THE ANAEROBIC TREATMENT OF SULFATE CONTAINING WASTEWATER

Promotor: Dr. ir. G. Lettinga
bijzonder hoogleraar in de anaërobe waterzuivering

Co-promotor: Dr. ir. L.W. Hulshoff-Pol
universitair docent Landbouwuniversiteit

André Visser

THE ANAEROBIC TREATMENT OF SULFATE CONTAINING WASTEWATER

Proefschrift
ter verkrijging van de graad van doctor
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op gezag van de rector magnificus,
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Voor Leonore, Iris, Dylan en Roos.

Stellingen

behorend bij het proefschrift "Anaerobic treatment of sulfate containing wastewater"
A. Visser.

Wageningen 12 september, 1995

1 De aanwezigheid van al of geen sulfaat heeft met betrekking tot de toepasbaarheid van anaërobe zuivering geen tot weinig invloed.

Dit proefschrift

2 Sulfidogene systemen zijn ongevoeliger voor veranderingen in milieu condities (pH temperatuur) dan methanogene systemen.

Dit proefschrift

3 Er wordt ten onrechte van uitgegaan dat de toxiciteit van sulfide uitsluitend bepaald wordt door ongedissocieerd waterstofsulfide

Dit proefschrift

4 In de interpretatie van de resultaten van het onderzoek naar het effect van sulfide op methanogene- en sulfaatreducerende bacteriën stellen McCartney en Oleskiewicz ten onrechte dat deze sulfide-toxiciteit bepalend is voor de competitie tussen deze bacteriën.

McCartney and Oleskiewicz (1991). Wat. Res. **25**: 203-209.
Oleskiewicz et al. (1989). Environ. Technol. Lett. **10**: 815-822

5 Het instellen van de functie van assistent in opleiding is een in feite niets anders dan een verkapte bezuinigings poging van het ministerie waarmee promotie onderzoek onmogelijk wordt gemaakt.

6 Het mondiale milieu zou er veel meer bij gebaat zijn indien een belangrijk deel van de inspanningen verricht in west-europa ter perfectionering van de kwaliteit van het milieu aldaar, aangewend zou worden om een begin te maken met de sanering van de milieu problemen in voormalige oostblok- en derde wereld landen.

7 Indien milieu problemen uitsluitend economisch benaderd worden blijven zij onopgelost, en daarmee ook onoplosbaar.

8 Het hanteren van positieve discriminatie bij het opvullen van vacatures benadrukt alleen maar de ongelijkheid van de betrokken groepering en leidt niet tot de nagestreefde integratie.

9 In een europa zonder grenzen zou het voor een land als Frankrijk onmogelijk moeten zijn om redenen van zogenaamd nationaal belang kernproeven uit te voeren.

10 "Morgen stop ik met roken".

11 Er bestaan slechts zogenaamde "verschillen" tussen Nederlanders en Belgen.

12 De beste houtlijm voor een lat-relatie is onderling vertrouwen

VOORWOORD

Het schrijven van dit proefschrift was niet mogelijk geweest zonder de medewerking van de vele personen die zowel direct als indirect hieraan hebben bijgedragen.

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ABSTRACT

Visser A (1995), The anaerobic treatment of sulfate containing wastewater, Doctoral thesis, Wageningen Agricultural University, Wageningen, The Netherlands.

In the anaerobic treatment of sulfate containing wastewater sulfate reducing bacteria (SRB) will compete with methanogenic- (MB) and acetogenic bacteria (AB) for the available substrates such as hydrogen, acetate, propionate and butyrate. The outcome of this competition will determine the endproduct of the anaerobic mineralisation proces: methane or sulfide.

The occurrence of the sulfate reduction proces is often considered unwanted due to the problems associated with the sulfide formed in the proces. These problems are: malodour, corrosion, toxicity, reduced removal of COD, reduced methane formation and higher levels of H_2S in the biogas. More recently the sulfate reduction proces is used in biological processes that aim at the removal of oxidised sulfur compounds from different waste streams.

In the competition between the SRB and AB this study shows that butyrate degrading AB can effectively compete with the SRB. The growth rates of both bacteria was found in the same range. On the contrary, propionate degrading AB are outcompeted by the SRB due to the better growth kinetic properties of the latter.

Concerning the competition between the SRB and MB for hydrogen the present study clearly shows that in anaerobic reactors hydrogenotrophic MB are outcompeted by the SRB. This apply both for mesophilic (30 °C) as for thermophilic (55 °C) conditions. However, the hydrogenotrophic MB are not expelled from the biomass but remain present in relative high number.

The competition between the acetotrophic MB (AMB) and acetotrophic SRB (ASRB) depends on several conditions. In this research the following items were investigated:

* The kinetic growth properties of AMB and ASRB under different conditions with respect to the pH and sulfide concentration.
* The ability of the ASRB and AMB to attach to granular sludge or a biofilm.
* The competition between the bacteria at higher temperatures (55 °C)

The results of these studies are:

* At neutral or acidic pH values the AMB can compete with the ASRB. The growth rates and acetate affinities for both bacteria are than in the same range. Moreover, at these pH values the AMB and ASRB are more or less equally inhibited by the toxic sulfide. At more alkalic pH values (pH > 7.5) the ASRB likely will outcompete the AMB. At these pH values the growth rates of the ASRB are significant higher then for the AMB and the ASRB are much less inhibited by the produced sulfide.
* Granulation experiments shows that the ASRB can maintain in granular sludge, resulting in the formation of sulfidogenic granular sludge. They are also effectively able to form a biofilm on pumice as a carrier. No significant difference between the attachment capacity of AMB or ASRB could be detected.
* Under thermophilic (55 °C) conditions the ASRB can compete with the AMB. At higher pH values than 7.5 the ASRB even become predominant. At more neutral pH values there exist an equilibrium between the ASRB and AMB.

CIP-DATA KONINKLIJKE BIBLIOTHEEK, DEN HAAG

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CHAPTER 1

Anaerobic treatment of wastewaters with high levels of sulfate-a literature review

1.1 Introduction

Prior to the 1970's anaerobic digestion processes were almost exclusively used for the digestion of sewage sludge and manure. Anaerobic wastewater treatment was not considered as an alternative to aerobic treatment systems. The anaerobic process was considered to be too sensitive and unstable for wastewater treatment and due to the low growth rate of anaerobic bacteria it was presumed that high loading rates would not be applicable. This situation changed with the development of new reactor types, based on sludge immobilisation and sludge retention, such as the anaerobic contact process, the anaerobic filter, the fluidised bed reactor and the upflow anaerobic sludge bed (UASB) reactor. In these systems, the solids retention time is uncoupled from the liquid retention time. As a result high biomass concentrations are maintained in the reactor and high space loading rates can be applied. Reviews of these systems are given by Henze and Harremoes (1983), Speece (1983), Van den Berg (1983) and Switzenbaum (1983).

The anaerobic wastewater treatment system with the widest application is the UASB reactor, a system developed in the Netherlands (Lettinga et al. 1980, 1983). In the UASB reactor the sludge is retained as a result of the formation of a well settleable highly active granular sludge. At present more than 400 full scale UASB reactors are used for the treatment of a variety of different types of wastewaters.

Several wastewater streams for which anaerobic treatment is considered to be economically attractive, have high concentrations of sulfate, sulfite or other oxidised sulfur compounds. Examples are wastewaters produced by the pulp- and paper-industry, wastewaters originating from the fermentation industry and wastewaters from the edible oil production. During the anaerobic treatment of these wastewaters, additionally to methanogenesis, sulfate reduction will occur as a second end step of the anaerobic mineralisation process. The occurrence of the sulfate reduction process has several advantages and disadvantages. The main disadvantages are :

* A part of the organic compounds in the wastewater will be used for the reduction of sulfate and is not available for the production of methane. This results in a lower methane yield per unit of organic waste and therefore negatively affects the overall energy balance of the process. Moreover, the quality of the biogas is reduced since a part of the produced sulfide ends up as H_2S in the biogas. Removal of H_2S from the biogas is therefore usually required.

* A part of the sulfide will be present in the effluent of the anaerobic reactor. This results in a lower overall treatment efficiency of the system. A post treatment system to

remove the sulfide from the wastewater is essential.

* Sulfide is an inhibiting compound for anaerobic bacteria, including methanogenic- (MB), acetogenic- (AB), and sulfate reducing bacteria (SRB). Accumulation of sulfide can result in a severe inhibition of the purification process, and might even cause a total process failure. Extra measures often are necessary to prevent a toxic accumulation of sulfide.

* The produced sulfide can cause malodour and corrosion problems to engines, boilers, pipes etc. Extra investments will be necessary to avoid these problems or the maintenance costs of the installation will increase.

Advantages of sulfate reduction are :

* The application of sulfate reduction in combination with sulfide removal techniques such as the biological sulfide oxidation to elemental sulfur, can be used for the removal of oxidised sulfur compounds from wastewaters.

* Heavy metals present in the wastewaters will precipitate as metalsulfides and can then be removed. The precipitation of metalsulfides will also reduce potential toxicity problems caused by heavy metals to the anaerobic digestion process.

* The reduction of the very toxic sulfite to the less toxic sulfide increase the potentials of anaerobic treatment of sulfite containing waste streams.

Occurrence of the sulfate reduction during the anaerobic treatment process often still is considered undesirable due to the problems associated with the process. In the past, attempts have been made to suppress sulfate reduction using the inhibitor molybdate. Molybdate has been used successfully for the inhibition of SRB in batch systems inoculated with sea- and freshwater sediments (Banat 1981, Smith and Klug 1981, Winfrey and Ward 1983). However, studies with continuously fed reactors showed that dosing of molybdate at inhibitory concentrations for SRB, also repressed MB (Hilton and Archer 1988, Lo and Liao 1990, Yadav and Archer 1989 ; Puhakka et al 1990). Furthermore, adaptation of SRB to molybdate occurred. Therefore, with time the concentration of molybdate had to be increased to maintain an effective suppression of the sulfate reduction process (Gao 1989, Yadav and Archer 1989). Consequently, application of molybdate seems little promising. Sofar, no selective inhibitor of SRB is known that can be used in full scale anaerobic reactors. This implies that sulfate reduction can not be prevented in practice.

There is a growing interest in the application of the sulfate reduction process. Some practical examples of the application of the process are the simultaneous removal of heavy metals and sulfate from groundwater (Barnes et al. 1991, Scheeren et al. 1991), sulfate removal from waste sulfuric acid (Hürzeler and Stucki 1990, Stucki et al. 1993), mining wastewaters (Maree et al. 1987, Maree et al. 1992) and other industrial sulfate-rich wastewater streams (Buisman et al. 1993, Särner 1989, Maree and Strydom 1985), the application of sulfate reduction in the bioleaching of contaminated soils (van Houten 1994) and desulfurization of waste gasses (C.N.J. Buisman, personal communication). In the Netherlands the standard for the discharge of sulfate on the sewer and the surface waster is

300 mg.l^{-1}. Therefore, removal of sulfate from the wastewater can be necessary. In addition to chemical and physical-chemical sulfate removal processes, such as precipitation with calciumhydroxide into gypsum, reversed osmosis and electrodialysis, biological treatment using the biological sulfur cycle is an alternative. The biological sulfur cycle is schematically shown in Fig 1.1.

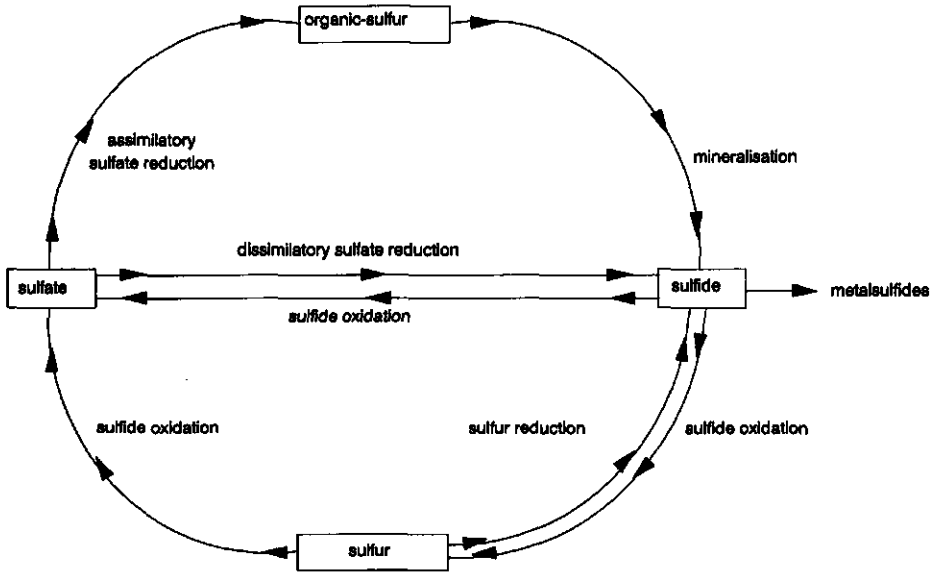

Fig 1.1. The biological sulfur cycle.

The cycle consists of several steps.

* *The assimilatory sulfate reduction.* In this proces sulfate is used as sulfur-source for the biosynthesis of organic-sulfur compounds in plants, fungi and micro-organisms.

* *Mineralisation.* The dissimilation of dead organic matter from plants or micro-organisms, resulting in the formation of sulfide. The produced sulfide can be oxidised or will precipitate, e.g. with heavy metals.

* *sulfide oxidation.* Under conditions of sufficient oxygen or nitrate the oxidation of sulfide can proceed spontaneously. Biological oxidation of sulfate proceeds under aerobic, anoxic and anaerobic conditions. In the presence of oxygen or nitrate the colourless sulfur bacteria oxidise sulfide until sulfur or sulfate. The electrons of sulfide are used to convert oxygen or nitrate into H_2O and N_2. The electrons can also be used for the reduction of CO_2. Anaerobic sulfide oxidation is performed by the fototrophic sulfur bacteria. These bacteria use the

sulfide electrons for the reduction and assimilation of CO_2. The energy is gained from the sunlight.

* *(Dissimilatory) sulfur reduction.* The biological reduction of elemental sulfur to sulfide. This reaction is done by the sulfur reducing bacteria. Often acetate is used as the electrondonor.

* *(Dissimilatory) sulfate reduction.* The biological reduction of sulfate by the sulfate reducing bacteria. These bacteria use H_2 and organic compounds as the electrondonor.

The biological treatment of waste streams with high levels of sulfate using the (biological) process of the sulfur-cycle consists of two steps. In the first part of this biological process, sulfate is (dissimilatory) reduced to sulfide. Important aspects in this first stage are the ability of the SRB to compete with the MB for the available organic substrate, and the sensitivity of the bacteria for toxic levels of sulfide. In the second part of the biological treatment process the sulfide from the first stage is biologically oxidized to elemental sulfur as end product, using the aerobic colourless sulfur bacteria. This process was developed at the Agricultural University of Wageningen (Buisman 1989).

The research described in this thesis deals with sulfate reduction and methanogenesis in anaerobic reactors, treating sulfate containing model substrates under mesophilic and thermophilic conditions.

1.2 The anaerobic digestion process at low and high sulfate concentrations

If the concentration of electron acceptors like sulfate or nitrate is negligible, anaerobic digestion of organic matter consists of several steps, by which complex organic matter is degraded into carbon dioxide and methane as the simple end products (Gujer and Zehnder 1983). The process is illustrated in Fig. 1.2, in which the following 5 steps can be distinguished :

1. *Hydrolysis :* liquefaction of complex insoluble organic matter. In this step carbohydrates, proteins and lipids are hydrolysed to the monomeric sugars, amino acids, polyols and long chain fatty acids, respectively ;
2. *Acidogenesis :* fermentation of the soluble compounds (sugars, amino acids and polyols) to volatile fatty acids, hydrogen, carbon dioxide and small amounts of ethanol and lactic acid ;
3. *β-oxidation :* oxidation of long chain fatty acids yielding acetate for even numbered fatty acids and acetate plus propionate for odd numbered fatty acids ;
4. *Acetogenesis :* oxidation of volatile fatty acids formed in the acidogenesis yielding acetate, hydrogen and depending on the chain length of the fatty acids also carbon dioxide.
5. *Methanogenesis :* formation of methane by decarboxylation of acetate by acetotrophic MB (AMB) and by hydrogenization of carbon dioxide by hydrogenotrophic MB (HMB).

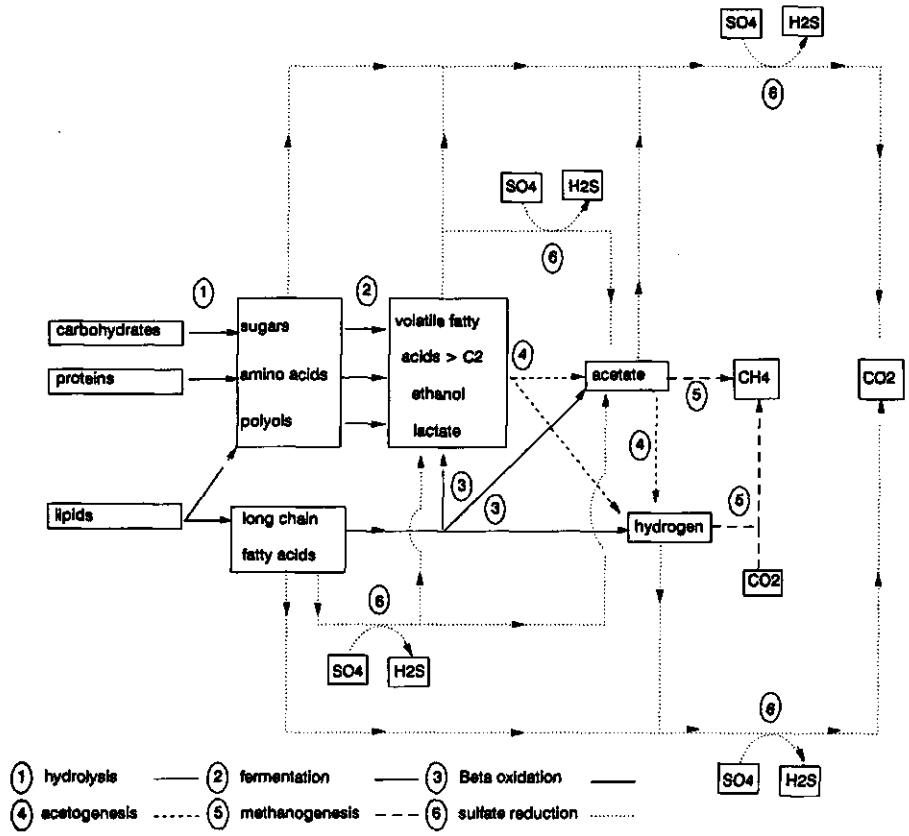

Fig 1.2. Scheme of the anaerobic degradation of organic compounds in the presence of sulfate

The anaerobic degradation of organic matter under methanogenic conditions has been extensively reviewed by others (Mc Carty 1982, Gujer and Zehnder 1983, Koster 1988), and will not be discussed in detail here.

The most important intermediates in the anaerobic digestion process are acetate, propionate and butyrate. About 70 % of the COD of anaerobically digested sewage sludge is transferred via acetate to methane (Gujer and Zehnder 1983). The contribution of propionate and butyrate to the produced methane depends on the nature of the organic compounds and is about 20 to 45 % (Mackie and Bryant 1981, Mah et al. 1990).

Fatty acids with more than two carbon atoms are degraded by the AB. Most of the reactions carried out by the AB are highly endergonic. However, if the partial- pressure of

H_2 is kept low, these reactions become exergonic. In methanogenic ecosystems the hydrogen concentration is maintained low by syntrophic consortia of AB and HMB (Dolfing 1988, Schink 1992). It has been shown that at 55 °C acetate can be degraded via an acetogenic oxidation to hydrogen and carbondioxide. This reaction is coupled with hydrogen consumption by the HMB (Zinder and Koch 1984). At 75 °C this pathway seems to be the major route for the degradation of acetate (J. van Lier, personal communication). The extent to which this route also occurs at 30 °C is unknown. Blomgren et al. (1990) reported that under the stress of high ammonia concentrations acetate conversion via a syntrophic association of AB and HMB is important. At low ammonia concentration the direct conversion of acetate by AMB was the most important route.

MB have a very limited substrate spectrum, consisting of hydrogen, acetate, formate, methanol and methylamines (Vogels 1988). Sofar, only *Methanosarcina* sp. and *Methanothrix* sp. are known to be able to degrade acetate (Jetten et al. 1992, Vogels 1992). *Methanothrix* species are of great importance in modern high rate anaerobic reactors. Observations in sludge granules and biofilms have indicated that *Methanothrix* is the predominant AMB in anaerobic reactors (Koorneef et al. 1990, Mac Leod et al. 1990, Grotenhuis 1992, Hulshoff Pol 1989). This has been explained by the higher growth rates of *Methanothrix* sp. at the low acetate concentrations, that are normally found in proper operated anaerobic reactors (Gujer and Zehnder 1983, Jetten et al. 1992).

When sulfate, sulfite or thiosulfate is present in wastewaters, SRB are able to use several intermediates of the anaerobic mineralisation process (Fig 1.2). Under sulfidogenic conditions the following reactions can occur :

* Oxidation of fatty acids with more than two carbon atoms by SRB. Two oxidation patterns can be distinguished here. Firstly an incomplete oxidation with acetate and sulfide as endproducts, and secondly a complete oxidation with CO_2 and sulfide as endproducts.
* Oxidation of acetate by acetotrophic SRB (ASRB) and molecular hydrogen by hydrogenotrophic SRB (HSRB).

SRB form a group of strict anaerobic bacteria, that share the ability to use sulfate as electron-acceptor for the oxidation of molecular hydrogen and a variety of organic compounds. The knowledge available about the SRB have recently extensively been reviewed (Widdel 1988, Widdel 1992, Widdel and Bak 1992, Widdel and Hansen 1992), and will not be discussed in detail. The SRB can be divided into two main groups :

- SRB oxidizing the substrate incompletely with acetate as the end-product. To this group belong the genera *Desulfovibrio*, *Desulfotomaculum*, *Desulfomonas*, *Thermodesulfobacterium* and *Desulfobulbus*.
- SRB oxidizing the substrate completely to carbon dioxide. To this group belong *Desulfobacter*, *Desulfococcus*, *Desulfosarcina*, *Desulfobacterium* and *desulfonema*.

SRB can use many substrates as electron-donor, such as molecular hydrogen, formate, acetate, propionate, butyrate and higher fatty acids, branched fatty acids, lactate, methanol,

ethanol and higher alcohols, fumarate, succinate, malate and aromatic compounds. In addition to the reduction of sulfate, reduction of sulfite and thiosulfate is also very common among SRB (Widdel and Hansen 1992). *Desulfovibrio* strains have been reported to be able to reduce thri-thionate, tetra-thionate and di-thionate (Fitz and Cypionka 1990, Postgate 1951).

A unique ability of some SRB is the dismutation of sulfite or thiosulfate, a process that may formally be termed an inorganic fermentation (Widdel and Hansen 1993). *Desulfovibrio dismutans* and *Desulfobacter curvatus* carry out the following reactions :

$4\ SO_3^{2-} + H^+ \rightarrow 3\ SO_4^{2-} + HS^-$ $\quad \triangle G^{o'} = -235.6$ KJ

$S_2O_3^{2-} + H_2O \rightarrow SO_4^{2-} + HS^- + H^+$ $\quad \triangle G^{o'} = -21.9$ KJ

In the absence of a sulfur containing electron-acceptor SRB are able to grow through a fermentative or acetogenic reaction. E.g., pyruvate, lactate, and ethanol are easily fermented by many SRB (Laanbroek et al. 1982 , Postgate 1984 , Stams et al. 1984, Stams et al. 1985, Widdel and Pfennig 1982). An interesting feature of the SRB is the ability to perform acetogenic oxidation in syntrophy with HMB. A syntrophic degradation of lactate and ethanol has been found for *Desulfovibrio* species which were co cultured with HMB (Bryant et al. 1977, Mc. Inerney and Bryant 1981, Traore et al. 1983, Yadav and Archer 1988, Tatton et al. 1989). More recently Wu et al. (1991) found that *Desulfobulbus*-like bacteria can degrade propionate, an important intermediate in the anaerobic digestion process, via an acetogenic oxidation in syntrophy with HMB. In the presence of sulfate the bacteria reacted as true SRB using propionate as electron-donor for the reduction of sulfate. Also Heppner et al. (1992) and Zellner et al. (1992) reported the acetogenic oxidation of propionate by *Desulfobulbus* sp. in experiments with a fluidized bed and a fixed bed reactor, respectively. In addition, Wu et al. (1992) found that in granular sludge, adapted to a VFA-mixture and brewery wastewater, in absence of sulfate, the SRB conduct the acetogenic oxidation of ethanol and especially propionate. So far no acetogenic oxidation of butyrate by SRB has been observed. However, according to Heppner et al. (1992) there are indications that this process also proceeds.

In the presence of sulfate obligatory hydrogen producing AB and MB have to compete with SRB for substrates such as hydrogen, acetate, propionate and other fatty acids. The outcome of this competition will determine to what extent sulfide and methane, the end-products of the anaerobic mineralization process, will be produced. The importance of this competition increases with a decrease in the COD/sulfate ratio of the wastewater.

1.3 Competition between SRB and MB or SRB and AB

The outcome of the competition between bacteria can be predicted using the kinetic growth and decay properties of the organisms involved. Growth of bacteria is generally described using Monod kinetics of bacterial growth, namely the specific growth rate (μ_{max}) and the

substrate affinity (k_s). In CSTR systems, if two bacteria are in competition for the same substrate, the organism with the higher μ_{max} will pre-dominate at high substrate concentrations. At low substrate concentrations the μ_{max}/k_s-ratio gives an indication of the outcome of the competition. Bacteria with a higher μ_{max}/k_s-ratio then have a kinetic advantage.

1.3.1 Competition for hydrogen

Tables 1.1 and 1.2 reveal that the thermodynamic and kinetic data predict an advantage of HSRB over HMB. HSRB gain more energy from the consumption of molecular hydrogen, have higher growth rates, a higher cell yield, and a better substrate affinity than the HMB. Consequently, provided sufficient sulfate is present, HSRB should be able to out-compete HMB. Several studies performed at marine and fresh-water sediments and with anaerobic reactors confirm this. In sediments, it is generally observed that in the presence of sufficient sulfate, hydrogen is oxidised by HSRB (Abram and Nedwell 1978, Lovley et al. 1982, Sörensen et al 1981, Winfrey and Ward 1983). Also, results of studies in anaerobic reactors show that HSRB will out compete HMB for hydrogen (Mulder 1984, Rinzema et al.1986, Rinzema and Lettinga 1988a).

