reliability of the system is low, as a result of unpredictable sludge flotation.

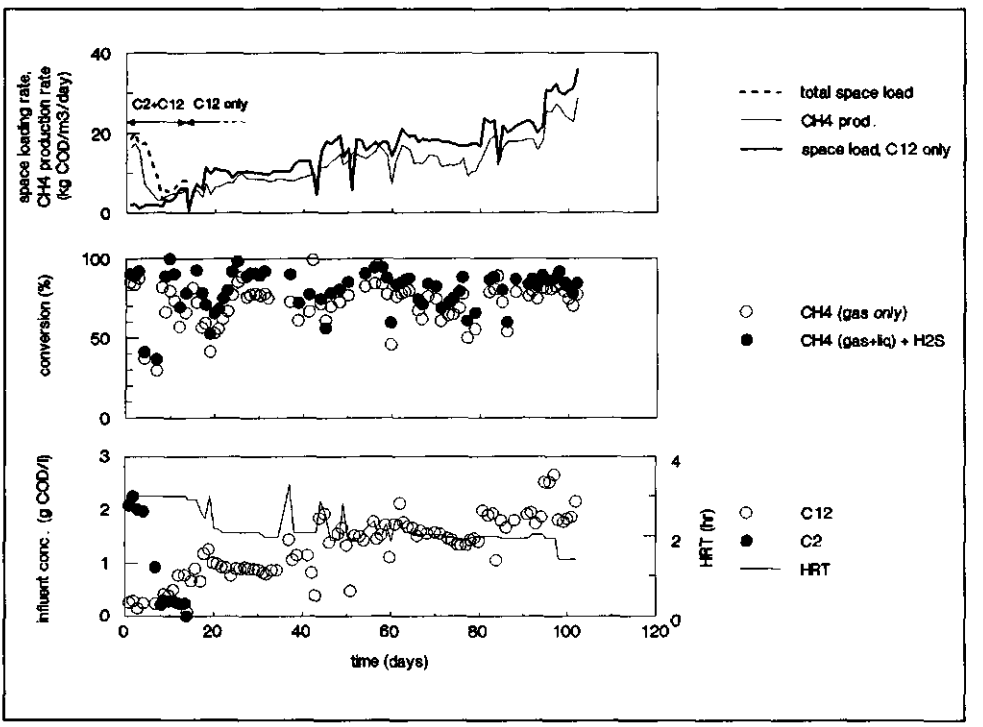

Figure 5. Results obtained with anaerobic digestion of lauric acid in an EGSB reactor (experiment 4)

Experiment 4: EGSB reactor - lauric acid

Figure 5 shows the results obtained in an EGSB reactor with an improved sludge separator, using lauric acid as sole substrate. After 105 days of operation, a space loading rate of 31.4 kg COD.m^{-3}.day^{-1} was reached (sludge loading rate 1 kg COD.kg^{-1} VSS.day^{-1}). Between days 25 and 102, on average 83% of the COD added was recovered as methane and sulfide (σ 14%, n 59, including methane dissolved in the effluent and organic matter mineralized by sulfate reducing bacteria). Figure 6 shows that the conversion efficiency was not significantly influenced by the

loading rate. COD analyses of the effluent indicated that the actual mineralization efficiency was higher than the recovery of methane and sulfide. This can be attributed to flotation of lipids and biomass production. COD analyses of the floating layers gave unreliable results, due to poor reproducibility of the sampling procedure.

Compared to capric acid, lauric acid is mineralized less efficiently. This is in accordance with the fact that heavy lipid flotation occurred during the entire experiment. After each increase in the loading rate, a transient increase of the rate of floating layer formation was observed. The floating layer had to removed intermittently, to avoid contamination of the effluent with floating material.

Wash-out of biomass in the effluent was insignificant, and only small quantities of biomass were removed with the floating layers. From the increase in the quantity of granular sludge in the reactor after 91 days of operation (from 53 g to 94 g VSS), an average net yield factor of ca. 0.01 kg VSS.kg^{-1} COD-mineralized can be calculated.

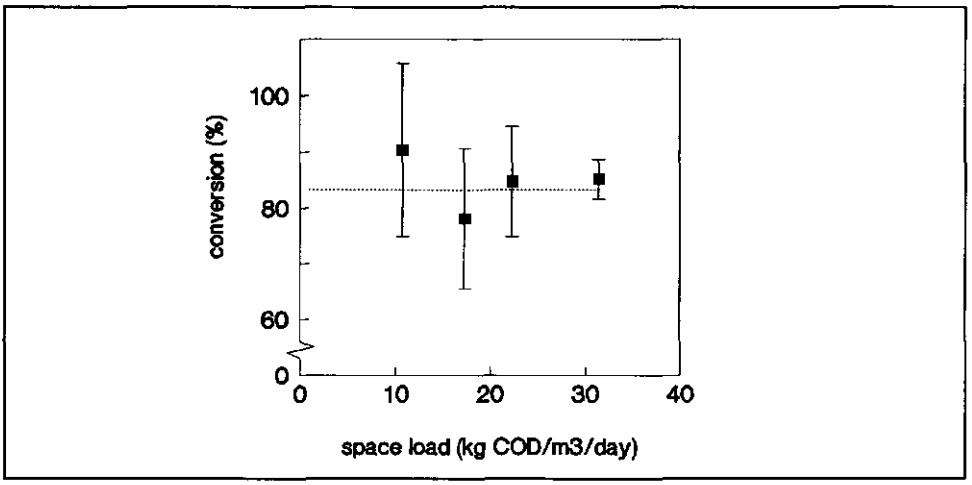

Figure 6. Conversion efficiency of lauric acid in an EGSB reactor as a function of the space loading rate (experiment 4; ■average; ⊢⊣standard deviation; ... average over entire period)

Comparison of UASB and EGSB Performance

From the results presented above, the conclusion can be drawn that complete mixing of the liquid in the digester, and efficient contact between substrate and all biomass are absolutely required for high-rate anaerobic digestion of LCFA. If one of these conditions is not met, local overloading will occur, even at moderate or low overall organic loading rates. This causes local accumulation of LCFA. As the calcium and sodium salts of these acids have a very limited solubility at neutral pH, the accumulation inevitably results in precipitation, a sharp drop in the conversion rate of the LCFA due to physical limitations, heavy sludge flotation, and ultimately heavy sludge wash-out.

Conventional UASB reactors cannot fulfill these requirements, and therefore cannot accommodate lipid loading rates exceeding ca. 4-5 kg COD.m^{-3}.day^{-1}, despite the high concentration of highly active, well settling biomass.

The precipitation problems observed during our UASB experiments are similar to those encountered by Sayed *et al* (1987) during anaerobic treatment of slaughterhouse wastewater in granular sludge UASB reactors. Sayed *et al* (1987) found a large difference between the methane production efficiency and the COD removal efficiency when the space loading rate in their UASB reactors was increased above a certain level, which could be attributed primarily to retention of fine suspended material (defined as colloidal). In our UASB experiments we observed an

accumulation of precipitated LCFA (salts) in the void space between the granules, as well as the formation of agglomerates of granular sludge and fatty acid precipitates. In adsorption experiments with inactive granular sludge, we demonstrated that LCFA salts accumulate on the granules in a solid-like layer (Keurentjes and Rinzema 1986). The encapsulation of granules in precipitated LCFA may even cause limitations in the transport of soluble substrates to the biomass, and consequently a decrease of the conversion capacity for these substrates, as is also indicated by the work of Sayed *et al* (1988) and Särner (1981).

However, EGSB reactors with very strong effluent recirculation do fulfill the requirements of mixing and contact between substrate and biomass. With capric and lauric acid as sole substrate, space loading rates up to ca. 30 kg $COD.m^{-3}.day^{-1}$ were achieved, at conversion efficiencies exceeding 91% and 83% respectively. The EGSB reactors could be operated at hydraulic residence times of 2 hours without any problems, which indicates very efficient biomass retention. The experiments were conducted with rather dilute LCFA solutions (< 3 kg $COD.m^{-3}$), but taking the good mixing characteristics resulting from the high recirculation ratio into account, we expect that the EGSB reactor can also handle more concentrated wastes at comparable loading rates. The results obtained with the EGSB reactors compare very favorably with those published for more conventional digesters (Kunst *et al* 1985). Although net biomass yields on LCFA are rather low, our results show that augmentation of granular sludge can be achieved. The new biomass has excellent settling properties.

Heavy lipid flotation occurs during anaerobic digestion of LCFA with more than ten carbon atoms. This can be attributed to the decrease in water solubility (Bell 1974), and the increase in surface activity with increasing chain length of the LCFA. The biogas produced by degradation of part of the lipids will cause flotation of another part. The production rate of the floating lipid layer will depend upon several factors, which cannot yet be quantified. Flotation of lipids contributes to water purification, and may therefore be beneficial, especially when the floating material is poorly biodegradable (Spies 1985). However, flotation clearly does not solve the waste problem. Therefore two alternative solutions deserve further attention for wastewater contaminated with readily biodegradable lipids: (a) a modification of the EGSB system allowing recycling of the floating lipids to the sludge bed, and (b) anaerobic digestion of the floating layer in a separate completely mixed anaerobic reactor. In the latter case, the high COD of the floating layer would even allow operation at thermophilic temperatures.

The application of the EGSB reactor on a larger scale still has to be demonstrated. On a lab scale, concentration gradients could easily be avoided by applying a very high effluent recirculation ratio. However, the applied recirculation ratios would cause inadmissibly high superficial liquid velocities in full scale reactors. The energy required for recirculation of large amounts of water may also limit the achievable recirculation ratio. Therefore, recirculation ratios in full scale reactors will have to be considerably lower than the values applied in our lab scale experiments (Table 1). There are some indications in the literature, that axial dispersion in three-phase fluidized beds increases strongly at increasing reactor diameter (Gommers *et al* 1986), which might compensate for the increase in the mixing time at lower recirculation ratios. Further research is clearly required, however, to determine the consequences of lower recirculation ratios and higher reactor diameters.

If insufficient mixing can be achieved in larger EGSB reactors, an anaerobic gas lift reactor may be considered as an alternative. Recirculation times in the order of magnitude of 10 seconds can be achieved in this type of reactor (Schoutens *et al* 1986), which is much shorter than the applicable hydraulic residence time and the time constant of the biological reaction. This means that the fluid phase can be regarded as completely mixed. The initial formation of aggregates of sucrose acidifying biomass in anaerobic gas lift reactors requires a carrier material (Beeftink and Van den Heuvel 1987, Beeftink *et al* 1988). However, once the first aggregates have been formed, the carrier material is no longer required to achieve augmentation of the aggregates (Beeftink and Staugaard 1986). This is confirmed by the results achieved with a pilot scale Internal Circulation reactor - a system that is analogous to a gas lift reactor (Hack *et al* 1988).

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CHAPTER 5

Anaerobic Digestion of Triglyceride Emulsions in Expanded Granular Sludge Bed Upflow Reactors with Modified Sludge Separators

(submitted for publication)

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Anaerobic Digestion of Triglyceride Emulsions in Expanded Granular Sludge Bed Upflow Reactors with Modified Sludge Separators

Arjen Rinzema, Herbert van Veen and Gatze Lettinga
Dept. of Water Pollution Control, Wageningen Agricultural University, Bomenweg 2, 6703 HD Wageningen, The Netherlands

ABSTRACT

Modification of the gas-solids separator of expanded granular sludge bed reactors is necessary, to prevent excessive sludge wash-out during anaerobic treatment of lipid emulsions. This paper describes two modifications: a hybrid reactor with a layer of floating carrier material (reticulated polyurethane foam) above the granular sludge bed, and a novel upflow reactor equipped with a sieve-drum separator.

Replacement of the conventional sludge separator by a layer of floating polyurethane foam, does not prevent sludge wash-out. Therefore, the hybrid reactor is unreliable in treatment of emulsified lipids.

An EGSB reactor equipped with a sieve-drum separator allows stable anaerobic digestion of emulsified lipids. An increase of the amount of granular sludge retained in the reactor can be achieved, provided that sludge granules with a sufficiently large diameter are used for inoculation. Especially if easily degradable organic contaminants are present in the wastewater besides the lipids, the maintenance of a sufficiently large inventory of granular sludge is no problem. The EGSB reactor with sieve-drum separator can accommodate higher organic and hydraulic space loading rates than previously described anaerobic filter reactors. The relatively poor conversion of organic material removed from the wastewater to methane, as a result of the flotation of lipids will require further research.

INTRODUCTION

Over 100 high-rate anaerobic digesters have been installed or commissioned for treatment of industrial wastewater (Lettinga *et al* 1984, Nyns 1985, Pohland 1985, De Zeeuw 1988). The majority of these reactors is of the Upflow Anaerobic Sludge Bed (UASB) reactor type (Lettinga *et al* 1984, De Zeeuw 1988). However, the application of anaerobic digestion is still largely limited to the treatment of wastewater containing mainly carbohydrates (Lettinga *et al* 1984, De Zeeuw 1988). The number of full scale applications to wastewater containing lipids or proteins is very limited (Samson *et al* 1985, Van Campen *et al* 1986, De Man and Piscaer 1986, De Zeeuw 1988), mainly because:

- there is a lack of experience with the application of anaerobic treatment systems to these types of wastewater,
- problems with sludge retention have been encountered during lab scale and pilot scale studies and full-scale applications, viz. the occurrence of sludge flotation and wash-out (De Zeeuw 1982, Liemburg and Van der Wal 1982, Sayed *et al* 1984, Samson *et al* 1985, Van Campen *et al* 1986),
- long-chain fatty acids may cause severe inhibition of methanogenic and acetogenic bacteria (Prins *et al* 1972, Chou *et al* 1978, Hanaki *et al* 1981, Koster and Cramer 1987, Rinzema, A., submitted), which is especially threatening in systems operated at a low hydraulic retention time.

Control of sludge wash-out and long chain fatty acid inhibition is a prerequisite for increased application of anaerobic digestion for the treatment of lipid containing wastewater. This requires a proper choice of reactor type. An evaluation of the currently existing high-rate reactor types

shows that a choice has to be made between: (1) reactors with mobile biomass aggregates, and (2) reactors with stationary biofilms (Rinzema 1988). This essentially boils down to a choice between: (1) the high biomass concentration that can be achieved in systems from category 1, and (2) the inherent safety against biomass wash-out of the systems in category 2. The difference in maximum sludge concentration between categories 1 and 2 is at least a factor 2. It should be emphasized that the risk of inhibition by long chain fatty acids is directly related to the overcapacity of the system, as these inhibitors are biodegradable (McCarty 1964). Furthermore, the relatively high costs associated with the support material in systems from category 2, and the lack of full scale experience with these systems are distinct disadvantages. The latter is especially important, regarding the relatively high risk of plugging and channeling associated with the use of stationary support materials.

Experimental data on anaerobic digestion of well defined lipid containing wastes are very scarce. Novak and Carlson (1970) investigated the kinetics of anaerobic digestion of long chain fatty acids in completely stirred tank reactors. Kunst *et al* (1985) investigated the mesophilic anaerobic digestion of soy bean oil emulsions in upflow anaerobic filter reactors. In a one-stage reactor for combined lipolysis, acetogenesis and methanogenesis, they achieved COD reduction efficiencies of ca. 82% at space loading rates up to 5 kg $COD.m^{-3}.day^{-1}$ (hydraulic retention time ca. 1 day). In a two-stage system, acetogenesis in the first stage stagnated when the space loading rate exceeded 7.5 kg $COD.m^{-3}.day^{-1}$. Backman *et al* (1985) investigated the performance of upflow anaerobic filter reactors in the mesophilic anaerobic treatment of diluted whole milk, with approximately 235-710 mg $fat.l^{-1}$. They found 61% COD removal at a loading rate of 7.6 kg $COD.m^{-3}.day^{-1}$ (hydraulic retention time 7.4 hours); 75% of the removed COD was recovered as methane. A tracer study revealed severe short circuiting and dead space in the random packed pall ring bed. The loading rates reported by Kunst *et al* (1985) and Backman *et al* (1985) are rather moderate for high-rate mesophilic anaerobic digesters.

We have reported previously that expanded granular sludge bed (EGSB) reactors can achieve COD removal efficiencies of 85-90% during treatment of long chain fatty acid solutions at space loading rates up to ca. 30 kg $COD.m^{-3}.day^{-1}$ (Rinzema *et al* submitted). The EGSB reactor is an expanded bed reactor inoculated with granular sludge from existing full scale UASB reactors; no carrier material is used in the EGSB system. Our studies show that strong effluent recirculation and sludge bed expansion are essential for high loading rates during digestion of lipids. Conventional UASB reactors could not accommodate loading rates in excess of ca. 5 kg $COD.m^{-3}.day^{-1}$.

Hydrolysis is usually not the rate-limiting step in anaerobic digestion of lipids (Heukelekian and Mueller 1958, O'Rourke 1968, Hanaki *et al* 1981, Rinzema, A. unpublished results). Therefore, we expected that the results obtained with long chain fatty acids could easily be extended to emulsified triglycerides. However, preliminary experiments indicated that triglyceride emulsions severely impaired the stability of the EGSB reactors. Strong wash-out of granular sludge occurred, even at very low organic space loading rates (1-2 kg $COD.m^{-3}.day^{-1}$, lipids as only substrate). Even aggregates of several granules showed very poor settling properties. Visual observations indicated that the problems were caused by gas bubbles adhering to the granules. These results were unexpected, because sludge flotation and wash-out occurred only after serious overloading during treatment of long chain fatty acid solutions.

Therefore, a modification of the gas-solids separator is required in upflow reactors treating emulsified lipids, in order to maintain the sludge retention at an acceptable level. Two modifications are described in this paper: (1) a hybrid expanded bed reactor with a layer of floating carrier material (reticulated polyurethane foam) above the granular sludge bed, and (2) an expanded bed reactor equipped with a sieve-drum separator. Several publications (Guiot and Van den Berg 1984, Guiot *et al* 1986, Oh and Yang 1985, Reynolds and Colleran 1986) suggest that the hybrid design can provide excellent sludge retention. The application of a sieve-drum separator has not been described previously, although the system used for this study shows some resemblance to a design suggested by Lettinga *et al* (1983).