An alternative explanation for the successful competition of HSRB with HMB has been given by Lovley et al. (1982) and Lovley (1985). Because of their more effective consumption of hydrogen, HSRB would maintain the hydrogen level below the threshold value of the HMB. In that case hydrogen utilisation by the HMB becomes energetically unfavourable.

1.3.2 Competition for acetate

From the thermodynamic and kinetic data presented in Tables 1.1 and 1.3 it can be seen that ASRB have an advantage over AMB in their competition for acetate.
ASRB gain more energy from the consumption of acetate than AMB. Furthermore, ASRB tend to have higher growth rates than AMB, especially at low acetate concentrations. Therefore, if sufficient sulfate is available, a pre-dominance of ASRB over AMB can be expected. Studies in sea- and freshwater sediments show, that in the presence of sufficient sulfate, acetate indeed mainly is scavenged by ASRB (Winfrey and Zeikus 1977, Banat 1981, Lovley and Klug 1982, Smith and Klug 1981). Also in CSTR reactors and in the contact process a pre-dominance of ASRB has been reported (Middleton and Lawrence 1977, Olthof et al. 1985). However, in modern high rate anaerobic reactors based on sludge immobilisation and a high sludge retention the situation is less clear. In several studies it was found, that acetate is completely converted into methane, even in the presence of an excess sulfate (Hoeks et al. 1984, Mulder 1984, Rinzema et al. 1986). However, other researchers reported a pre- dominance of ASRB (Rinzema and Schultz 1987, Choi and Rim 1991, Stucki et al 1992). The outcome of the competition between the two bacteria therefore seems unpredictable (Rinzema 1988).

Table 1.1. Standard free enthalpy change for methanogenesis, and sulfate reduction. (Data from Thauer et al. 1977)

Reaction	ΔG° KJ/mole
$4\ H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O$	-32.7
$CH_3COOH + H_2O \rightarrow CH_4 + HCO_3^-$	-28.2
$4\ H_2 + HSO_4^- \rightarrow HS^- + 4\ H_2O$	-38.0
$CH_3COOH + SO_4^{2-} \rightarrow HS^- + 2\ HCO_3^-$	-39.5

Table 1.2. Kinetic Monod parameters for hydrogen utilization by mesophilic HSRB and HMB.

	Ks μM	μ_m day^{-1}	Y g VSS.mol^{-1} H_2	pH	T °C	Ref.
SRB						
Desulfovibrio						
sp. G11	3.3	1.37	0.85	6.7	37	1
vulgaris (marburg)		4.75	2.50	7.0	37	2
vulgaris (hil)		3.33	2.25	7.0	37	2
vulgaris	4		1.10	7.0	30	3
desulfuricans		2.56	2.00	7.0	37	2
gigas		1.39	1.75	7.0	34	2
MB						
Methanospirillum						
hungatei (JF1)	6.6	1.27	0.20	6.7	37	1
Methanosarcina						
barkeri		1.11	1.60	7.0	37	4
Methanobacter						
bryantii		0.34				5
formicicum	2.0	2.0	0.80			6
Methanobacterium sp	14		0.60	7.0	30	3
Methanobrevibacter						
arbophilus	38	1.4	0.65	7.0	33	7

1 Robinson and Tiedje 1984 ; 2 Brandis and Thauer 1981 ; 3 Lupton and Zeikus 1984 ; 4 Weimar and Zeikus 1978 ; 5 Roberton and Wolfe 1970 ; 6 Shauer and ferry 1980 ; 7 Zehnder and Wuhrmann 1977

Table 1.3. Kinetic Monod parameters for acetate utilization by mesophilic ASRB and AMB.

	Ks mM	μ_m day^{-1}	Y g VSS.mol^{-1} C2	pH	T °C	Ref.
SRB						
Desulfobacter						
postagei		1.03	2.56		28	1
Desulfotomaculum						
acetoxidans		0.55	5.52	7.1	36	2
acetoxidans		1.44	7.55	7.1	36	3
Desulfonema						
limicola		0.55		7.6	30	4
Enrichment culture	0.10	0.51			31	5
Enrichment culture	0.17	0.015	3.7	7.5	30	6
MB						
Methanothrix						
soehngenii	0.44	0.11	1.47	7.6	37	7
concilii	1.20	0.69	1.15	7.2	35	8
Methanosarcina						
barkeri	0.69	2.4		6.3	35	9
Enrichment culture	5.60	0.26	3.2		30	10
Enrichment culture	0.55	0.037		7.5	30	6

1 Brandis-Heep et al. 1983 ; 2 Widdel and Pfennig 1977 ; 3 Widdel and Pfennig 1981 ; 4 Widdel 1980 ; 5 Middleton and Lawrence 1977 ; 6 Yoda et al. 1987 ; 7 Huser 1981 ; 8 Patel 1984 ; 9 Powell et al. 1983 ; 10 Lawrence and Mc Carty 1969

Factors that could influence the competition for acetate are discussed below.

Acetate concentration. Yoda et al. (1988) calculated from their experiments with a fluidized bed reactor kinetic growth properties of AMB and ASRB growing in a biofilm (see table 1.3). According to their findings the AMB have a higher K_s value but also a higher maximum specific growth rate. Consequently at high and at low concentrations of acetate a pre-dominance of respectively AMB and ASRB can be expected. According to the data of Yoda et al., AMB grow faster than ASRB at acetate concentrations exceeding 8 mg.l^{-1}. However, in their calculations of the maximum specific growth rates, decay rates and yield coefficients, Yoda et al. made several assumptions without verification. Moreover, from table 1.3 it can be seen that the parameters published by Yoda et al. differ significantly from other published values. In general it is assumed that ASRB grow faster than AMB, both at low and high acetate concentrations.

Sulfate concentration. Growth of ASRB depends on both the acetate and the sulfate concentration, whereas growth of AMB is dictated solely by the acetate concentration. At low sulfate concentrations growth of ASRB will be sulfate limited, which could enable AMB to

out-compete ASRB. Furthermore, at low sulfate concentrations different types of SRB will compete for the available sulfate. Laanbroek et al. (1984) showed that in chemostats operated under sulfate limiting conditions the acetate degrading *Desulfobacter postagei* is a poor competitor for the available sulfate with the propionate degrading *Desulfobulbus propionicus* and the hydrogen oxidizing *Desulfovibrio (Desulfomicrobiom) baculatus*. Reported sulfate affinities for the SRB also reveal, that at low sulfate concentrations ASRB are poor sulfate-scavengers as compared to the hydrogen-degrading *Desulfovibrio* species (Table 1.4). Therefore, at low sulfate concentrations or high COD/sulfate-ratios, the oxidation of compounds such as hydrogen and propionate by SRB is likely, while acetate is then mainly left for the AMB.

Table 1.4. sulfate affinities of mesophilic SRB.

	K_{SO4} mg.l^{-1}	Ref
Desulfovibrio vulgaris (Marburg)	0.5	1
Desulfovibrio vulgaris (Hildenborough)	3	1
Desulfovibrio sapovorans	0.7	1
Desulfovibrio salexigens	7	1
Desulfobacter postagei	19	2
enrichment culture[a]	45	3
enrichment culture[b]	30	4

1. Ingvorsen and Jörgensen 1984, 2. Ingvorsen et al. 1984, 3. Yoda et al. 1987, 4. Middleton and Lawrence 1977

[a] biofilm, electrondonor acetate. [b] suspended sludge, electrondonor acetate

It was shown that in granular sludge adapted to COD/sulfate-ratios exceeding 20, only few ASRB are present. At COD/sulfate-ratios below 20, the number of ASRB in the sludge increases with decreasing COD/sulfate-ratio (C.N.J. Buisman, personal communication). Choi and Rim (1991) reported that for COD/sulfate ratios exceeding 2.7, AMB predominate, whereas at COD/sulfate ratios below 1.7 ASRB become the pre-dominant organisms. At COD/sulfate ratios between 1.7 and 2.7 there is an active competition between the AMB and ASRB. For reactors operated at an excess of sulfate, sulfate limitation of ASRB and sulfate competition among different types of SRB probably is less important. However, sulfate limitation in an anaerobic biofilm or sludge granule still might occur due to mass transfer limitation of sulfate into the biomass. Nielsen (1987) reported that a biofilm of only a few hundred μm thickness will already become sulfate limited at sulfate concentrations in the bulk liquid below 50 mg.l^{-1}. Lens (1994) calculated that sludge granules will become sulfate limited at bulk liquid sulfate concentration less than 300 mg/l.

Immobilisation properties. Sludge retention in modern high rate anaerobic reactors is

usually based on the ability of the bacteria to immobilize on inert solid particles or into sludge granules. Bacteria with a poor attachment ability will be washed out from these reactors. Therefore, in addition to the growth properties of the bacteria, also their ability to immobilize will affect the competition between organisms. The role of the attachment capacity of SRB and MB was investigated by Isa et al (1986 a,b). They studied the competition between SRB and MB in an anaerobic filter treating a synthetic sulfate containing wastewater. Acetate, ethanol and formate were found to be largely converted into methane. Cell counts and activity tests showed that the ratio of the number of bacteria present in the packing material of the filter and in the liquid fase was much lower for the SRB than for the MB. Isa et al. concluded that the colonisation ability of the MB is superior over that of the SRB. As a result MB can successfully compete with the SRB for the available substrate. However, as indicated by Rinzema (1988), the cell count technique used by Isa et al., only has limited accuracy for immobilised biomass, and the results of the activity tests were based on incomplete conversion of the substrate, which can result in a incorrect estimation of the ratio.

Type of substrate. Methanothrix, presumably the predominant AMB in anaerobic reactors, can only grow on acetate (Vogels 1988). The pre-dominant ASRB in anaerobic granular sludge was isolated recently by Oude Elferink et al. (1993). This bacterium uses a wide range of substrates including hydrogen, fatty acids, ethanol and lactate. With the exception of the specialized *Desulfobacter* species, for many completely oxidizing SRB, growth with acetate as the sole substrate may be relatively low whereas it is considerably higher if other substrates (e.g. butyrate) are also present (Widdel and Hansen 1993). If acetate and other substrates are used simultaneously by the SRB, the outcome of the competition for acetate between the nutrionally limited *Methanothrix* and the nutrionally versatile sulfate reducer might be affected. The sulfate reducer then would have an advantage over the MB. To what extent this is also valid for a complex eco-system such as granular sludge is not yet clear. In these systems the ASRB have to compete for the available other substrates with AB and other non acetate degrading SRB.

Type of seed sludge and experimental runtime. A disadvantage of studying the competition between bacterial species in modern high rate anaerobic reactors, is the long period of time needed to reach a steady state situation. This is due to the fact that in these reactors very high biomass retention times are common. In UASB reactors the sludge retention time can be as high as 0.5-1 year (Hulshoff Pol 1989). Consequently, a long period of time may be needed for one type of bacteria to out-compete other species. This will especially be true if the quantity of one of the competing species is very low in comparison with the other. In this respect, the choice of the seed sludge is very important. In sludge adapted to wastewaters with low sulfate concentrations or even negligible sulfate concentrations a (very) low number of ASRB can be expected. It was found that in sludge obtained from full-scale UASB reactors adapted to COD/sulfate-ratios exceeding 20, no significant amount of ASRB are present (C.N.J. Buisman, personal communication).

Nutrient concentrations. The SRB are known to have a high demand for iron (Postgate 1984). However, results of Isa et al (1986 a,b) show that dosing extra iron to an anaerobic filter treating acetate, ethanol and formate does not exert a beneficial effect on the SRB. Accurate assessment of the role of the nutrient concentration is difficult, because apart from the concentration also the biological availability of the nutrients has to be considered. Precipitation of iron (e.g. FeS) and complex formation can reduce its availability for micro-organisms. Finally, a limitation of iron will affect both the MB and SRB.

Temperature. The temperature range and temperature optimum for growth of mesophilic SRB and MB is about the same. Results of batch experiments performed at our department with a granular sludge adapted to 30 °C, show that the course of the specific methanogenic and sulfidogenic activity within a temperature range 10-50 °C is virtually the same (A. Visser, unpublished results). With respect to temperature variations we found that SRB are less sensitive to temperature shocks than MB (Visser et al. 1993b).
Relatively little is known about the thermophilic anaerobic treatment of sulfate containing wastewater, and the competition between the SRB and MB under these conditions. In experiments with UASB reactors, Rintala and Lettinga (1992) observed that at 37 °C acetate was converted into methane on a synthetic wastewater with high sulfate concentrations. However, when operated under thermophilic conditions (55 °C) the acetate was mainly degraded by the ASRB.

pH. Until now insufficient data are available to predict the effect of the pH or temporary pH variations on the competition between ASRB and AMB. In addition to a direct pH-effect, also an indirect influence of the pH is possible, e.g. the toxic effects of sulfide and ammonia are pH dependent.

Toxic compounds. Since sulfate reduction always results in the formation of sulfide, sulfide toxicity could be an important factor. Some wastewater streams, like those produced in the paper and pulp production, contain the toxic compound sulfite. The toxicity of sulfate itself is low, but wastewaters with very high sulfate concentrations will also contain high amounts of cations. These cations, like sodium or potassium, can become inhibitory to the bacteria at high concentrations. Toxicity phenonema related with wastewaters with high levels of oxidised sulfur compounds will be discussed in paragraph 1.4.

1.3.3 Competition for propionate and butyrate

Table 1.5 lists the kinetic data for growth of SRB and AB on propionate and butyrate. From this table it can be seen, that SRB exert a much higher maximum growth rate than syntrophic consortia if neither sulfate nor the substrate are limiting. Therefore, a predominance of SRB can be expected. Unfortunately, hardly any information is available about K_s-values for propionate and butyrate sofar. A comparison of the growth rates of syntrophic cultures and SRB at low substrate concentrations therefore is not possible. Studies on ecosystems in sea water sediments have shown that here a direct oxidation of propionate and butyrate by SRB is a significant route (Banat and Nedwell 1983, Smith and Klug 1981,

Table 1.5. Specific growth rates for mesophilic AB in co culture with HMB or HSRB, and SRB growing on propionate and butyrate.

	μ_{max} day^{-1}		
	- sulfate	+ sulfate	Ref
propionate			
SRB			
Desulfobulbus propionicus 1pr3		0.89-1.66	1,2
Desulfobulbus propionicus		2.64	3
Desulfobulbus elongatus		1.39	4
Desulfococcus multivorans		0.17-0.23	5
AB			
syntrophobacter wolinii	0.02-0.10	0.18-0.21	6,7
strain MPOB	0.15-0.17		8
strain KoProp1	0.07		6
Culture PW	0.10		9
butyrate			
SRB			
Desulfovibrio sapovorans		1.58	3
Desulfococcus multivorans		0.17-0.23	5
Desulfotomaculum acetoxidans		1.11	10
Desulfotomaculum strain Gro11		1.19-1.3	11
Desulfobacterium autotrophicum		0.67-1.11	12
AB			
Syntrophospora (Clostridium) bryantii BH	0.25		9,13,14
Syntrophomonas sapovorans OM	0.6		15
Syntrophomonas wolfei	0.19-0.20	0.31	6,16,17
Syntrophomonas strain FSM2	0.32		151

1. Widdel and Pfennig 1982, 2. Stams et al. 1984, 3. Nanninga and Gottschal 1987, 4. Samain et al. 1984, 5. Stieb and Schink 1989, 6. Dörner 1992, 7. Boone and Bryant 1980, 8. Stams et al. 1993, 9. Wu et al. 1992, 10. Widdel and Pfennig 1981, 11. Kuever et al. 1993, 12. Brysh et al. 1987, 13. Stieb and Schink 1985, 14. Zhao et al. 1990, 15. Roy et al. 1986, 16. McInerney et al. 1981, 17. McInerney et al. 1979, 18. Zhao et al 1993.

Sörensen et al. 1981). However, similar data are not available yet for anaerobic reactors. Most reports mention that the reducing equivalents formed in the conversion of fatty acids into acetate are used by the SRB (Mulder 1984, Rinzema et al. 1986). However, these studies don't distinguish between syntrophic oxidation of fatty acids by acetogens growing in syntrophy with HSRB, or a direct oxidation of these fatty acids by SRB. Nevertheless, several studies reveal that SRB play an important role in the degradation of fatty acids such as propionate and butyrate. According to Mulder (1984), the degradation of propionate and butyrate will not proceed in anaerobic sludges adapted to high sulfate concentrations, when

the sulfate concentration in the influent is decreased to low values. Mc Cartney and Oleskiewicz (1991) found in a mixed culture growing on lactate and acetate, that the presence of SRB is essential for the conversion of propionate. Ueki et al. (1988, 1992), found that in the anaerobic digestion of sewage, sulfate reduction depends on the degradation of propionate. An addition of propionate greatly enhanced the sulfate reduction. Quatabi et al. (1990) studied the degradation of lactate and propionate in a mixed culture obtained from an anaerobic fermenter treating wine distillery wastewater. In the absence of sulfate the propionate degradation proceeded slowly, but in the presence of sulfate it proceeded smoothly. At relatively low sulfate concentrations the degradation of propionate and butyrate can proceed in different ways. Part of the fatty acids may be oxidised directly by SRB, while the remainder is degraded by syntrophic consortia of AB and HMB. It is also possible that the fatty acids are degraded by AB growing in syntrophy with HSRB and HMB. Since HSRB have a higher affinity for sulfate than the fatty acid oxidizing SRB (Laanbroek et al. 1984), it is likely that at low sulfate concentrations the degradation of fatty acids proceeds mainly by AB growing in syntrophy with HSRB and HMB. Finally, it is also possible that SRB perform the acetogenic oxidation of propionate and butyrate, at least partially. It has been shown that in granular sludge adapted to a mixture of fatty acids, propionate is degraded acetogenically by SRB. (Wu et al. 1991, Wu et al. 1992). Moreover, SRB also seems capable to grow acetogenically on butyrate (Heppner et al. 1992).

1.4 Inhibition phenonema in the anaerobic digestion of waste streams with high levels of oxidised sulfur compounds

1.4.1 Inhibition by sulfide

Sulfate reduction results in the production of sulfide, which is toxic at higher concentrations. The inhibitory effect of sulfide is presumed to be caused by undissociated hydrogen sulfide because only neutral molecules can permeate the cell membrane (Schlegel 1981). The exact mechanism of the inhibition by H_2S has not been cleared up yet. Once H_2S has passed the cell wall it may denature native proteins through the formation of sulfide and disulfide cross-links between the polypeptide chains (Conn et al. 1987). The H_2S may also interfere with the various key metabolic coenzyme A and M through the formation of sulfide linkages. The acetyl coenzym A pathway for CO_2 fixation is common for both SRB and MB (Stouthamer 1988). H_2S may also interfere with in some way or another the assimilatory metabolism of sulfur, while it possibly may also affect the internal cell pH.

The concentration of undissociated hydrogen sulfide in an anaerobic reactor is determined by the chemical and physical equilibria :

$$H_2S(l) \leftrightarrow HS^- + H^+$$
$$H_2S(l) \leftrightarrow H_2S(g)$$

The pKa value of the dissociation equilibrium of H_2S is about 7.04 at 18 °C (Weast

1977). Consequently, small pH-variations in the pH range 6-8 will significantly affect the H_2S concentration.

The gas-liquid distribution coefficient for H_2S is about 2.27 at 30 °C (Wilhelm et al. 1977). As a result of stripping of H_2S by the biogas produced, the H_2S concentration in the liquid fase can become significantly lower.

Most studies dealing with toxicity of sulfide focus on the inhibition of the AMB. Relatively little attention has been paid sofar to the effect of sulfide on AB and SRB.
In the 50's many researchers studied the inhibiting effect of sulfide on methanogenesis (Bannink and Muller 1951, Rudolfs and Amberg 1952, Aulenbach and Heukelkian 1955, Butlin et al. 1956). However, unfortunately the role of the pH and stripping of H_2S by the biogas was not taken into account in these studies. Therefore, the results of these studies are less useful for interpretation of the effect of H_2S. In more recent studies these factors received more attention. Table 1.6 list some literature results for the inhibition of anaerobic bacteria by sulfide. The data show that for suspended sludge systems the inhibition of the AMB correlates well with the H_2S concentration, both at low and high pH values. A 50 % inhibition was found at about 100-130 mg $H_2S.l^{-1}$. For AMB immobilized in granules the dependency of the toxicity of sulfide appeared to be more complex. According to Koster et al. (1986), at high pH values (7.8-8.0) the inhibition caused by H_2S is significantly higher than in the pH range 6.2-7.2. In the pH-range 6.2-7.2 the inhibition of AMB is dictated by the H_2S concentration, whereas at higher pH values (7.0-8.0) it seems to correlate with the total-sulfide concentration. The 50 % inhibition values reported by Koster et al. are 250 mg $H_2S.l^{-1}$ at pH 6.2-7.2 and 825 $mg.l^{-1}$ total sulfide at pH 7.0-8.0.

Comparison of the results of the different researches (see table 1.6) learns that a low and neutral pH values granular sludge is less inhibited by H_2S than suspended sludges, whereas at high pH values the inhibition is very similar for suspended and granular sludge. This also applies for thermophilic granular and suspended sludges (Visser et al. 1993a).

AMB growing in immobilized form are less affected by sulfide than AMB growing in suspended form. This has been confirmed by Maillacheruvu et al. (1993) and Parkin et al. (1991) in experiments with anaerobic filters and CSTR's.

In order to be able to predict maximal allowable sulfide concentrations in anaerobic treatment processes, also the effect of sulfide on AB and SRB should be known. For this reason, increasingly emphasize has been put on the latter systems more recently.

In a UASB reactor fed with propionate and sulfate a sharp decrease in the propionate conversion rate at H_2S concentrations exceeding 100 $mg.l^{-1}$, viz. a 50 % inhibition was found at 140 mg $H_2S.l^{-1}$ at a pH range of 7.0-7.4 (Rinzema and Lettinga 1988b). The granular sludge used by these researchers was adapted to low sulfate concentrations. In an earlier research Koster et al. (1986) found a 50 % inhibition of the acetoclastic methanogenic activity at 250 $mg.l^{-1}$. Apparently, the oxidation of propionate is the most sulfide sensitive step.

Table 1.6. H_2S concentrations ($mg.l^{-1}$) causing a 50 % inhibition of the methanogenesis, sulfate reduction and degradation of specific substrates.

Biomass	substrate	T °C	pH	H_2S $mg.l^{-1}$	Ref
methanogenesis					
sludge suspension	acetate	--	----	50	1
sludge suspension	distellery wastewater	37	7.0-7.2	130	2
sludge suspension	acetate	35	6.5-7.4	125	3
			7.7-7.9	100	
sludge suspension	lactate	35	7	100	4
			8	100	
sludge granules	acetate	30	6.2-6.4	246	5
			7.0-7.2	252	
			7.8-8.0	90	
Sulfate reduction					
Desulfovibrio sp.	lactate	37	6.2-6.6	450	6
Desulfovibrio desulfuricans	lactate	35	7	250	7
sludge suspension	lactate	35	7.2-7.6	80	8
sludge suspension	lactate	35	7	> 300	9
			8	185	
Specific substrates					
sludge granules	propionate	30	7.0-7.5	140	10
sludge suspension	propionate	35	6.5-7.4	100	3
			7.7-7.9	60	
sludge suspension	butyrate	35	6.5-7.4	235	3
			7.7-7.9	> 200	
sludge suspension	lactate	35	6.5-7.4	320	3
			7.7-7.9	390	

1. Kroiss and Plahl-Wabnegg (1983), 2. Karhadkar et al. (1987), 3. Oleskiewicz et al. (1989), 4. Mc Cartney and Oleskiewicz (1993), 5. Koster et al. (1986), 6. Reiss et al. (1992), 7. Okabe et al. (1992), 8. Mc Cartney and Oleskiewicz (1991), 9. Mc Cartney and Oleskiewicz (1993), 10. Rinzema and Lettinga (1988b).

For a suspended sludge adapted to low sulfate concentrations Oleskiewicz et al (1989) found that the H_2S inhibition increased for different electrondonors in the following sequence : lactate, butyrate, acetate, propionate. Again propionate degradation is the most sulfide inhibited step. McCartney and Oleskiewicz (1991, 1993) studied the degradation of lactate by flocculant types of sludge adapted to a COD/sulfate ratio of 3.7, 1.6 and 0.8. For the sludge cultivated at a COD/sulfate ratio of 3.7, propionate accumulation was observed at H_2S concentrations exceeding 110 $mg.l^{-1}$. For the other two sludges any accumulation of

propionate was not found at H_2S concentrations up to 325 mg.l^{-1}. Apparently, in sludges adapted to COD/sulfate ratios less than approximately 1.6 the conversion of propionate is much less affected by sulfide than sludges cultivated at higher COD/sulfate ratios. Possibly in sludge adapted to high COD/sulfate ratios, propionate is degraded by AB growing in syntrophy with HMB or HSRB. Apparently, in these systems the propionate oxidizers are seriously inhibited by sulfide. In sludge cultivated at low COD/sulfate ratios the direct oxidation of butyrate and propionate by SRB very likely is the pre-dominant route. The degradation of acetate then will become most inhibited by sulfide. Research at our laboratory has shown that for sludge adapted to COD/sulfate ratios of 0.5, the degradation of propionate and butyrate in the presence of sulfate was less inhibited by sulfide than the conversion of acetate by SRB or MB (A. Visser, unpublished results).

Sofar, only very few data are available for the effect of sulfide on the SRB, especially the ASRB. Reiss et al (1992) found a complete inhibition of the growth of *Desulfovibrio* sp on lactate at H_2S concentration of 550 mg.l^{-1} and pH 6.2-6.6. The extent of the inhibition correlated well with the H_2S concentration. Okabe et al. (1992) found a 50 % inhibition of the growth of *Desulfovibrio desulfuricans* on lactate at a H_2S level of 250 mg.l^{-1} at pH 7. According to Hilton and Oleskiewicz (1988), in the degradation of lactate, the inhibition of SRB is directly related to the total-sulfide concentration whereas that of the MB by the H_2S concentration. Therefore, according to these findings high pH values would be relatively favourable for the MB. For a flocculant sludge adapted to lactate, acetate and sulfate (COD/sulfate 3.7), McCartney and Oleskiewicz (1991) found a higher inhibition of the SRB relative to that of the MB, in the degradation of lactate. On the contrary, McCartney and Oleskiewicz (1993) found for a sludge cultivated on lactate, acetate and sulfate at COD/sulfate 1.6 and 0.8, respectively, that the SRB were less sensitive for sulfide than the MB. According to the authors, the lactate degradation pathway was dependent upon the COD/sulfate ratio used. A ratio of 3.7 resulted in a pathway with propionate and acetate as the products, but did not result in a significant sulfate reduction. Here, the sulfate reduction was associated with the degradation of propionate which was more inhibited by sulfide than the MB. As said before, the inhibition of the SRB here, was probably due to an inhibition of propionate degrading AB. At COD sulfate ratios $\leq$ 1.6, the lactate degradation resulted in an SRB pathway with acetate as the endproduct. Here, the SRB were much less inhibited than the MB.