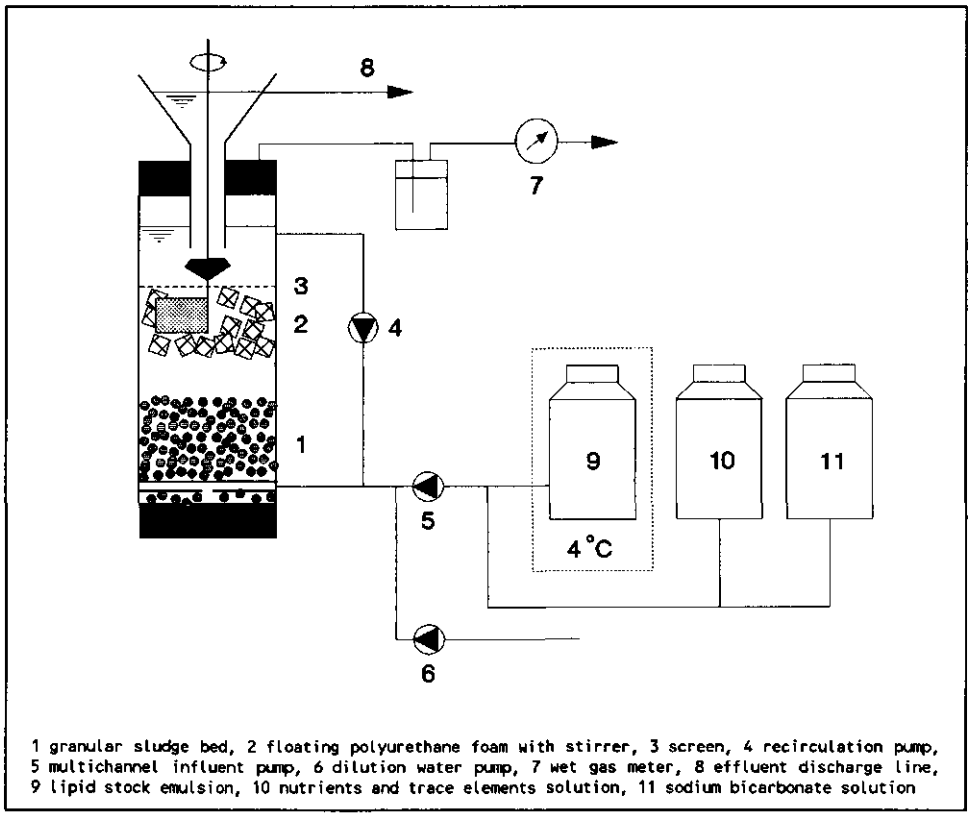

Figure 1. Schematic representation of the hybrid EGSB reactor

MATERIALS AND METHODS

Hybrid EGSB reactor

Reactor. The reactor consisted of a perspex tube (internal diameter 92 mm, height 720 mm, liquid volume 4.75 liters) closed with a butyl rubber stopper at both ends (Figure 1). A layer of floating reticulated polyurethane (PUR) foam cubes above the granular sludge bed acted as a filter (Recticel, T40, edge 5 mm, packed volume ca. 0.1 liter). The PUR foam was kept in place by a perforated steel plate (1 mm holes), and was intermittently mixed (30 seconds every 5 minutes, 60 rpm). The influent flow rate was 10 $l.day^{-1}$ during the entire experiment, corresponding to a hydraulic retention time of 11.4 hours. To ensure adequate mixing and sludge bed expansion, an effluent recirculation flow rate of 168 $l.day^{-1}$ was applied, resulting in a superficial liquid velocity of 1.12 $m.hr^{-1}$. The suction line of the recirculation pump was mounted directly below the gas-liquid interface. Gas tight butyl rubber tubing was used for the recirculation line, to avoid losses of methane and inhibition by oxygen.

Biomass. The reactor was inoculated with 3 liters (180 g VSS) of granular sludge from a full scale UASB reactor treating sugar beet refinery wastewater (Suiker Unie, Roosendaal, The Netherlands); ca. 33% of the inoculum had been adapted to triglycerides (triolein) in a previous experiment.

the packed zone, and the return of sludge granules to the sludge bed was severely hindered. After 34 days of operation, 35% of the inoculum had been washed out of the reactor. Although the loss of biomass did not yet affect the treatment efficiency, we terminated the experiment at this point. The hybrid system clearly does not give sufficient improvement over the conventional EGSB system described earlier (Rinzema *et al* submitted), and cannot be regarded as a suitable treatment system for lipid containing wastes. The layer of packing material did not prevent sludge wash-out, but instead it aggravated this undesirable phenomenon, and thus reduced the stability of the reactor to an unacceptable level.

Possibly, the choice of another kind of packing material might give better performance of a hybrid reactor. However, the results of Backman *et al* (1985) indicate that pall rings also do not give satisfactory sludge retention during treatment of wastewater with a high lipid content. Therefore, we decided to choose a completely different approach, viz. a system equipped with a vertical sieve-drum as sludge-water separator.

Performance of the EGSB reactor with sieve-drum separator

The EGSB reactor equipped with a sieve-drum separator was investigated with three influent media: (1) diluted cream, (2) diluted cream with calcium chloride, and (3) a mixture of diluted cream and acetate. Calcium was added in experiment 2, because it can prevent inhibition of methanogenic and acetogenic bacteria by long chain fatty acids (Hanaki *et al* 1981, Koster 1987), and increase the rate of lipolysis (Rinzema, A. unpublished results). Acetate was added in experiment 3, as a substitute for rapidly degradable organic contaminants, which will usually accompany lipids in wastewater. It can be expected that the biogas production from these rapidly degradable compounds will affect the performance of the anaerobic reactor, e.g. by increasing the amount of lipids and biomass accumulating in a floating layer, and by altering the mixing characteristics as well.

A brushing system was included in the sieve-drum system, in order to prevent plugging of the perforated cylinder (Figure 2). Our experiments indicate that intermittant brushing is indeed required, as a film of lipid material was rapidly deposited on the sieve-drum, which caused total plugging within several hours of operation. The scum breaker connected to the brushing device also proved to be valuable, because it diminished the inclusion of sludge granules in a layer of floating lipids, as will be outlined below.

Treatment results

Table 1 summarizes the average operating conditions and treatment results in the three EGSB reactors. Figure 3 shows the COD removal efficiency as a function of the space loading rate. At a space loading rate of 7-8 kg $COD.m^{-3}.day^{-1}$, a COD removal efficiency of 80-85% could be achieved in all three reactors. The space loading rates and the COD removal efficiencies indicated in Table 1 and Figure 3 are based on the cream only, i.e. essentially on the lipids. Consequently the loading rate applied to reactor 3, and the COD removal efficiency achieved in this reactor were considerably higher than indicated in Table 1. Reactor 3 accommodated a total space loading rate up to 40 kg $COD.m^{-3}.day^{-1}$ (cream plus acetate), with a COD removal efficiency in excess of 85%.

At a space loading rate of 13-16 kg $COD.m^{-3}.day^{-1}$, the lipid removal efficiency in reactor 2 - which received an influent with increased calcium concentration - was clearly superior to that in the other two reactors.

Compared to the anaerobic filter reactors investigated by Kunst *et al* (1985) and Backman *et al* (1985), the EGSB reactor with sieve-drum separator can accommodate higher organic and hydraulic space loading rates. It should be noted, however, that the conversion of removed organic material to methane was relatively low (50-60%) in the EGSB reactor. Both the efficiency of methanogenesis and the COD removal efficiency are considerably lower than the values we have achieved previously with long chain fatty acid solutions (Rinzema *et al* submitted). It is very unlikely that this difference must be attributed to poor hydrolysis of the esters. Unpublished results of batch experiments conducted by the present authors, as well published results (Heukelekian and Mueller 1958, O'Rourke 1968, Hanaki *et al* 1981) indicate that anaerobic oxidation of long chain fatty acids is the rate limiting step in lipid digestion. The relatively poor results obtained with triglyceride emulsions may be the result of the high percentage of fatty acids with 16 and 18 carbon atoms in the milk fat. The sodium salts of these acids are less soluble than those of capric and lauric acid, which were used in our previous study. The poor solubility of the long chain fatty acids from the milk fat will presumably limit the mass transfer to the biomass

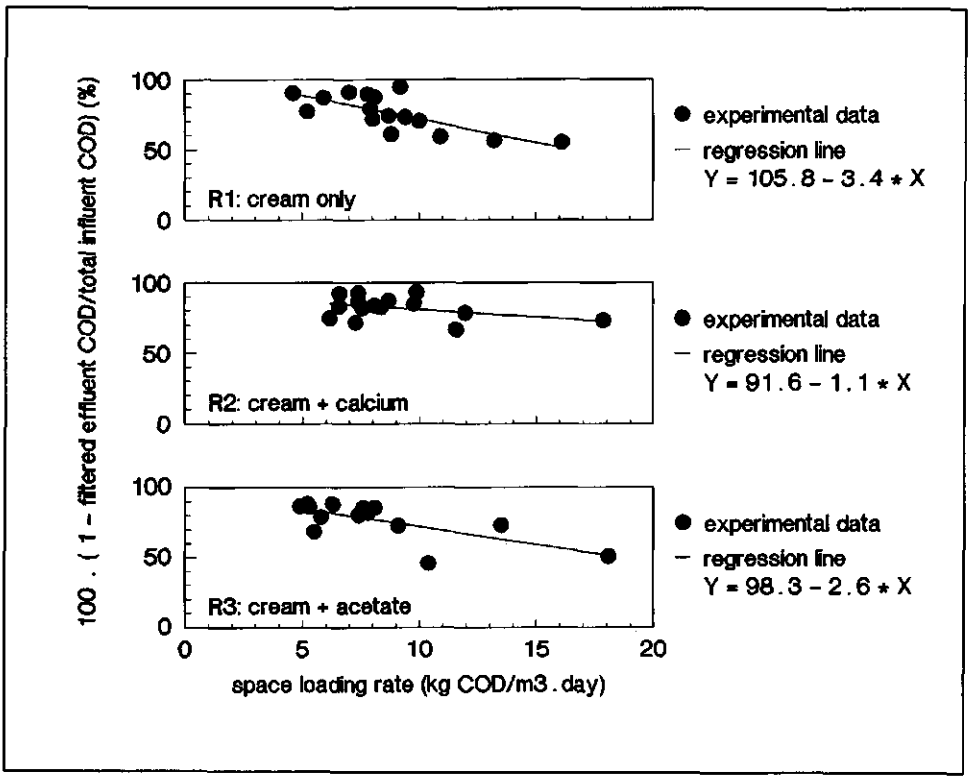

Figure 3. COD removal efficiency as a function of the space loading rate in EGSB reactors equipped with sieve-drum separators. (Note: for reactor 3 the efficiency and space loading rate are based on the cream only, c.f. Table 1).

granules, and stimulate the flotation of lipids. This is in accordance with the exceptionally strong floating layer production, which will discussed below.

Sludge retention

Table 2 summarizes the sludge concentrations in the three reactors, at the beginning and the end of the experiment. In all three reactors, a small amount of sludge was lost due to the removal of floating layers, and occasional accidents. Table 2 indicates that only in reactor 3, an increase of the amount of sludge was achieved. Regarding the higher amount of organic material (cream plus acetate) converted in this reactor, obviously a larger biomass production can be expected.

The amount of sludge washed out of reactors 1 and 2 corresponds closely to the amount of small granules (Suiker Unie) added as inoculum. Wash-out of these granules was to be expected, regarding their diameter. Therefore, we do not consider the loss of biomass observed in reactors 1 and 2 as a sign of serious malfunctioning of the sieve-drum separator. Furthermore, in practice a higher biomass production may be expected, as a result of the digestion of more easily degradable contaminants that are usually present in lipid containing wastewater. Therefore, it is justified to conclude that the EGSB reactor with a sieve-drum separator has sufficient potential for treatment of lipid containing wastes to warrant further research, provided that it is inoculated with sufficiently large granules.

Table 1. Process conditions and treatment results in EGSB reactors equipped with sieve-drum separators

reactor[a]	HRT[b]	v_{ls}[b]	v_{gs}[b]	L_v[b,c]	η_{CH4}[b,d]	η_{COD}[b,e]
	(hr)	($m.hr^{-1}$)		($kg\ COD.m^{-3}.d^{-1}$)	(%)	(%)
day 7-54						
1	5.9	3.45	0.11	8.2	43	80 (10,13)
2	6.5	3.13	0.11	7.4	49	84 (7,12)
3	6.3	3.23	0.58	7.6[f]	43[f]	81 (8,13)[f]
day 55-64						
1	3.2	6.36	0.15	15.0	32	57 (2,3)
2	3.1	6.56	0.17	15.7	35	72 (5,3)
3	3.6	5.65	1.01	13.5[f]	39[f]	57 (12,3)[f]

a reactor 1 diluted cream; reactor 2 diluted cream + calcium chloride; reactor 3 diluted cream + acetate

b HRT hydraulic retention time, v_{ls} superficial liquid velocity, v_{gs} superficial gas velocity, L_v space loading rate, η_{CH4} conversion efficiency added COD to methane, η_{COD} COD reduction efficiency

c $\Sigma(L_v.\Delta t)/\Sigma(\Delta t)$

d $\Sigma((r_{CH4}/L_v).\Delta t)/\Sigma(\Delta t)$

e $\Sigma(\eta)/n$ (between brackets the standard deviation and the number of determinations)

f for reactor 3, the efficiencies and the space loading rate are based on the cream only, assuming a methane production of 1 mole CH_4 per mole acetate, and neglecting biomass production

Table 2. Quantities of total suspended solids (TSS) and volatile suspended solids (VSS) in the EGSB reactors equipped with sieve-drum separators, at the start and the end of the experiment

reactor[a]	beginning (day 0)			end (day 65)				
	TSS (g)	VSS (g)	VSS/TSS (%)	TSS (g)	(%)[b]	VSS (g)	(%)[b]	VSS/TSS (%)
1	617	480	78	464	75	380	79	82
2	617	480	78	864	140	370	77	43
3	617	480	78	671	109	560	117	84

a reactor 1 diluted cream; reactor 2 diluted cream + calcium chloride; reactor 3 diluted cream + acetate

b % of the amount on day 0

Floating layer formation

The organic material removed from the water phase, was only partially converted to methane (Table 1). This must be attributed to the fact that a large, and rather variable amount of lipids accumulated in a thick floating layer. The formation of a floating layer is in accordance with the results we have obtained previously in EGSB experiments with long chain fatty acids (Rinzema *et al* submitted). Contrary to the previously described hybrid reactor system, the EGSB reactors with sieve-drum separators had no provisions for recirculation of the floating layer to the sludge bed. The floating lipids were degraded only very slowly, because there was virtually no contact with the biomass in the granular sludge bed. In all three reactors periodic removal of the floating material was necessary, because insufficient head-space was available to store the lipid layer.

Practically no inclusion of granular sludge in the floating layer was observed. Some floating granules accumulated beneath the lipid layer, but contrary to the lipids, these granules settled rapidly after the intermittent operation of the scum breaker. Clearly, the use of a scum breaker is essential in the EGSB reactor used in the present study.

Although flotation of lipids contributes to water purification, it clearly does not solve the waste problem. The formation of the floating layer requires a change of the design of the EGSB system, compared to that used in the present study. The results obtained with the hybrid design discussed above, indicate that the suspended lipids can be degraded efficiently by granular anaerobic sludge, provided that sufficient contact is achieved. Therefore, recycling of the floating lipids to the sludge bed may be a solution for the waste problem. The technological possibilities for this approach have been discussed in our previous publication (Rinzema *et al* submitted). An alternative solution is automatic removal of the floating lipids from the EGSB reactor, and anaerobic digestion of this material in a separate completely mixed anaerobic reactor. Our results indicate that this can be achieved without unacceptable loss of biomass granules, although the current design of the EGSB reactor with sieve-drum separator does not allow a complete separation between floating lipids and (temporarily) floating sludge granules. Further investigation of the formation rate and digestion kinetics of the floating layer, as well as the technological solutions mentioned above, is warranted.

Effect of calcium addition

Calcium addition improved the COD removal efficiency at higher space loading rates (Table 1), but it also caused a strong increase of the ash content of the granular sludge (Table 2). The increased ash content was also evident from the appearance of a whitish precipitate on the granules after day 50. In the long run, this may lead to a decrease in the capacity of the reactor, because the accumulating calcium precipitates expel biomass and scaling of the granules may cause transport limitations (Lettinga *et al* 1987). Consequently, further optimization of the calcium addition is required.

CONCLUSIONS

Emulsified triglycerides cause very severe flotation and wash-out of granular anaerobic sludge in expanded bed reactors, even when only a very low organic space loading rate is applied. The performance of the expanded granular sludge bed (EGSB) reactor during treatment of triglyceride emulsions is clearly inferior to the performance during treatment of long chain fatty acid solutions. Therefore, a modified sludge separator is required to guarantee the stability of this reactor system during treatment of lipid containing wastewater.

Replacement of the conventional sludge separator by a layer of floating polyurethane foam cannot prevent sludge wash-out. The hybrid reactor combining an expanded granular sludge bed and a polyurethane foam layer is unreliable in treatment of emulsified lipids. The floating support material does not prevent strong sludge wash-out, in fact it may aggravate the wash-out of floating sludge granules.

An EGSB reactor equipped with a sieve-drum separator allows stable anaerobic digestion of emulsified lipids. An increase of the amount of granular sludge retained in the reactor can be achieved, provided that sludge granules with a sufficiently large diameter are used for inoculation. Especially if easily degradable organic contaminants are present in the wastewater besides the lipids, the maintenance of a sufficiently large inventory of granular sludge is no problem. If the wastewater is contaminated only with lipids, the choice of the inoculum is probably a critical factor.

The following results can be obtained with anaerobic digestion of emulsified lipids (milk fat) in the EGSB reactor equipped with a sieve-drum separator: 80% COD removal (filtered effluent/unfiltered influent) at a space loading rate of 8 kg $COD.m^{-3}.day^{-1}$ (hydraulic retention time 6 hours), 57% COD removal at a space loading rate of 15 kg $COD.m^{-3}.day^{-1}$ (hydraulic retention time 3.2 hours). This drop in efficiency can be prevented by adding calcium chloride to the wastewater. The optimal amount of calcium requires further investigation. Furthermore, the incomplete conversion to methane of the organic material removed from the wastewater should be a point of further investigations. The incomplete mineralization must be attributed to the accumulation of a large, and rather variable amount of lipids in a thick floating layer. Although inclusion of granular sludge in the floating layer can be prevented effectively with a scum breaker, and although flotation of lipids contributes to water purification, further modification of the design of the EGSB system used in the present study is required to solve the floating layer problem. Results obtained with the hybrid reactor design show that recirculation of the floating lipids to the granular sludge bed enhances their conversion to methane.