Unfortunately, hardly any data are available about the effect of sulfide on ASRB. Stucki et al. (1992) observed a process failure in a sulfidogenic fixed bed reactor treating a mixture of acetate and sulfate at H_2S concentration exceeding 50 mg.l^{-1}, indicating to rather high susceptibility of ASRB.

1.4.2 Inhibition by sulfite

From the few information available it seems that sulfite exerts a strong inhibitory effect on anaerobic bacteria. In batch experiments, sulfite causes a lag fase in the methane

production, the length of which depends on the history of the biomass. Yang et al. (1979) observed a lag fase of more than 60 hours and 12 days after dosing 25 and 75 mg SO_3^{2-}/l, respectively, to an enrichment-culture of AMB. Eis et al.(1983), however, did not find any lag fase, even after addition of 100 mg SO_3^{2-}/l to an adapted sludge. Maaskant and Hobma (1981) and van Bellegem et al. (1979) found a 50 % inhibition of the methanogenic activity at about 150-200 mg SO_3^{2-}/l. However, they also showed that upon repeated dosing of sulfite the inhibition become significant less as a result of adaptation of the sludge. This adaptation of the sludge presumably can be contributed to the development of SRB in the sludge. They will reduce sulfite to sulfide. Inhibition by sulfite probably will be insignificant in continuous reactors, because here a sulfate reducing population will develop, which will eliminate the sulfite (Rinzema and Lettinga 1988a). It has been shown that wastewaters with sulfite concentrations up to 800 mg SO_3^{2-}/l can be treated in anaerobic reactors satisfactory (Eis et al 1983, Ferguson et al 1983, Särner 1986, Särner 1989).

1.4.3 Inhibition by Cations

Although sulfate by itself is a non-toxic compound, in wastewaters with high sulfate concentrations high cations concentrations like calcium or sodium can inhibit the anaerobic bacteria. Although the calcium ion does not exert a severe direct toxic effect it has been found that $CaCO_3$ and/or $Ca_3(PO_4)_2$ precipitates may entrap the biomass (scaling). This may result in substrate transport limitation (Lettinga et al. 1987). Serious scaling of biomass by calcium-precipitates may already occur at calcium concentrations of 400 mg.l^{-1}. The precipitates will gradually accumulate in the reactor and in the piping system which results in clogging problems, and ultimately it even may lead to a complete loss of the activity of the sludge granules due to the fact that a calcium-layer completely blocks the transport of substrate (Visser 1987, Pereboom 1984). Moreover, calcium-fosfate precipitation may cause fosfate deficiency (Callander and Bardford 1983, Lettinga et al. 1987, Alphenaar 1994).

The effect of sodium on the anaerobic digestion has been studied extensively. However, the results published concerning the effect of sodium on methanogenesis show many inconsistencies. Reported values for the 50 % inhibition of MB by sodium range from 6 to 40 g.l^{-1} (de Baere et al. 1984, Kugelmann and Mc Carty 1964, Lettinga and Vinken 1981, van den Berg et al 1976,). These differences presumably can be attributed to the history of the sludge, antagonistic and synergistic effects and the test method used. Sludge adapted to high sodium levels undoubtedly will be much less sensitive than non-adapted sludge. The presence of other cations like potassium cause antagonistic or synergistic effects, resulting in a significant change in the sodium sensitivity (Kugelmann and Chin 1971, McCarty and McKinney 1961). Rinzema et al. (1988) observed for a granular sludge a 10 %, 50 % and 100 % inhibition of the acetoclastic methanogenic activity at 5, 10 and 14 g.l^{-1}. The sludge used in their experiments did not show any clear adaptation after continuous exposure to 13.7 g Na^+.l^{-1} for a 12 week period. Experiments conducted at our laboratory revealed that granular sludges adapted to sodium concentrations of 1.5-2 and 5.5-6 g.l^{-1} respectively,

become inhibited at sodium concentrations exceeding 6 $g.l^{-1}$. A 50 % inhibition of the activity was observed at 7 to 8 g $Na^{+}.l^{-1}$ for both sludges (A. Visser, unpublished results).

The presence of high concentrations of sodium is essential for the growth of many marine SRB (Widdel 1988). On the Contrary, freshwater SRB may be inhibited by high sodium concentrations. Sofar, relatively little data are available regarding the effect of sodium on the sulfate reduction process in anaerobic reactors. Results of experiments performed at our laboratory show that the sulfidogenic activity of granular sludge adapted to sodium levels of 1.5-2 and 5.5-6 $g.l^{-1}$ respectively, becomes inhibited at sodium concentrations exceeding 11 $g.l^{-1}$. A 50 % inhibition of the activity was observed at about 15 $g.l^{-1}$ of sodium for both sludges (A. Visser, unpublished results), which really are exceptionally high concentrations.

1.5 Technological aspects of anaerobic treatment of sulfate containing wastewaters

The strategy to be applied in order to accomplish a successful anaerobic treatment of a sulfate containing waste stream is related to the objective of the treatment. The goals of the treatment process can be :

* *The removal of organic matter.* Considering the disadvantages, viz. the potential problems related to the sulfate reduction process, a complete suppression of the sulfate reduction and a complete conversion of the organic substrate into methane could be considered as an optimal process configuration. However, as already mentioned in chapter 1.1, sulfate reduction to some extent will always occur.

* *The removal of organic matter and/or sulfate.* Generally for wastewaters containing organic matter and sulfate, the removal of the organic matter will proceed via sulfate reduction and methanogenesis, in a ratio that depends on the COD/sulfate ratio of the wastewater. For waste streams with a COD/sulfate ratio of 0.67 in principle enough sulfate is available to accomplish a complete removal of the organic matter by a sulfidogenic biomass only. For COD/sulfate ratios less than 0.67, the amount of organic matter for a complete reduction of the sulfate doesn't suffice, and then extra substrate should be added. Otherwise, for COD/sulfate ratios exceeding 0.67 a complete removal of the organic matter can only be achieved if, in addition to sulfate reduction also methanogenesis will occur. To what extent sulfate reduction or methanogenesis will proceed, more knowledge should be obtained about the processes, especially with respect to the competition between SRB and MB.

Sulfide toxicity certainly can be a serious problem in the anaerobic treatment of wastewaters with high levels of oxidised sulfur compounds. Sofar, insufficient data are available to predict the conditions where a process failure by sulfide inhibition will not occur. Moreover, the results which have been reported sofar show big discrepancies. For example, reported H_2S-values causing a 50 % inhibition of the methanogenesis vary between 50 and 270 mg/l (See chapter 1.4). In general, higher sulfide concentrations can be tolerated in high

rate anaerobic reactors based on the formation of a biofilm or sludge granules (anaerobic filter, UASB reactor), than reactors operated with suspended sludge (CSTR, contact process). Speece (1983) estimated that for a stable methanogenic process the H_2S concentration should not exceed 150 mg/l. For the sulfate reduction process such guidelines have not yet been given. Based on literature data Rinzema and Lettinga (1988a) concluded that at COD to sulfate ratios higher than 10 anaerobic treatment always proceeds successfully. Using a model based on chemical and physical equilibria they calculated, that for these wastewaters the H_2S concentration in the anaerobic reactor will never exceed the presumed critical value of 150 mg/l. At COD/sulfate ratios lower than 10 process failures of anaerobic reactors have been reported, while in other cases the process proceeds successfully though only after additional precautions were taken to prevent sulfide toxicity (Rinzema and Lettinga 1988a). Suitable measures to prevent sulfide inhibition are (Rinzema and Lettinga 1988a) :

- dilution of the wastewater,
- elevation of the pH in the reactor,
- extending of the anaerobic treatment step with a sulfide removal step.

The last option implies the installation of a extra treatment unit in the treatment system. There exist several options to integrate the anaerobic treatment step with a sulfide removal unit (Fig 1.3).

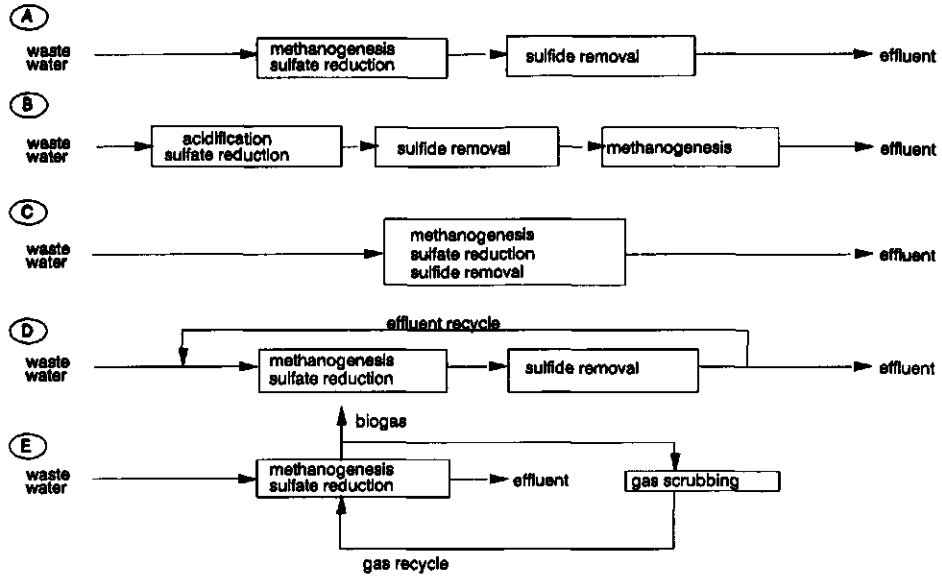

Fig 1.3. Process configurations integrating sulfide removal, sulfate reduction and MB

If sulfide toxicity is not of major concern, the sulfide removal process can be placed after the anaerobic step (Fig 1.3a). If a severe sulfide inhibition is expected, different process configurations can be used, viz. :

- *Anaerobic digestion in two stages, a pre-acidification step with sulfate reduction, followed by a methanogenic stage.* The sulfide can be removed in the first stage or between the two stages (fig 1.3b). Because of the relatively low pH of the effluent of an acidification reactor, stripping of H_2S would proceed relatively easy. The critical facts in this configuration is to assure a complete reduction of sulfate in the first stage. Results obtained sofar show that a complete sulfate reduction in the acidification stage does not occur (Mulder 1982, Rinzema et al. 1986, Rudolfs and Amsberg 1952). A complete removal of sulfate will only be possible if a sufficient amount of hydrogen is generated, which implies relatively high COD/sulfate ratios in the wastewater.
- *The precipitation of sulfide in the anaerobic digester* (Fig 1.3c). The most common heavy metal used for sulfide precipitation is iron. Research of Särner (1986) showed that supply of iron was effective for maintaining the sulfide concentration in the anaerobic reactor at low values. However, important drawbacks for this method are the costs associated with iron dosage, and the accumulation of precipitated FeS in the reactor, which could result in a low VSS/TSS- ratio of the sludge and an increase in the total sludge production. Recently, also organic H_2S-scavengers on the market have been introduced. The effectiveness of these chemicals still needs to be established.
- *The removal of sulfide from the effluent of the anaerobic reactor, combined with the recirculation of the effluent* (Fig 1.3 d). In this configuration any of the present known sulfide removal techniques, which will be discussed later in this chapter, can in principle be used.
- *The stripping of sulfide from the anaerobic reactor with the biogas, using gasscrubbing and gasrecirculation* (Fig 1.3e). Stripping of H_2S has been investigated in the past (Olthof et al. 1985, Särner 1986). Särner (1989) used an anaerobic trickling (antric) filter in which sulfate and sulfite were reduced, as a pre-treatment step. In the antric filter the sulfide was stripped from the liquid by gas that passed a recirculation system in which the H_2S in the gas gas was removed in a scrubber. After passing the antric filter the wastewater was treated in an anamet (a contact process followed by an activated sludge plant) system. To what extent stripping of H_2S combined with gasscrubbing and gasrecirculation is well feasible for modern high rate anaerobic reactors is still uncertain.

Presently, several sulfide removal techniques, including precipitation, stripping, and chemical or biological oxidation can be used along with anaerobic treatment of wastewaters containing high levels of oxidised sulfur compounds. The selection of the most suitable method depends also on factors like the operation and investment costs of the process. If sulfate removal is not required, an aerobic post-treatment system can be used converting the sulfide into sulfate. If no or only limited amounts of sulfur compounds can be discharged other techniques must be used. Methods which are suitable are :

* *precipitation of sulfide.* This option has already been discussed in this chapter.

* *Stripping of sulfide from the reactor combined with gasscrubbing and gasrecirculation.* Sämer (1989) applied a gas-washing system which uses a solution of ferric ions. The Fe^{3+}-ions react with the H_2S in the biogas producing elemental sulfur. A chelating agent was added to the liquid to prevent FeS, $Fe(OH)_2$ or $Fe(OH)_3$ precipitation. After separation of the elemental sulfur, the Fe^{2+} ion was oxidised to Fe^{3+} using air oxygen. In this way the iron could be re-used in the gas washing circuit. The process is quite attractive because it enables the recovery of sulfur. On the other hand, in the oxidation of Fe^{2+} to Fe^{3+}, part of the of the oxygen will be used for oxidizing organic compounds, including the chelating agent. This means that a part of the chelating agent, which is a very essential compound in the process, will be lost and must again be replaced. Since chelating agents, like EDTA, HEDTA or NTA are quite expensive, this will affect the economic feasibility of the process seriously.

* *Biological oxidation of sulfide to elemental sulfur.* This process, developed at the Department of Environmental Technology of the Agricultural University of Wageningen, is based on the biological conversion of the sulfide by the colourless sulfur bacteria. In fact, this biological oxidation of sulfide is an incomplete oxidation, because the ultimate endproduct is sulfate. However, by imposing the proper process conditions, such as oxygen supply and sulfide loading rate, sulfide can be converted almost completely into elemental sulfur (Buisman 1989). The very attractive feature of this process is that it also enables the recovery of sulfur. Presently the process is already applied successfully at full-scale (Buisman et al. 1993 a,b).

1.6 Scope and organisation of this thesis

As described before there exist interesting new applications for the sulfate reduction process as a wastewater treatment system. With respect to the possible application of sulfate reduction and the suppress of this process in anaerobic treatment systems, a better understanding of the process is becoming increasingly important.

The research conducted for this thesis deals with sulfate reduction and methanogenesis in anaerobic reactors under mesophilic and thermophilic conditions. The objectives of the investigations were :

- to asses the role of sulfate reduction in the anaerobic degradation of organic matter in the anaerobic treatment of sulfate containing wastewater.
- to gain a better understanding of the competition between the SRB, MB and AB in anaerobic reactors, i.e of the role of the kinetic growth properties of the bacteria, the immobilisation properties of the bacteria, and the effect of environmental conditions.
- To define reliable guidelines with respect to :
  (1) conditions that must be imposed to maximize or minimize the sulfate reduction and/or methanogenesis and,
  (2) maximal allowable sulfide concentrations in sulfidogenic and methanogenic systems.

Investigations on the anaerobic treatment of sulfate containing wastewater at the Department of Environmental Technology were started in 1983 by A. Rinzema. These investigations focused on the inhibition of MB and AB by sulfide and the anaerobic treatment of acid water, a wastewater from the edible oil refineries. Laboratory and pilot scale experiments were conducted. The research in the framework of this thesis began in 1988 and was financed by the Agricultural University of Wageningen and by the Netherlands technology Foundation (NETFo). Presently, part of the work is continued on the basis of financial support by Senter-IOP.

Many of the results presented in this thesis have already been published elsewhere (Alphenaar et al. 1993, Visser et al. 1992 , Visser et al. 1993 c,d,e, Visser et al. 1994)

Chapter 2 of the thesis gives an overview of the materials and methods used in the investigations.

Chapter 3 present the results obtained in investigations dealing with the degradation of acetate and fatty acids in the presence of sulfate using UASB reactors.

The results obtained in investigations dealing with the kinetic growth properties of AMB and ASRB at different pH levels and sulfide concentrations, are presented in chapter 4, while that concerning the immobilisation of SRB and MB in UASB and fluidized bed reactors is discussed in chapter 5.

Investigations concerning the degradation of fatty acids at different COD/sulfate ratios, and the degradation of fatty acids in the presence of sulfate under thermophilic conditions are discussed in chapters 6 and 7, respectively.

A summary and the conclusion of the investigations are provided in chapter 8.

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CHAPTER 2

Materials and methods

2.1 General

The following main research-issues were investigated in this thesis:

* The long-term competition between sulfate reducing bacteria (SRB) and methanogenic bacteria (MB) in laboratory UASB reactors operated at 30 °C and fed with (1) acetate and sulfate, and (2) a mixture of acetate, propionate, butyrate and sulfate.
* The assessment of the maximal net specific growth rates, acetate- and sulfate affinities of acetogenic SRB (ASRB) and acetogenic MB (AMB) using batch experiments.
* The assessment of the specific methanogenic and sulfidogenic sludge activities for granular sludge at 30 °C and different pH values and sulfide concentrations.
* The granulation/immobilisation of MB and SRB in laboratory 1.1 l UASB reactors operated at 30 °C and fed with sucrose, fatty acids and sulfate. The investigations were focused on :
  (1) The role of the immobilisation process on the competition between the SRB and MB.
  (2) The effect of the upward liquid velocity (in the range 0.05 to 0.7 $m.h^{-1}$) and the hydraulic retention time (in the range 6 to 45 h) on the granulation process and competition between SRB and MB.
  (3) The immobilisation of the SRB on an inert carrier and granular sludge.
* The effect of the COD/sulfate ratio, in the range from about 10 to 0.5 on the competition between the SRB and acetogenic bacteria (AB), and SRB and MB in 1.7 l UASB reactors operated at 30 °C and fed with acetate, propionate, butyrate and sulfate.
* The competition between the SRB and MB under thermophilic conditions (55 °C). For this purpose a 5.75 l UASB reactor was started up using mesophilic granular sludge as the inoculum. The effect of the pH-value on sulfate reduction and methanogenesis in UASB reactors fed with acetate, propionate, butyrate and sulfate were investigated.

A more detailed description of the different experiments is given in chapters 3 till 7.

2.2 UASB Reactors

The experiments with the reactors were performed in laboratory UASB reactors ranging in volume from 0.15 to 20 liter reactor volume. The general design of the reactors is shown in Fig 2.1. The experiments under mesophilic conditions were performed in a temperature controlled room of 30 $\pm$ 1 °C. For the experiments under thermophilic conditions the reactors were equipped with a double wall through which heated water was circulated, or normal reactors were placed in a temperature controlled water bath.

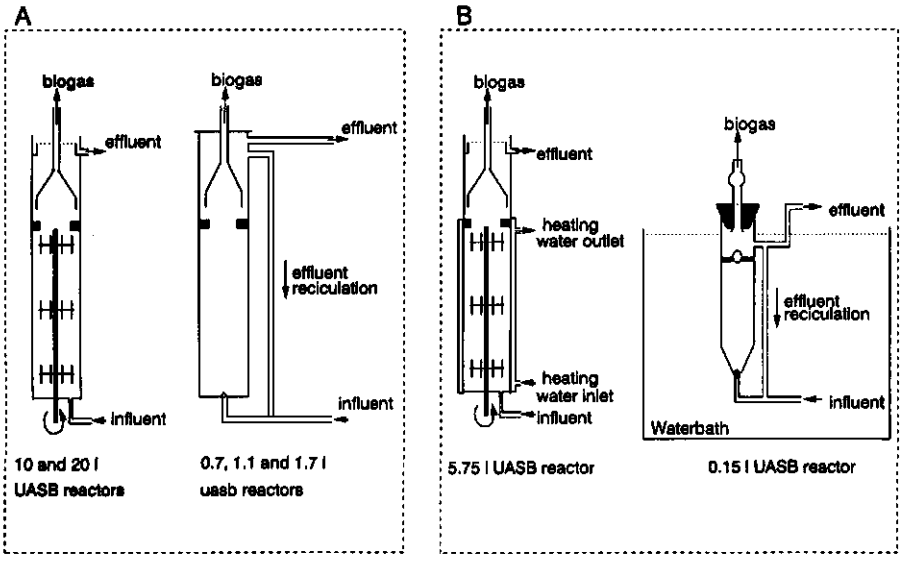

Fig 2.1. Schematic diagram of the 0.15 and 5.75 l UASB reactors used in the experiments under thermophilic conditions and the temperature shock experiments (2.1a) and of the 0.7 till 20 l UASB reactors used in the experiments under mesophilic conditions (2.1B).

The biogas produced in the reactors was led through a 3 % NaOH solution and a column of soda lime pellets to remove the CO_2 and H_2S from the gas. The (wet) methane gas was measured in a wet-test gasmeter (Meterfabriek Schlumberger, Dordrecht, The Netherlands). In most experiments effluent recirculation was applied in order to improve mixing and to avoid concentration gradients over the reactor. In the experiments without effluent recirculation the reactors were equipped with a central axis stirring blade, which was operated intermittently (5 seconds at 100 rpm every 30 minutes). The seed sludge used in the different experiments consisted of granular sludge pre-cultivated in laboratory UASB reactors, or granular sludge obtained from full-scale UASB reactors. The conditions used in the cultivation of the granular sludges in the laboratory are described in chapter 3. The granular sludges obtained from the full scale UASB reactors originated from a UASB reactor treating potato wastewater at the Aviko potato processing factory at Steenderen (The Netherlands), or from a full scale UASB reactor treating distillery wastewater at Nedalco, Bergen op Zoom (The Netherlands).

All the reactors were fed with a basal medium consisting of ($mg.l^{-1}$) : NH_4Cl (1044), KCl (270), KH_2PO_4 (169), $MgCl_2.6H_2O$ (150), yeast extract (18) and a trace element solution (1 $ml.l^{-1}$) consisting of ($mg.l^{-1}$) : $FeCl_2.4H_2O$ (2000), $MnCl_2$ (500), resazurin (500), EDTA

(500), Na_2SeO_3 (100), H_3BO_3 (50), $ZnCl_2$ (50), $(NH_4)_6Mo_7O_{24}.4H_2O$ (50), $AlCl_3$ (50), $NiCl_2.6H_2O$ (50), $CoCl_2.2H_2O$ (50), $CuCl_2.2H_2O$ (50) and HCl 36 % (1 ml/l). The media were prepared using tap water which contains about 30 mg Ca^{++}/l.

The organic substrate used in the different experiments consisted of (1) acetate, (2) a mixture acetate, propionate and butyrate, (3) a mixture of sucrose, acetate and propionate, and (4) a mixture of sucrose, acetate, propionate and butyrate. To the substrate-solution sulfate was added as Na_2SO_4. In the different experiments COD/sulfate ratios ranging from 10 to 0.5 were used. The feed solutions were neutralized with NaOH up to the desired pH-value.

2.3 Sludge characteristics

Assessment of kinetic growth properties

Batch reactors made of poly vinyl chloride with a liquid volume of 2.5 litre were used to asses the maximal net specific growth rates and the acetate affinity of ASRB and AMB in anaerobic granular sludge. Also the sulfate affinity of the ASRB was determined. The general experimental setup is shown in Fig. 2.2.

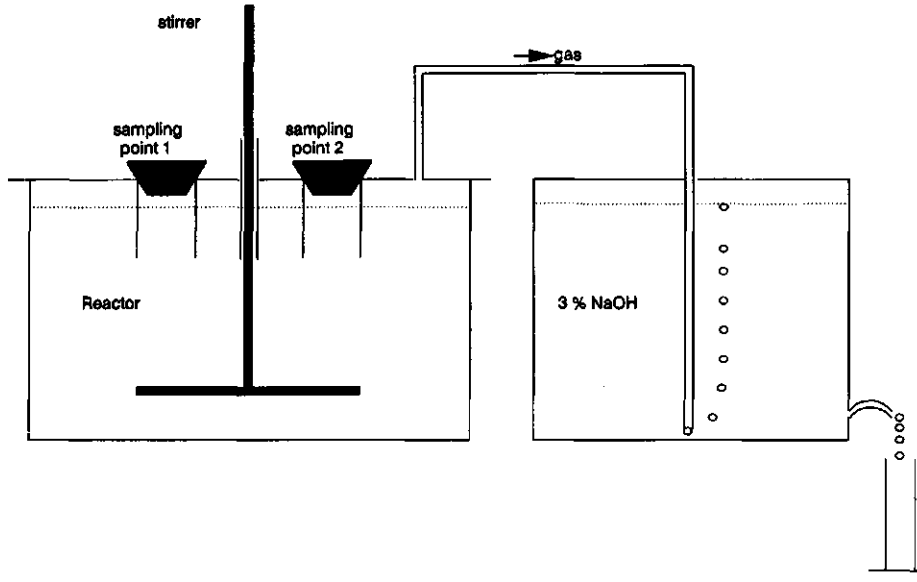

Fig 2.2. Schematic diagram of the batch reactors used in the experiments to asses the kinetic growth properties.

In the experiments for the assessment of the specific growth rates the batch reactors were stirred intermittently (15 seconds at 140 rpm per 30 seconds). During the measurements of the acetate and sulfate affinities the reactors were stirred continuously.

In all experiments the basal medium solution consisted of ($mg.l^{-1}$) : $NaHCO_3$ (2000), $NaH_2PO_4.2H_2O$ (795), K_2HPO_4 (600), NH_4Cl (280), $MgSO_4.7H_2O$ (111), $CaCl_2.7H_2O$ (10), yeast extract (20), and a trace element solution (1 $ml.l^{-1}$) consisting of ($mg.l^{-1}$) : $FeCl_2.4H_2O$ (2000), $MnCl_2$ (500), resazurin (500), EDTA (500), Na_2SeO_3 (100), H_3BO_3 (50), $ZnCl_2$ (50), $(NH_4)_6Mo_7O_{24}.4H_2O$ (50), $AlCl_3$ (50), $NiCl_2.6H_2O$ (50), $CoCl_2.2H_2O$ (50), $CuCl_2.2H_2O$ (50) and HCl 36 % (1 ml/l).