ACKNOWLEDGEMENTS

We want to thank Toon Helmink for analytical assistance. This work was supported by the Dutch Government, Clean Technology Program grant no. (F.41)LH511, and the Dutch Seed Crushers and Oil Processors Association (Vernof). Granular sludge was provided by Aviko B.V., Steenderen, The Netherlands, and Suiker Unie N.V., Roosendaal, The Netherlands.

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CHAPTER 6

Sulfide Inhibition of the Methanogenic Activity of Granular Sludge at Various pH-Levels

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erratum
page 89, column 1, line 20: "approx. 100 mg S l^{-1} would be tolerable" should be "approx. 1000 mg S l^{-1} would be *tolerable*"

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SULFIDE INHIBITION OF THE METHANOGENIC ACTIVITY OF GRANULAR SLUDGE AT VARIOUS pH-LEVELS

I. W. KOSTER, A. RINZEMA, A. L. DE VEGT and G. LETTINGA
Wageningen Agricultural University, Department of Water Pollution Control, De Dreijen 12, 6703 BC Wageningen, The Netherlands

(*Received September 1985*)

Abstract—The effect of sulfide on the formation of methane from acetate in granular sludge originating from a UASB reactor has been determined using a new batch anaerobic toxicity assay. The assay is based on measurement of the methane concentration in the closed head space of a serum bottle, thus allowing operation at constant pH and without loss of sulfide via off-gases.

Sulfide toxicity appeared to be correlated with the free hydrogen sulfide concentration in the pH range 6.4–7.2. However this correlation did not hold at pH = 7.8–8.0. Free hydrogen sulfide concentrations leading to 50% inhibition were 250 mgS l^{-1} in the pH range 6.4–7.2 and 90 mgS l^{-1} at pH = 7.8–8.0.

The high tolerance for sulfide toxicity exhibited by the granular sludge can probably be attributed to the existence of a pH gradient in the sludge granules leading to an increased internal pH.

Key words—sulfide, toxicity, inhibition, methanogenesis, anaerobic digestion, Methanothrix, granular sludge, anaerobic toxicity assay

INTRODUCTION

In dark anaerobic environments hydrogen sulfide is the metabolic end-product of the degradation of sulfur containing compounds, either organic or inorganic (Zinder and Brock, 1978; Dunnette *et al.*, 1985). The sulfur content of methanogenic bacteria is unusually high when compared to aerobic microorganisms (Speece, 1983). Sulfide is the major sulfur source of methanogenic bacteria (Zehnder and Wuhrmann, 1977; Zeikus, 1977; Mountfort and Asher, 1979; Scherer and Sahm, 1981; De Zeeuw, 1984). At concentrations exceeding the ppm range in which sulfide stimulates methanogenesis it has an inhibitory effect. When considering that sulfide-inhibition of methanogenesis is one of the major factors that have prevented the commercial application of the anaerobic digestion process to the wastewaters of several major sectors of international industries, sulfide might be ranked as one of the most important inhibitors (Anderson *et al.*, 1982).

Sulfide toxicity is very much dependent on pH, because unionized hydrogen sulfide is able to pass through the cell membrane (Schlegel, 1981; Speece, 1983). The effect of pH on the unionized fraction, of the total amount of sulfide present, is shown in Fig. 1. Below pH = 9 all ionized sulfide will be present as bisulfide (Mosey and Jago, 1977).

Inhibition of methanogenesis by sulfide has been reported several times (Bannink and Muller, 1951; Rudolfs and Amberg, 1952; Aulenbach and Heukelekian, 1955; Butlin *et al.*, 1956, Lawrence *et al.*, 1964; Yang *et al.*, 1979; Kroiss and Plahl-Wabnegg, 1983; Parkin *et al.*, 1983) but lack of precise pH control and variations of sulfide concentration (caused by removal of hydrogen sulfide with the biogas leaving the test reactor) during most of these experiments has prevented a clear interpretation of sulfide toxicity data so far.

In the present article we describe an anaerobic toxicity assay which enables accurate measurement of the specific methanogenic activity (viz. methane production rate per unit biomass) at a constant pH and a constant sulfide concentration. In this assay acetate is used as substrate, because in the anaerobic di-

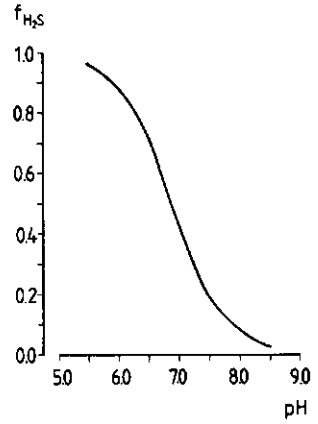

Fig. 1. The fraction fH_2S of the total dissolved sulfide present as free hydrogen sulfide as a function of pH. Calculated using data from Lawrence *et al.* (1964).

gestion of organic material approx. 70% of the methane is produced via acetate (Zehnder, 1978). Usually, industrial wastewaters emenable to sulfide toxicity during anaerobic treatment will contain a lot of sulfate. Although the use of acetate for sulfate reduction is thermodynamically favored over cleavage of acetate to produce methane (Archer, 1983), in anaerobic wastewater treatment processes applied to sulfate-rich wastewaters almost all acetate is used for methanogenesis (Hoeks *et al.*, 1983, 1984; Mulder, 1984; Rinzema and Lettinga, 1986). As source of active methanogenic biomass to be exposed to sulfide we used granular sludge from an Upflow Anaerobic Sludge Bed (UASB) reactor because to date the UASB reactor is by far the most widely applied high-rate anaerobic wastewater treatment system (Van den Berg, 1984; Lettinga *et al.*, 1984). At present granular sludge to be used as seed material for full scale digesters can be obtained on the free market.

MATERIALS AND METHODS

Biomass

The methanogenic mixed culture used in our experiments originated from the UASB reactor of the Aviko potato processing factory at Steenderen, The Netherlands. From this sludge a clean fraction of granules with a diameter in the range 1–2.5 mm was obtained by means of elutriation. The elutriation procedure has been described elsewhere (Tramper *et al.*, 1984). These granules were stored in an acetate-fed batch reactor at 30°C. Apart from the sulfide, the environment in this reactor was similar to the environment in the anaerobic toxicity assay. The predominant acetoclastic methanogenic bacterium in Aviko granular sludge strongly resembles *Methanothrix*, a genus which has such a strong affinity for acetate that it will outcompete all other known acetoclastic methanogens at conditions prevailing in anaerobic high rate wastewater treatment systems (Gujer and Zehnder, 1983). At present two different species have been isolated and characterized (Huser *et al.*, 1982; Patel, 1984).

Chemicals

The basal medium used in the anaerobic toxicity assay contained (mg l^{-1}): $NaHCO_3$ (1000), NH_4Cl (40), KCl (25), $MgCl_2 \cdot 6H_2O$ (11), KH_2PO_4 (9), Na_2SO_4 (9), yeast extract (50). The medium was made up in Wageningen tap water, which contains approx. 35 mg l^{-1} calcium ions. Per litre of medium one ml of a trace element solution according to Zehnder *et al.* (1980) was added. All chemicals were of analytical grade and were supplied by Merck, Darmstadt, F.R.G., except for the gases, which were supplied by Hoekloos, Schiedam, The Netherlands. The yeast extract was supplied by Gist-brocades, Delft, The Netherlands.

Analyses

Volatile Suspended Solids (VSS) were determined according to *Standard Methods* (APHA, 1975). Sulfide was determined photometrically (Trüper and Schlegel, 1964). Acetic acid was determined with a gaschromatograph equipped with a 2 m × 4 mm (i.d.) glass column packed with Supelcoport (100–120 mesh) coated with 10% Fluorad FC 431. The temperature of the column, the injection port and the flame ionization detector were 130, 220 and 240°C respectively. Nitrogen saturated with formic acid was used as carrier gas at a flow rate of 50 ml min^{-1}. Methane was determined with a gaschromatograph equipped with mol sieve 5A (mesh 60–80). The temperature of the column, the injection port and the flame ionization detector were 120, 200 and 250°C respectively. Nitrogen was used as a carrier gas at a flow rate of 20 ml min^{-1}.

The pH was determined with a pH-electrode which was put in the liquid immediately after opening the reaction bottle, in order to avoid a pH rise before measurement due to release of carbon dioxide from the liquid.

Anaerobic toxicity assay

At each pH-range to be tested at least two test runs of six activity measurements were performed. Each test run consisted of two activity measurements at "blank" conditions and four activity measurements at different sulfide concentrations. Activity tests were performed in 1.16 l. glass serum bottles sealed with a 4 mm rubber septum kept in place by a screw-cap. Each serum bottle contained 500 ml of the basal medium and substrate plus a known amount of granular sludge in the range of 0.04–0.20 g VSS per bottle, depending on the expected inhibition.

Taking into account the pH change to be induced by the subsequent flushing operation, before closing a serum bottle the pH was corrected to the desired level by adding sodium hydroxide from a concentrated stock solution. At the start of each experiment the liquid was flushed with nitrogen if the pH had to be near 8 or with a 70% nitrogen/30% carbon dioxide mixture for all other pH's. Flushing was continued until the redox indicator (resazurin) present in the trace element solution changed from light pink to colourless. The serum bottles were placed in a reciprocating shaker situated in a temperature controlled room kept at 30 ± 1°C. The shaker was operated 1 min each 5 min at a frequency of 150 strokes per min.

Except for two test runs at pH = 7.0–7.2 with substrate concentrations of 500 and 2000 mg l^{-1} acetate all activity measurements were performed at 1000 mg l^{-1} acetate. After one day of incubation the pH and the acetate concentration were measured and corrected if necessary. At this time in four out of six serum bottles sulfide was added from a 1 M Na_2S stock solution. After flushing of the gas phase the bottles were incubated one more day. The actual measurement of the specific methanogenic activity was performed on the third day after incubation had been started. The concentration of acetic acid and the pH were measured and corrected if necessary. Sulfide was also measured so that after having calculated the amount of sulfide present in the gas phase Na_2S could be added to make up for the loss of sulfide during subsequent flushing of the gas phase. During a period of 2–3 h, starting one h after flushing of the gas phase, every 20 min two 25 μl samples of the head space of each serum bottle were taken with a gas tight syringe and instantly injected in the gas chromatograph to be analysed for methane. From the slope (S) of the resulting progress line of relative methane concentration and the accessory amount of VSS the specific methanogenic activity expressed as kg COD turnover per unit VSS per day can be calculated using the following equation:

$$A = \frac{S}{100} \cdot V \cdot 24 \cdot f \cdot \frac{1}{\text{VSS}}$$

where

A = specific methanogenic activity (kg CH_4-COD kg^{-1} VSS d^{-1})
S = slope of progress line (% h^{-1})
V = volume of head space (m^3)
f = conversion factor for m^3 CH_4 to kg COD
VSS = Volatile Suspended Solids (kg).

At 30°C the value of f is 2.57 kg COD m^{-3}.

RESULTS

In order to check whether or not an acetate concentration of 1000 mg l^{-1} was high enough to avoid substrate diffusion induced rate limitation,

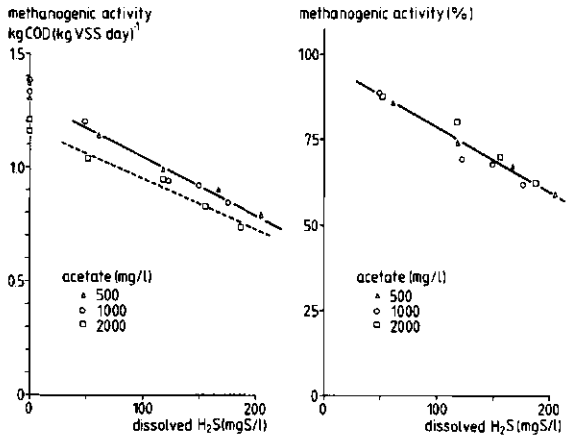

Fig. 2.(a) (left) and 2(b) (right). The specific methanogenic activity of granular sludge as a function of the free hydrogen sulfide concentration in the liquid at pH = 7.0–7.2, determined at various acetate concentrations. The dotted line in Fig. 2(a) refers to measurements at 2000 mg l^{-1} acetate, the continuous line refers to measurements at the other two concentrations. In Fig. 2(b) the remaining activity is given as a percentage of the mean uninhibited activity at the concomitant acetate concentration.

three test runs at pH = 7.0–7.2 with 500, 1000 and 2000 mg l^{-1} acetate respectively were performed. The result of this series of experiments is shown in Fig. 2. In Fig. 2(a) the absolute values of the specific methanogenic activities are given, whereas in Fig. 2(b) the remaining specific methanogenic activity is given as a percentage of the average value of the two blanks at a similar acetate concentration.

In Table 1 the specific methanogenic activities in the experiments without exposure to sulfide in the various pH-ranges tested are tabulated. Because of the apparent (very slight) pH dependency of the uninhibited specific methanogenic activity the effect of sulfide at a particular pH-range should be related to the specific methanogenic activity at that particular pH-range. Therefore we will give the results of our anaerobic toxicity assay as the proportionally remaining specific activity, which means the specific methanogenic activity at a certain sulfide concentration expressed as the fraction of the uninhibited specific methanogenic activity in the same pH-range. This reference specific methanogenic activity is the average value of the results of all blank experiments performed at a certain pH-range.

In Figs 3 and 4 the proportionally remaining specific methanogenic activity at three different pH-ranges is shown as a function of the total sulfide concentration and the free hydrogen sulfide concentration respectively.

Table 1. Uninhibited maximum specific methanogenic activity (kg COD kg^{-1} VSS d^{-1}) of acetate-fed granular sludge at three different pH ranges

pH	Maximum specific methanogenic activity	SD	No. of tests
6.4–6.6	1.23	0.04	4
7.0–7.2	1.29	0.09	6
7.8–8.0	1.25	0.06	4

DISCUSSION

Because in anaerobic digestion the pH should always be in the range 6–8 (Clark and Speece, 1971; Zehnder *et al.*, 1982), the general practice is to incubate batch experiments at approx. neutral pH

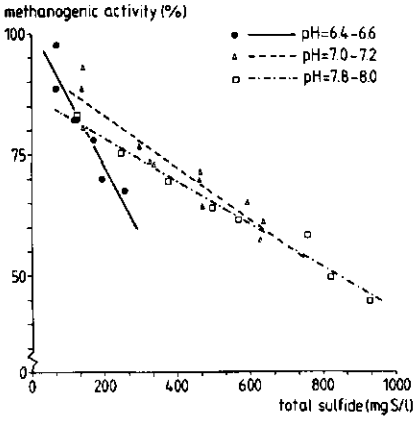

Fig. 3. The max. specific methanogenic activity (expressed as percentage of the uninhibited max. specific methanogenic activity at the concomitant pH range) of acetate-fed granular sludge incubated at 30°C at various pH ranges as a function of the total sulfide concentration. For each pH range a linear regression line has been drawn.

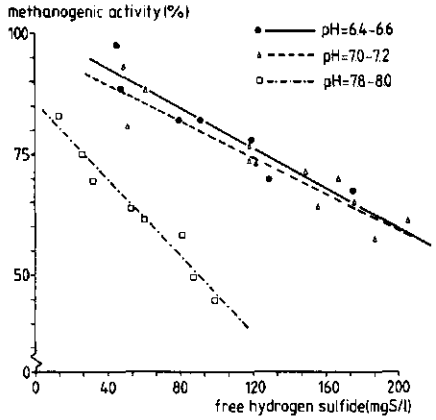

Fig. 4. The max. specific methanogenic activity (expressed as percentage of the uninhibited max. specific methanogenic activity at the concomitant pH range) of acetate-fed granular sludge incubated at 30°C at various pH ranges as a function of the free hydrogen sulfide concentration. For each pH range a linear regression line has been drawn.

values. Consequently a considerable fraction of the total sulfide will be present in the form of free hydrogen sulfide, as is shown in Fig. 1. Considering the value of the dimensionless Henry constant (representing the ratio of the concentration in the gas phase and the concentration in the liquid phase), which is 0.439 at 30°C and a partial gas pressure of 1 atm (Wilhelm *et al.*, 1977), this implies that much of the sulfide added in a toxicity assay applied for methanogenic bacteria will end up in the head space above the reactor liquid containing the biomass to be tested. Consequently in the commonly used anaerobic toxicity assays which are based on monitoring the gas production for several days (or weeks sometimes) the sulfide concentration will continuously decrease because of sulfide transport out of the system with the biogas. This phenomenom will occur in batch reactor toxicity tests from which the biogas is continuously removed (McCarty and McKinney, 1961; Koster and Lettinga, 1984) as well as in Hungate technique derived serum bottle toxicity tests from which the biogas is removed at intervals (Owen *et al.*, 1979; Parkin *et al.*, 1983; Witkowski and Jeris, 1983). In the batch anaerobic toxicity assay of Kroiss and Wabnegg (1983) the loss of sulfide from the liquid is less severe because their method makes use of a reactor head space which is 10 times bigger than the expected gas production.

Normally in batch anaerobic toxicity assays a considerable increase in pH due to the conversion of acetic acid into methane and carbon dioxide occurs. The carbon dioxide will partly diffuse to the gas phase; the carbonic acid remaining in the liquid phase is weaker than acetic acid.

Since in our serum bottle technique all gas produced during the actual period of measurements was kept in the head space which had a constant volume, the sulfide concentration in the liquid remained constant. The very low detection level (approx. 0.5 ppm) of the gaschromatographic methane determination we used made it possible to operate the toxicity assay at an acetate conversion rate of approx. 30 μmol per hour per serum bottle. Such a very small conversion in relation with the amount of acetate present guarantees a nearly constant pH during the tests. The excess pressure resulting from the gas production in a constant volume head space was only some millibars. Elevation of the pressure itself has no effect on the specific methanogenic activity (Ariga *et al.*, 1984) but it should be kept low in order to prevent a change in sulfide concentration in the liquid.

The fact that the maximum specific acetoclastic methanogenic activities measured at 2000 mg l^{-1} acetate are somewhat lower than the maximum specific acetoclastic methanogenic activities measured at 500 and 1000 mg l^{-1} acetate might be attributed to substrate toxicity, but the amount of unionized acetic acid is too low to make this very probable. At pH = 7 and an acetate concentration of 2000 mg l^{-1} the amount of unionized acetic acid present is 11 mg l^{-1}. Duarte and Anderson (1982) reported toxicity at unionized acetic acid concentrations exceeding 10–25 mg l^{-1}, whereas Kroeker *et al.* (1979) reported 30–60 mg l^{-1} as the toxicity range.

The results shown in Fig. 2(b) of the tests performed at different acetate concentrations clearly indicate that in the concentration range of 500–2000 mg l^{-1} acetate does not influence sulfide toxicity.

From Fig. 2(a) the conclusion can be drawn that 1000 mg l^{-1} is sufficient to avoid a mass transfer resistant effect with respect to the substrate on the specific acetoclastic methanogenic activity of the granular sludge. So the inhibitory effect of sulfide on the biomass activity will not be masked by a partial abolition of substrate transfer limitation. The absence of substrate transfer limitation means that all bacteria present in the granules are saturated with substrate, so that the observed specific acetoclastic methanogenic activities are the maximum achievable. The absence of substrate transfer limitation at acetate concentrations exceeding 500 mg l^{-1} is in accordance with results obtained with granular methanogenic sludge cultivated on wastewater from beet sugar producing and processing factories (Tramper *et al.*, 1984; Dolfing, 1985).

In the often cited reviews concerning toxicity in anaerobic digestion of McCarty (1964) and Kugelman and Chin (1971) an upper limit of tolerable sulfide concentrations of 200 mgS l^{-1} is advocated. This is based on research with sewage sludge digesters which completely failed when the total sulfide concentration was increased from 200 to 390 mgS l^{-1} (Lawrence *et al.*, 1964). From the results

Table 2. Concentrations of the various sulfide forms at 50% inhibition of the maximum specific methanogenic activity of granular sludge fed with acetate. The values are calculated from the linear regression lines shown in Figs 3 and 4

pH	Total sulfide (mgS l^{-1})	Free hydrogen sulfide (mgS l^{-1})	Bisulfide (mgS l^{-1})
6.4–6.6	357	246	111
7.0–7.2	810	252	558
7.8–8.0	841	90	751

shown in Fig. 3 and Table 1 it can be seen that in our experiments at 200 mgS l^{-1} a maximum specific acetoclastic methanogenic activity of 0.9–1.1 kg COD kg^{-1} VSS d^{-1} remained. Such a maximum specific methanogenic activity is well in the range of activities which have been reported for mixed cultures present in one-stage anaerobic wastewater treatment systems treating a wide variety of wastewaters (Henze and Harremoës, 1983). With the modern high-rate anaerobic wastewater treatment systems, which are characterized by a very good sludge retainment (Van den Berg and Kennedy, 1983; Callander and Barford, 1983), much lower maximum specific methanogenic activities can be allowed. In such systems the high biomass concentration makes up for the loss of activity caused by inhibition. If we assume that a maximum specific *acetoclastic* methanogenic activity of 0.5 kg COD kg^{-1} VSS d^{-1} suffices to economically operate a UASB-reactor a sulfide concentration of approx. 100 mgS l^{-1} would be tolerable at neutral or alkaline pH values. It should be noted that in the case of sulfide toxicity in the anaerobic treatment of sulfate containing wastewater the hydrogenotrophic methanogenic bacteria will be outcompeted by the sulfate reducing bacteria, resulting in a decrease of the overall maximum specific methanogenic activity of the sludge. However, the maximum specific *acetoclastic* methanogenic activity will not be affected by the ingrowth of sulfate reducing bacteria, since these sulfate reducing bacteria do not consume acetate and have a growth yield which is comparable to the growth yield of the hydrogenotrophic methanogenic bacteria which they replace (Rinzema and Lettinga, 1986).

The results shown in Fig. 3 clearly show that an acidic pH intensifies the inhibitory effect of sulfide on acetoclastic methanogenesis, as compared to the effect of sulfide at neutral or alkaline pH values. If the relationship between the maximum specific acetoclastic methanogenic activity and the free hydrogen sulfide concentration at various pH-levels is contemplated (Fig. 4) it appears that the free hydrogen sulfide concentration cannot be used as the sole parameter to describe sulfide inhibition in anaerobic digestion, as is suggested by Kroiss and Plahl-Wabnegg (1983). A good correlation between the free hydrogen sulfide concentration and the maximum specific acetoclastic methanogenic activity exists at neutral and acidic pH values. At pH = 7.8–8.0, where the fraction of free hydrogen sulfide is only approx. 10%, the maximum specific acetoclastic methanogenic activity decreased much faster with an increasing free hydrogen sulfide concentration than at the other pH values tested. In the alkaline pH range the total sulfide concentration appears to dictate the inhibition (Fig. 3). This phenomenon might be caused either by an inhibitory effect of the bisulfide ion which becomes apparent only at increased concentrations or by an increased susceptibility for free hydrogen sulfide of the acetoclastic methanogens near the limit of their physiological pH range.

Notwithstanding the lack of an unambiguous explanation for the role of the bisulfide ion in sulfide toxicity, from our work it can be concluded that 50% inhibition of the maximum specific acetoclastic methanogenic activity of granular sludge occurred at a free hydrogen sulfide concentration of 250 mgS l^{-1} or a total sulfide concentration of 825 mgS l^{-1}, depending of the pH range (Table 2).

The similarity of the relationship between maximum specific acetoclastic methanogenic activity and total sulfide concentration at pH = 7.8–8.0 and pH = 7.0–7.2 might be attributed to the existence of a pH gradient in the granula. In the granula, apart from methane, bicarbonate is being produced. The diffusional resistance against transport of the bicarbonate out of a granule causes a bicarbonate gradient in the granule, with the highest concentration near the center. Since in methanogenic environments bicarbonate is the principal buffering agent this results in a pH gradient with the highest pH near the centre of the granule. The existence of such pH gradients has been confirmed for denitrifying biofilms (Arvin and Kristensen, 1979; Arvin and Kristensen, 1982). Some indications for the existence of a pH gradient in granular methanogenic sludge have been reported recently (Ten Brummeler *et al.*, 1985). The existence of a pH gradient in biofilms such as granular sludge might also explain the fact that in our study the effect of free hydrogen sulfide on methanogenesis appeared to be less severe than had been reported in previous studies (all concerning dispersed sludges). Another explanation might be the existence of a sulfide gradient as the result of sulfide stripping by biogas leaving the biofilm.

CONCLUSIONS

The serum bottle anaerobic toxicity assay presented in this article is a relatively simple technique for quantifying the effect of certain chemicals on acetate cleaving methanogenic bacteria. The technique enables tests to be run at a constant pH without the necessity of a pH-controlling apparatus.

The effect of sulfide on the maximum specific acetoclastic methanogenic activity of granular sludge depends on the pH. In the pH range 6.4–7.2 a good correlation was found between the unionized hydrogen sulfide concentration and the maximum specific

acetoclastic methanogenic activity. However at pH = 7.8–8.0 the maximum specific acetoclastic methanogenic activity decreased faster with an increasing concentration of unionized hydrogen sulfide than in the neutral and acidic pH ranges. At pH = 7.0–7.2 and pH = 7.8–8.0 the same correlation between maximum specific acetoclastic methanogenic activity and total sulfide concentration was found.

The average uninhibited specific maximum acetoclastic methanogenic activity of the granular sludge was 1.26 kg COD kg^{-1} VSS d^{-1}. This was slightly influenced by pH, the neutral value being optimal. Unionized hydrogen sulfide concentrations leading to a 50% decrease of the maximum specific acetoclastic methanogenic activity were found to be 250 mgS l^{-1} in the pH range 6.4–7.2 and 90 mgS l^{-1} at pH = 7.8–8.0.

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CHAPTER 7

The Effect of Sulphide on the Anaerobic Degradation of Propionate

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THE EFFECT OF SULPHIDE ON THE ANAEROBIC DEGRADATION OF PROPIONATE

Arjen Rinzema* and Gatze Lettinga

Wageningen Agricultural University, Department of Water Pollution Control, De Dreijen 12, 6703 BC Wageningen, The Netherlands

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ABSTRACT

The anaerobic degradation of propionate in the presence of excess sulphate was studied in an Upflow Anaerobic Sludge Bed reactor inoculated with granular sludge. A stable and efficient propionate degradation could be achieved (space load 5 kg COD m^{-3} day^{-1}, VFA removal 96%). Only 23% of the added COD was recovered as sulphide in the effluent and biogas. H_2S concentrations above approximately 100 mg l^{-1} S caused a sharp drop of the VFA removal efficiency and resulted in process instability, possibly aggravated by propionate inhibition. Recovery took 3-4 weeks. Acetoclastic methanogenesis was not the rate limiting step under sulphide inhibition.

INTRODUCTION

During the anaerobic treatment of socalled 'acid water', an extremely sulphate rich waste stream from edible oil refineries, serious inhibition problems can occur as a result of the formation of hydrogen sulphide (1). In experiments with acid water we observed that severely upset anaerobic digesters frequently had high concentrations of propionic acid in the effluent. One of the major organic components in acid water is glycerol, which can be fermented to propionic acid during anaerobic digestion (2-4). In the aforementioned experiments the degradation of propionate seemed to be affected more seriously than the degradation of acetate by methanogenic bacteria, although the latter conversion step is usually postulated to be the most sensitive in anaerobic digestion.

In anaerobic digestion of sulphate free wastes, propionate is normally fermented to acetate, hydrogen and carbon dioxide by obligate hydrogen producing bacteria (5-7), e.g. *Syntrophobacter wolinii* (8). The fermentation products are used by acetotrophic and hydrogenotrophic methanogenic bacteria. However when sulphate is present in the waste, these three groups of bacteria will have to compete for substrates with sulphate reducing bacteria. In sulphate rich sediments propionate is oxidized to acetate and carbon dioxide by sulphate reducers incapable of complete fatty acid oxidation, e.g. *Desulfobulbus propionicus* (9,10). Recently this microorganism has also been isolated from anaerobic digesters (11). Widdel (12) also isolated sulphate reducers capable of complete oxidation of propionate, as well as acetate oxidizing sulphate reducers from sediments and manure. Hydrogen oxidizing sulphate reducing bacteria, e.g. *Desulfovibrio* spp., have been described extensively (12-17). Apparently, in principle complete oxidation of organic waste material by sulphate reducers is possible when sufficient sulphate is present, as indicated by several sediment studies (18-24).

The literature (1,25-28) indicates that in anaerobic digesters based on immobilized biomass, acetate remains available for methanogenesis during treatment of sulphate containing wastes. However the hydrogen that is normally generated during acetogenesis in the absence of sulphate, is largely used for sulphate reduction. Either the hydrogenotrophic methanogens are outcompeted by hydrogenotrophic sulphate reducers, or the obligate hydrogen producers are outcompeted by sulphate reducers incapable of complete oxidation of fatty acids with more than two carbon atoms.

A poor degradation of propionate can be attributable either to inhibition of the propionate splitting microorganisms (sulphate reducers or obligate hydrogen producers) or of hydrogen oxidizing microorganisms (hydrogenotrophic sulphate reducers or methanogens). Information on the sulphide sensitivity of all three groups of bacteria is rather scarce. Boone and Bryant (8) report 23% and 51% inhibition of a co-culture of *Syntrophobacter wolinii* and *Desulfovibrio* sp. growing on propionate, at 5 and 10 mM total sulphide respectively. Grotenhuis (29) found 50% reduction of the propionate conversion capacity at 7.2 mM total sulphide (pH 7.2, 30°C), in batch experiments with granular sludge cultivated on propionate in an Upflow Anaerobic Sludge Bed (UASB) reactor. The available information on the sensitivity of sulphate reducers is rather conflicting: according to Badziong *et al* (30) *Desulfovibrio* spp. do not grow exponentially in batch culture if sulphide is allowed to accumulate, but several other reports indicate that sulphate reducers are relatively insensitive towards sulphide compared with other bacteria participating in the anaerobic digestion process (8,31-34).

This paper describes an experiment directed at the assessment of the sulphide sensitivity of granular sludge from a UASB reactor, degrading propionate in the presence of excess sulphate. A continuous experiment was chosen rather than a batch assay (35), because short term batch assays would not include long term shifts in the microbial population as a result of the increased sulphate concentration, and also the determination of the rate limiting step would hardly be possible in batch assays.

MATERIALS AND METHODS

Biomass - Elutriated granular sludge from the UASB reactor of the Aviko potato processing factory at Steenderen, The Netherlands, was used (35). In the absence of sulphate this sludge had a maximum specific propionate degrading activity of 0.25 kg COD kg^{-1} VSS day^{-1} (s.d. 0.03, triplicate batch experiment with 400 mg l^{-1} propionate, pH 7.5, 30°C). The maximum specific acetoclastic methanogenic activity of the sludge was 1.26 kg COD kg^{-1} VSS day^{-1} (>500 mg l^{-1} C_2, pH 6.5-8, 30°C) (35).

Apparatus - A 1.5 litre plexiglass UASB reactor similar to the 2.5 litre reactor described by Hulshoff Pol *et al* (36) was used. No mechanical mixing or effluent recycling was applied. Methane production was measured with a wet gas meter (Meterfabriek Dordrecht, The Netherlands). At the start of the experiment the reactor was inoculated with 0.6 litre (47 g VSS) well settled granular sludge.

Chemicals - The influent was prepared from a stock solution containing propionic acid (66 g l^{-1}), NH_4Cl (16 g l^{-1}), KH_2PO_4 (4 g l^{-1}) and yeast extract (3.5 g l^{-1}). This solution was diluted to the desired level with tap water, and neutralized to approximately pH 6.5-7 with NaOH. Na_2SO_4 (7.4 or 14.8 g l^{-1}, see text), KCl (25 mg l^{-1}), $MgCl_2.6H_2O$ (11 mg l^{-1}), and 1 ml l^{-1} of a trace element solution (37) were added to the influent. All chemicals were of analytical grade (Merck, Darmstadt, F.R.G.), yeast extract was supplied by Gist-Brocades, Delft, The Netherlands.

Analysis - Effluent samples were taken from the bottom of the conical settler on top of the reactor. The pH was also determined at the bottom of this settler using a pH-electrode. Methods for the analysis of volatile fatty acids (VFA), sulphide, gas composition, and volatile suspended solids (VSS) have been described elsewhere (1,35,37). The concentration of undissociated hydrogen sulphide in the reactor liquid was calculated from the total sulphide concentration and the pH, assuming chemical equilibrium (pK_a = 6.87 for H_2S at 30°C (38)). The total sulphide production was calculated from data on the total sulphide concentration ($H_2S + HS^- + S^{2-}$) in the liquid phase, the pH and the biogas production, assuming chemical and physical equilibrium (ratio between the H_2S concentration in the liquid phase and in the gas phase 2.276 (-) at 30°C (39)).

RESULTS

The reactor was operated at a space load of approximately 5 kg COD m^{-3} day^{-1}. Starting on the fourth day after inoculation, 5 g l^{-1} sulphate was added to the influent solution, and from day 8 onwards the sulphate concentration was increased to 10 g l^{-1}. Sulphate addition resulted in an immediate decrease of the methane production efficiency (the ratio between methane production rate and space load), and a concomitant strong sulphide production (figure 1). Initially, also the propionate degradation efficiency decreased, but three days after the addition of 10 g l^{-1} sulphate the propionate degradation efficiency exceeded 90% again. Between day 19 and 73 the average removal efficiency of VFA (C_2 and C_3) was 96% (s.d. 3%, n = 22). On average only 23% (s.d. 3%, n = 23) of the added COD was recovered as sulphide in the effluent and the biogas.

From day 54 onwards the propionate concentration in the influent was increased gradually - keeping the space load unchanged - in order to obtain higher sulphide levels. At undissociated H_2S concentrations exceeding approximately 70 mg l^{-1} S the VFA removal efficiency dropped below 90% (figure 1). No strong accumulation of acetate in the effluent was observed (figure 1), consequently the drop in VFA removal efficiency can be attributed almost entirely to the retarded degradation of propionate. The lower degree of acetogenesis caused a distinct decrease of the sulphide production. Nevertheless the undis-

sociated H_2S concentration in the liquid still increased, which can be attributed to the pH decrease in the reactor caused by the higher VFA concentration. Illustrative of the strong effect of the pH is the temporary pH drop at day 101, which resulted in a marked decrease in efficiency. From day 120 onwards the propionate removal efficiency dropped sharply. Figure 2 shows the relationship between the propionate degradation rate and the H_2S concentration in the reactor liquid between day 11 and 153 (based on the same data as figure 1).

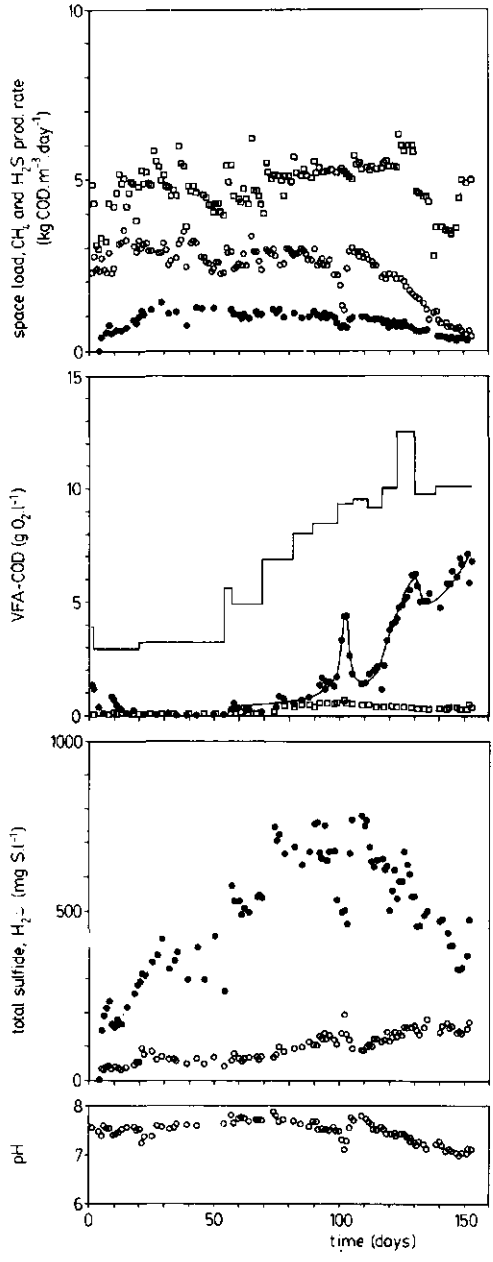

Figure 1.
(a) The applied space load (□), the methane production rate (○) and the sulphide production rate (●)

(b) the VFA concentration in the influent (—C_3) and effluent (-●-C_3, □ C_2)

(c) the total sulphide (●) and H_2S (○) concentration in the digester liquid

(d) the pH

On day 154 the influent propionate concentration was reduced to 4 g COD l^{-1} and the space load was reduced by 50%. This resulted in a decrease of the effluent VFA levels (data not shown). One day peak loads (4 kg COD m^{-3} day^{-1}) applied on days 160 and 164 showed however that the biomass had not yet recovered its original propionate conversion capacity (figure 2). Around day 180 the system again accommodated a space load of approximately 5 kg COD m^{-3} day^{-1} at an H_2S concentration between 120 and 130 mg l^{-1} S (figure 2).