* *maximal net specific growth rates*

At the start of the experiments 5 $g.l^{-1}$ of acetate was added to the batch fermenter. For the experiments with the SRB additionally 10 g $SO_4^{2-}.l^{-1}$ and 3.2 $g.l^{-1}$ of 2 Bromo-ethane sulfonic acid (Besa) was added. Besa was added to inhibit the methanogenesis. Preliminary sludge activity assays showed that a Besa concentration of 3.2 $g.l^{-1}$ totally inhibited the methane production after 1 day of incubation without affecting the sulfate reduction process. The pH and the sulfide concentrations were measured and controlled twice daily. The pH was controlled by addition of HCl or NaOH. The sulfide concentration was regulated by addition of a Na_2S or $FeCl_2$ solution. During the experiment the acetate concentration was monitored twice a day. Furthermore the methane-gasproduction and sulfate concentration were measured regularly. The maximal net specific growth rates were calculated from the acetate depletion curve as described in paragraph 2.5.4.

* *Acetate affinities*

At the start of the experiments about 500 $mg.l^{-1}$ acetate was added to the batch reactor. For the experiments with the SRB additionally 2000 $mg.l^{-1}$ sulfate and 3.2 $g.ll^{-1}$ Besa was added. The pH was controlled continuously at the desired pH value. The sulfide concentrations were measured every hour, and all produced sulfide was precipitated by
dosing the appropriate amount of a $FeCl_2$ solution. During the experiment the acetate concentration was monitored. The acetate affinities were calculated from the acetate consumption curve as described in paragraph 2.5.4

* *Sulfate affinities*

At the start of the experiments about 500 $mg.l^{-1}$ sulfate, 2000 $mg.l^{-1}$ acetate and 3.2 $g.l^{-1}$ Besa was added to the batch system. The pH was controlled continuously at the desired pH value. The sulfide concentrations were measured every hour, and all produced sulfide was precipitated by dosing the appropriate amount of a $FeCl_2$ solution. The sulfate concentration was monitored during the course of the experiment. The sulfate affinities were calculated from the sulfate depletion curve as described in paragraph 2.5.4.

Sludge activity tests

The specific sludge activities were determined in glass serumbottles. Three different activity measurements were used : the specific methanogenic activity, the specific

sulfidogenic activity, and the simultaneous measurement of the methanogenic, sulfidogenic and total sludge activity.

In all the activity tests the basal medium consisted of ($mg.l^{-1}$) : $NaHCO_3$ (2000), $NaH_2PO_4.2H_2O$ (795), K_2HPO_4 (600), NH_4Cl (280), $MgSO_4.7H_2O$ (111), $CaCl_2.7H_2O$ (10), yeast extract (20), and a trace element solution (1 $ml.l^{-1}$) consisting of ($mg.l^{-1}$) : $FeCl_2.4H_2O$ (2000), $MnCl_2$ (500), resazurin (500), EDTA (500), Na_2SeO_3 (100), H_3BO_3 (50), $ZnCl_2$ (50), $(NH_4)_6Mo_7O_{24}.4H_2O$ (50), $AlCl_3$ (50), $NiCl_2.6H_2O$ (50), $CoCl_2.2H_2O$ (50), $CuCl_2.2H_2O$ (50) and HCl 36 % (1 ml/l).

As substrates in the activity test were used : (1) acetate, (2) propionate, (3) butyrate and (4) a mixture of acetate, propionate and butyrate (1:1:1, based on COD-values)

* *The specific methanogenic sludge activity.*

The activity tests were performed in 315 ml glass serumbottles sealed with a rubber septum kept in place by a screw cap. Each serum bottle contained 150 ml of basal medium, substrate and a known amount of anaerobic sludge. The activity was measured at a substrate concentration of 2 g $COD.l^{-1}$. Before closing the serumbottles the pH was corrected by adding a NaOH or HCl solution. After flushing the gasphase of the serumbottles with nitrogen gas if the pH was near 8 or higher, or with a 70 % N_2/30 % CO_2 mixture at lower pH values, the serumbottles were placed in a waterbath of the desired temperature. After one day of incubation the pH and substrate concentration were measured and corrected if necessary. For the assessment of the activity as function of the sulfide level, sulfide was added as a Na_2S solution up to the desired level. After flushing of the gasphase, the serumbottles were placed again in the waterbath and the sulfide concentrations were measured. After about 1.5 hour of incubation the activity was measured by monitoring the methane concentration in the gasphase for a 2-3 hour period. Directly after this measurement the pH, sulfide concentration and the volatile suspended solids (VSS) amount were determined. The specific methanogenic activity was calculated from the slope of the measured progress line (relative methane concentration) and the amount of VSS.

* *The specific sulfidogenic sludge activity.*

The activity tests were performed in 115 ml glass serumbottles sealed with a rubber septum kept in place by a screw cap. Each serum bottle contained 100 ml of basal medium, substrate (2 g $COD.l^{-1}$), sulfate (4 $g.l^{-1}$) and 3.2 $g.l^{-1}$ Besa. The Besa was added to totally inhibit the methanogenic activity in the sludge. A known amount of anaerobic sludge was added to the serumbottles to obtain a substrate degradation rate of about 50 $mg.l^{-1}.h^{-1}$. Before closing the serumbottles the pH was corrected by adding a NaOH or HCl solution. After flushing the gasphase of the serumbottles with nitrogen gas if the pH was near 8 or higher, or a 70 % N_2/30 % CO_2 mixture for lower pH values, the serumbottles were placed in a waterbath at the desired temperature. After 1 day of incubation the medium in the bottles was replaced by an identical medium. For the assessment of the sulfidogenic activity at different sulfide levels the new medium also contained sulfide. After flushing of the gasphase the serumbottles were again placed in the waterbath and the sulfide concentration was measured.

After about 1.5 hour of incubation the activity was measured by monitoring the decrease of the substrate concentration over a 4-6 hour period. The sulfide concentration was measured at the start, about halfway and at the end of the test. At the end of the experiment the pH and the amount of VSS were determined. The activity was calculated from the course of the substrate concentration and the amount of VSS.

* *The simultaneous assessment of the methanogenic, sulfidogenic and total sludge activity.*

The activity tests were performed in 115 or 575 ml glass serumbottles sealed with a rubber septum kept in place by a screw cap. Each serum bottle contained 100 or 500 ml of basal medium, 2 g COD.l^{-1} substrate, and 4 g.l^{-1} sulfate. Before closing the serumbottles the pH was set at 7 by adding a NaOH or HCl solution. After flushing the gasphase of the serumbottles with a 70 % N_2/30 % CO_2 gas mixture, the serumbottles were placed in a waterbath of the desired temperature. The substrate concentration, sulfate concentration and sulfide concentration were monitored in time. The methane production rate was monitored with a modified Mariotte flask containing 3 % NaOH. At the end of the test the amount of VSS in each serumbottle was measured. The total sludge activity was calculated from the course of substrate concentration with time. The sulfidogenic activity was obtained from the course of the sulfate and sulfide concentration. The cumulative methane production was used to calculated the specific methanogenic activity.

Most probable number (MPN) countings

MPN countings were used to asses the number of specific bacteria present in the sludge of the reactors. 10 ml of reactor sludge was diluted with 90 ml of basal medium without substrate. The composition of the medium was as described previously (Grotenhuis et al. 1991a). The sludge granules were disintegrated by pressing them several times through a syringe needle (Microlance 23G¼ 0.6 X 30), and the different physiological types of bacteria (SRB, MB, AB) were counted using the most probable number (MPN) technique (n=3). The test was done in 20 ml Hungate tubes sealed with rubber stoppers. The tubes contained 4.5 ml basal medium with 20 mM acetate, 20 mM propionate or 20 mM butyrate as substrate, and with and without 20 mM sulfate. The gasphase consisted of N_2/CO_2 (4:1). For quantification of hydrogenotrophic bacteria H_2/CO_2 (4:1) gas was used as substrate, both in the presence and in the absence of sulfate. In cases where propionate or butyrate in the absence of sulfate were the substrates, 1 ml of a H_2-grown culture of *Methanobacterium formicicum* (DSM 2639) was added, to establish a steady hydrogen consumption. After 8 weeks of incubation in the dark at 30 °C, methane production, substrate depletion and sulfide formation were determined. Additionally, the cultures were examined by microscopy for cell morphology.

Sludge size distribution

The size distribution of the granular sludge was determined by an image analyzing technique. For sampling, the total sludge bed was removed from the reactors and then mixed so that a representative sludge sample could be obtained. A sludge sample of approximately

1 ml was brought in to a 3.5 cm petri dish. The sludge particles were fixed in Kaisergelatin (Merck AG, Darmstadt, Germany). Pictures of the dishes (minimal 4 plates per sample and minimal 500 particles per sample) were digitalized and analyzed by image analyzing software (TEA Image Manager (TIM), Difa Measuring Systems BV, Breda, the Netherlands). Assuming ideal spherical shapes of the particles, the radius and volume were calculated from the two-dimensional projection of the particles. This method was introduced by Grotenhuis et al. (1991b).

Granular strength

The granular strength was measured in terms of the resistance against compression forces using a dynamic overload apparatus, as described by Hulshoff Pol et al. (1986).

Scanning electron microscopic observations.

Electron microscopy was used to study differences in morphology of the bacteria present in the sludge from the UASB reactors. Sludge samples were fixed for 2 hours in a 2.5 % glutaraldehyde solution. After washing, fixation of the sludge samples continued in 1 % osmium tetraoxide for 1.5 hours. Subsequently, the sludge samples were dehydrated in a graded ethanol series (10, 30, 50, 70, 90, 96 and 100 % ethanol) , critical point dried, mounted on stubs and sputter coated with gold/palladium after which they were observed in a scanning electron microscope (JEOL 5200).

2.4 Analysis

Volatile suspended solids (VSS) and total suspended solids (TSS) were analyzed according to standard methods (American Public Health Assoc. 1975).

The COD was determined spectrometrically according to the micromethod (Jirka and Carter 1975). Prior to the COD measurement, the sulfide present in the effluent samples from the reactors was removed by adding a few drops of 98 % sulfuric acid and flushing the liquid with nitrogen gas.

Volatile fatty acids were analyzed with a Hewlett Packard 5890A gaschromatograph equipped with a 2m x 4mm (internal diameter) glass column packed with Supelcoport (100-120 mesh) and coated with 10% Fluorad FC 431. Temperature of the column, injection port and flame ionization detector were 130, 200 and 280°C respectively. Nitrogen saturated with formic acid was used as carrier gas at a flow rate of 50 ml/min. Samples were centrifuged first and fixed by diluting the sample 3 time with a 3 % formic acid solution.

Sulfate was measured with a high pressure liquid chromatograph (Spectra Physics), equipped with a VYDAC ion chromatography colum (cat # 302 IC, 250 x 4.6 mm). Samples were fixed by adding 0.5 ml of a 0.1 M Zinc Acetate solution to 0.5 ml of sample, centrifuged and diluted with demineralised water to the appropriate concentration (< 500 mg $SO_4^{2-}.l^{-1}$). 20 μl of sample was injected. The eluent was 0.018 M potassium biphtalate with 2.5 % (v/v) acetonitrile at a flow rate of 1.2 $ml.min^{-1}$. The temperature of the column and

the detector (conductivity meter, Waters 431) was 30 °C.

Methane was measured with a Chrompack packard model 438S gaschromatograph equipped with a molecular sieve column (2m x 0.635 cm x 4.3 mm). The injection volume was 100 μl. 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 of 20 ml.min^{-1}.

Sulfide was measured colorimetrically, using a method adapted from Trüper and Schlegel (1964).

Protein was measured according to Lowry et al. (1951) as described by Grotenhuis et al. (1991c).

2.5 Calculations

2.5.1 Sludge growth, sludge yield

The sludge production or sludge growth was assessed by measuring the sludge amount in the reactors on different sampling days and by regurarly determining the sludge washout between the sampling days.

The amount of sludge produced between days t2 and t1 can be calculated according to :

$$\Delta X = X_{t2} - X_{t1} + \sum_{t1}^{t2} (Q_i \, X_{effl}) + X_{discharge} \qquad (2.1)$$

With	$\triangle X$	=	sludge produced between days t1 and t2	g VSS
	X_{t1}, X_{t2}	=	sludge amount in reactor on day t1 and t2	g VSS
	X_{effl}	=	sludge concentration in effluent	g VSS.l^{-1}
	$X_{discharge}$	=	sludge discharged between days t1 and t2	g VSS
	Q_i	=	flow rate	l.day^{-1}

The amount of COD degraded between days t2 and t1 is calculated according to :

$$\Delta COD = \sum_{t1}^{t2} Q_i \, (COD_{infl} - COD_{effl}) \qquad (2.2)$$

with	$\triangle$COD	=	COD degraded between days t1 and t2	g COD
	COD_{infl}	=	COD concentration in the influent	g COD.l^{-1}
	COD_{effl}	=	COD concentration in the effluent	l.day^{-1}

The net sludge yield is then given by :

$$Y = \frac{\Delta X}{\Delta COD} \qquad (2.3)$$

with	Y	=	net sludge yield	g VSS.g^{-1} COD degraded

2.5.2 Percentage of COD used by the SRB and MB

In the anaerobic degradation of organic matter in the presence of sulfate, the released electrons (in terms of COD) are used by the SRB and the MB. The electron flow can be calculated using the following equations :

Methanogens :

$4\ H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3\ H_2O$

$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$

The COD of methane is given by :

$CH_4 + 2\ O_2 \rightarrow CO_2 + 2\ H_2O$

Thus 1 mole of CH_4 produced ≡ 64 g COD. The substrate COD used by the MB (A) is than given by :

A = moles methane produced x 64 (g COD)

Sulfate reducers :

$4\ H_2 + H^+ + SO_4^{2-} \rightarrow HS^- + 4\ H_2O$

$CH_3COO^- + SO_4^{2-} \rightarrow HS^- + 2\ HCO_3^-$

The COD of H_2S is given by :

$H_2S + 2\ O_2 \rightarrow H_2SO_4$

Thus 1 mole of sulfate reduced ≡ 1 mole of H_2S produced ≡ 64 g COD. The substrate COD used by the SRB (B) is than given by :

B = moles of sulfate reduced x 64 (g COD)

The percentage of the COD used by the SRB and MB is given by :

$$\textit{Percentage of COD used by MB} = \frac{A}{A + B}\ 100\ \% \qquad (2.4)$$

$$\textit{Percentage of COD used by SRB} = \frac{B}{A + B}\ 100\ \%$$

2.5.3 Percentage of hydrogen and acetate used by the SRB and MB

At known substrate composition of the waste water the amount of hydrogen and acetate used by the SRB and MB can easily be calculated.

The substrates used in the experiments consisted of :

a. acetate plus sulfate,
b. acetate, propionate and butyrate plus sulfate,
c. acetate, propionate and sucrose plus sulfate,
d. acetate, propionate, butyrate and sucrose plus sulfate.

In this paragraph we describe the calculation of the percentage of hydrogen and acetate used by SRB and MB for the substrate composition as presented above under d. The

equations given in this paragraph can also be used for the experiments with the other substrate compositions a,b and c, by discarding the equations for the degradation of the substrates that are not present.Table 2.1 lists the various reactions of the degradation of the substrates in mixture d.

Table 2.1. Anaerobic degradation reactions for a mixture of sucrose, butyrate, propionate acetate in the presence of sulfate.

sucrose	
$C_{12}H_{22}O_{11} + 5\ H_2O \rightarrow 4CH_3COOH + 4\ CO_2 + 8H_2$	(1)
butyrate	
$CH_3CH_2CH_2COO^- + 2\ H_2O \rightarrow 2\ CH_3COO^- + H^+ 2H_2$	(2)
$CH_3CH_2CH_2COO^- + 0.5\ SO_4^{2-} \rightarrow 2\ CH_3COO^- + 0.5\ HS^- + 0.5H^+$	(3)
$CH_3CH_2CH_2COO^- + 2.5\ SO_4^{2-} + 0.25\ H_2O \rightarrow 4\ HCO_3^- + 2.5\ HS^- + 0.75\ H^+ + 0.25\ OH^-$	(4)
propionate	
$CH_3CH_2COO^- + 3\ H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 3\ H_2$	(5)
$CH_3CH_2COO^- + 0.75\ SO_4^{2-} \rightarrow CH_3COO^- + HCO_3^- + 0.75\ HS^- + 0.25\ H^+$	(6)
$CH_3CH_2COO^- + 1.75\ SO_4^{2-} + 0.25\ H_2O \rightarrow 3\ HCO_3^- + 1.75\ HS^- + 0.5\ H^+ + 0.25\ OH^-$	(7)
acetate	
$CH_3COO^- + SO_4^{2-} \rightarrow 2\ HCO_3^- + HS^-$	(8)
$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$	(9)
hydrogen	
$4\ H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4\ H_2O$	(10)
$4\ H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3\ H_2O$	(11)

From the degradation reactions it can seen that the oxidation of propionate and butyrate can proceed via an acetogenic oxidation followed by acetate and hydrogen conversion by either SRB or MB, or via a direct oxidation of these fatty acids by SRB. Using mass balances only, no distinction between these different reactions can be made.

Table 2.1 shows that an incomplete oxidation of propionate or butyrate by the SRB (reactions 3 and 6) results in the same amount of sulfate reduced than the acetogenic oxidation of propionate and butyrate (reactions 2 and 5) followed by hydrogen oxidation by SRB (reaction 10). Similarly, a complete oxidation of propionate and butyrate by the SRB (reactions 4 and 7) will give the same amount of sulfate reduced as the acetogenic oxidation of propionate and butyrate (reactions 2 and 5), followed by acetate and hydrogen oxidation

by the SRB (reactions 8 and 10). In the rest of this paragraph we will therefore speak only of acetate and/or hydrogen oxidation by the SRB. This can either be a direct oxidation of acetate and hydrogen (reactions 8 and 10), or a complete or incomplete oxidation of propionate and/or butyrate by the SRB (reactions 3,4,6 and 7)

The equations used to calculate the amount and percentage of acetate and hydrogen used by the SRB and MB are listed in Table 2.2.

Table 2.2. Equations for calculating substrate utilisation by SRB and MB

1 *conversion rates*

$COD_{conv,tot} = Q_l \, (\, COD_i - COD_e \,)$

$C_{2,conv} = Q_l \, [\, (C_{2,i} - C_{2,e}) + 0.57 \, (C_{3,i} - C_{3,e}) + 0.8 \, (C_{4,i} - C_{4,e}) + 0.7 \, (suc_i - suc_e) \,]$

$H_{2,conv} = Q_l \, [\, 0.43 \, (C_{3,i} - C_{3,e}) + 0.2 \, (C_{4,i} - C_{4,e}) + 0.3 \, (suc_i - suc_e) \,]$

$COD_{conv,mb} = p_{CH4} \, Q_g \, f_{CH4} + g \, Q_l$

$COD_{conv,srb} = 2 \, Q_l \, (\, SO_{4,i} - SO_{4,e} \,)$

2 *Assumptions made :*

a. acetate used by SRB and MB

$COD_{conv,mb} \leq C_{2,conv} \Rightarrow C_{2,conv,mb} = COD_{conv,mb}$

$COD_{conv,mb} > C_{2,conv} \Rightarrow C_{2,conv,mb} = C_{2,conv}$

$COD_{conv,srb} \leq H_{2,conv} \Rightarrow C_{2,conv,srb} = 0$

$COD_{conv,srb} > H_{2,conv} \Rightarrow C_{2,conv,srb} = COD_{conv,srb} - H_{2,conv}$

b. hydrogen used by SRB and MB

$COD_{conv,mb} \leq C_{2,conv} \Rightarrow H_{2,conv,mb} = 0$

$COD_{conv,mb} > C_{2,conv} \Rightarrow H_{2,conv,mb} = COD_{conv,mb} - C_{2,conv}$

$COD_{conv,srb} \leq H_{2,conv} \Rightarrow H_{2,conv,srb} = COD_{conv,srb}$

$COD_{conv,srb} > H_{2,conv} \Rightarrow H_{2,conv,srb} = H_{2,conv}$

3 *percentage of hydrogen and acetate used by SRB and MB*

$$\%\text{-}H_{2,mb} = \frac{H_{2,conv,mb}}{H_{2,conv,srb} + H_{2,conv,mb}} \, 100 \, \%$$

$$\%\text{-}C_{2,mb} = \frac{C_{2,conv,mb}}{C_{2,conv,mb} + C_{2,con,srb}} \, 100 \, \%$$

$$\%\text{-}H_{2,srb} = \frac{H_{2,conv,srb}}{H_{2,conv,srb} + C_{2,conv,mb}} \, 100 \, \%$$

$$\%\text{-}C_{2,srb} = \frac{C_{2,conv,srb}}{C_{2,conv,mb} + C_{2,conv,srb}} \, 100 \, \%$$

Symbols : COD = organic-COD ($g.l^{-1}$), C2 = acetate (g $COD.l^{-1}$), H2 hydrogen (g $COD.l^{-1}$), C3 propionate (g $COD.l^{-1}$), C4 butyrate (g $COD.l^{-1}$), Suc = sucrose (g $COD.l^{-1}$), SO_4 sulfate ($g \, S^{-1}$), Q_l flow rate ($l.day^{-1}$), P_{CH4} = partial pressure CH_4, Q_g gasproduction ($l.day^{-1}$), f_{CH4} conversion factor from liter CH_4 to g COD, g = solubility CH_4 in water (g $COD.l^{-1}$).

Subscripts : conv = converted, tot = total, mb = by methanogens, srb = by sulfate reducers

calculation $C_{2,conv}$, $H_{2,conv}$: equations (1), (2) and (5) from table 2.2 show the degradation of sucrose, butyrate and propionate. From these equations it can be calculated that in the degradation of 1 g COD sucrose, butyrate and propionate respectively 0.7, 0.8 and 0.57 g COD is degraded via acetate, and that 0.3, 0.2 and 0.43 g COD is degraded via hydrogen.

In these calculations the following assumptions were made :

- The biological conversion reactions describing the system are according to those in table 2.1.
- SRB primarily use hydrogen as the electron donor. As long as the amount of converted hydrogen exceeds the equivalent amount of sulfate reduced, it is assumed that no acetate by the SRB is utilized. If the amount of reduced sulfate exceeds the amount of converted hydrogen the additional amount of reduced sulfate is considered to be acetate oxidation by
- MB primarily use acetate as the substrate. If the amount of converted acetate exceeds the equivalent amount of produced methane, hydrogen utilisation by the MB is not to occur. If the amount of produced methane exceeds the amount of acetate degraded the difference is the result of hydrogen consumption by the MB.

The last 2 assumptions are based on the results of several studies. As has been discussed in chapter 1, in the anaerobic treatment of wastewater with sufficient sulfate the Hydrogenotrophic SRB (HSRB) generally will out compete the hydrogenotrophic MB (HMB) for hydrogen. As a result hydrogen will be completely oxidised by the HSRB. On the other hand, acetate will be partly or completely be degraded by either the ASRB or AMB.

2.5.4 Maximal net specific growth rates, acetate and sulfate affinities.

The growth rates and affinities of the ASRB and AMB were measured in batch reactors. The growth of bacteria in a batch reactor with e.g. acetate as the substrate is given by :

$$\frac{dX}{dt} = \mu X - K_d X \qquad (2.5)$$

$$\frac{dS}{dt} = -\frac{1}{Y} \mu X \qquad (2.3)$$

With	X	= biomassconcentration	g VSS.l^{-1}
	μ	= growth rate	d^{-1}
	K_d	= decay rate	d^{-1}
	S	= substrate concentration	g.l^{-1}
	Y	= yield factor	g VSS.g^{-1} substrate

The specific growth rate according to the Monod equation is :

$$\mu = \mu_{max} \frac{S}{K_s + S} \qquad (2.7)$$

For the ASRB the following modified Monod equation is used :

$$\mu = \mu_{max} \frac{S}{K_s + S} \frac{SO_4}{SO_4 + K_{SO4}} \quad (2.8)$$

With	μ_{max}	= maximal growth rate	d^{-1}
	K_s	= substrate affinity	$g.l^{-1}$
	K_{SO4}	= sulfate affinity	$g.l^{-1}$
	SO_4	= sulfate concentration	$g.l^{-1}$

* *Specific maximal net growth rates*

The measurement of the net growth rate was performed at high acetate ($S >> K_s$) and (for the ASRB) high sulfate concentration ($SO_4 >> K_{SO4}$). Equations (2.5) and (2.6) can then be written as :

$$\frac{dX}{dt} = \mu_n X \quad (2.9)$$

$$\frac{dS}{dt} = - \frac{1}{Y} \mu_{max} X \quad (2.10)$$

With $\mu_n = \mu_{max} - K_d$, the specific maximal net growth rate. d^{-1}

Integration of equations (2.9) and (2.10) yields :

$$S = S_o + \frac{V_a}{\mu_n} (1 - e^{\mu_n t}) \quad (2.11)$$

with	V_a =	$\mu_{max} X_o/Y$ representing the initial acetate degradation rate in the batch fermenter	$g.l^{-1}.d^{-1}$
	S_o =	substrate concentration at t=0	$g.l^{-1}$

In equation (2.11) μ_n and V_a are the only unknown parameters. These parameters have to be calculated from the course of the acetate consumption occurring in the batch experiments.

Estimation of growth rates using batch procedures is generally based on the assumption of a normal gaussian distribution of the error in the measured substrate concentration. However, in our experiments the error in the acetate concentration is characterised by a relative distribution.

The optimal set parameters μ_n and V_a is then found for those values of μ_n and A_o where the function Z is minimal, where Z is defined by :

$$Z = \sum_1^n (\frac{U(t) - f(t,\gamma)}{f(t,\gamma)})^2 \quad (2.12)$$

with U(t) = measured acetate concentration

$f(t,\tau)$ = calculated acetate concentrations according to equation (2.8).

τ = parameters μ_n and A_o

t = time

Since equation (2.12) can not be solved analytically, numerical methods must be used to approximate the optimal solution. A minimalisation method of the simplex type was used. The calculations were done using the Nelder-Mead algorithm which is commercially available from the Math works Inc., Natrick, Massachusets, USA.

* *acetate and sulfate affinities.*

The measurement of the acetate affinity was conducted at a relative low acetate concentration and a high biomass concentrations. For the ASRB also a high sulfate concentration ($SO_4 >> K_{SO4}$) was provided. Due to the high biomass concentration each experiment only lasted 6-8 hours. Consequently, the increase in biomass can be neglected, i.e. $dX/dt = 0$. Integration of equations (2.5) to (2.8) then yields :

$$K_s \ln(\frac{S}{S_o}) + S - S_o = - V_a t \qquad (2.13)$$

For the measurement of the sulfate affinity a low sulfate and high acetate concentration ($S >> K_s$) was used. The course of the sulfate concentration during the experiment is then analogue to equation (2.13) :

$$K_{SO4} \ln(\frac{SO_4}{SO_{4,o}}) + SO_4 - SO_{4,o} = - V_a t \qquad (2.14)$$

With $SO_{4,o}$ = sulfate concentration at time t=0 $g.l^{-1}$

The estimation procedure used for the determination of the growth rates was also applied to obtain the affinities. Here, equations (2.13) and (2.14) were used.