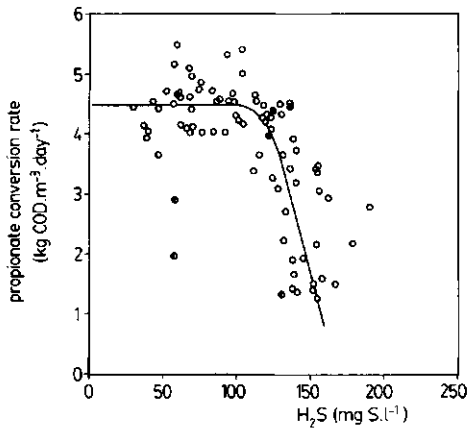

Figure 2.
The propionate conversion rate as a function of the H_2S concentration in the liquid (O day 10-153, ◐ day 156-164, ● day 179-181)

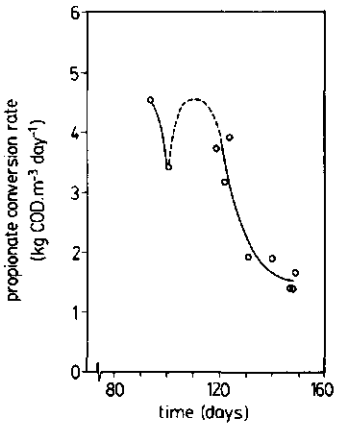

Figure 3.
The time course of the propionate conversion rate at comparable H_2S levels (136-145 mg l^{-1} S)

DISCUSSION

Stable propionate degradation could be achieved in the presence of excess sulphate, despite the fact that the total sulphide concentration reached values around 700 mg l^{-1} S. At the imposed sludge load of 0.16 kg C_3-COD kg^{-1} VSS day^{-1} (which is 64% of the initial specific activity on propionate, and corresponds to a space load of 5 kg COD m^{-3} day^{-1}) an average VFA removal efficiency of 96% was obtained. At the same time only 23% of the added COD was recovered as sulphide, indicating that sulphate reducers did not completely outcompete either the obligate hydrogen producers or the hydrogenotrophic methanogens. This is in agreement with results obtained by Isa *et al* (27,28) with hydrogen and ethanol as electron donors.

The propionate conversion rate dropped off sharply when the undissociated H_2S concentration in the liquid exceeded approximately 100 mg l^{-1} S: at approximately 140 mg l^{-1} S already a 50% decrease of the conversion rate was found (figure 2). It should be stressed that the plateau in the conversion rate (approximately 4.5 kg COD m^{-3} day^{-1}, figure 2) was imposed by the feeding rate (i.e. the conversion efficiency is 96%) and not by the maximum conversion capacity of the sludge. This seriously hampers a comparison of our results with those of other authors. Grotenhuis (29) found 50% inhibition at a calculated H_2S concentration of 74 mg l^{-1} S in batch experiments with sludge cultivated on propionate in the absence of sulphate. Our results seem to indicate a slightly higher H_2S tolerance, even if we assume that the maximum propionate conversion rate of the uninhibited sludge at the start of the experiment can be regarded as the 100% level (0.25*47/1.5 = 7.8 kg COD m^{-3} day^{-1}). However, the validity of a comparison between the batch experiments conducted by Grotenhuis and our continuous experiments should be questioned, because of possible differences in the microflora, and in the reference points used for calculating the percentage remaining activity under inhibition (46). A comparison with the results reported by Boone and Bryant (8) on a coculture of *Syntrophobacter wolinii* and *Desulfovibrio* sp. growing on propionate, is in our opinion not justified, because no data on the concentration of undissociated H_2S can be deduced from their publication.

The fact that a decrease of the total sulphide concentration between day 100 and 153 coincided with the decrease of the conversion capacity (figure 1) clearly indicates that the inhibition must be

attributed to undissociated H_2S rather than to HS^- or S^{2-}. Relatively little accumulation of acetate occurred in the inhibited system, indicating that the acetogenic oxidation of propionate was the rate limiting step. Taking into account that the applied sludge load corresponded to only 13% of the initial maximum acetoclastic methanogenic activity, this observation is not surprising. It is also in accordance with our previous finding that the acetoclastic methanogenic activity of the granular sludge used in this study decreases by only 50% at approximately 250 mg l^{-1} H_2S-S (35).

No attempt was made to determine the microbial consortium degrading the propionate. It is therefore not clear whether the inhibition must be attributed to bacteria converting propionate to acetate, or to those oxidizing hydrogen.

Figure 2 shows a relatively large variance in the propionate degradation rate at high H_2S levels. This must probably be attributed in part to a certain degree of retention time distribution. However a progressive decrease of the activity of one of the bacterial species involved in the degradation of propionate, at H_2S concentrations of approximately 140 mg l^{-1} S, also seems to play a role (see figure 3). At this point the possibility of a secondary inhibitor, viz. propionate itself, must be considered. Although data on the sensitivity of obligate hydrogen producers and fatty acid oxidizing sulphate reducers towards propionate are not available, information on propionate inhibition of acidogenic bacteria (7,40,41), hydrogenotrophic sulphate reducers (42), acetotrophic methanogens (43,44) and *Bacillus* sp. (45) indicate that propionate itself may severely retard bacterial growth at the concentrations occurring in this experiment after day 120. Due to the coupling between efficiency and propionate concentration in the effluent that is inherent to the current experimental approach, no definite conclusions on the effect of propionate can be drawn. It seems justified however to conclude that sulphide triggered the accumulation of propionate, which in turn may have aggravated the inhibition.

Dilution of the influent with the purpose of reducing the sulphide concentration, did not result in a complete recovery of the propionate degradation capacity within 10 days. However after approximately 30 days the reactor apparently had regained its original capacity (figure 2). In view of the long period of time required for recovery it seems advisable to maintain the H_2S concentration in the liquid below 100 mg l^{-1} S, in order to avoid instability of the process caused by a progressive decrease of the propionate conversion capacity of the sludge.

CONCLUSIONS

Anaerobic degradation of propionate in the presence of excess sulphate is stable at total sulphide concentrations up to approximately 700 mg l^{-1} S, provided the concentration of undissociated H_2S is kept below 100 mg l^{-1} S by pH regulation or dilution. The fraction of the organic matter oxidized by sulphate reducers is far lower than expected on the basis of the hydrogen production from propionate during normal acetogenesis, viz. only 23% instead of 47%.

When the H_2S concentration exceeds approximately 100 mg l^{-1} S, the propionate degradation capacity drops off sharply and the process becomes unstable. Sulphide inhibition may have been aggravated by the propionate itself. Recovery after a severe process disturbance caused by H_2S takes 3-4 weeks.

Methanogenesis from acetate is not the rate limiting step in the degradation of propionate under H_2S inhibition. The drop in sludge activity must be attributed to inhibition of either propionate splitting organisms (sulphate reducers or obligate hydrogen producers) or hydrogen oxidizing organisms (hydrogenotrophic sulphate reducers or methanogens).

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CHAPTER 8

Sodium Inhibition of Acetoclastic Methanogens in Granular Sludge from a UASB Reactor

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Sodium inhibition of acetoclastic methanogens in granular sludge from a UASB reactor

Arjen Rinzema, Jules van Lier and Gatze Lettinga

Department of Water Pollution Control, Wageningen Agricultural University, De Dreijen 12, 6703 BC Wageningen, The Netherlands

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The effect of sodium on the formation of methane from acetate in granular sludge from a UASB reactor has been determined at various acetate concentrations and pH-levels. At neutral pH sodium concentrations of 5, 10 and 14 g Na^+ l^{-1} caused 10, 50 and 100% inhibition respectively, relative to the maximum specific acetoclastic methanogenic activity of the granular sludge. These values reflect the sensitivity of Methanothrix *sp. towards sodium, as this is the predominant acetoclastic methanogen in the granular sludge used in this study. The pH did not affect the inhibitory action of sodium significantly in the range 6.5–7.2, but at pH-levels near 8 the inhibition was more pronounced. At acetate concentrations below 500 mg l^{-1} diffusion limitation partly masked the influence of sodium on the specific activity of the granular sludge. No adaptation of* Methanothrix *sp. to high sodium concentrations could be obtained in a period of 12 weeks. Net growth of* Methanothrix *sp. could be obtained at sodium concentrations of approx. 10 g l^{-1}. At equal sodium concentrations, sodium chloride had a somewhat stronger effect on the specific activity than sodium sulfate, but the difference is unimportant for design purposes. The results obtained with short term activity measurements can reliably be used for predictions of the effect of sodium salts on continuously fed UASB reactors.*

Keywords: Sodium; toxicity; inhibition; methanogenesis; granular sludge; Methanothrix; anaerobic digestion; UASB reactor

Introduction

During the past decade, the application of anaerobic wastewater treatment under suboptimal conditions has received increasing interest, mainly as a result of the development of several new reactor types with immobilized biomass, i.e., the Upflow Anaerobic Sludge Bed (UASB) reactor, the Fluidized Bed reactor and Stationary Fixed Film reactors.[1,2,3] With these systems a very good sludge retention can be achieved. Generally the sludge residence time can be one to two orders of magnitude higher than the hydraulic residence time. Therefore these systems tolerate higher concentrations of inhibitory compounds than conventional completely mixed reactor systems.[4]

Sodium salts represent an important inhibition problem during anaerobic treatment of various types of industrial wastewater and agricultural waste, e.g., wastewater from sauerkraut production,[5] rayon spinning,[6] production of chemicals,[7] shell-fish tinning,[8] tomato canning,[9] edible oil refining,[10] and dairy waste.[11] Although sodium salts have a distinct negative effect upon the metabolic activity of methanogenic bacteria in these cases, anaerobic treatment of these wastes still has to be considered as an alternative for conventional aerobic treatment methods, because the latter are also negatively affected by high sodium levels.[12–14]

A low concentration of sodium appears to be essential for methanogenic bacteria, probably because of its role in a chemiosmotic coupling mechanism.[15,16] Perski *et al.*[15] measured apparent K_S-values ranging from 9–25 mg l^{-1} Na^+ for five species of (hydrogenotrophic) methanogens. Patel and Roth[17] reported optimal growth and methane production at 350 mg l^{-1} Na^+ for a hydrogenotrophic methanogen strongly resembling *Methanobacterium formicicum*. Methanogens possess a mechanism for active removal or uptake of sodium, as demonstrated by the observation that *Methanospirillum hungatei* established an intracellular sodium concentration below that in the cultivation medium[18] while *Methanobacterium thermoautotrophicum* and *Methanobrevibacter arboriphilus* concentrated so-

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methanogenic activity was performed on the third day. Before this measurement, the acetate concentration and pH were corrected if necessary without opening the bottles. The gas phase was flushed, and the bottles were incubated again. The actual measurement started 30 min after reincubation and lasted 2–4 hours, depending on the methane production rate. Every 20 min two 25 μl samples of the headspace of each serum bottle were taken, using a gas tight syringe, and instantly analysed for CH_4. Immediately after the termination of the activity measurement, the acetate concentration and pH were measured, samples for sodium and sulfate or chloride analysis were taken, and the amount of VSS was determined. The specific acetoclastic methanogenic activity was calculated from the slope of the measured progress line (relative methane concentration vs. time) and the amount of VSS. Except where indicated otherwise, percentually remaining activities have been calculated relative to the average value of the two blanks in the same series.

Substrate dependant activity

Two additional activity tests were performed to determine the specific acetoclastic methanogenic activity as a function of the acetate concentration between ≈1000 and 0 mg l^{-1}. Conditions were as described above, except that in this case the acetate concentration was deliberately allowed to decrease, and was measured regularly.

Prolonged exposure to sodium

Granular sludge was exposed to sodium sulfate (10 g Na^+ l^{-1}) during 12 weeks, in a 5 l batch reactor at 30°C. This reactor was fed semicontinuously with acetate, using an automatic feeding system coupled to a wet gas meter, to ensure a relatively constant substrate level of 500 mg l^{-1}. Sludge samples for activity tests were taken from this batch reactor after 6 and 12 weeks.

UASB experiments

Continuous experiments were performed in 0.2 l glass reactors of 35 mm internal diameter, as described by Rinzema *et al.*[10] All reactors were inoculated with a known amount of granular sludge, and were initially fed with a neutralized acetate solution containing nutrients and trace elements as described above. The reactors were maintained at an effluent acetate concentration above 600 mg l^{-1}, to allow the determination of the maximum specific activity of the sludge. One reactor served as a control (0–1 g l^{-1} Na^+) during the whole experiment, while the other three were operated at sodium levels of 5–6, 9–10 and 13–14 g l^{-1} Na^+ respectively from day 5–21. Sodium sulfate was used to increase the sodium concentration to the desired level. During the first 2–4 days after sodium addition, the overloading was continued, to allow the determination of the maximum specific activity of the sludge under inhibition. Thereafter the reactors were operated at lower space loads. From day 21 onwards all reactors were again overloaded (effluent acetate concentration above 1000 mg l^{-1}) with a sodium-free medium, to establish the final maximum uninhibited specific activity. The experiment was terminated after 24 days, and the amount of biomass in all the reactors was determined. All activities were calculated using an estimate of the amount of biomass based on a constant yield (g VSS g^{-1} COD-removed) calculated from the cumulative methane production and the cumulative increase in the amount of biomass.

Results

Influence of the substrate concentration

Previous work indicated that—for the granular sludge used—an acetate concentration of 500 mg l^{-1} was suffi-

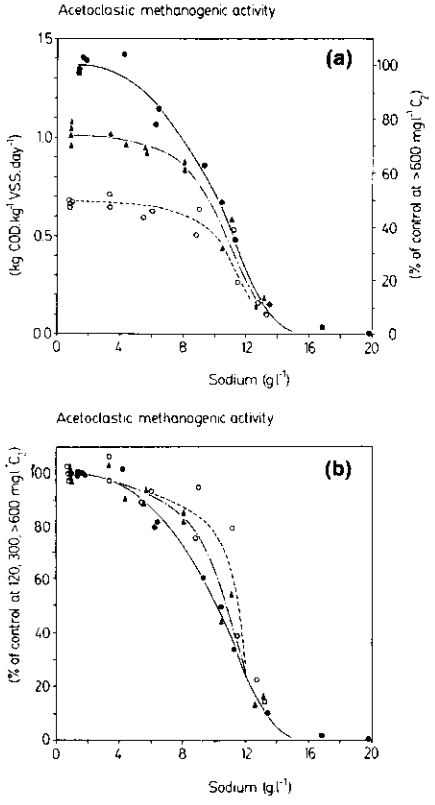

Figure 1a and **1b** The specific acetoclastic methanogenic activity of granular sludge as a function of the sodium concentration at pH 6.5–7.0, determined at three acetate concentrations (●——, above 600 mg l^{-1}; ▲—·—, 300 mg l^{-1}; ○---, 120 mg l^{-1} acetate). *Figure 1a* shows the activities both as absolute values and as a percentage of the average blank activity at acetate concentrations above 600 mg l^{-1}. *Figure 1b* shows the same results expressed as a percentage of the average blank activity at each acetate concentration

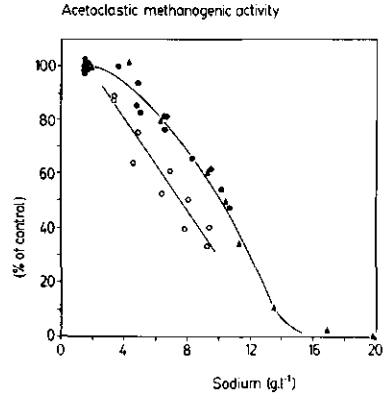

Figure 2 The maximum specific acetoclastic methanogenic activity of granular sludge as a function of the sodium concentration in three pH ranges. (▲, pH 6.5–6.8; ●, pH 6.8–7.2; ○, pH 7.8–8.1, >600 mg l^{-1} acetate)

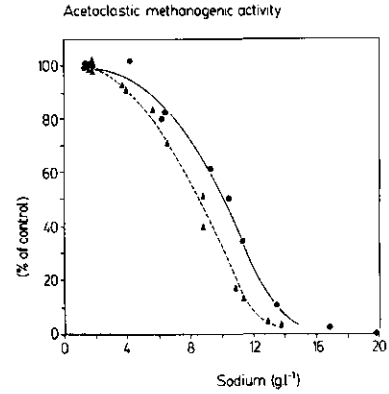

Figure 3 The maximum specific acetoclastic methanogenic activity of granular sludge as a function of the sodium concentration at pH 6.5–7.0, determined with sodium sulfate (●——) and sodium chloride (▲----)

cient to avoid a rate limitation caused by diffusion of the substrate into the granules.[44] Most tests described in this paper were directed at the determination of the maximum specific methanogenic activity, and were therefore performed at acetate concentrations above 600 mg l^{-1}. In two series of tests, the substrate concentration was significantly lower than 500 mg l^{-1}. *Figure 1* shows the specific methanogenic activity as a function of the sodium concentration at 120, 300 and >600 mg l^{-1} of acetate.

Influence of the pH

The effect of sodium on the maximum specific methanogenic activity was determined at three pH levels. The results of these tests are given in *Figure 2*. Because of a slight pH dependency of the uninhibited activity, *Figure 2* shows the remaining activity for each pH range as a percentage of the average uninhibited activity in the same pH range.

Influence of the anion

The influence of sodium sulfate was compared with that of sodium chloride in the pH range 6.5–7.0. *Figure 3* shows the remaining activity at various sodium concentrations.

Influence of prolonged exposure

Granular sludge was exposed to sodium sulfate (10 g l^{-1} Na^+) during 12 weeks. The influence of sodium sulfate on the specific methanogenic activity was determined after 6 and 12 weeks. *Figure 4a* shows the

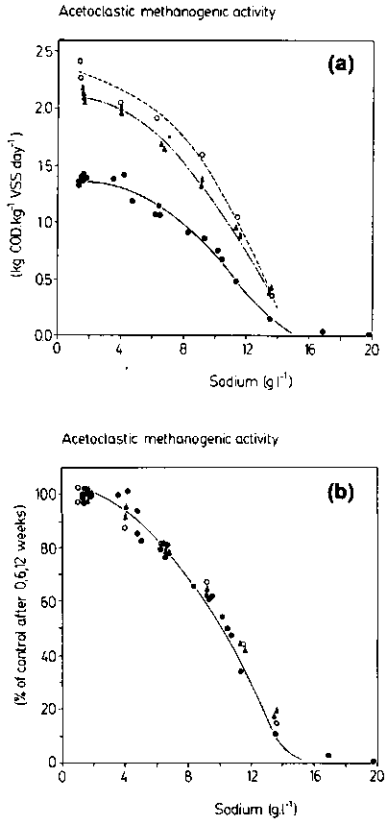

Figure 4a and 4b The maximum specific acetoclastic methanogenic activity of granular sludge as a function of the sodium concentration at pH 6.5–7.2, determined after 0 (●——), 6 (▲—·—) and 12 weeks (○----) of exposure to 10 g l^{-1} Na^+. In *Figure 4b* the remaining activity is shown as a percentage of the mean uninhibited activity after the concomitant exposure period

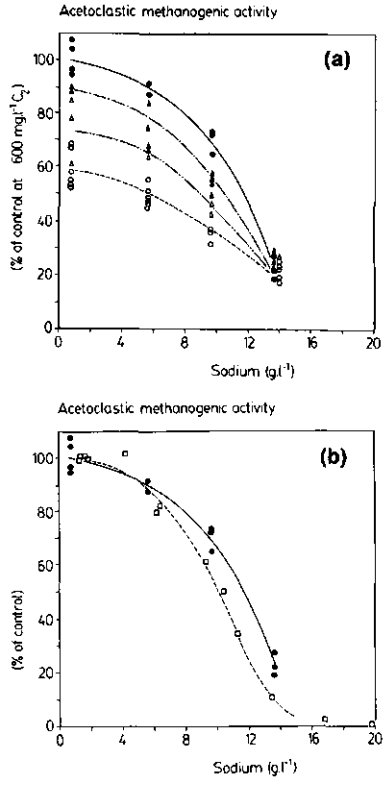

Figure 5a and 5b The specific acetoclastic methanogenic activity of granular sludge as a function of the sodium concentration at effluent acetate concentrations of 100–200 (○----), 200–300 (△—·—), 400–500 (▲——) and >600 (●——) mg l^{-1}, determined in UASB reactors. In *Figure 5b* the maximum specific activity found in the UASB reactors (●——) is compared with that found in the batch experiments (□----) (in both experiments acetate concentrations exceeded 600 mg l^{-1})

absolute values of the specific activity as a function of the sodium concentration, *Figure 4b* shows the remaining activity as a percentage of the average blank activity for each exposure time. Microscopical examinations after 9 weeks exposure revealed that the dominant methanogen in the granules was still a *Methanothrix* sp., indicating no shift in the population.

Continuous experiments

Four UASB reactors were operated at different sodium sulfate levels (≈0.8, 5.6, 9.7 and 13.7 g l^{-1} Na^+). *Figure 5a* shows the results obtained at different effluent acetate concentrations, *Figure 5b* gives a comparison between the maximum activities found in the batch activity measurements and the UASB reactors. *Table 2* summarizes the results obtained in the UASB experiments. Microscopical examinations showed that no important shift in the methanogenic population occurred in these experiments.

In all experiments described above, the sulfide concentration in the liquid phase ranged from 30–50 mg S l^{-1}. No indications were found for significant sulfate reduction with concomitant acetate oxidation.

Discussion

The results of the short-term activity tests show that sodium sulfate hardly affects the maximum specific acetoclastic methanogenic activity of the granular sludge used in this study, at concentrations below 5 g l^{-1} Na^+ (10% inhibition), whereas the activity is practically reduced to zero at 14 g l^{-1} Na^+ (*Figure 1, Table 3*). The UASB experiments indicate slightly more favorable figures, i.e., 10% and 100% inhibition at 5.5 and 15 g l^{-1} Na^+ respectively. The differences are, however, relatively small (*Figure 5b*). The main difference between the batch activity tests and the UASB experiments was the abruptness of the exposure to high sodium levels: In the batch tests the biomass was instantly subjected to the concentration to be tested,

Table 2 Summary of results obtained with UASB reactors

Reactor		1	2	3	4
Sludge quantity (g VSS)					
	start	2.8	2.8	2.8	2.8
	end[a]	5.5	4.4	3.7	3.4
Maximum uninhibited specific activity[b] (g C_2–COD/g VSS/d)					
	start	1.57 (.08,4)	1.51 (.01,2)	1.54 (.07,2)	1.59 (.06,2)
	end[a]	2.37 (– ,1)	2.35 (.11,2)	1.88 (.02,2)	1.65 (.08,2)
Maximum specific activity under inhibition[b] (g C_2–COD/g VSS/d)		1.57 (.08,4)	1.40 (.03,2)	1.10 (.06,3)	0.36 (.05,4)
(% of uninhibited activity)[c]		100	92	71	22
Sodium concentration (g Na^+ l^{-1})[b]		0.76 (.19,15)	5.64 (.19,15)	9.68 (.24,15)	13.71 (.72,15)

[a] The uninhibited activity was determined after 17 days exposure to sodium sulfate; the sludge quantity was determined after another two days of operation without sodium addition
[b] Determined at effluent acetate concentrations above 600 mg l^{-1}, between brackets the standard deviation and the number of measurements
[c] Expressed as % of the maximum uninhibited specific activity measured in the same reactor

Table 3 Sodium concentrations leading to various degrees of reduction of the specific acetoclastic methanogenic activity of granular sludge

Acetate concentration (mg l^{-1})	Exposure time (days)	pH	Anion	Reference concentration (g Na^+ l^{-1})	Sodium concentration (g l^{-1}) leading to % of reduction in activity		
					10	50	100
Activity tests							
120	1	6.5–7.0	SO_4^{2-}	0.84	7	12	14
300	1	6.5–7.0	SO_4^{2-}	0.85	6	11	14
>600	1	6.5–7.0	SO_4^{2-}	1.55	5	10	14
>600	1	6.5–6.8	SO_4^{2-}	1.55	5	10	14
>600	1	6.8–7.2	SO_4^{2-}	1.53	5	10	14
>600	1	7.8–8.1	SO_4^{2-}	1.57	2.5	7.5	12
>600	1	6.5–7.0	Cl^-	1.65	4	8.5	13
>600	43[a]	7.0–7.2	SO_4^{2-}	1.61	5–6	11	14
>600	85[a]	6.9–7.1	SO_4^{2-}	1.41	6	11	14
UASB reactors							
>600	2–4	6.7–7.2	SO_4^{2-}	0.76	5.5	12	15

[a] Exposure to 10 g l^{-1} Na^+ during 42 and 84 days, respectively, plus one day to the concentrations to be tested

while in the UASB reactors this concentration was more gradually approached, i.e., within 5–10 hours (the hydraulic residence time was 5 h). Possibly the extremely rapid increase in the salt concentration during the batch tests caused a larger part of the bacteria to die.

Because of the lack of knowledge about the mixing characteristics of the UASB reactors used in this study, a meaningful comparison between the activities measured in batch and UASB experiments is only possible at (effluent) acetate concentrations high enough to guarantee maximum metabolic activity of the entire amount of biomass, viz. concentrations over 500 mg l^{-1}. This also implies that the lines presented in *Figure 5a* for acetate concentrations below 600 mg l^{-1}, cannot be used to predict the performance of any UASB reactor, irrespective of its scale and mode of operation.

Regarding the small differences between the effect on the maximum specific activity found in batch tests and UASB experiments (*Figure 5b*), it can be concluded that the short-term activity tests predict the effect of high salt levels on the maximum activity of methanogenic bacteria with sufficient accuracy. The measured values reflect the sensitivity of *Methanothrix* sp. towards sodium, as these are the predominant acetoclastic methanogens in the granular sludge used in this study.

The inhibitory effect of sodium seems to be affected by the acetate concentration (*Figure 1b, Table 3*). A comparison of the measured uninhibited specific activity of the granular sludge at various acetate levels (*Figure 1a* and two additional experiments) with the growth rate of *Methanothrix* sp. (*Figure 6*), indicates that the substrate conversion capacity of the granular sludge drops off relatively fast at decreasing acetate concentrations. The reason for this discrepancy is that below ≈500 mg l^{-1} C_2 the capacity of the granules is limited by the diffusion rate of substrate into the granules, a phenomenon that is also described by others.[40,45] As a result of this limitation, insufficient acetate penetrates to the centre of the uninhibited granules. Consequently, the inhibitory effect of sodium at low substrate levels is masked by a partial removal of this physical rate limitation. Obviously substrate diffusion limitation can have important implications for toxicity experiments. For instance, data from underloaded continuous reactor systems with immobilized biomass should be employed carefully, because a serious underestimation of the degree of inhibition can be made.

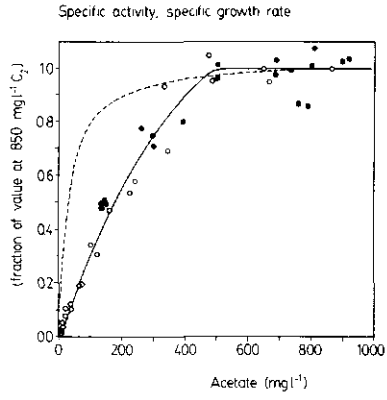

Figure 6 A comparison between the calculated growth rate of *Methanothrix soehngenii* (-----, $\hat{\mu}$ 0.1 d^{-1}, K_s 30 mg l^{-1} C_2^{50}) and the measured uninhibited specific activity of the granular sludge (——, ● blanks from toxicity assays maintained at a constant acetate concentration; ○ additional experiments with variable acetate concentration), as a function of the acetate concentration, both expressed as a fraction of their value at 850 mg l^{-1} acetate

The pH does not affect the inhibitory action of sodium significantly in the range 6.5–7.2, but at pH levels near 8 the inhibition is distinctly higher (*Figure 2*). We observed a similar effect for hydrogen sulfide.[44] It might be that the tolerance of the bacteria for adverse environmental conditions is lower at pH 8, which is near the upper pH limit for methanogens,[29,46–49] especially for *Methanothrix* sp.[32,41]

Contrary to earlier reports in the literature,[26,27] the anion seems to have a distinct influence on the inhibitory effect of sodium ions (*Figure 3*). The differences between sodium sulfate and sodium chloride are however comparatively small, and can be neglected for design purposes.

No clear adaptation of *Methanothrix* sp. to high sodium concentrations could be demonstrated after 12 weeks of continuous exposure (*Figure 4b*). Earlier investigations suggest adaptation of methanogens to sodium.[30,31,34] We have reasons to believe, however, that the word 'adaptation' is inappropriately used in these earlier publications. Kugelman and McCarty[34] concluded that methanogens can be adapted to sodium chloride, but their Continuously Stirred Tank Reactors (CSTR) were clearly not in a steady state. Results of comparable experiments performed by Kugelman and Chin[19] also indicate that no increase in the tolerance towards sodium is obtained after prolonged exposure. Yang *et al.*[30] suggested that the 'adaptation potential'—defined as the ratio between the concentrations causing 50% inhibition in an anaerobic filter, and in batch experiments—for sodium is 1.5. The comparison between batch and continuous experiments, as made by Yang *et al.*,[30] is very dubious, however, because it is not certain that the same microorganisms dominated in both systems, and the filters were probably severely underloaded. De Baere *et al.*[31] came to the conclusion that adaptation is possible by comparing two reactors containing a clearly different microflora, due to a pH drop occurring in one reactor after a shock load with sodium chloride. Consequently, in our opinion, their conclusion is not justified.

A very significant net growth of *Methanothrix* sp. at high sodium concentrations is clearly demonstrated by the increase in the specific activity of the granules during prolonged exposure to 10 g Na^+ l^{-1} in a batch reactor (*Figure 4*), as well as in a UASB-reactor (*Table 2*). Calculation of specific growth rates is impossible due to variations in the effluent acetate concentration.

The results from our activity measurements compare well with those reported by other authors, as far as comparable methods (i.e., activity measurements) have been employed (*Table 1*, *Table 3*). All comparable studies used *Methanothrix* sp. (This can be deduced from the experimental conditions applied in the chemostat for cultivation of the enrichment culture used by Van den Berg *et al.*[29] and by Yang *et al.*,[30] and from the origin of the granular sludge used by Dolfing and Bloemen.[32]) Similar results have also been reported for an undefined culture by Georgacakis and Sievers.[11] The results of Kugelman and Chin[19] indicate that the maximum specific acetoclastic activity of *Methanosarcina* sp. is only slightly affected (≈10% decrease) at a sodium concentration of 8 g l^{-1}.

Table 4 Cation composition of media used by various authors

Reference	Cation concentration (mg l^{-1})			
	K^+	NH_4^+[a]	Ca^{2+}	Mg^{2+}
Kugelman and McCarty[28] and Kugelman and Chin[19]	6	56	–	–
Van den Berg *et al.*[29]	331	806	33	21
Yang *et al.*[30]	210	157	–	40
Toldra *et al.*[9]	507	1369[b]	–	–
De Baere *et al.*[31]	1311	337	80	12
Dolfing and Bloemen[32]	252	168	0	0
This study	190	398	35	18

[a] mg l^{-1} NH_4^+
[b] Total nitrogen

A comparison with results obtained in continuous reactors with immobilized biomass shows extremely large differences (*Table 1*, *Table 3*). Besides the uncertainties concerning the applied sludge loading rate and the maximum capacity of the biomass in these systems, the possibility of antagonistic and synergistic effects must be taken into account when evaluating these results (*Table 4*). It should be remembered that Lettinga and Vinken[5] worked with sauerkraut wastewater with unknown quantities of cations. De Baere *et al.*[31] worked with a synthetic medium containing a relatively high concentration of potassium, viz. 1311 mg l^{-1}, which is well within the range where potassium acts as an antagonist of sodium.[27]

If we assume that a maximum specific acetoclastic methanogenic sludge activity of 0.5 kg COD/kg VSS/day suffices for the economic and safe operation of a UASB-reactor, it can be deduced from our results that a sodium concentration of 11–12 g l^{-1} would be tolerable for reactors treating acidified wastewater, where a maximum uninhibited activity of 2–3 kg COD/kg VSS/day can be expected. For a UASB reactor treating unacidified wastewater the maximum uninhibited activity would be ≈0.6–1 kg COD/kg VSS/day, and the maximum tolerable sodium concentration is then 6.5–10 g l^{-1} (all values at 30°C).

In the anaerobic treatment of 'acid water' from edible oil refineries, inhibition by sodium will in many cases necessitate dilution of the waste. The guidelines discussed above cannot be directly applied, however, because the large quantities of sulfide produced by sulfate reducing bacteria growing on other electron donors than acetate must also be taken into account.[10,44]

Conclusions

At neutral pH, sodium concentrations of 5, 10 and 14 g Na^+ l^{-1} cause 10, 50 and 100% inhibition respectively, relative to the maximum specific acetoclastic methanogenic activity of the granular sludge used in this study. These values reflect the sensitivity of *Me-*

thanothrix sp. towards sodium, as this is the predominant acetoclastic methanogen in the granular sludge used in this study. The pH does not significantly affect the inhibitory action of sodium in the range 6.5–7.2, but at pH levels near 8 the inhibitory effect is more pronounced. At equal sodium concentrations, sodium chloride has a somewhat stronger effect on the specific activity than sodium sulfate, but the difference between these two sodium salts is of minor importance for design purposes. No adaptation of *Methanothrix* sp. to high sodium concentrations can be obtained in a period of 12 weeks. Net growth of *Methanothrix* sp. can be obtained at sodium concentrations of ≈ 10 g l^{-1}.

At acetate concentrations below 500 mg l^{-1}, diffusion limitation partly masks the influence of sodium on the specific activity of the granular sludge used in this study.

The results obtained with short-term activity measurements can be used reliably for predicting the effect of sodium salts on continuously fed UASB reactors.

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CHAPTER 9

Summary

Samenvatting

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Summary

Since the early 1980s, anaerobic wastewater treatment is more and more accepted as an attractive alternative to conventional aerobic biological purification. Several anaerobic treatment systems based on biomass aggregate or biofilm formation have been rapidly developed during the 1970s. The most successful example of these high rate systems is the Upflow Anaerobic Sludge Bed reactor (UASB), that was developed in the Netherlands.

Worldwide, over 100 anaerobic wastewater treatment reactors are in operation or under construction at this moment. Most of these installations treat wastewaters contaminated with rapidly biodegradable organic compounds, which contain only low concentrations of inhibitory compounds, such as sulfate. This must be ascribed to the fact that serious problems were encountered in laboratory scale investigations with more complex wastewaters.

This thesis describes research on anaerobic treatment of wastewaters contaminated with lipids or sulfate. The experimental results can be subdivided into two groups: Chapters 2-5 deal with research on anaerobic treatment of lipid containing wastewater, and Chapters 6-8 describe research on problems that result from high sulfate concentrations. The study of two subjects is based on the wastewater problems occurring at edible oil refineries. Wastewater from the edible oil refining industry may have a high lipid concentration, as well as a high sulfate concentration. Both contaminants were assessed separately, to enhance the general applicability of the results.