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CHAPTER 3

The anaerobic degradation of acetate, propionate and butyrate in the presence of sulfate in UASB reactors

3.1 Introduction

In anaerobic reactors treating sulfate containing wastewater, there will be a competition between the sulfate reducing bacteria (SRB) and methanogenic bacteria (MB) for the substrates hydrogen and acetate, and between the SRB and acetogenic bacteria (AB) for substrates such as propionate, butyrate and other fatty acids.

Based on the thermodynamics of the sulfate reduction, methanogenesis and acetogenesis, and the kinetic properties of the bacteria involved it is expected that SRB will out-compete MB and AB (Rinzema and Lettinga 1988, Widdel 1988). Observations of the process in anaerobic reactors showed that for hydrogen, generated as intermediate during the anaerobic digestion process, the hydrogenotrophic SRB (HSRB) out-compete the hydrogenotrophic MB (HMB) (Mulder 1984, Rinzema et al. 1986, Rinzema and letting 1988). However, with respect to the outcome of the competition for acetate the literature shows inconsistencies. A complete conversion of acetate by acetotrophic MB (AMB) (Mulder 1984, Hoeks et al. 1984, Rinzema et al. 1986) as well as a predominance of the acetotrophic SRB (ASRB) (Rinzema and Schultz 1987, Stucki et al. 1993) have been reported. With respect to fatty acids such as propionate and butyrate it has been shown that if there is no sulfate limitation, the reducing equivalents formed during the oxidation of these fatty acids to acetate are completely used by the SRB (Rinzema and Lettinga 1988). However, it can not be distinguished whether or not the fatty acids are degraded via a syntrophic association of AB and HSRB or via a direct oxidation by the SRB. Competition between the SRB and AB will be discussed in more detail in chapter 6.

A better understanding and possible control of the competition between the different organisms is important in order to achieve an optimal anaerobic treatment of wastewater with high levels of sulfate.

The goal of this research was to study the competition between the SRB and MB in UASB reactors.

3.2 Material and methods

general

The experiments were performed with two different substrates, namely :

1. acetate plus sulfate.
2. a mixture of acetate, propionate, butyrate and sulfate.

operation of the UASB reactors

1. The degradation of acetate plus sulfate

During the experiment, two UASB reactors as described in chapter 2, with a liquid volume of 10 and 1.7 l were used.

During the first part of the experiment the 10 l UASB reactor was used. The reactor was seeded with 3 l of elutriated sludge obtained from a pilot-plant UASB reactor treating acid-water, a wastestream from the edible oil production, at Unimills, Zwijndrecht, the Netherlands. The characteristics of this sludge have been described elsewhere (Rinzema and Schultz 1987). Prior to the experiment, the sludge was stored at 4°C for about 1 year. After about 775 days of operation the reactor was stopped and the sludge was stored at 4 °C. At day 875, approximately 0.5 l of this sludge was used to start up a 1.7 l UASB reactor.

The acetate and sulfate concentrations in the influent were 1.5 g COD.l^{-1} and 0.75 g.l^{-1} during days 0-520, 3.0 g COD.l^{-1} and 2.9 g.l^{-1} during days 520-775, and 2.1 g COD.l^{-1} and 3.4 g.l^{-1} during days 875-1300.

2. The degradation of acetate, propionate, butyrate and sulfate

A 20 l UASB reactor, as described in chapter 2, was used in this experiment. The reactor was seeded with 8 l of the same sludge as used in experiment 1. The reactor was fed with a mixture of volatile fatty acids consisting of acetate, propionate, butyrate (C2:C3:C4 = 5:3:2, based on COD values) and sulfate. After completion of the experiments, the sludge was stored at 4 °C.

At day 364, about 0.4 l of sludge was taken from the reactor and used as an inoculum for the start-up of a 0.7 l UASB reactor. This reactor was fed with a medium also consisting of acetate, propionate, butyrate (C2:C3:C4 = 5:3:2, based on COD values) and sulfate.

The organic-COD and sulfate concentration in the influent of the 20 l UASB reactor was 3.3 and 1.6 g.l^{-1}, respectively. In the 0.4 l UASB reactor, the organic-COD and sulfate concentration was 4.0 and 8.3 g.l^{-1}, respectively.

sludge characterization

The sludge in the reactors was characterised by means of sludge activity assays. The activity of the sludge was measured with acetate, propionate and butyrate as substrates. The simultaneous measurement of the total-, methanogenic, and sulfidogenic activity was assessed as described in chapter 2.4.

simulation of the competition between the ASRB and AMB

A simple growth model was used to simulate the competition between the ASRB and AMB in anaerobic reactors fed with acetate and sulfate. In the model we assumed a completely mixed high rate anaerobic reactor with the sludge retention time being uncoupled or independent of the hydraulic retention time. Using a mass balance for the reactor, and assuming that the influent does not contain any biomass, the sludge concentration of AMB and ASRB can then be written as :

$$\frac{dX_{mb}}{dt} = \mu_{mb} X_{mb} - K_{d,mb} X_{mb} - \frac{1}{SRT_{mb}} X_{mb} \quad (3.1)$$

$$\frac{dX_{srb}}{dt} = \mu_{srb} X_{srb} - K_{d,srb} X_{srb} - \frac{1}{SRT_{srb}} X_{srb} \quad (3.2)$$

With	X_{mb}	= biomassconcentration of AMB	g VSS.l^{-1}
	μ_{mb}	= growth rates of AMB	day^{-1}
	$K_{d,mb}$	= decay rates AMB	day^{-1}
	SRT_{mb}	= biomass retention time AMB	day
	X_{srb}	= biomassconcentration of ASRB	g VSS.l^{-1}
	μ_{srb}	= growth rates of ASRB	day^{-1}
	$K_{d,srb}$	= decay rates ASRB	day^{-1}
	SRT_{srb}	= biomass retention time ASRB	day

The substrate and sulfate concentration is given by :

$$\frac{dS}{dt} = \frac{1}{HRT} S_i - \frac{1}{HRT} S - \frac{1}{Y_{mb}} \mu_{mb} X_{mb} - \frac{1}{Y_{srb}} \mu_{srb} X_{srb} \quad (3.2)$$

$$\frac{dSO_4}{dt} = - \frac{1}{HRT} SO_{4,i} - \frac{1}{HRT} SO_4 - \frac{1}{Y_{srb}} \mu_{max,sr} \frac{96}{60} X_{srb} \quad (3.4)$$

With	S	= acetate concentration in reactor	g.l^{-1}
	S_i	= acetate concentration in influent	g.l^{-1}
	SO_4,	= sulfate concentration in reactor	g.l^{-1}
	$SO_{4,i}$	= sulfate concentration in influent	g.l^{-1}
	HRT	= Hydraulic retention time	day
	Y_{mb}	= biomass yield AMB	g VSS.g^{-1} COD degraded
	Y_{srb}	= biomass yield ASRB	g VSS.g^{-1} COD degraded

The growth rate of the bacteria in equations (3.1) to (3.4) is according to the Monod kinetics given as :

$$\mu_{mb} = \mu_{max,mb} \frac{S}{S + K_{s,mb}} \quad (3.5)$$

$$\mu_{srb} = \mu_{max,srb} \frac{S}{S + K_{s,srb}} \frac{SO_4}{SO_4 + K_{SO4,srb}} \quad (3.6)$$

With	$\mu_{max,mb}$	= maximal growth rates of AMB	day^{-1}
	$K_{s,mb}$	= acetate affinities of AMB	$g.l^{-1}$
	$\mu_{max,srb}$	= maximal growth rates of ASRB	day^{-1}
	$K_{s,srb}$	= acetate affinities of ASRB	$g.l^{-1}$
	K_{SO4}	= sulfate affinity of ASRB	$g.l^{-1}$

equations (3.1) to (3.6) were solved for different conditions using the SIMNON programm which is commercially available from the Department of automatic control, Lund institute of technology, Lund, Sweden.

3.3 Results

3.3.1 The degradation of acetate plus sulfate in UASB reactors

reactors

Fig 3.1 shows the loading rate, the conversion rates, the sulfide concentrations and the effluent-pH during the experiment.

In the experiments three periods can be distinguished, namely days 0-520, 520-775 and 875-1300. During these periods the COD/sulfate ratio of the feed was 2, 1 and 0.62, respectively. This means that until day 775 there was an excess of substrate relative to the sulfate, whereas from day 875 onwards theoretically sufficient sulfate was available to oxidise all acetate via sulfate reduction.

During the first 50 days of operation all acetate was converted by the AMB ; sulfide could not be detected. From days 50 onwards a slow increase in the amount of acetate used by the ASRB was observed. After about 200 days of operation a steady state with respect to the removal of acetate by ASRB and AMB was reached. From day 200 till 500 the performance of the reactor remained fairly constant. Approximately 77 and 23 % of the degraded acetate was removed by the AMB and ASRB, respectively. The total-sulfide and sulfate effluent concentration during this period was about and 135 and 230 $mg.l^{-1}$, respectively. The sulfate removal efficiency was approximately 70 %. During this stage of the experiment, the sulfate reduction process probably was sulfate limited due to sulfate transport limitation into the sludge granules. This prevented a further increase of the acetate utilisation by the ASRB. Therefore, at day 520 the sulfate concentration in the influent was increased, which resulted in an increase of the utilisation of acetate by the ASRB. At day 775 the reactor was stopped. At day 875 the experiment was restarted and the sulfate concentration was further increased so that theoretically all acetate could be degraded by the ASRB. During this stage of the experiment the amount of acetate used by the ASRB steadily increased further. At day 1200 a complete removal of the acetate by the ASRB was accomplished.

An average net sludge yield of about 0.038 g $VSS.g^{-1}$ COD degraded was found during the experiment.

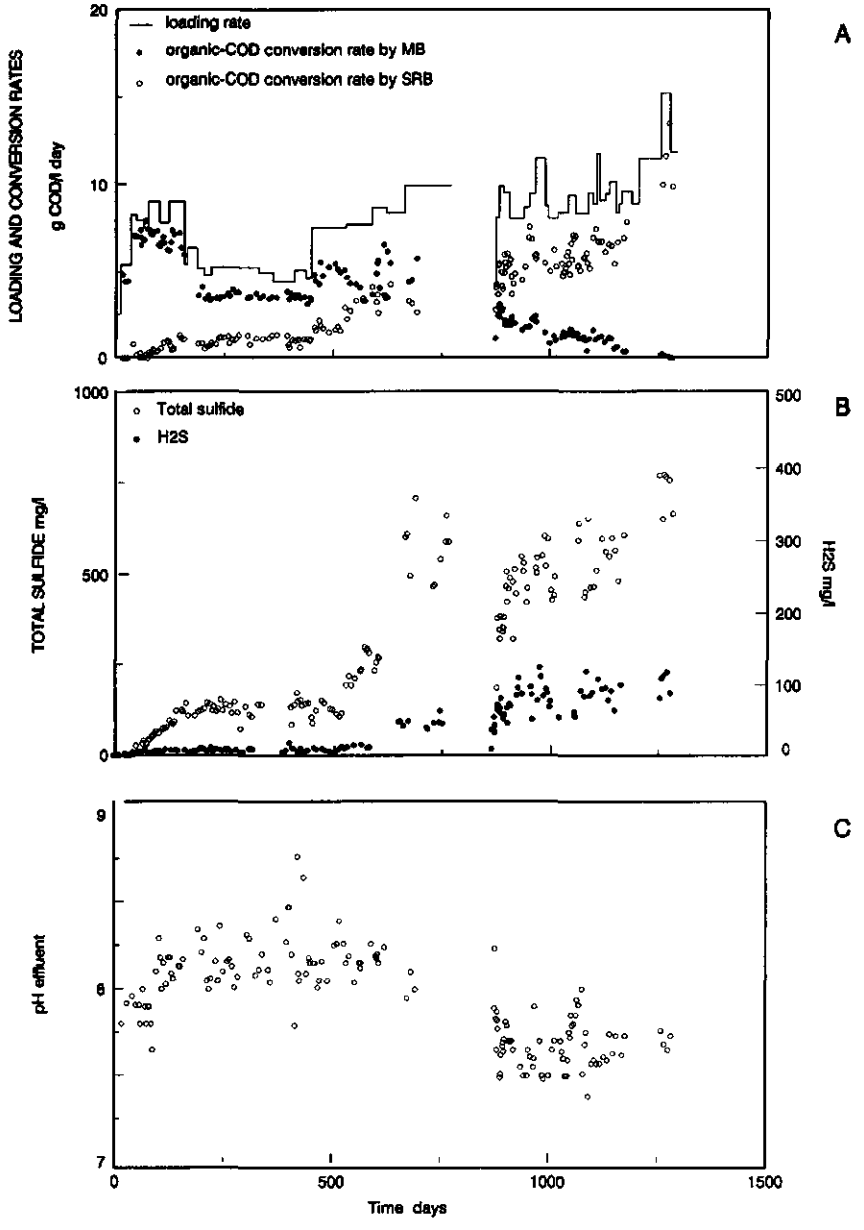

Fig 3.1. The loading- and conversion rate (a), the total-sulfide and free H_2S concentrations (b) and the effluent-pH (c) in the UASB reactor fed with acetate and sulfate. The acetate and sulfate concentrations of the influent were 1.5 g COD.l^{-1} and 2.9 g^{-1}, during days 0-520, 3.0 g COD.l^{-1} and 2.9 g.l^{-1} during days 520-775 and 2.1 g COD.l^{-1} and 3.4 g.l^{-1} during day 875-1300.

sludge characterisation

The development of the sludge activity during the experiment is shown in Fig 3.2.

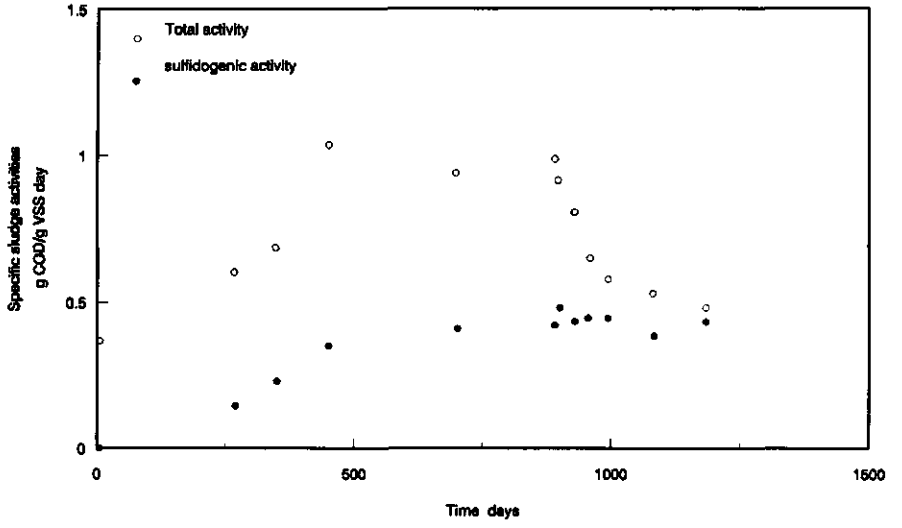

Fig 3.2. The total and sulfidogenic sludge activity of sludge samples taken at different moments of the UASB reactor fed with acetate and sulfate. The acetate and sulfate concentrations of the influent of the reactor were 1.5 g COD.l^{-1} and 2.9 g^{-1}, during days 0-520, 3.0 g COD.l^{-1} and 2.9 g.l^{-1} during days 520-775 and 2.1 g COD.l^{-1} and 3.4 g.l^{-1} during day 875-1300.

In the initial seed sludge no acetotrophic sulfidogenic activity was detected, indicating that only a low number of acetate degrading ASRB were present. During the first part of the experiment, when the reactor was operated at relatively low sulfate influent concentrations, a slow increase in the methanogenic activity was observed. On the contrary, the sulfidogenic activity increased relatively fast. At the end of the first part of the experiment at day 750, the specific methanogenic as well as the sulfidogenic sludge activity were about 0.5 g COD.g^{-1} VSS.day^{-1}. Activity tests with other substrates showed that besides acetate also propionate, butyrate, ethanol and lactate were well degraded by the sludge. The specific degradation rates for these substrates were respectively 0.41, 0.54, 0.77 and 0.73 g COD.g^{-1} VSS.day^{-1}. However, methanol was not degraded within one week.

During the second part of the experiment, when the reactor was operated at an excess of sulfate, the methanogenic activity decreased whereas the sulfidogenic activity remained rather constant. At the end of this period the methanogenic activity was almost zero. These results

indicate that the ASRB became the predominant organisms in the sludge, and that a sulfidogenic granular type of sludge had developed.

3.3.2 The degradation of acetate, propionate, butyrate and sulfate in UASB reactors

reactors

Fig 3.3 shows the loading rate, conversion rates, the sulfide concentrations and the pH during the experiment with the 20 l UASB reactor, which was operated under sulfate limiting conditions (COD/sulfate = 2).

The sulfate reduction process started almost immediately after the start-up of the reactor and increased steadily until from days 75 onwards a steady-state situation, with respect to the ratio of substrate removed via sulfate reduction and methanogenesis was reached. Under these conditions about 68 and 32 % of the organic-COD was removed via methanogenesis and sulfate reduction, respectively. Using mass balances it was calculated that during days 0-30 all acetate was converted into methane. On the other hand, all the reducing equivalents formed during the oxidation of propionate and butyrate to acetate, in the following termed 'hydrogen', were completely used by the SRB during the whole experiment. From days 30 onwards the oxidation of acetate by the ASRB steadily increased until from days 75 onwards about 82 and 18 % of the acetate was removed via methanogenesis and sulfate reduction respectively. Under these pseudo steady-state conditions the sulfate and total-sulfide effluent concentrations amounted to 175 and 375 $mg.l^{-1}$, respectively. The reactor has been in operation for 700 days.

At days 364 about 0.4 l of sludge was taken from the 20 l UASB reactor for the start up of a 0.7 l UASB reactor, which was operated under conditions of sufficient sulfate (COD/sulfate = 0.5). Fig 3.4 shows the loading rates, conversion rates, the sulfide concentrations and the pH values obtained in the reactor.

In the UASB reactor an increase in the amount of organic-COD used by the SRB with time was observed. At the end of the experiment the SRB were the predominant species. The sulfide concentrations in the effluent at the end experiment was 2000 $mg.l^{-1}$.

sludge characterisation

In Fig 3.5 the course of the sludge activities for sludge samples taken from the 20 l UASB reactor, which was operated at a COD/sulfate ratio of 2, is shown. The seed sludge had no acetotrophic sulfidogenic activity, indicating that only a low number of ASRB were present in this sludge.

During the experiment the sulfidogenic activity with acetate as substrate increased slowly. At the end of the experiment the sulfidogenic and methanogenic sludge activities on acetate were about 0.25 and 0.5 g COD. g^{-1} VSS day^{-1}, respectively. With Propionate and butyrate as substrates, sludge activities increased with time until approximately day 300. At the end of the experiment the assessed sulfidogenic on propionate and butyrate were 0.42 and 0.5, g $COD.g^{-1}$ $VSS.day^{-1}$, respectively. The methanogenic activities on propionate and butyrate were 0.5 and 0.4 g $COD.g^{-1}$ $VSS.day^{-1}$, respectively.

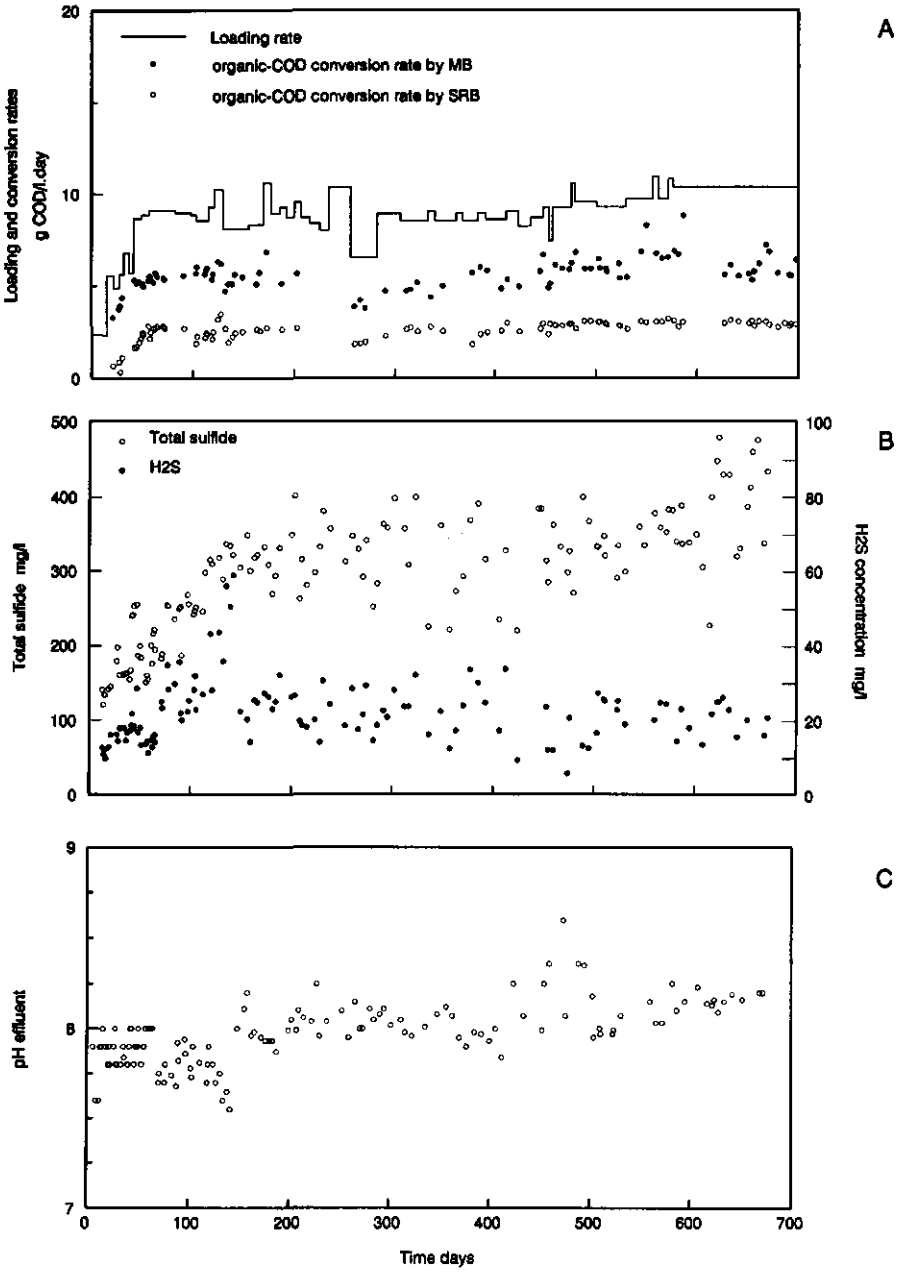

Fig 3.3. The loading- and conversion rate (a), the total-sulfide and H_2S concentrations (b) and the effluent-pH (c) in the 20 l UASB reactor fed with a mixture of acetate, propionate and butyrate (5:3:2, based on COD values) and sulfate. The organic-COD and sulfate concentration of the influent of the reactor were 3.3 g $COD.l^{-1}$ and 1.6 $g.l^{-1}$.

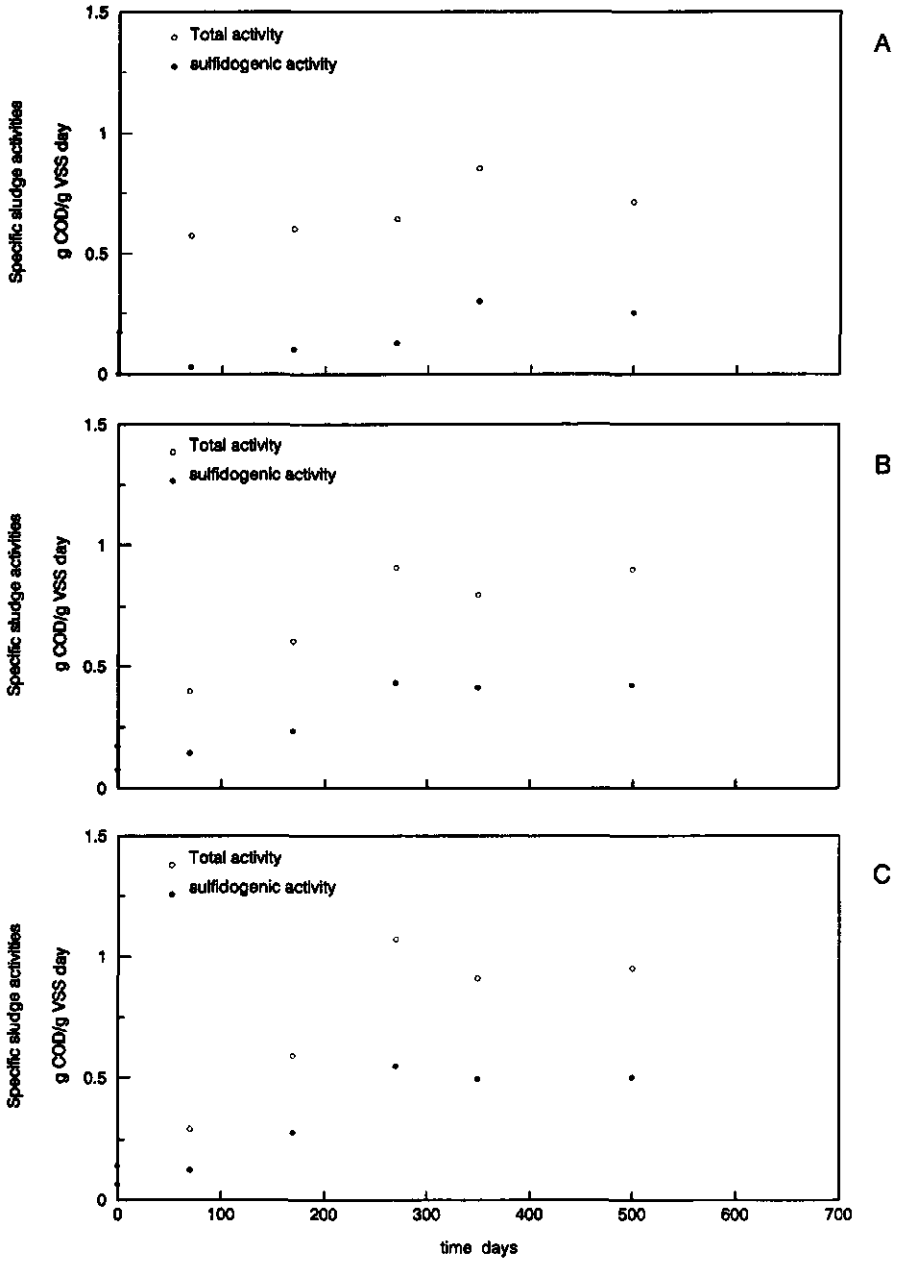

Fig 3.4. The total and sulfidogenic sludge activity of sludge samples of the 20 l UASB with acetate (a), propionate (b) and butyrate (c) as substrates. The reactor was fed with a medium consisting of a mixture of acetate, propionate and butyrate (5:3:2, based on COD values) and sulfate. The organic-COD and sulfate concentration of the influent of the reactor were 3.3 g COD.l^{-1} and 1.6 g.l^{-1}.