The goals of the research were:

- to develop an efficient and reliable high rate treatment system for purification of lipid containing wastewater, and
- to determine the conditions that will allow anaerobic treatment of wastewater with a high sulfate concentration.

The UASB reactor inoculated with granular sludge was chosen as the starting-point for the investigations, because it has significant advantages over other reactor systems.

Anaerobic treatment of lipid containing wastewater

In principle, lipids are readily biodegradable. However, two problems have to be dealt with to guarantee the efficiency and reliability of the anaerobic treatment system at high loading rates: (1) inhibition of the methanogenic and acetogenic bacteria by long chain fatty acids, and (2) wash-out of the biomass due to flotation.

Chapter 2 deals with the inhibitory effect of long chain fatty acids. The start-up period of an anaerobic digester on a lipid containing wastewater, and peak loads of lipids during continuous operation were simulated in stirred batch reactors, with capric acid solutions. The results indicate that a lag phase in the degradation of long chain fatty acids will occur during the start-up period. Overloading of the anaerobic digester during this lag phase, is likely to result in strong inhibition of the anaerobic sludge. Several weeks will be required for recovery when the sludge has been poisoned. The lag phase can be shortened by lowering the concentration of long chain fatty acids in the influent.

A peak load of lipids during continuous operation may also result in inhibition of the anaerobic biomass, especially of the acetotrophic methanogens. The results of our experiments indicate that the inhibition is primarily related to the concentration of long chain fatty acids, not to the lipid:biomass ratio, as was assumed until now. A threshold long chain fatty acid concentration can be distinguished. If this threshold is exceeded, over 99.9% of the acetotrophic methanogens is killed, and recovery will take approximately 10 weeks. This period is too long for practical purposes.

The threshold concentration for capric acid is approximately 1 kg/m^3. Acetotrophic methanogens do not adapt to long chain fatty acids, neither after prolonged exposure to sub-lethal concentrations, nor after repeated exposure to lethal concentrations.

The threshold level found in different sets of experiments varied slightly. This can be ascribed to differences in the mass transfer rate, and the particle size and specific activity of the biomass granules. This means that the effect of peak loads of lipids on continuous treatment systems can hardly be predicted from batch experiments. It also means that the sensitivity of various reactor systems will probably be different.

Phospholipids have a synergistic effect on long chain fatty acids. Furthermore, phospholipids and long chain fatty acids mutually reduce each others degradation rate. Long chain fatty acids will usually be mixed with phospholipids in industrial wastewaters.

Chapter 3 describes research on the effect of peak loads of long chain fatty acids on acetate degradation in continuously fed UASB reactors. The granular sludge which was used as an inoculum had not been previously exposed to lauric acid, and did not degrade this acid within the duration of the experiments. In the absence of calcium, lauric acid completely inhibits acetotrophic methanogens above a threshold level of 100 mg/l, whereas no inhibition occurs below this threshold concentration. Addition of an equivalent amount of calcium to wastewater containing lauric acid, prevents inhibition of acetotrophic methanogens up to a lauric acid concentration of at least 1500 mg/l. Addition of less than an equivalent amount of calcium apparently removes more than a stoichiometric amount of lauric acid, as 50% inhibition occurs at a concentration of approximately 700 mg 'free' or excess lauric acid per litre. This must probably be attributed to co-precipitation of lauric acid and calcium laurate.

Besides inhibition, peak loads of long chain fatty acids have a second negative effect. Complete flotation of granular sludge occurs within 2-8 hours, if the system is overloaded with an influent containing more than 100 mg lauric acid per litre. In a conventional UASB reactor this will result in virtually complete sludge wash-out. Calcium does not prevent flotation of the sludge.

Chapter 4 describes the anaerobic degradation of long chain fatty acids in conventional and modified granular sludge bed UASB reactors. Again, capric and lauric acid were selected as model substrates. The results indicate that rapid and efficient degradation of long chain fatty acids is only possible when the incoming wastewater is rapidly mixed with the digester contents, and efficient contact between biomass and substrate is guaranteed. Conventional UASB reactors cannot fulfill these requirements, and therefore cannot accommodate lipid loading rates exceeding ca. 4-5 kg COD/m^3.day. A higher space load or a small disturbance of the treatment system results in local overloading of the anaerobic biomass, which causes accumulation of long chain fatty acids. As a consequence the acids precipitate, which further retards their degradation. Eventually the precipitates will float, and this causes strong biomass wash-out.

Efficient degradation of long chain fatty acids can be achieved in an Expanded Granular Sludge Bed (EGSB) reactor. In this modified UASB system, effluent recycling is applied to achieve rapid dilution of the influent and expansion of the sludge bed. An EGSB reactor can mineralize at least 85-90% of the added long chain fatty acids at space loading rates up to ca. 30 kg COD/m^3.day. No significant flotation of granular sludge occurred during long term continuous operation. No wash-out of granular sludge occurred at hydraulic residence times as low as 2 hours. Augmentation of granular sludge in lab scale EGSB reactors was demonstrated.

After the successful experiments with long chain fatty acids, we attempted to treat triglyceride emulsions in the EGSB reactor (Chapter 5). We expected good results, because the hydrolysis of triglycerides is not the rate limiting step in their mineralization. However, triglyceride emulsions already caused very strong flotation of granular sludge at low loading rates. Modification of the gas-solids separator of EGSB reactors is necessary, to prevent excessive sludge wash-out. Two modifications have been investigated: (1) a hybrid EGSB reactor with a layer of floating reticulated polyurethane foam above the granular sludge bed, and (2) an EGSB reactor equipped with a sieve-drum separator.

The hybrid EGSB reactor does not prevent the wash-out of granular sludge sufficiently, and is therefore unsuitable for treatment of lipid containing wastewater.

The EGSB reactor equipped with a sieve-drum separator can retain sufficient granular sludge to allow a stable treatment process. An increase of the amount of granular sludge retained in the reactor can be achieved, provided that sludge granules with a sufficiently large diameter are used for inoculation. Especially if easily degradable organic contaminants are present in the wastewater besides the lipids, the maintenance of a sufficiently large inventory of granular sludge is no problem. The EGSB reactor with sieve-drum separator can accommodate higher organic and hydraulic loading rates than previously described anaerobic filter reactors.

A problem that remains to be solved, is the flotation of lipids in the EGSB reactor. It is estimated that approximately 10% of the added long chain fatty acid accumulated in a floating layer, in the experiment with lauric acid. In the experiments with triglyceride emulsions, this percentage was considerably higher. This problem will also manifest itself during anaerobic treatment of industrial wastewater. The floating layer primarily consisted of undigested lipids and contained only minor amounts of biomass granules. Although the floating lipids have been removed from the wastewater, the conversion rate to methane is only very low, because there is no contact with the granular sludge bed. A modification of the EGSB system is required to achieve either recycling of the floating lipids to the sludge bed, or their removal from the reactor and additional treatment outside the EGSB reactor.

With respect to the practical application of anaerobic treatment of lipid containing wastewater, the following conclusions can be drawn:

- *The lag phase during the start-up of an anaerobic treatment system with lipid containing wastewater can be shortened by lowering the lipid concentration in the influent. Overloading during the lag phase may cause strong inhibition by long chain fatty acids.*
- *Emulsified esters of long chain fatty acid have a synergistic effect on long chain fatty acids.*
- *A peak load of lipids during continuous operation affects especially the acetotrophic methanogens. Lipid concentrations below 1 kg/m^3 may already kill 99.9% of these bacteria. The sensitivity of various reactor systems towards long chain fatty acids is probably different as the result of different mass transfer characteristics.*
- *Wash-out of biomass granules is a more serious threat to the stability of an anaerobic treatment system than inhibition. Complete sludge flotation can occur within only a few hours if the long chain fatty acid concentration in the digester rises to only 100 mg/l. after a shock load. Inhibition can be prevented by addition of calcium salts to the wastewater, but this does not prevent sludge flotation.*
- *The EGSB reactor equipped with a sieve-drum separator offers good perspectives for high rate anaerobic treatment of lipid containing wastewaters. This system can achieve a very high purification efficiency at high space loading rates. Wash-out of granular sludge can be reduced to an acceptable level.*
- *Full scale application of the EGSB reactor is, however, not yet possible. Further research on upscaling and lipid flotation is required.*

Anaerobic treatment of sulfate containing wastewater

High sulfate concentrations can cause significant problems in anaerobic digesters. Sulfate reducing bacteria can utilize several intermediates in the anaerobic mineralization process as electron donor, which leads to the formation of hydrogen sulfide.

Hydrogen sulfide is a known inhibitor of methanogenic bacteria. Previous investigations were based on the assumption that the inhibition is completely determined by the concentration of undissociated hydrogen sulfide, irrespective of the pH value. This would imply that the inhibition can be reduced simply by increasing the pH.

Chapter 6 deals with the inhibition of acetotrophic methanogens by hydrogen sulfide. The influence of hydrogen sulfide levels up to approximately 200 mg/l was assessed at various pH levels, in short term batch experiments. Extrapolation of the results indicates that approximately 250 mg H_2S per litre causes a 50% decrease of the maximum specific activity at neutral pH levels. However, the inhibitory effect of undissociated hydrogen sulfide is not completely independent of the pH level. From pH 6.4-7.2 the pH level does not affect the effect of a given H_2S concentration, but at the same concentration the inhibition increases significantly if the pH is increased to a level near 8. Consequently, an increase of the pH level in the anaerobic digester up to ca. 7.2 gives a reduction of the inhibition, but a further increase of the pH level will not reduce the inhibition any further.

The pH effect indicated by our results may be the result of physiological or physical phenomena, i.e. increased sensitivity under suboptimal conditions, or pH gradients in granular sludge respectively. Our research does not clarify the mechanism.

We found a considerably lower inhibitory effect of hydrogen sulfide, compared to the results obtained by others. This may indicate a protective effect of the granular structure, for example resulting from an internal pH gradient.

Previously published research on sulfide inhibition deals almost exclusively with inhibition of acetotrophic methanogens. It was implicitly assumed that methanogenesis from acetate is the most sensitive step in the anaerobic degradation process.

Chapter 7 describes research on the effect of sulfide on propionate degradation. The results indicate that the activity of the propionate degrading population drops very sharply at undissociated hydrogen sulfide concentrations exceeding ca. 100 mg/l, i.e. at concentrations that cause hardly any inhibition of acetotrophic methanogens. Acetate degradation is not the rate limiting step in the mineralization of propionate.

A high sulfate concentration in an industrial wastewater is usually the result of the use of large quantities of sulfuric acid in the production process. Neutralization is required, before such a wastewater can be treated in a biological process. This results in a high salt concentration. Sodium hydroxide is the most appropriate chemical for neutralization of wastewater with a high sulfuric acid concentration, especially because it does not cause excessive precipitation and scaling during the subsequent biological treatment.

The effect of sodium sulfate on acetotrophic methanogens is described in Chapter 8. The inhibitory effect of sodium sulfate was assessed in short term batch experiments, at various substrate and pH levels. At neutral pH levels, sodium concentrations up to 5 g/l cause no inhibition. A sodium concentration of 10 g/l causes a 50% decrease of the maximum specific acetotrophic methanogenic activity, 14 g/l causes complete inhibition. The inhibitory effect of sodium sulfate increases at pH levels near 8, as was the case with hydrogen sulfide. A physiological mechanism is the only logical explanation in the case of sodium salts. The results of the short term batch experiments were confirmed by those obtained in continuously fed UASB reactors.

Contrary to other authors, we found no adaptation of acetotrophic methanogens to high sodium concentrations. We did find growth of these bacteria at a sodium concentration of 10 g/l. Our results indicate that diffusion limitation in biomass granules may mask the inhibition. Consequently, the results of previously published investigations with continuous reactor systems with biofilms or biomass aggregates are considered to be unreliable.

The following guidelines for the maximum permissible concentrations of hydrogen sulfide and sodium salts in anaerobic treatment systems with biofilms or biomass aggregates can be derived from Chapters 6-8:

	acidified wastewater	non-acidified wastewater	
uninhibited sludge activity [a]	1.5 - 2.0	0.6 - 1.0	kg C_2/kg VSS.day
maximum permissible concentration:			
hydrogen sulfide	>> 8.5	2.8 - 8.5 [b]	mol% H_2S in gas
sodium salts	ca. 11.5	6.5 -10.1	kg sodium/m^3

[a] sludge cultivated on the indicated type of wastewater

[b] the lower limit applies to the lowest sludge activity, the upper limit to the highest activity

The following assumptions were made in the derivation of these guidelines: (1) the treatment system operates at 30 °C, (2) hydrogen sulfide or sodium is the only inhibitor, and (3) economical and safe operation of the anaerobic digester is possible when the specific conversion capacity of the biomass is 0.5 kg acetic acid per kg VSS per day. A distinction is made between acidified and non-acidified wastewater, because this may affect the composition and the uninhibited activity of the biomass significantly. Realistic values have been assumed for the uninhibited activity.

The table clearly shows that no general guidelines can be given for unacidified wastewater, because the range for the uninhibited activity is relatively close to the minimum allowable value.

An increase of the pH level in the anaerobic digester up to ca. 7.2 gives a reduction of the inhibitory effect of hydrogen sulfide, but a further increase of the pH level will not reduce the inhibition any further.

It was also assumed that acetotrophic methanogenesis is the most sensitive step in the anaerobic mineralization process. Chapter 7 clearly shows that degradation of propionate may be a more important bottle neck than acetate degradation, when the waste contains organic contaminants that are fermented to propionate, like glycerol and certain amino acids. General guidelines for propionate degradation cannot be given, because insufficient data are available on the uninhibited conversion capacity of the relevant propionate degrading microbial populations.

Samenvatting

Anaerobe biologische zuivering wordt sinds het begin van de jaren '80 in toenemende mate beschouwd als een aantrekkelijk alternatief voor de meer traditionele aerobe biologische zuivering. Vanaf 1970 zijn in snel tempo verschillende technische uitvoeringsvormen voor anaerobe zuiveringsreactoren ontwikkeld, die berusten op het principe van korrel- of biolaagvorming. Het meest succesvolle voorbeeld is de in Nederland ontwikkelde Upflow Anaerobic Sludge Bed reactor (UASB).[1]

Op dit moment zijn wereldwijd al meer dan 100 anaerobe zuiveringsreactoren in bedrijf of in aanbouw. Het merendeel van deze installaties zuivert echter afvalwater met relatief eenvoudig af te breken verontreinigingen en met lage concentraties storende componenten, zoals bijvoorbeeld sulfaat. Dit heeft te maken met problemen die bij eerder verricht laboratoriumonderzoek met complexe afvalwatertypes naar voren zijn gekomen.

Dit proefschrift beschrijft onderzoek naar de anaerobe zuivering van vet- en sulfaathoudend afvalwater. De beschreven experimenten kunnen in twee groepen worden onderverdeeld: de hoofdstukken 2 tot en met 5 beschrijven onderzoek naar de anaerobe zuivering van vethoudend afvalwater, de hoofdstukken 6 tot en met 8 onderzoek naar de problemen die ontstaan bij de zuivering van sulfaathoudend afvalwater. De aanleiding voor de bestudering van twee onderwerpen is de afvalwaterproblematiek in de spijsolieindustrie. Hier kunnen zich zowel hoge vetgehaltes als extreme sulfaatgehaltes voordoen. Beide componenten zijn afzonderlijk bestudeerd, om de algemene toepasbaarheid van de resultaten te verhogen.

Het onderzoek had als doelstellingen:

- de ontwikkeling van een snelle, efficiënte en betrouwbare techniek voor de anaerobe zuivering van vethoudend afvalwater, in eventueel daarop aangepaste anaerobe zuiveringsreactoren, en
- het vaststellen van de condities waaronder afvalwater met een hoge sulfaatconcentratie anaeroob kan worden gezuiverd.

De UASB reactor geënt met korrelslib is als uitgangspunt voor het onderzoek genomen, omdat deze reactor een aantal belangrijke voordelen biedt t.o.v. andere anaerobe reactorsystemen.

Anaerobe zuivering van vethoudend afvalwater

Vetten zijn in principe goed anaeroob afbreekbaar. Bij de technische realisatie van een systeem dat een hoge capaciteit combineert met een zeer efficiënte afbraak en voldoende bedrijfszekerheid kunnen zich echter twee problemen voordoen: (1) remming van de methaanproducerende bacteriën en de vetzuuroxiderende bacteriën door hogere vetzuren, en (2) uitspoeling van de biomassa uit de reactor ten gevolge van flotatie.

Hoofdstuk 2 beschrijft onderzoek naar de remmende werking van hogere vetzuren. In ladingsgewijs gevoede, geroerde tankreactoren zijn de opstartperiode en piekbelastingen bij continu-bedrijf nagebootst met een oplossing van caprinezuur. Het blijkt dat bij de opstart een zekere vertraging (lagfase) in de afbraak van hogere vetzuren optreedt. Wanneer met deze lagfase onvoldoende rekening wordt gehouden, kan het anaerobe slib vergiftigd raken, waarna een wekenlange herstelperiode nodig is. De lagfase duurt korter naarmate de vetzuurconcentratie in het afvalwater lager is.

Een piekbelasting met hogere vetzuren tijdens continu-bedrijf kan eveneens tot vergiftiging leiden. De azijnzuursplitsende methaanproducerende bacteriën zijn de meest kwetsbare groep. De verrichte experimenten tonen aan dat de remming afhankelijk is van de concentratie van de hogere vetzuren, en niet zoals tot nu toe werd aangenomen van de verhouding tussen de hoeveelheid vetzuren en de hoeveelheid bacterieslib. Boven een drempelconcentratie van de hogere vetzuren wordt meer dan 99.9% van de azijnzuursplitsende methaanproducerende

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[1] Opstroom Anaeroob Slibbed reactor

bacteriën gedood, waarna een herstelperiode van ca. 10 weken nodig zal zijn. Deze periode is voor de praktijk ontoelaatbaar lang.

De drempelconcentratie ligt voor caprinezuur in de orde van grootte van 1 kg/m^3. De tolerantie van azijnzuursplitsende methaanproducerende bacteriën blijkt noch bij langdurige blootstelling aan concentraties onder de drempelwaarde, noch bij herhaalde blootstelling aan toxische concentraties toe te nemen.

Tussen verschillende series experimenten traden verschillen in de drempelconcentratie op, die kunnen worden toegeschreven aan verschillen in de stofoverdrachtssnelheid en de deeltjesgrootte en specifieke activiteit van het anaerobe korrelslib. Dit betekent dat het effect van piekbelastingen met hogere vetzuren op continu bedreven anaerobe zuiveringsreactoren nauwelijks kan worden voorspeld op basis van ladingsgewijze experimenten in geroerde tankreactoren. Het betekent ook dat de gevoeligheid van diverse types anaerobe zuiveringsreactoren hoogstwaarschijnlijk zal verschillen.

Mengsels van hogere vetzuren en fosfolipiden blijken sterker te remmen dan hogere vetzuren alleen. Bovendien blijkt de afbraaksnelheid van de hogere vetzuren en de fosfolipiden in het mengsel lager te liggen dan voor de afzonderlijke componenten is vastgesteld. Hogere vetzuren zullen in de praktijk meestal voorkomen in dergelijke mengsels.

Hoofdstuk 3 beschrijft onderzoek naar het effect van stootbelastingen met laurinezuur op de afbraak van azijnzuur in continu gevoede UASB reactoren. De experimenten zijn uitgevoerd met korrelslib dat niet eerder aan laurinezuur was blootgesteld, en daardoor binnen de tijdsduur van de experimenten niet in staat was tot afbraak van dit vetzuur. In een calciumvrije oplossing blijkt laurinezuur vrijwel volledige remming van de azijnzuursplitsende methaanproducerende bacteriën te veroorzaken, indien de concentratie in het inkomende water hoger dan 100 mg/l is. Lagere waarden veroorzaken geen remming. Toevoeging van een equivalente hoeveelheid calciumchloride aan het water voorkomt de remming tot een laurinezuurconcentratie van tenminste 1500 mg/l. Een kleinere hoeveelheid calciumchloride blijkt meer effect te hebben, dan op grond van de vorming van onoplosbaar calciumlauraat was verwacht: een overschot van 700 mg laurinezuur per liter ten opzichte van het calciumzout, geeft nog maar 50% remming van de azijnzuursplitsende methaanproducerende bacteriën. Dit is veel minder dan op basis van de experimenten met calciumvrije oplossingen was verwacht. Waarschijnlijk is er sprake van coprecipitatie van laurinezuur en calciumlauraat.

Behalve remming veroorzaakt een stootbelasting met hogere vetzuren nog een tweede zeer nadelig effect. Bij concentraties boven 100 mg/l in het inkomende water gaat binnen 2 tot 8 uur alle korrelslib drijven. In een conventionele UASB reactor zal dit drijvende slib vrijwel volledig uitspoelen. De slibflotatie kan niet worden voorkomen door toevoeging van calciumzouten.

Hoofdstuk 4 beschrijft de anaerobe afbraak van hogere vetzuren in conventionele en aangepaste UASB reactoren met korrelslib. Caprinezuur en laurinezuur zijn wederom als modelsubstraten gebruikt voor deze experimenten. Een snelle en efficiënte afbraak van hogere vetzuren blijkt alleen mogelijk te zijn, indien het inkomende water zeer snel met de reactorinhoud wordt gemengd en goed in contact wordt gebracht met het korrelslib. In conventionele UASB reactoren kan moeilijk aan deze voorwaarden worden voldaan, waardoor al bij een relatief lage belasting (4-5 kg CZV/m^3.dag) problemen ontstaan. Bij hogere belastingen of bij een kleine storing treedt plaatselijk overbelasting van het anaerobe korrelslib op, waardoor de vetzuren zich ophopen. De ophoping leidt tot de vorming van neerslagen, waardoor de afbraak nog verder wordt bemoeilijkt. De neerslagen gaan uiteindelijk drijven en dit veroorzaakt sterke uitspoeling van korrelslib.

Een efficiënte afbraak van hogere vetzuren bij hoge belastingen is wel mogelijk in een Expanded Granular Sludge Bed (EGSB)[2] reactor. Dit is een aangepaste UASB reactor, waarin gezuiverd water wordt terug gepompt naar de invoer, om een snelle verdunning van het inkomende water te bereiken, en een hogere opwaartse vloeistofsnelheid in de reactor, waardoor het korrelslibbed expandeert. Met caprinezuur en laurinezuur kan in de EGSB reactor bij belastingen van ca. 30 kg CZV/m^3.dag tenminste 85-90% van het toegevoerde vetzuur worden gemineraliseerd. Er deden zich tijdens langdurig continu bedrijf geen noemenswaardige problemen voor met drijvend slib. De verblijftijd van het afvalwater kon worden verlaagd tot 2 uur, zonder dat uitspoeling van korrelslib optrad. Bovendien bleken nieuwe slibkorrels gevormd te worden.

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2 Geëxpandeerd Korrelslibbed reactor

Na de succesvolle experimenten met hogere vetzuren in EGSB reactoren is deze reactor onderzocht voor de zuivering van triglyceriden-emulsies (hoofdstuk 5). Hiermee werden ook goede resultaten verwacht, omdat de hydrolyse van triglyceriden sneller verloopt dan de afbraak van de hogere vetzuren. De triglyceriden-emulsies bleken echter al bij zeer lage belastingen sterke slibflotatie te veroorzaken. Een aanpassing van de slibafscheider van de EGSB reactor is vereist om de uitspoeling van het anaerobe korrelslib terug te brengen naar een aanvaardbaar niveau. Twee aanpassingen zijn onderzocht: (1) een hybride EGSB reactor met een laag drijvend polyurethaanschuim boven het korrelslibbed, en (2) een EGSB reactor met een trommelzeef-afscheider.

De hybride EGSB reactor met drijvend polyurethaanschuim blijkt de uitspoeling van drijvend korrelslib onvoldoende te beperken. Dit systeem is dus niet geschikt voor anaerobe zuivering van vethoudend afvalwater.

De EGSB reactor met trommelzeef-afscheider kan wel voldoende korrelslib terughouden om een stabiel zuiveringsproces mogelijk te maken. Een toename van de hoeveelheid korrelslib in de reactor door vermenigvuldiging van de bacteriën is mogelijk, indien een entslib met voldoende grote korrels wordt gekozen. Vooral als het afvalwater naast vetten ook eenvoudig afbreekbare organische verontreinigingen bevat, zal het geen probleem zijn om voldoende korrelslib in de reactor te houden. De EGSB reactor met trommelzeef-afscheider kan hogere belastingen verwerken dan in de literatuur beschreven gepakt bed reactoren.

Een probleem dat nog om een oplossing vraagt, is de flotatie van vet in de EGSB reactor. In de experimenten met laurinezuur hoopte naar schatting 10% van het toegevoerde vetzuur zich op in een drijflaag. In de experimenten met triglyceriden-emulsies was dit percentage aanzienlijk hoger. Dit probleem zal zich ook met industrieel afvalwater voordoen. De gevormde drijflaag bestaat grotendeels uit neergeslagen vetzuren, en bevat slechts weinig anaeroob korrelslib. Het drijvende vet is weliswaar uit het water verwijderd, maar het wordt niet snel genoeg omgezet in methaan, omdat het niet meer in contact komt met het korrelslibbed. Dus is nog een verdere aanpassing van de EGSB reactor nodig, zodat het drijvende materiaal hetzij naar het korrelslibbed kan worden teruggevoerd, hetzij uit de reactor kan worden verwijderd en in een aparte reactor kan worden vergist.

Met betrekking tot anaerobe zuivering van vethoudend afvalwater in de praktijk kunnen uit het onderzoek de volgende conclusies worden getrokken:

- *De lagfase bij de opstart van een anaerobe zuiveringsinstallatie voor vethoudend afvalwater kan worden verkort door de vetconcentratie in het inkomende water te verlagen. Overbelasting tijdens de lagfase kan tot vergiftiging door hogere vetzuren leiden.*
- *Geëmulgeerde vetzuuresters versterken de remmende werking van hogere vetzuren.*
- *Een piekbelasting met vetten tijdens continu-bedrijf treft vooral de azijnzuursplitsende methaanproducerende bacteriën. Al bij vetconcentraties lager dan 1 kg/m^3 kan 99.9% van deze bacteriën worden gedood. De gevoeligheid van verschillende reactortypes voor hogere vetzuren zal waarschijnlijk uiteenlopen ten gevolge van verschillen in de stofoverdrachts-karakteristieken.*
- *Slibuitspoeling is een grotere bedreiging voor de stabiliteit van de anaerobe zuivering dan remming. Indien de concentratie van hogere vetzuren na een piekbelasting oploopt tot slechts 100 mg/l kan al binnen enkele uren volledige slibflotatie optreden. Remming kan in principe worden voorkomen door toevoeging van calciumzouten, maar flotatie van korrelslib niet.*
- *De EGSB reactor met trommelzeef-afscheider biedt goede perspectieven voor de hoogbelaste anaerobe zuivering van vethoudend afvalwater. Met dit systeem kan een zeer hoge zuiveringsgraad worden bereikt bij een hoge belasting. De uitspoeling van korrelslib kan tot een aanvaardbaar nivo worden beperkt.*
- *De EGSB reactor is echter nog niet rijp voor toepassing op industriële schaal. De schaalvergroting van de EGSB reactor en de verwerking van floterende vetten vereisen nog nader onderzoek.*

Anaerobe zuivering van sulfaathoudend afvalwater

Hoge concentraties sulfaat kunnen belangrijke problemen veroorzaken in een anaerobe zuiveringsreactor. Sulfaatreducerende bacteriën zijn in staat diverse tussenprodukten in het anaerobe vergistingsproces om te zetten, waardoor waterstofsulfide ontstaat.

Het is bekend dat waterstofsulfide een sterke remmende werking heeft op de methaanproducerende bacteriën. Bij voorgaand onderzoek is men er steeds van uitgegaan dat de remming volledig wordt bepaald door de concentratie van het ongedissocieerde waterstofsulfide, en dat dit effect niet afhangt van de zuurgraad. Dit zou betekenen dat sturing van de zuurgraad in de anaerobe reactor een effectieve en eenvoudige mogelijkheid biedt om de remmende werking van waterstofsulfide te beperken.

Hoofdstuk 6 beschrijft onderzoek naar de remmende werking van waterstofsulfide op de methaanvorming uit azijnzuur. In kortdurende experimenten met ladingsgewijs gevoede reactoren is de invloed van ongedissocieerd waterstofsulfide tot een concentratie van ca. 200 mg/l onderzocht bij verschillende waarden van de zuurgraad. Door extrapolatie van de meetresultaten kan worden berekend dat bij neutrale pH-waarden 50% remming optreedt bij ca. 250 mg ongedissocieerd waterstofsulfide per liter. Het blijkt echter, dat de remmende werking van waterstofsulfide niet volledig onafhankelijk is van de zuurgraad. Bij pH-waarden tussen 6.4 en 7.2 is geen invloed van de zuurgraad meetbaar, maar pH-waarden in de buurt van 8 versterken het remmende effect van een bepaalde concentratie waterstofsulfide. Dit betekent dat een verhoging van de pH in de anaerobe reactor tot ca. 7.2 tot een vermindering van de remming leidt, maar dat een verdere pH-verhoging geen vermindering van de remming meer oplevert.

Het gevonden pH-effect kan zowel een gevolg zijn van fysiologische als van fysische factoren, d.w.z. een grotere gevoeligheid onder ongunstige omstandigheden, respectievelijk pH-gradiënten in de anaerobe slibkorrels. Ons onderzoek geeft geen uitsluitsel over de precieze oorzaak.

Het door ons vastgestelde effect van waterstofsulfide is veel geringer dan door andere onderzoekers is gevonden. Dit kan wijzen op een beschermende werking van de korrelstructuur, bijvoorbeeld door een interne pH-gradient.

Eerder beschreven onderzoek naar de remmende werking van sulfide is vrijwel uitsluitend gericht geweest op de remming van azijnzuursplitsende methaanproducerende bacteriën, waarbij impliciet is aangenomen dat de methaanproduktie uit azijnzuur de gevoeligste stap in het totale afbraakproces is.

In hoofdstuk 7 wordt onderzoek naar de gevoeligheid van propionzuurafbrekend korrelslib voor waterstofsulfide beschreven. Het blijkt dat de activiteit van de propionzuur afbrekende bacteriepopulatie scherp daalt bij waterstofsulfideconcentraties boven ca. 100 mg/l, d.w.z. bij concentraties die nog nauwelijks remming van de azijnzuursplitsende methaanproducerende bacteriën geven. De afbraak van azijnzuur is niet de snelheidsbepalende stap bij de mineralisatie van propionzuur.

Zeer hoge sulfaatgehaltes in afvalwater zijn meestal een gevolg van het gebruik van grote hoeveelheden zwavelzuur in het produktieproces. Voor de biologische zuivering van dergelijk afvalwater is neutralisatie vereist, waardoor grote hoeveelheden zouten in het afvalwater terecht komen. Natronloog komt het meest in aanmerking voor neutralisatie van afvalwater met een hoog zwavelzuurgehalte, met name omdat hierdoor bij de biologische zuivering geen problemen met neerslagvorming zullen ontstaan.

De invloed van natriumsulfaat op de methaanproduktie uit azijnzuur door anaeroob korrelslib komt aan de orde in hoofdstuk 8. In kortdurende experimenten met ladingsgewijs gevoede reactoren is de invloed van natriumsulfaat onderzocht bij verschillende waarden van de azijnzuurconcentratie en de zuurgraad. Bij neutrale pH-waarden hebben natriumconcentraties tot ca. 5 g/l geen belangrijk nadelig effect. Bij 10 gram natrium per liter treedt een daling van 50% in de maximale specifieke azijnzuursplitsende methanogene activiteit op, en bij 14 g/l volledige remming. De invloed van natriumsulfaat neemt toe indien de zuurgraad wordt verhoogd tot pH 8, zoals ook voor waterstofsulfide is gevonden. Een fysiologische oorzaak is in het geval van natriumzouten de enige aannemelijke verklaring. Controle-experimenten met continu gevoede UASB reactoren geven aan dat bovengenoemde kortdurende proeven een betrouwbaar beeld geven van het effect van natrium.

In tegenstelling tot andere onderzoekers vinden wij geen aanpassing van azijnzuursplitsende methaanproducerende bacteriën aan hoge natriumconcentraties. Wel blijken deze bacteriën

zich bij een natriumconcentratie van 10 g/l nog te vermenigvuldigen. Ons onderzoek geeft aan dat beperkingen in de aanvoer van substraat naar de bacteriën binnen in de korrels (diffusielimitatie) remmende effecten kunnen maskeren. Eerder gepubliceerde resultaten van experimenten in continue reactoren met biofilms of korrelslib zijn daarom onbetrouwbaar.

Uit hoofdstukken 6-8 kunnen onderstaande richtlijnen worden afgeleid voor de maximaal toelaatbare concentraties van waterstofsulfide en natriumzouten in een anaerobe waterzuiveringsinstallatie met korrelslib of biolagen op een dragermateriaal:

	voorverzuurd afvalwater	niet-verzuurd afvalwater	
ongeremde slibactiviteit [a]	1.5 - 2.0	0.6 - 1.0	kg C_2/kg VSS.dag
maximaal toelaatbaar:			
waterstofsulfide	>> 8.5	2.8 - 8.5 [b]	mol% H_2S in gas
natriumzouten	ca. 11.5	6.5 -10.1	kg natrium/m^3

[a] voor slib gekweekt op het betreffende afvalwater
[b] de laagste grenswaarde geldt bij de laagste slibactiviteit, de hoogste grenswaarde bij de hoogste slibactiviteit

Bij het opstellen van bovenstaande richtlijnen zijn de volgende aannames gedaan: (1) de zuiveringsinstallatie wordt bedreven bij 30 °C, (2) waterstofsulfide of natriumsulfaat is de enige remmende stof, en (3) een economische en veilige procesvoering is mogelijk als de anaerobe biomassa een omzettingscapaciteit heeft van 0.5 kg azijnzuur per kg organische stof per dag. Er wordt onderscheid gemaakt tussen de zuivering van voorverzuurd en niet-verzuurd afvalwater, aangezien dit een sterke invloed heeft op de samenstelling en daardoor op de activiteit van de biomassa in de anaerobe reactor. Voor de ongeremde activiteit van het gekweekte slib zijn realistische waarden aangenomen.

Uit de tabel blijkt dat met name voor de zuivering van niet-verzuurd afvalwater geen algemene grenswaarde is aan te geven, omdat het traject voor de ongeremde slibactiviteit hier relatief dicht bij de aangenomen minimumwaarde ligt.

Verhoging van de pH in de anaerobe reactor tot ca. 7.2 geeft een vermindering van de remmende werking van waterstofsulfide, maar verdere verhoging van de pH levert geen verdere vermindering van de remming meer op.

Bij de berekening is ook aangenomen dat de methaanproduktie uit azijnzuur de meest gevoelige omzetting is. Hoofdstuk 7 geeft aan dat de propionzuurafbraak een belangrijker knelpunt kan zijn dan de azijnzuurafbraak, indien het afvalwater organische verontreinigingen bevat die via propionzuur als voornaamste tussenprodukt worden omgezet, zoals bijvoorbeeld glycerol en bepaalde aminozuren. Algemeen geldige richtlijnen voor de propionaatafbraak zijn niet te geven, omdat onvoldoende gegevens bekend zijn over de ongeremde omzettingscapaciteit van de relevante propionaat afbrekende consortia.

Curriculum vitae

De auteur van dit proefschrift is op 15 mei 1958 geboren in Drachten. In 1976 behaalde hij het diploma gymnasium ß aan de Rijksscholengemeenschap Het Drachtster Lyceum. In hetzelfde jaar begon hij met de studie Technische Scheikunde aan de Rijksuniversiteit Groningen, met als bijvak Milieukunde. In 1983 legde hij *cum laude* het doctoraalexamen af. Van september 1983 tot januari 1988 voerde hij als tijdelijk wetenschappelijk medewerker bij de vakgroep Waterzuivering van de Landbouwuniversiteit Wageningen het onderzoek uit dat in dit proefschrift wordt beschreven. Vanaf januari 1988 is hij als wetenschappelijk researchmedewerker werkzaam bij de afdeling Milieutechnologie van de Hoofdgroep Maatschappelijke Technologie van TNO te Apeldoorn.