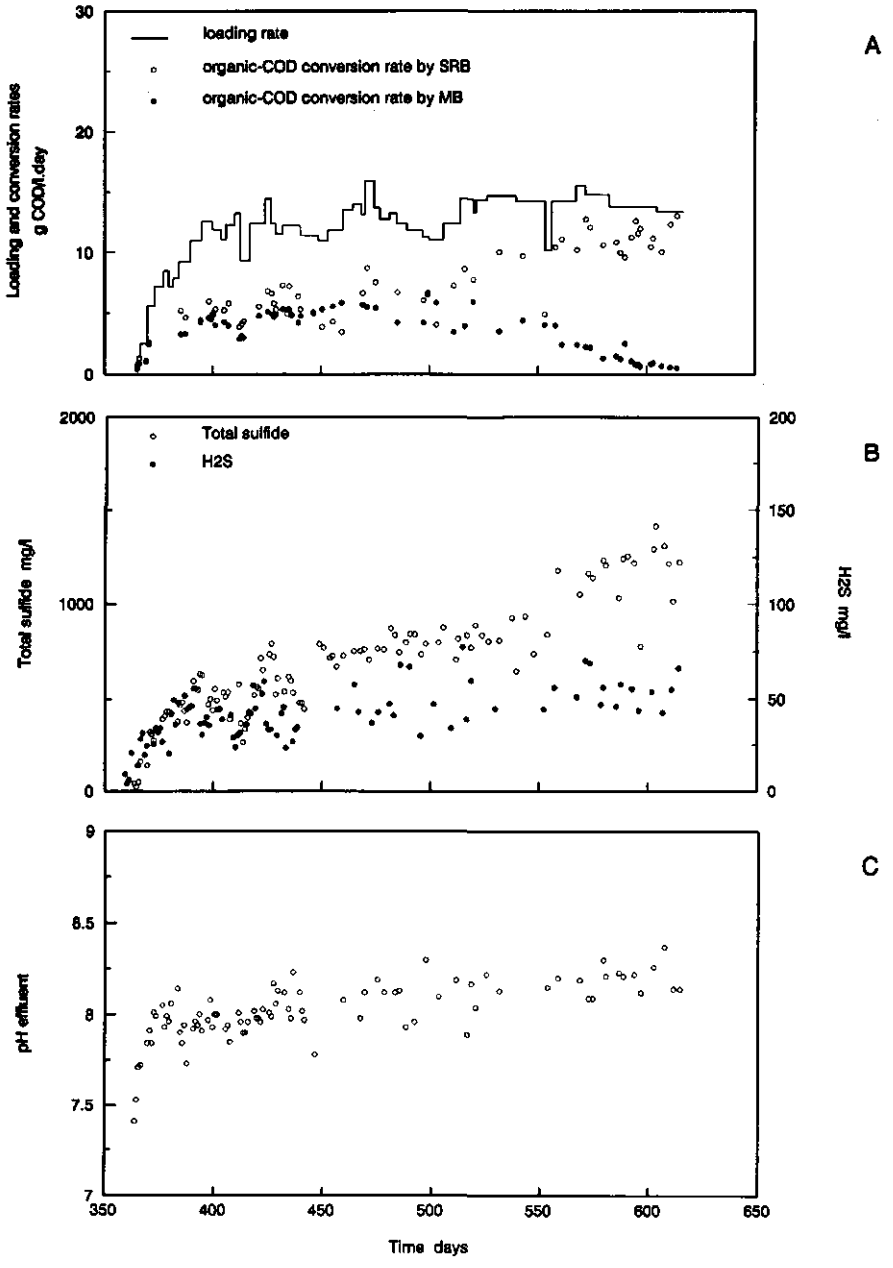

Fig 3.5. The loading- and conversion rate (a), the total-sulfide and H_2S concentrations (b) and the effluent-pH (c) in the 0.7 l UASB reactor, fed with a mixture of acetate, propionate, butyrate (5:3:2, based on COD values) and sulfate. The organic-COD and sulfate concentration of the influent of the reactor were 4.3 g $COD.l^{-1}$ and 8.3 $g.l^{-1}$.

Activity tests in the absence of sulfate showed that under these conditions propionate and butyrate were not degraded within one week. Activity tests with other substrates showed that in the presence of sulfate also ethanol and lactate were well degraded by the sludge. The specific degradation rates for lactate and ethanol were 0.76 and 0.9 g COD.g^{-1} VSS.day^{-1}. However, methanol was not degraded within one week.

Fig 3.6 shows the sludge activities found of the sludge from the 0.7 l UASB reactor which was operated at a COD/sulfate ratio of 0.5.

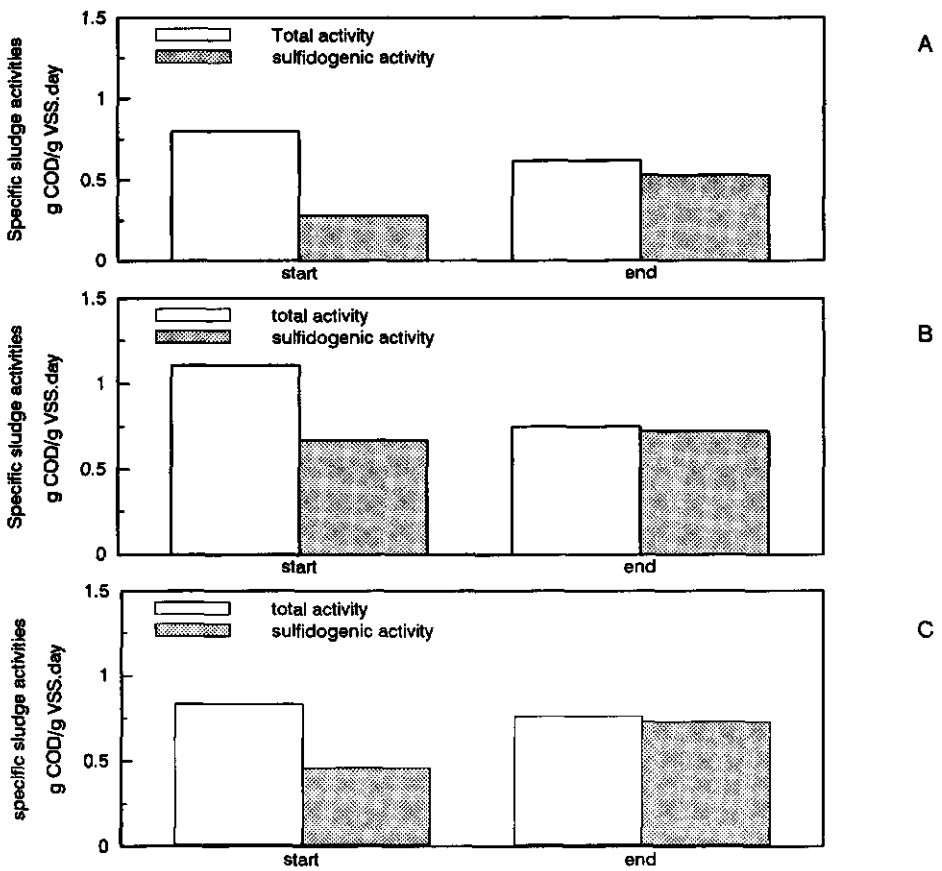

Fig 3.6. The total and sulfidogenic sludge activity of sludge samples of the 0.7 l UASB reactor with acetate (a), propionate (b) and butyrate (c) as substrates. The reactor was fed with a medium consisting of a mixture of acetate, propionate and butyrate (5:3:2, based on COD values) and sulfate. The organic-COD and sulfate concentration of the influent of the reactor were 4.3 g COD.l^{-1} and 8.3 g.l^{-1}.

The activity assays clearly reveal that at the end of the experiment the SRB were the predominant species. The final activities on acetate, propionate and butyrate were 0.79, 0.75 and 0.75 g $COD.g^{-1}$ $VSS.day^{-1}$, respectively.

3.3.3 Simulation of the competition between the ASRB and AMB for acetate

Table 3.1 shows the results of the simulation studies. The growth rates, acetate and sulfate affinities used in these simulations were measured in our laboratory (see chapter 4). The other parameters were taken as an average value from the literature.

Table 3.1B clearly shows that if the growth rate of the ASRB is only slightly higher than the growth rate of the AMB, it takes a very long time before AMB are out-competed by ASRB. The impact of the composition of the seed sludge on the course of the competition is illustrated in table 3.1C. If the seed sludge contains hardly any ASRB, the time before the ASRB out-compete the AMB is very long. The effect of the SRT is shown in table 3.1D. This table shows that at longer SRT's it takes more time before the ASRB will become the predominant species.

3.4 Discussion

The results of this study clearly show that ASRB can out-compete AMB as the predominant acetotrophic organisms. Reports on the competition between ASRB and AMB are not consistent. Both a pre-dominance of ASRB (Rinzema and Schultz 1987, Stucki et al 1992), and AMB (Hoeks et al. 1984, Mulder 1984, Rinzema et al. 1986) are reported. The results presented here show that, although ASRB eventually become the pre-dominant species, a long time is required for the ASRB to actually out-compete the AMB in the sludge. This is in accordance with Harada et al. (1994) who also reported that only after long-term operation of UASB reactors, ASRB became pre-dominant.

The seed sludge that was used in this study contained only a low number of ASRB . This was reflected by the fact that no sulfidogenic activity on acetate as substrate was detected. In the acetate fed reactor, it took 50 days before acetate degradation by the ASRB was observed. After 100 days, 10 % of the acetate was degraded by the ASRB. The simulation studies showed that a long time may be involved before a 10 % degradation of the acetate by the ASRB is realized. This depends on the number of ASRB and AMB present in the seed sludge at the start of the experiment.

In the acetate fed reactor it took about 400 days to increase the share of acetate used by the ASRB from 50 to 90 %. In the reactors fed with a VFA mixture, this took about 250 days. These observations clearly illustrate that the population shift in the reactor in the number of ASRB and AMB proceeds slowly.

Table 3.1. Results of the simulation of the competition between ASRB and AMB. Growth rates and affinities were assessed experimentally (see chapter 4). Other parameters are average values from the literature.

A : simulation data

Acetate affinities K_s ($mg.l^{-1}$)	: ASRB (55), AMB (55).
Sulfate affinity K_{SO4} ($mg.l^{-1}$)	: ASRB (33)
Decay rates (day^{-1})	: ASRB (0.005), AMB (0.005)
Yield (g VSS.g^{-1} C2)	: ASRB (0.05), AMB (0.025)
Influent ($g.l^{-1}$)	: C2 (2), SO_4 (4)
Others	: HRT 0.25 day, $(X_{mb})_{t=0}$ 10 g VSS.l^{-1}

B : Effect of growth rates of ASRB and AMB

$\mu_{max,mb}$ $\mu_{max,srb}$	Time (days) before the SRB degrade 10, 50 and 90 % of the acetate		
day^{-1}	10	50	90
0.111[a] 0.119[b]	7200	10100	13800
0.091[b] 0.122[b]	1300	1925	2650
0.051[c] 0.131[b]	180	311	530

[a]pH 7, 30 °C ; [b]pH 7.5, 30 °; [c]pH 8, 30 °C
SRT_{mb} = SRT_{srb} = 150 days, $(X_{mb}/X_{srb})_{t=0}$ = 1 e3.

C : Effect of number of AMB and ASRB at the start

	Time (days) before the SRB degrade 10, 50 and 90 % of the acetate		
$(X_{mb}/X_{srb})_{t=0}$	10	50	90
1 e3	180	311	530
1 e5	410	541	760
1 e7	460	771	980
1 e9	1160	1100	1520

$\mu_{max,mb}$ = 0.051 day^{-1} , $\mu_{max,srb}$ = 0.131 day^{-1} , SRT_{mb} = SRT_{srb} = 150 days

D : Effect of the solid retention time

	Time (days) before the SRB degrade 10, 50 and 90 % of the acetate		
SRT_{mb},SRT_{srb}	10	50	90
50	115	175	280
150	180	311	530
300	220	400	700

$\mu_{max,mb}$ = 0.051 day^{-1} , $\mu_{max,srb}$ = 0.131 day^{-1} , $(X_{mb}/X_{srb})_{t=0}$ = 1 e3

This is also confirmed by the results of the simulations. In the case where it was assumed that the growth rates of the ASRB and AMB were almost the same, ie respectively 0.119 and 0.11 day^{-1}, we calculated that at a SRT of 150 days, about 3700 days are needed for an increase in the percentage of acetate degradation by ASRB from 50 to 90 %. If the growth rates of ASRB and AMB are respectively 0.131 and 0.051 day^{-1}, 219 days are needed for such an increase. These results indicate that the kinetic growth properties of the ASRB and AMB under the imposed conditions are very important. The effect of the environmental conditions on the growth rates of ASRB and AMB will be discussed in chapter 4. Another reason for the slow shift in the utilisation of the acetate from AMB to ASRB lays in the long SRT normally found in high-rate anaerobic reactors. In UASB reactors the SRT can be as high as ½-1 year (Hulshoff Pol 1989).

Apart from the competition for acetate it was observed that 'hydrogen' was completely oxidized by the SRB in the reactors. The oxidation of 'hydrogen' by SRB takes place either by a direct oxidation of molecular hydrogen during an acetogenic oxidation of fatty acids coupled with the oxidation of the produced hydrogen by HSRB, or during the incomplete oxidation of the fatty acids by SRB. However, based on the present experiments no distinction between these two reactions is possible. The pre-dominance of 'hydrogen' degrading SRB observed in this study is in agreement with earlier observations showing that in anaerobic reactors SRB will out-compte HMB or AB for 'hydrogen' (Mulder 1984, Rinzema et al. 1986, Rinzema and Lettinga 1988). In this study no hydrogenotrophic methanogenic activity was detected during the whole experimental period. This shows that already in the seed sludge, which was adapted to a wastewater with an excess of sulfate, hardly any HMB were present. In anaerobic reactors with immobilized biomass and treating a wastewater with sufficient sulfate, the activity of the HMB is normally completely suppressed within a few weeks. Even in cases where reactors were seeded with sludge adapted to no or low sulfate concentrations, it is observed that within a short time all the hydrogenotrophic methanogenic activity in the reactor has stopped (See chapter 6, Oude-Elferink et al. 1994). As the HMB likely are still present in high numbers in the sludge, these observations can not be simply explained by only the difference in Monod kinetics of the HSRB and HMB. Therefore, presumably the competition is also affected by the threshold value for hydrogen of the HMB and HSRB. The outcome of the competition then can be explained by a lower threshold value for hydrogen of the HSRB than the HMB, and the assumption that the HSRB keeps the hydrogen concentration below the threshold value of the HMB. In that case hydrogen consumption by the HMB would energetically be a unfavourable process. The role of this mechanism in competition between HSRB and HMB was suggested by Lovley et al. (1982). These authors found for a eutrophic lake sediment a minimum threshold value for hydrogen of approximately 0.0088 $\mu mol.l^{-1}$ under methanogenic conditions. After the addition of sulfate this value was lowered to 0.0013 $\mu mol.l^{-1}$. An alternative explanation for the rapid suppression of the conversion of hydrogen by HMB is that in the presence of sulfate hydrogen is no longer an important intermediate anymore. In

the absence of sulfate, hydrogen and acetate are formed by the acetogens. In the presence of sulfate the substrates for the AB can be directly oxidized by SRB without the intermediate formation of hydrogen. In that case the HMB are out-competed due to the fact that the SRB out-compete the AB. Competition between the SRB and AB will be discussed in more detail in chapter 6. However, in more complex wastewater some hydrogen will always be formed by the fermentative bacteria.

3.5 references

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CHAPTER 4

Kinetic growth properties of acetotrophic sulfate reducers and methanogens at different pH values and sulfide concentrations

4.1 Introduction

The outcome of the competition of acetotrophic sulfate reducing bacteria (ASRB) and acetotrophic methanogenic bacteria (AMB) in anaerobic reactors depends on the environmental conditions imposed on the bacteria. In this chapter the effect of the pH and the sulfide concentrations is discussed.

The pre-dominant acetotrophic methanogen in anaerobic reactors is considered to be *Methanothrix soehngenii* (Grotenhuis 1992, Hulshoff Pol 1989). The optimal, minimal and maximal pH for growth of this organism are about 7.1-7.8, 6.8 and 8.3, respectively. For *Methanosarcina* sp., the other acetotrophic methanogen, the optimal, minimal and maximal pH values for growth are about 6.5-7.5, 5.5 and 8.0, respectively (König and Stetter 1984). The acetate degrading SRB that are sofar isolated, show an optimal pH in the range of 7.3 to 7.6. The minimal and maximal pH values for growth is about 6.0 and 9.0, respectively (Sneath 1984, Widdel and Pfennig 1984). In general, it is seen that the optimal pH values for the ASRB and AMB are in the same range. However, the ASRB can tolerate higher pH values than the AMB.

The toxicity of sulfide for the AMB has been investigated extensively. It is generally assumed that the toxicity of sulfide is caused by the undissociated H_2S. A 50 % inhibition of the methanogenesis in suspended and granular sludges has been reported at H_2S concentrations ranging of 50 to 250 $mg.l^{-1}$ (Kroiss and Plahl-Wabnegg 1983, Karhadkar et al. 1987, Koster et al. 1986, Oleskiewicz et al. 1989). Contrarily to the AMB hardly any data are presented regarding the inhibition by sulfide of ASRB. A complete inhibition of the growth of *Desulfovibrio* species has been found at 550 and 350 $mg.l^{-1}$ H_2S at pH values 6.2-6.7 and 7, respectively (Reiss et al. 1992, Okabe et al. 1992). Widdel (1980) reported an inhibition of the acetate degrading *Desulfotomaculum acetoxidans* at H_2S concentrations exceeding 85 $mg.l^{-1}$. Stucki et al. (1993) reported a process failure in a sulfidogenic fixed bed reactor in which a mixture of acetate and sulfate was treated, at H_2S concentrations above 50 $mg.l^{-1}$. These results suggest that acetate degrading SRB are rather sensitive to sulfide.

The goal of this study was to asses the effect of the sulfide concentration and the pH on the kinetic growth properties of AMB and ASRB and to assess the impact of these environmental conditions on the competition between the two bacterial species.

4.2 Material and Methods

General

The kinetic parameters of ASRB and AMB that were determined at different pH values and sulfide concentrations were the maximal specific net growth rate, the acetate affinity, the sulfate affinity and the maximal specific methanogenic and sulfidogenic activity.

Batch experiments

The assessment and calculations of the different kinetic parameters are described in chapter 2. The sludge used originated from a 10 l UASB reactor fed with a mixture of acetate and sulfate with a ratio of 1 g acetate-COD.g^{-1} sulfate, as described in chapter 3. The sludge was stored at 4 °C, and then re-activated in a 1.7 l UASB reactor fed with acetate (2 g COD.l^{-1}) and sulfate (2 g.l^{-1}). The sludge samples used in the batch experiments were taken from the 1.7 l UASB reactor which was continuously in operation during the batch tests.

4.3 Results

In this study the course of kinetic growth properties of ASRB and AMB was determined at different pH values and sulfide concentrations.

The kinetic data were assessed from batch experiments. Fig 4.1 shows an typical example of the measured and calculated acetate concentrations during a measurement of the net specific growth rate and the acetate affinity found during a batch assay.

The role of the pH

The course of the maximal net specific growth rates of ASRB and AMB in granular and crushed granular sludge at different pH values is shown in Fig 4.2. Fig 4.3 shows the specific methanogenic and sulfidogenic sludge activity of granular sludge at different pH-values.

The AMB have a maximal growth rate at pH-values of 6.5-7. If the AMB are located in sludge granules, however, growth can take place at higher pH-values. The optimal pH value with respect to the specific methanogenic activity of the granular sludge was about 7-7.5. At pH values above the optimal pH, a relative slow decrease in the specific activity was observed even though the growth rate of the bacteria decreased relatively fast. On the other hand at lower pH values both the activity and the growth rate decreased relative rapidly.
The optimal pH for growth of the ASRB was found to be at pH 8.5 till 9. The ASRB in sludge granules grow in a wider pH range than ASRB in suspended sludge. In the granular sludge good growth was observed in a wide pH range of 7 to 9 ; at pH 7 the growth rate was only slightly less than the rate at pH 9. The optimal pH for the sulfidogenic activity of granular sludge was in the pH-range of 7.5-8.5. During the assessments of the sulfidogenic sludge activities it was observed that, at pH values below 6.5 and above 8, an increase and/or decrease of the pH of 0.2 till 0.5 pH unit occurred.

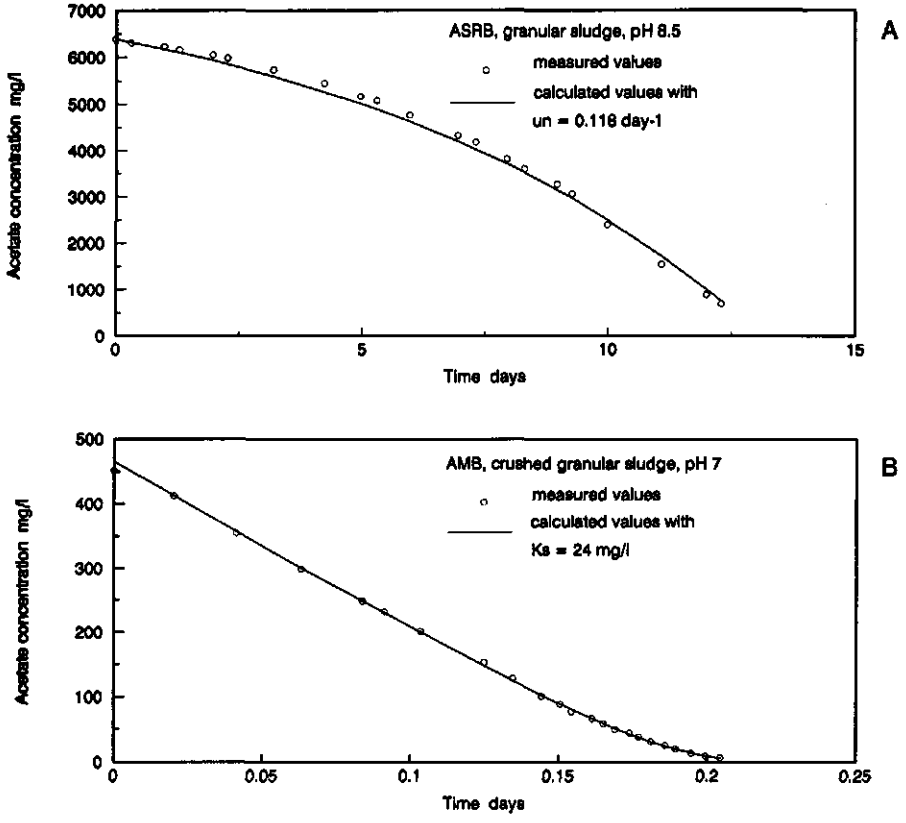

Fig 4.1. Measured and calculated acetate concentrations during a batch assay for the assessment of the net growth rate (A) and the acetate affinity (B)

For data interpretation the average pH values during the assays were considered. During the determination of the growth rates the pH was rather constant during the experiments. Fig 4.2 and 4.3 show that there was a reasonable correlation between the course of the sulfidogenic sludge activity and the growth rates of the ASRB.

The acetate and sulfate affinities of ASRB and AMB were measured at different pH values ranging from 6 till 8. No significant different in the affinities was found at the different pH values. The affinities seemed independent of the pH. In Table 4.1 the average affinities are listed.

Table 4.1. Acetate and sulfate affinities ASRB and AMB in granular sludge and suspended sludge.

		K_s-acetate mg.l^{-1}	K_s-sulfate mg.l^{-1}
MB			
	granular sludge	54 ± 14	--
	suspended sludge	29 ± 5	--
SRB			
	granular sludge	55 ± 11	33 ± 7
	suspended sludge	10 ± 5	18 ± 5

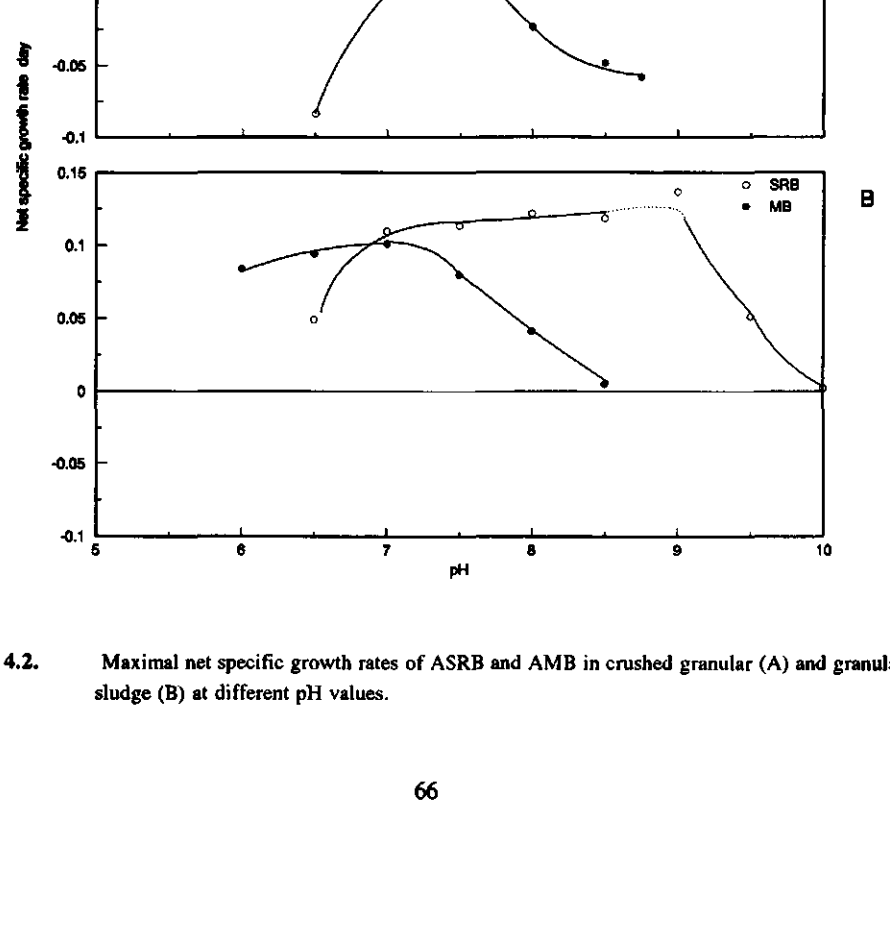

Fig 4.2. Maximal net specific growth rates of ASRB and AMB in crushed granular (A) and granular sludge (B) at different pH values.

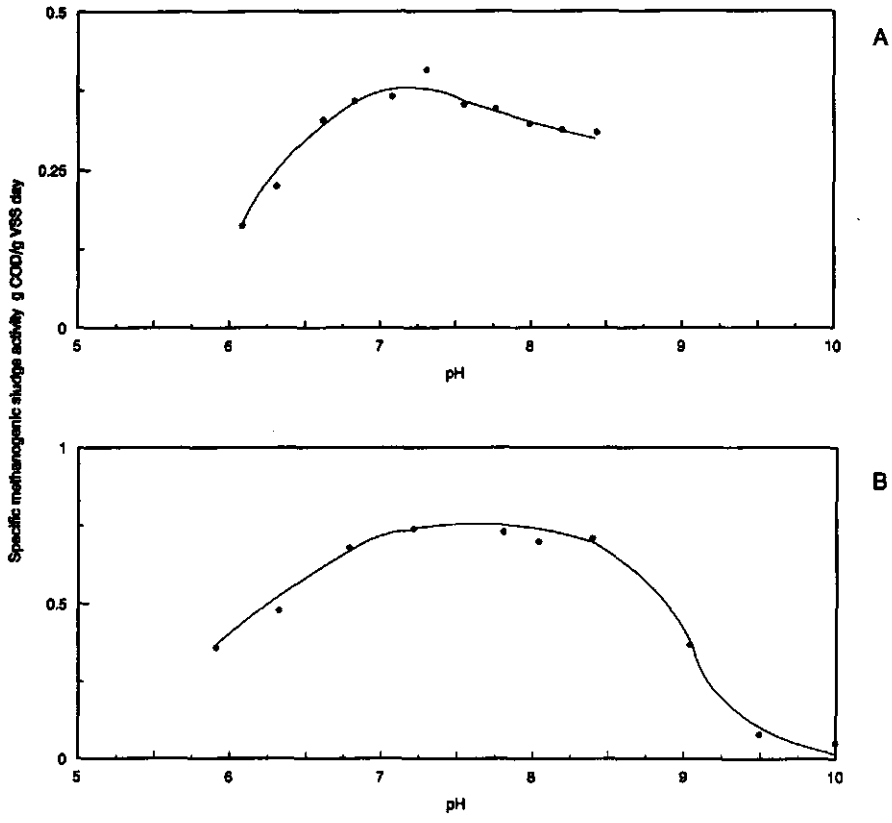

Fig 4.3. *Specific methanogenic (A) and sulfidogenic (B) activity in granular sludge at different pH values.*

The results show that the ASRB from crushed granular sludge have a higher affinity for acetate than the AMB. In sludge granules, however, the acetate affinities of the ASRB and AMB were the same, and lower than the affinities measured in the suspended sludge. The sulfate affinity of the ASRB in suspended sludge was higher than for ASRB in granular sludge.

The actual growth rate of the AMB and ASRB in anaerobic reactors is dependent of the acetate and/or sulfate concentration in the digester. Based on the maximal growth rates and the affinities we calculated that at pH values lower than about 7.4 suspended AMB have higher growth rates than suspended ASRB at low and high acetate concentrations. Contrarily, at pH values exceeding about 7.7, ASRB grow faster than the AMB. At pH values between

7.4 and 7.7, both the acetate and sulfate concentration will determine whether ASRB or AMB have better growth properties, as is illustrated in Fig 4.4A.

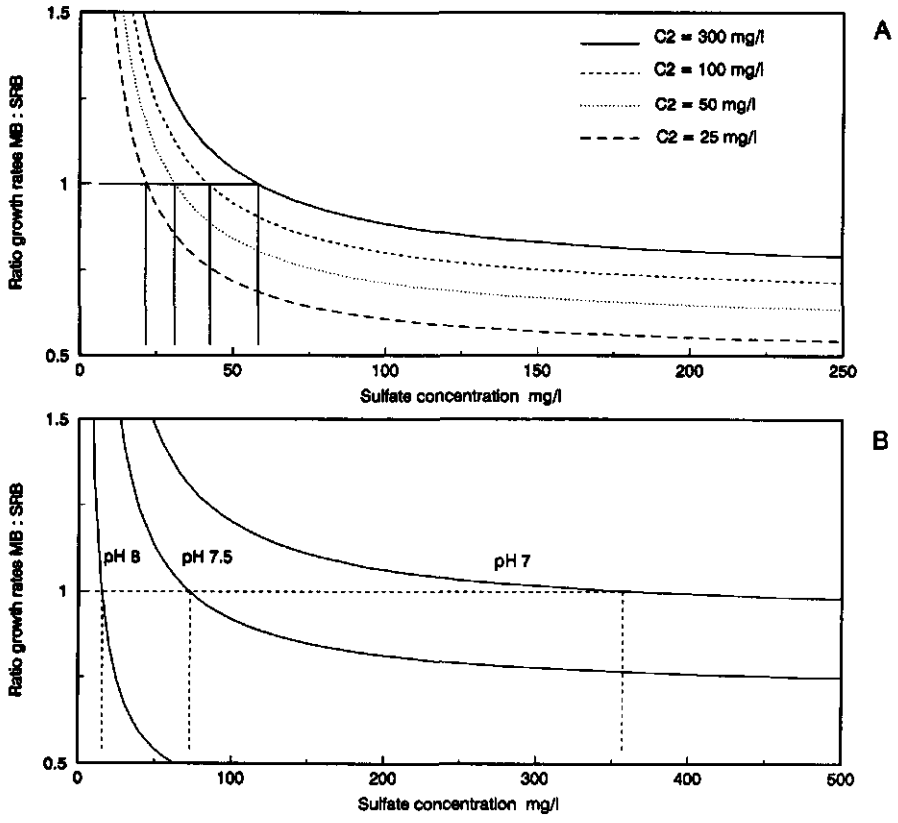

Fig 4.4. Effect of the sulfate concentration on the ratio of the growth rates of AMB and ASRB in suspended sludge at pH 7.5 (A) and granular sludge at pH of 7, 7.5 and 8 (B).

The acetate affinities of the ASRB and AMB in granular sludge are the same. Consequently, the acetate concentration has no influence on the ratio of the growth rates of the ASRB and AMB. From fig 4.2 it can be seen that at pH values lower than about 6.9 the AMB have higher growth rates than the ASRB. On the other hand, at pH values exceeding 8.5 the ASRB have better growth properties than the AMB. In the pH range of 6.9 to 8.5 the sulfate concentration in the reactor determines whether ASRB or AMB have the have the

highest growth rate, as is shown in Fig 4.4B. At pH values of 7, 7.5, and 8 ASRB have higher growth rates than AMB only if the sulfate concentration is higher than 360, 75 and 15 $mg.l^{-1}$, respectively.

The role of the sulfide concentration

Fig 4.5 and 4.6 show the effect of the free H_2S and the total sulfide (TS) concentration on the growth rates and specific activity of AMB, respectively.

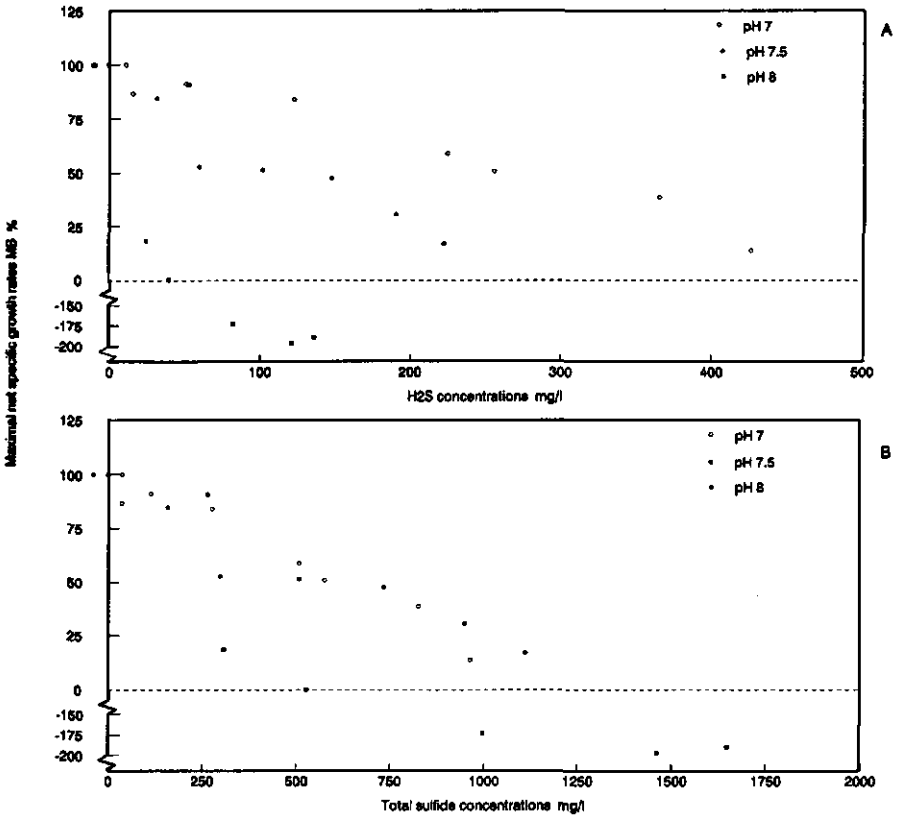

Fig 4.5. Growth rates of AMB at different H_2S (A) and TS concentrations (B) at pH values 7, 7.5 and 8. The growth rates are expressed as the percentage of the not-inhibited maximal growth rates at 0 $mg.l^{-1}$ sulfide.

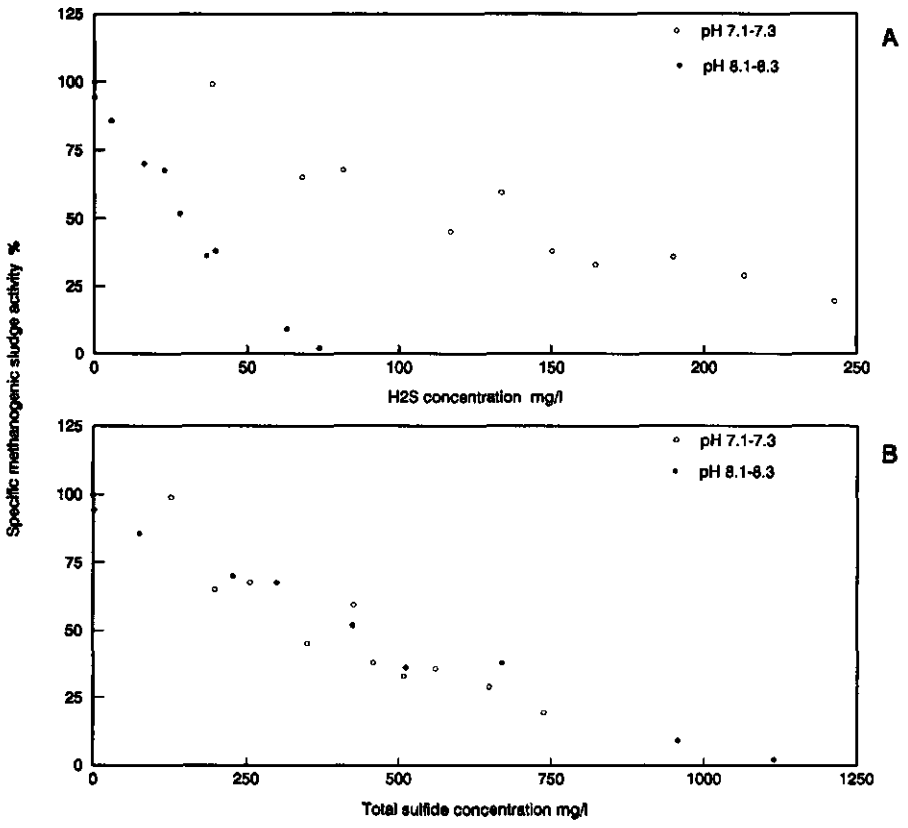

Fig 4.6. The specific methanogenic activity at different H_2S (A) and TS concentrations (B) at pH values 7.2-7.4 and 8.1-8.3. The activities are expressed as the percentage of the not-inhibited maximal specific activity at 0 $mg.l^{-1}$ sulfide.

On the contrary, at pH values of about 8 the growth rate of AMB was more inhibited by sulfide than the specific methanogenic activity. The specific methanogenic activity was equally inhibited by the TS concentration at pH 7.2-7.4 and 8.1-8.3, respectively. However, the growth rate of AMB was more inhibited by the TS concentration at pH 8 than at pH 7-7.5. At pH values of 7-7.5 there is a good correlation between the decrease in the specific methanogenic activity and in the growth rates with increasing sulfide concentrations.

Fig 4.7, 4.8 and 4.9 show the effect of the sulfide concentration on the growth rates and the specific activity of the ASRB.

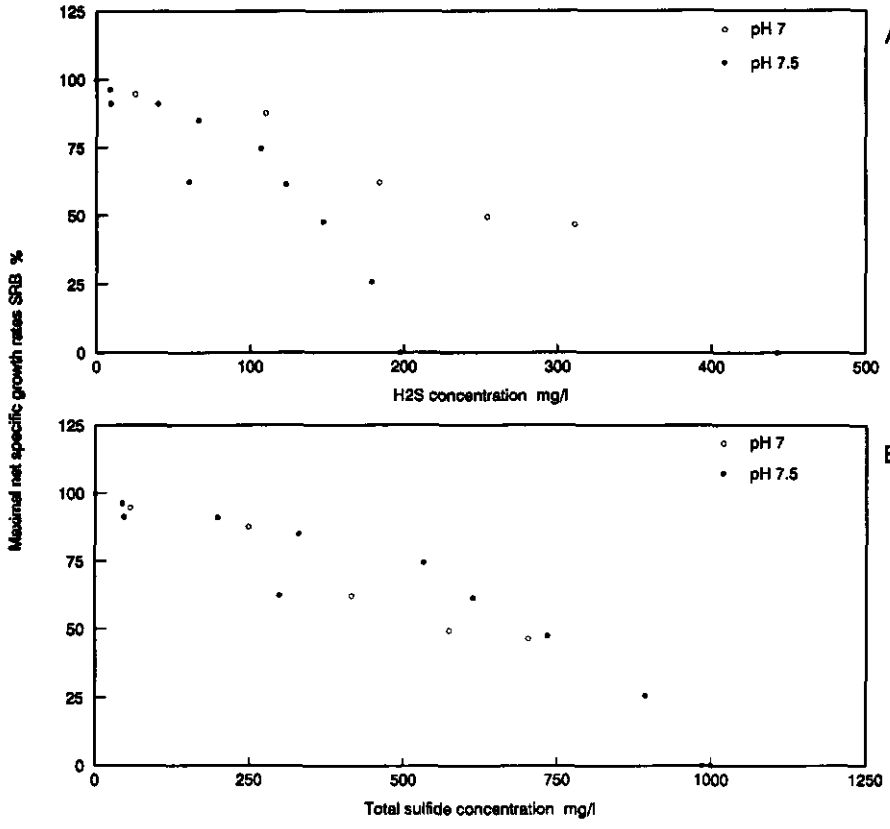

Fig 4.7. Growth rates of ASRB at different H_2S (A) and TS concentrations (B) at pH values 7 and 7.5. The growth rates are expressed as the percentage of the not-inhibited maximal growth rates at 0 $mg.l^{-1}$ sulfide.

At pH values of 7-7.5 a good correlation between the effect of sulfide on the growth rate and specific sulfidogenic activity was observed. At pH 8 the decrease in the growth rate was a little higher than the decrease in the specific activity with increasing sulfide concentrations. Both the growth rates and the specific activities were less inhibited by the TS concentration at pH values of 8-9 than at a pH of 7 to 7.5.

The results clearly show that the inhibiting effect of undissociated H_2S on AMB as well as ASRB was stronger at higher pH values.

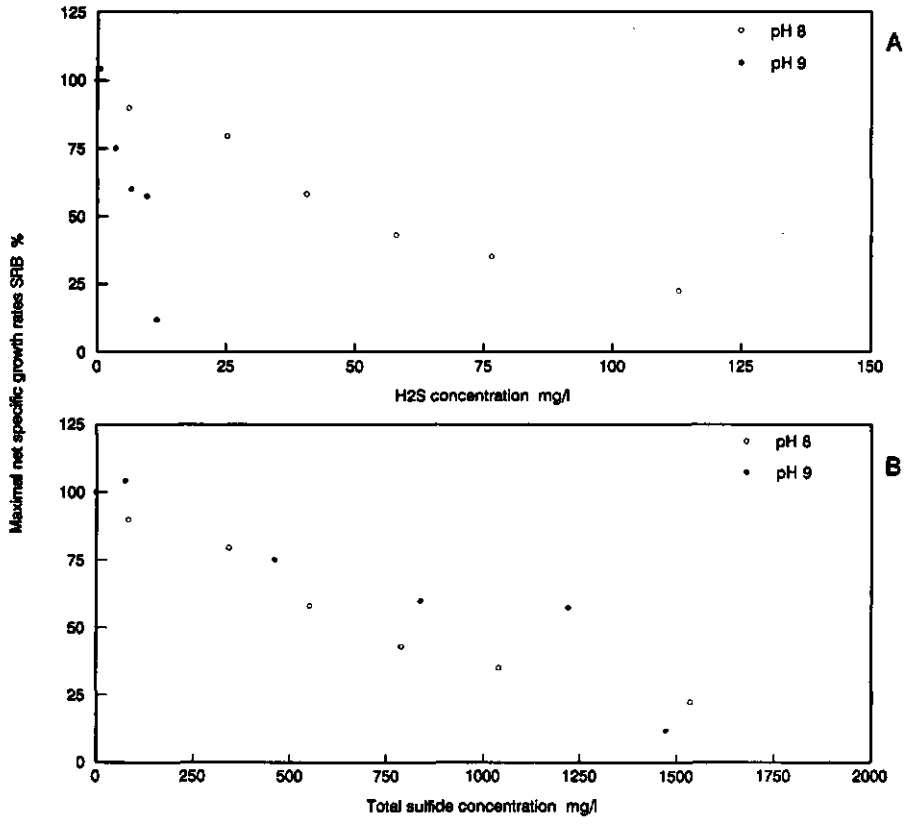

Fig 4.8. Growth rates of ASRB at different H_2S (A) and TS concentrations (B) at pH values 8 and 9. The growth rates are expressed as the percentage of the maximal growth rates at 0 $mg.l^{-1}$ TS.

Table 4.2 lists the free H_2S and TS concentrations that cause a 50 % inhibition of the growth rates and specific activities of ASRB and AMB.

Table 4.2. TS and H_2S concentrations causing a 50 % inhibition of the specific methanogenic activity, the sulfidogenic activity, and the growth rates of ASRB and AMB at different pH values. The sulfide concentrations were calculated by linear regression of the activity or growth rate versus the sulfide or free H_2S concentration.

		AMB		ASRB	
	pH	H_2S $mg.l^{-1}$	TS $mg.l^{-1}$	H_2S $mg.l^{-1}$	TS $mg.l^{-1}$
50 % of activity	7.2-7.4	184	564	171	615
	8.1-8.3	38	590	57	1125
50 % of maximal growth rate	7	248	561	231	521
	7.5	131	650	114	569
	8	20	246	68	921
	9	---	---	7.4	943

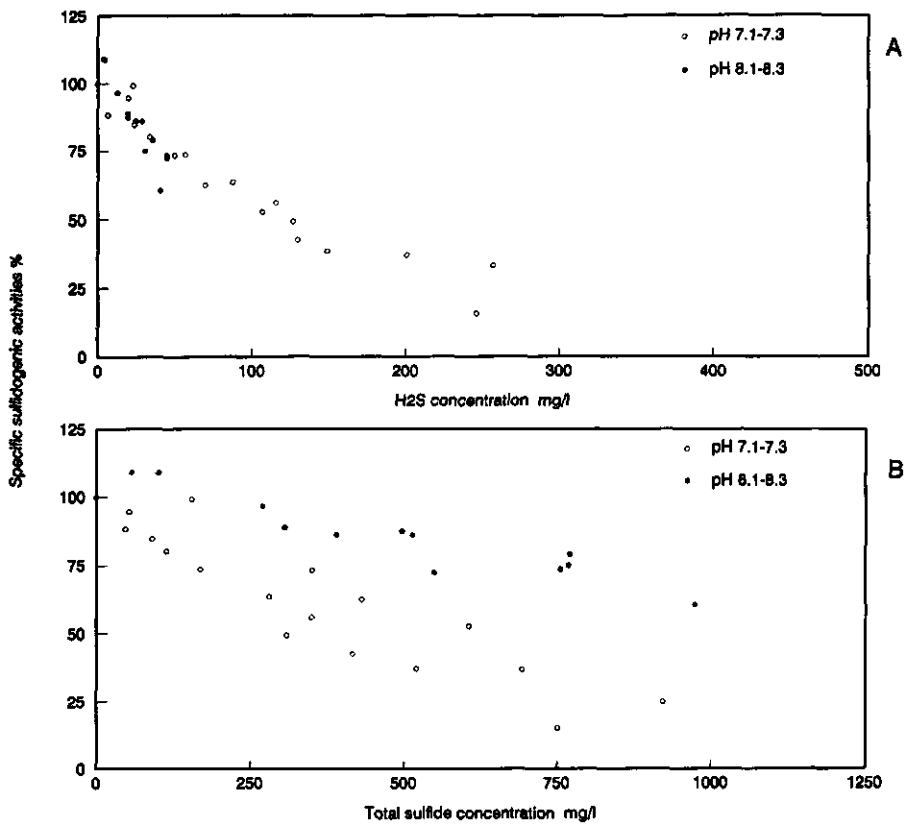

Fig 4.9. Specific sulfidogenic activity at different H_2S (A) and TS concentrations (B) at pH values 7.2-7.4 and 8.1-8.3. The activities are expressed as the percentage of the maximal specific activity at 0 $mg.l^{-1}$ TS.

At pH values of 7-7.5 the inhibition of both free H_2S and TS on AMB and ASRB is about the same. These results indicate that at these pH values the presence of sulfide has no important additional effect on the competition between ASRB and AMB. At pH values of 8 and higher the ASRB are considerably less sensitive to sulfide than the AMB. Consequently, at these pH values ASRB have in the presence of sulfide an advantage over AMB.

In Table 4.3 the kinetic parameters determined in this study as well as literature values are presented.

Table 4.3. Growth rates, acetate- and sulfate affinities of ASRB and AMB at different pH values and temperatures as determined in this study and depicted from the literature.

	μ_{max} day^{-1}	K_s mg.l^{-1}	K_{SO4} mg.l^{-1}	pH	T °C	Reference
ASRB						
granular sludge	0.11	55	33	7	30	this study
	0.12	55	33	7.5	30	this study
suspended sludge	0.001	10	18	7	30	this study
	0.05	10	18	7.5	30	this study
biofilm	0.015	10	45	7.5	30	1
suspended sludge	0.58	6	30		31	2
Desulfobacter Postagei	0.84			7.3	32	3
		14		6.9	30	4
Desulfotomaculum acetoxidans	0.55			7.1	36	5
Desulfonema limicola	0.55			7.6	30	3
AMB						
granular sludge	0.12	54		7	30	this study
	0.09	54		7.5	30	this study
suspended sludge	0.09	29		7	30	this study
	0.03	29		7.5	30	this study
biofilm	0.037	33		7.5	30	1
Methanothrix soenghenii	0.11	26		7.6	37	6,7
Methanothrix concilii	0.69	72		7.2	30	8
Methanosarcina barkeri	0.69	144		6.3	35	9
	0.50	300			36	10

1. Yoda et al. 1986, 2. Middleton and Lawrence 1977, 3. Widdel 1980, 4. Schönheit et al. 1982, 5. Widdel and Pfennig 1977, 6. Huser 1981, 7. Zehnder et al. 1980, 8. Patel 1984, 9. Powell et al. 1983, 10. Smith and Mah 1980.

The growth rates and affinities of the AMB in the sludge used in this study are similar to the values reported for *Methanothrix soenghenii* (see table 4.3). The growth rates found for the ASRB in this sludge are however lower than the values presented in the literature for

pure cultures of *Desulfobacter Postagei*, *Desulfotomaculum acetoxidans* or *Desulfonema limicola* (see table 4.3). The acetate and sulfate affinities are in the same range as the values normally observed for the ASRB. The maximal pH value found in this study for growth of the ASRB in the sludge is about 10 which is higher than the maximal pH of 9 reported in literature (Widdel and Pfennig 1984).

4.3 Discussion

This study clearly shows that the pH and the sulfide concentration play an important role in the competition between the ASRB and AMB. At pH values below 6.9 AMB can out-compete ASRB in granular sludge, due to the better growth properties of AMB under these conditions. At pH values of 6.9 to 7.7 AMB and ASRB are very competable. The growth rates of the bacteria are in the same range, and ASRB and AMB are equally inhibited by sulfide. The outcome of the competition is dependent on the sulfate concentration in the bulk solution. At low sulfate concentrations growth of ASRB will be sulfate limited, giving AMB sufficient advantage over the ASRB. On the contrary, at sufficient high sulfate concentrations, ASRB can out-compete AMB and will become the predominant species. In addition to a direct effect of the sulfate concentration on the growth rates of ASRB, different types of SRB will also compete for the available sulfate at low sulfate concentrations . Laanbroek et al (1984) showed that in chemostats operated under sulfate limiting conditions, the acetate degrading *Desulfobacter postagei* is a poor competitor to the propionate degrading *Desulfobulbus propionicus* and the hydrogen oxidizing *Desulfovibrio (Desulfomicrobiom) baculatus* in their competitiom for the available sulfate. As reported in chapter 1, ASRB have lower sulfate affinities than the hydrogen degrading *Desulfovibrio* species. For a complex wastewater with a low sulfate concentration or a high COD/sulfate ratio it therefore can be expected that compounds such as hydrogen or propionate will be oxidised by the SRB while acetate will mainly be degraded by the AMB. It has been observed that in granular sludges obtained from different full scale UASB reactors and adapted to COD/sulfate ratios exceeding 20, hardly any ASRB were present (C.N.J. Buisman, personal communication). Choi and Rim (1991) reported that if the COD/sulfate ratios exceeds 2.7 AMB predominate, whereas at COD/sulfate ratios lower than 1.7 ASRB became the more pre-dominant organisms. At COD/sulfate ratios between 1.7 and 2.7 there was an active competition between the AMB and ASRB.

For reactors operating with excess sulfate, sulfate limitation and sulfate competition is not relevant. However, sulfate limitation for ASRB in an anaerobic biofilm or sludge granule might still occur even at relatively higher sulfate concentrations in the bulk liquid, due to mass transfer limitation of sulfate into the biomass. Nielsen (1986) reported that a biofilm of only a few hundred μm thickness will already be sulfate limited if the sulfate concentration in the bulk liquid is below 50 $mg.l^{-1}$. The apparent sulfate affinity of ASRB in the granular

sludge measured in this study indicate that at sulfate concentrations lower than about 350 $mg.l^{-1}$, growth of the ASRB in the sludge granules will be limited by the sulfate. This is in accordance with Overmeitre et al. (1994) who calculated that sludge granules will become sulfate limited at bulk liquid sulfate concentration less than 300 mg/l. In chapter 3 it was shown that, in reactors fed with acetate and sulfate at sulfate concentration in the bulk solution of approximately 230 $mg.l^{-1}$, the ASRB became sulfate limited.

Our findings indicate that at pH values exceeding about 7.7 the ASRB will out-compete the AMB, because under these conditions the ASRB have a higher maximal specific growth rate and are less inhibited by sulfide than AMB. The sulfidogenic bacteria still grow until pH values of 10, whereas the maximal pH value for growth of the AMB is about 8.5.
In the batch assays for the assessments of the growth rates in this study, high acetate concentrations of approximately 5 $g.l^{-1}$ were used at the start experiments. Such high acetate levels can, especially at lower pH values, inhibit AMB or ASRB due to high concentrations of undissociated acetate. Recent research at our laboratory provides evidence that the acetate concentrations used in this study can inhibit the AMB and ASRB at pH values ≤ 6 and ≤ 6.5, respectively (A. Visser, unpublished results). The results obtained at these low pH values should therefore be interpreted with care. It should also be noted that the results in this study are based on short term batch experiments. The predictions of the pattern of the competition between ASRB and AMB based on the presented results of our experiments should be validated in long term experiments in continuous systems before any definite conclusions can be drawn. However, in chapter 3 it was already shown that it can take a very long time, up to a few years, before ASRB will out-compete AMB in UASB reactors. On the contrary, batch assays as used in the presented study, provides useful indications about the competition between ASRB and AMB under different environmental conditions in the short term.

Sofar only little is known about the role of the pH and sulfide level on the competition between ASRB and AMB. Data on sulfide toxicity of AMB show a big variation. A 50 % inhibition of the methanogenic activity has been reported at H_2S concentrations ranging from 50 $mg.l^{-1}$ to 125 $mg.l^{-1}$ at pH values of 7-8 for suspended sludge and 250 $mg.l^{-1}$ and 90 $mg.l^{-1}$ at pH values 6.4-7.2 and 7.8-8.0, respectively, for sludge granules (Kroiss and Plahl-Wabnegg 1983, Oleskiewicz et al. 1989, McCartney and Oleskiewicz 1993, Koster et al. 1986). Sofar no data are available from the literature about the effect of sulfide on ASRB. For lactate degrading SRB, a complete inhibition of the sulfate reduction has been reported at H_2S concentrations ranging from 230 to 550 mg $H_2S.l^{-1}$ at pH values 6.2-8 (McCartney and Oleskiewicz 1991, 1993, Reiss et al. 1992, Okabe et al. 1992). Hilton and Oleskiewicz (1988) concluded from their experiments with a suspended sludge growing on lactate, that the inhibition of SRB correlate with the TS concentration, whereas the inhibition of the MB was caused by the concentration of undissociated H_2S. According to these findings high pH values combined with high sulfide concentrations, would be favourable for the MB, provided that the H_2S concentration is sufficient low. McCartney and Oleskiewicz (1991) reported for

a suspended sludge, adapted to a mixture of lactate, acetate and sulfate (COD/sulfate ratio 3.7) that SRB were more inhibited by sulfide than MB. In contrast, McCartney and Oleskiewicz (1993) observed for a suspended sludge adapted to lactate, acetate and sulfate at COD/sulfate ratios of 1.6 and 0.8, that the SRB were less sensitive to sulfide than the MB. These results show the impact of the history of the sludge. Possibly, as has been indicated in chapter 1, for sludges adapted to relative high COD/sulfate ratios a syntrophic degradation of lactate, propionate and other fatty acids coupled with hydrogen oxidation by the SRB is the predominant route. The effect of sulfide on sulfate reduction is then likely caused by a an inhibition of the acetogens which then results in less intermediate hydrogen formation and less sulfate reduction. For sludges adapted to lower COD/sulfate ratios a direct oxidation of lactate, propionate and other fatty acids by SRB is a more important degradation route. The effect of sulfide can then be directly be attributed to the inhibition of the SRB. The investigations of McCartney and Oleskiewicz (1991, 1993) showed that in all their experiments acetate was completely used by the AMB. It is therefore likely that in the biomass used by these authors, little if any ASRB were present. Their results can therefore not be used to evaluate the toxicity of sulfide for ASRB or the effect of the sulfide concentration in the competition between ASRB and AMB.

In this study some interesting differences between the granular and the suspended, crushed granular sludge were observed. For AMB and ASRB it was shown that they can grow at a wider pH range within in sludge granules than when they are present in suspended sludge. It is likely that this can be attributed to the existence of pH and substrate gradients in sludge granules. The occurrence of pH gradients has been observed in denitrifying biofilms (Arvin and Christensen 1979, 1982) and sludge granules (De Beer and van den Heuvel 1988). The difference in acetate- and sulfate affinities of ASRB and AMB in suspended and granular sludge as determined in this study, indicate that in sludge granules the apparent affinities are dependent on the mass transfer of the substrate and/or sulfate into the sludge granule, rather then the biological conversion rates in the sludge granule. As a result, the acetate affinities of ASRB and AMB in sludge granules are about the same, whereas for suspended sludge the ASRB have a lower K_s value than the AMB.

With respect to the toxic effect of sulfide on ASRB and AMB in granular sludge we observed that in the pH-range of 7 till 9 inhibition by sulfide is dictated by the TS concentration rather than the H_2S concentration. Similar observations were reported by Koster et al (1986) for AMB in sludge granules and by Oleskiewicz et al. (1989) for SRB in suspended sludge. Oleskiewicz et al. (1989) and McCartney and Oleskiewicz (1993) found on the other hand that inhibition of the MB in a suspended sludge was caused by the H_2S concentration. Visser et al. (1993) reported, that the inhibition of thermophilic AMB by sulfide at pH values of 6.2 to 8.3, depends on the H_2S concentration for a suspended sludge, whereas for granular sludge in a pH range 7.1-8.3, the inhibition related to the TS level. All these observations indicate that the inhibition of the SRB at pH values exceeding 7, is

determined by the TS level. For the sulfide inhibition of MB the sludge characteristics seems to be important. In suspended sludges inhibition depends on the H_2S concentration, whereas in sludge granules inhibition is dictated by the TS concentration. In the literature an increase in the reactor pH is suggested as an effective method to reduce the toxicity of sulfide. The idea is that by raising the pH, the H_2S concentration, which is considered to be the toxic form of sulfide, will be reduced (Särner 1986, Rinzema and Lettinga 1988). The effect of the pH on the H_2S and TS concentration is illustrated in Fig. 4.10. From Fig 4.10 it can be seen that a significant reduction in the H_2S concentration is only achieved if the pH is increased above 7.5. The reason for this is that if the pH increased below 7.5 the shift in the dissociation equilibrium of H_2S is compensated by a less effective stripping of H_2S with the biogas. However, the results of our study show that at pH values higher than 7 the inhibition of the AMB and ASRB in granular sludge correlated more with the TS concentration and not the free H_2S concentration. Therefore, a reduction in the inhibition by sulfide at higher pH-values is not expected. On the contrary, as can be seen from Fig 4.10, at higher pH values the TS concentration in the reactor will increase and, consequently, a higher degree of inhibition will occur. This will especially be true for the MB, since the results of our study show that the growth of the MB was much more affected by sulfide at pH 8 than at pH 7 and 7.5.

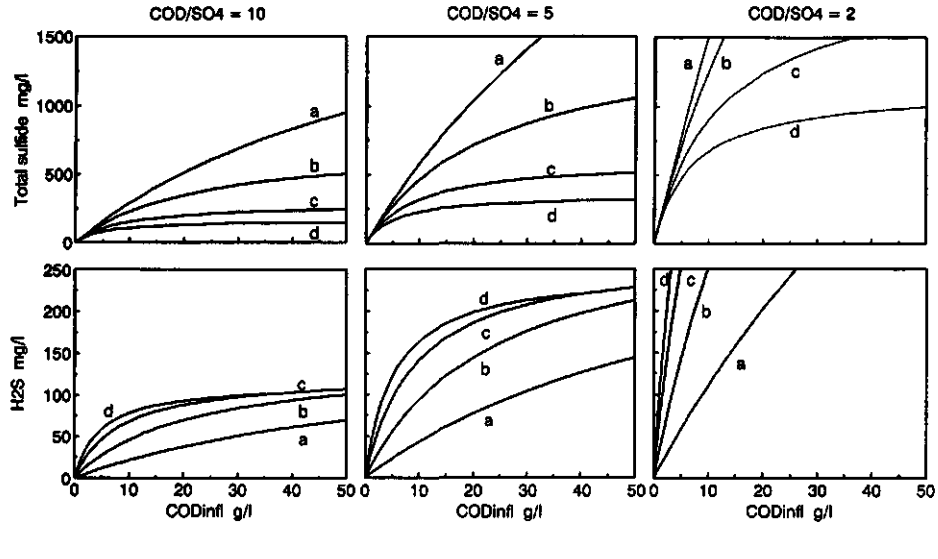

Fig 4.10. The effect of the pH on the H2S and TS concentration at different COD/sulfate ratios. The TS and H_2S concentrations were calculated according to the model given by Rinzema and Lettinga (1988a).

In this study the effect of the pH and sulfide concentration on the growth rate and the substrate degradation rate showed some differences. It was found that at high pH values, and especially at a high pH in combination with a high sulfide concentration, growth of AMB is strongly inhibited, whereas the specific methanogenic activity is relatively slightly affected. Similar observations were made with ASRB. These findings can be explained by assuming that under conditions of "stress", all energy derived from the dissimilation is required for cell maintenance and is not available for cell growth. In the literature activity tests often are used to predict toxic effects. However, according to our findings such tests may result in an underestimation of the long term toxicity. Activity assays seem proper to predict a reactor response to toxic compounds on a short term, e.g. during a temporary exposure. The different response to the growth rate and activity to high pH and high sulfide levels show that for long term predictions growth rates measurements seems more suitable.

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CHAPTER 5

Immobilisation and granulation of sulfate-reducing and methanogenic bacteria in anaerobic reactors

5.1 Introduction

Immobilization of bacteria is a process widely used in anaerobic digestion. The bacteria are immobilized, for example, by the formation of a biofilm on inert solid particles or by a spontaneous formation of bacterial aggregates (sludge granules).

So far, most research concerning immobilization and granulation of bacteria in anaerobic reactors has been focused on methanogenic systems and the role of the methanogenic bacteria (MB). Recently, the immobilisation and granulation process in anaerobic reactors has been extensively reviewed (Hulshoff Pol 1989, Grotenhuis 1992) and will not be discussed in detail further here.

In contrast to the methanogenic systems only little is known about the immobilization in sulfidogenic systems. Although pure cultures of sulfate reducing bacteria (SRB) cultivated in the laboratory often aggregates in clumps or stick to surfaces (Widdel 1988), the ability of the SRB to form a biofilm or a sludge granule in anaerobic reactors is still not clear.

The ability of bacteria to immobilize is critical in modern high rate anaerobic reactors. Bacteria insufficient capable to attach to granular sludge or a biofilm will be washed out of the reactor, and will be present in low numbers only. Therefore, the immobilisation properties of bacteria is an important aspect in the competition between bacterial species, such as the SRB and MB. According to Isa et al. (1986 a,b) a superior colonisation capacity of MB would enable these bacteria to successfully compete with the SRB. Contrary to these authors, Yoda et al. (1987) assumed an equal attachment ability of the SRB and MB. So far, insufficient data are available to properly evaluate the importance of the immobilisation of SRB and MB in the competition between the two species. Therefore, we studied the granulation process and the formation of a biofilm on a carrier in anaerobic reactors treating waste water with high levels of sulfate. The goals of this study were :

- To investigate the ability of the MB and SRB to form sludge granules or a biofilm in different systems, namely a methanogenic system, a mixed methanogenic/sulfidogenic system and a sulfidogenic system.
- To asses the role of the immobilisation/granulation process in the competition between the SRB and MB.
- To asses the effect of the hydraulic retention time and the liquid upward velocity on the granulation process in UASB reactors treating sulfate containing waste waters.

5.2 Materials and methods

General

Three different types of experiments were performed :

1. Granulation of SRB and MB in a methanogenic, sulfidogenic and mixed methanogenic/sulfidogenic system.
2. The effect of the liquid upward velocity (v_{upw}) and hydraulic retention time (HRT) on granulation of SRB and MB in a mixed methanogenic/sulfidogenic system.
3. The effect of the carrier material on the immobilisation of SRB in a sulfidogenic system.

Operation of the reactors

1. Granulation in a sulfidogenic, methanogenic and mixed methanogenic/sulfidogenic system.

Three UASB reactors as described in chapter 2 with a liquid volume of 1.1 l were used. In all reactors effluent recycle was applied. The upward liquid velocity in the reactors amounted to about 0.35 $m.h^{-1}$. The average HRT in the reactors was about 5 h.

The reactors were inoculated with a blend of two types of granular sludge. Approximately 20 % of the volqtile suspended solids (VSS) originated from a 20 l lab-scale UASB reactor adapted to a mixture of acetate, propionate, butyrate (C2:C3:C4 = 5:3:2, based on COD-values) and sulfate (COD/sulfate = 2, with COD and sulfate both expressed in $g.l^{-1}$). The characteristics of this sludge has been described in chapter 3. The remaining sludge originated from a full Scale UASB reactor treating distillery waste water (Nedalco, Bergen op Zoom, The Netherlands). Before seeding the reactor the sludges were mixed, and then crushed under anaerobic conditions by pressing the sludge through a syringe needle (Microlance 21g½ 0.8x40).

The organic substrate of the medium consisted of acetate, propionate, butyrate, and sucrose, in a ratio of 3:3:3:1 based on COD values. Sulfate was added up to a COD/sulfate ratio of 0.5 in two of the three reactors. The third reactor, which was fed without sulfate, is called the methanogenic system. In one of the two reactors fed with sulfate, 5 $mg.l^{-1}$ of chloroform was added during days 1-5 in order to terminate the methanogenic activity of the sludge. This reactor is called the sulfidogenic system. The other sulfate fed reactor is called the mixed methanogenic/sulfidogenic system.

2. The effect of V_{upw} and HRT on the granulation of SRB and MB.

Two UASB reactors as described in chapter 2 with a liquid volume of 1.1 l were used. Both reactors were operated at an HRT of about 7-7.5 h. In one of the two UASB reactors effluent recycle (recycle ratio 10) was applied. This resulted in a liquid upward velocity of about 0.65 $m.h^{-1}$. In the non recirculated UASB reactor the upward liquid velocity was about 0.05 $m.h^{-1}$. In addition to the two UASB reactors, one 1.1 l UASB reactor placed in series with a 5 l CSTR was used. This system was operated at an HRT of about 40-45 h. In the system effluent recycling (recycle ratio 10) was applied. The upward liquid velocity in the

UASB reactor of this system was about 0.65 $m.h^{-1}$.

The reactors were inoculated with the same seed sludge as described for the previous experiment. The organic substrate consisted of acetate, propionate, and sucrose, in a ratio of 5:4:1 based on COD values. Sulfate was added up to a COD/sulfate ratio of 0.5.

3. The effect of the carrier material on the immobilisation of SRB.

Two UASB reactors as described in chapter 2 with a liquid volume of 1.1 l were used. In one reactor, 300 ml of pumice was added as an inert carrier material. In order to achieve an expansion of the pumice a high recirculation flow of 800 $l.d^{-1}$, resulting in a V_{upw} of about 12 $m.h^{-1}$, was used at the start of the experiment. This resulted in an expansion of the pumice from 300 to 500 ml of volume. The reactor was seeded with the suspended sludge obtained from the sulfidogenic system described in experiment 1. This reactor is called the sulfidogenic biofilm reactor. The other reactor was inoculated with the same sludge as described in experiment 1. However, the sludge was not crushed. An effluent recycle flow of about 50 $l.d^{-1}$, resulting in a V_{upw} of about 0.8 $m.h^{-1}$, was applied. In this reactor, about 5 $mg.l^{-1}$ chloroform was added to the feed during days 1-5 in order to terminate the methanogenic activity. This reactor is called the sulfidogenic UASB reactor. In addition a third reactor with the same condition as the sulfidogenic UASB reactor was used. However, in this reactor no chloroform was added during the start up period. This reactor is called the mixed methanogenic/sulfidogenic UASB reactor.

In all reactors the feed was as described in experiment 1. The average HRT in the reactors was about 5 h.

Sludge characterization

To improve the insight into the granulation process and the type of bacteria involved, the sludge development in the different experiments was followed by means of :

- Sludge activity assays. The activity of the sludge for a mixture of acetate, propionate and butyrate (1:1:1, based on COD values) in the absence and presence of sulfate, was followed with time. In addition, the activity was also assessed for different sludge fractions, namely the more flocculant sludge fraction and the more granular sludge fraction. The activity was assessed as described in chapter 2.4 for the simultaneous measurement of the total-, methanogenic, and sulfidogenic activity.
- Sludge size distribution.
- Granular strength of the sludge.
- Scanning electron microscopic observations.

5.3 Results

5.3.1 Granulation in a methanogenic, sulfidogenic and mixed methanogenic/

sulfidogenic system.

Reactors

In Fig 5.1 the loading and removal rates in the reactors are shown. The average performance of the reactors at the end of the experiment is listed in table 5.1.

Table 5.1. Average performance of the sulfidogenic, methanogenic and mixed methanogenic/sulfidogenic system at the end of the experiment

	sulfidogenic system	methanogenic system	mixed system
Loading rate			
g COD.l^{-1}.d^{-1}	13.7	6.5	7.0
g COD.g^{-1} VSS.d^{-1}	1.4	0.6	0.7
Removal rate			
g COD.l^{-1}.d^{-1}	8	6.1	6.6
% COD degraded used by :			
SRB Start	100	0	51
end	100	0	78
MB Start	0	100	49
end	0	100	22
% C2COD degraded used by :			
SRB Start	100	0	40
end	100	0	69
MB Start	0	100	60
end	0	100	31

The sulfidogenic system was started up using 5 mg.l^{-1} $CHCl_3$ during days 1-5 in order to stop the methanogenesis. In the sulfidogenic system no methane production was detected during the whole experiment. The substrate degradation was completely sulfidogenic in nature. During the course of the experiment the organic-COD removal rate in the reactor increased with time. This increase was mainly due to an increase in the acetate removal rate.

Propionate and butyrate were almost completely degraded during all stages of the experiment. During days 115-118 a second chloroform dose of 5 mg.l^{-1} was imposed on the sulfidogenic system. This resulted in an inhibition of the organic-COD removal. In particularly, the oxidation of acetate was inhibited strongly. The breakdown of propionate and butyrate was only slightly affected. Recovery of the substrate degradation after the chloroform dose proceeded fairly quickly. At the end of the experiment a removal rate of about 0.9-1.0 g organic-COD.g^{-1} VSS.day^{-1} was obtained.

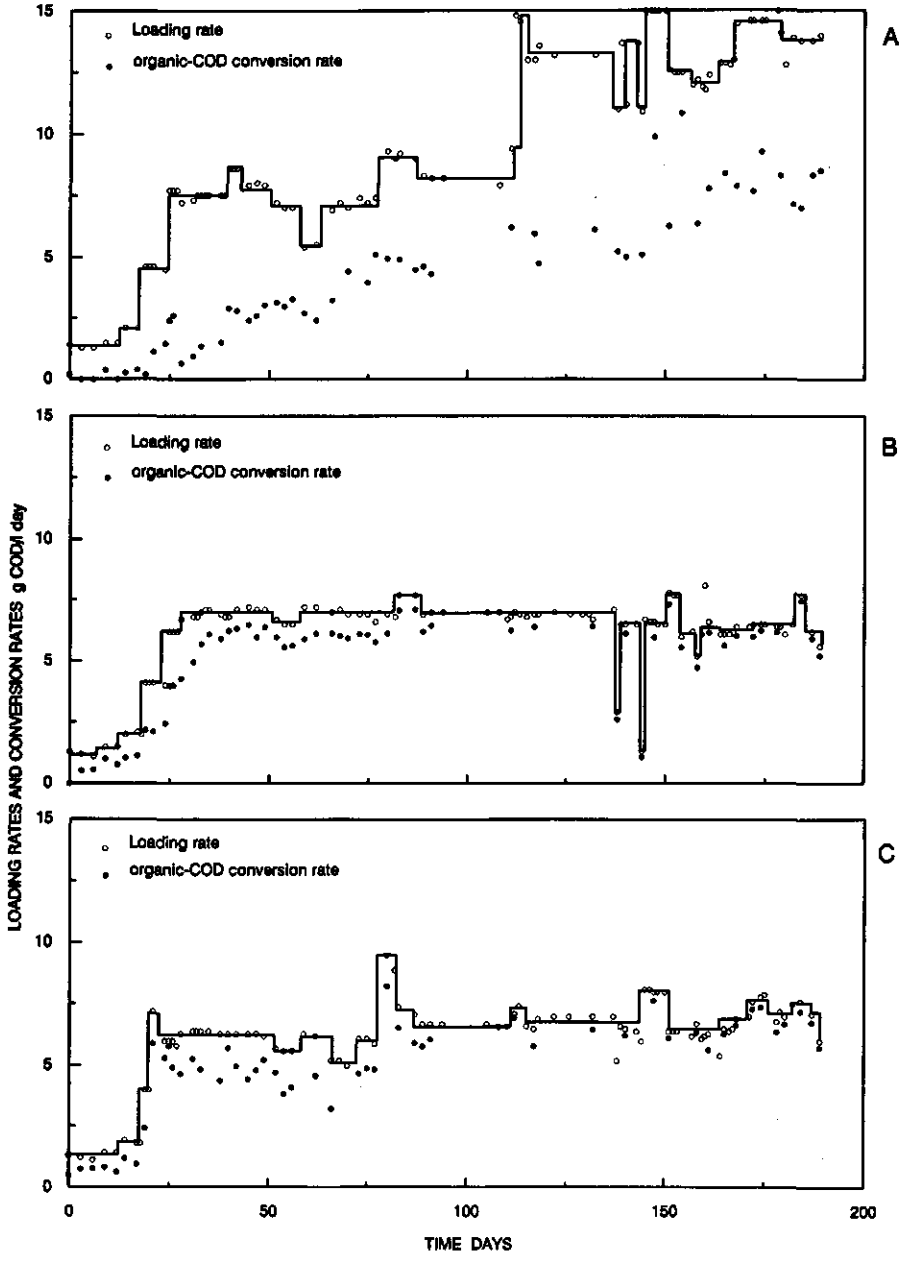


Fig. 5.1. The organic loading rate and the organic-COD conversion rate in the sulfidogenic system (5.1a), the methanogenic system (5.1b) and the mixed methanogenic-/sulfidogenic system (5.1c).

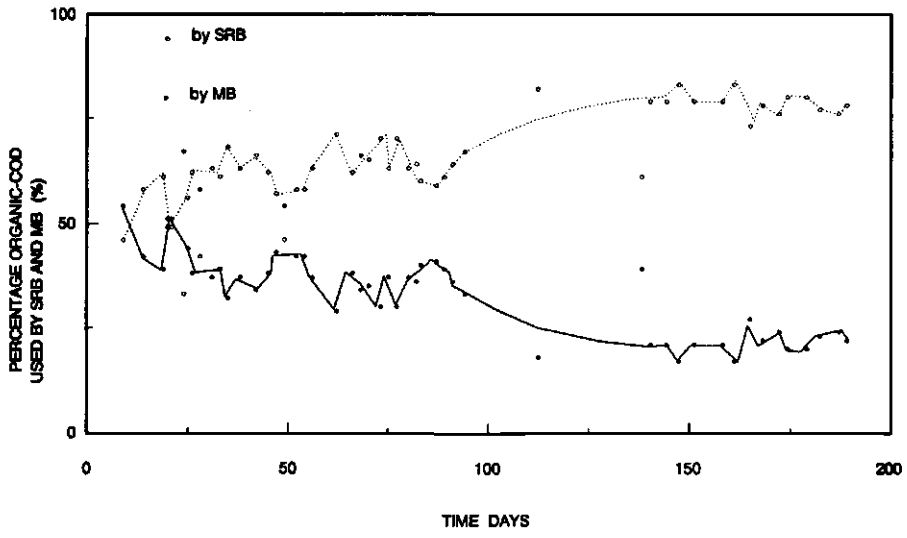


Fig 5.2. The percentage of the degraded organic-COD used by the SRB and the MB in the mixed methanogenic/sulfidogenic system.

In the methanogenic- and the mixed methanogenic/sulfidogenic system a very good removal of the organic substrate was accomplished. In the mixed methanogenic/sulfidogenic system a shift in the percentage of the organic-COD degraded by SRB and MB was found, resulting in a pre dominance of the SRB (Fig 5.2). At the end of the experiment the ratio of the organic COD removed by SRB relative to MB was about 3.5. In the mixed methanogenic/sulfidogenic system the reducing equivalents formed during the degradation of propionate, butyrate and sucrose to acetate, was found to be completely converted by the SRB during the whole experiment. Therefore, the observed shift in the organic-COD removal by SRB and MB in this system is a results of a shift in the acetate utilisation by the MB and SRB. At the end of the experiment the ratio of acetate used by the SRB relative to the MB was about 2.2.

In all three reactors an average net sludge yield of about 0.04 g VSS.g^{-1} organic-COD degraded was found.

Sludge characterization

The characteristics of the sludge was followed by means of sludge activity tests, the sludge size distribution, and the granular strength at the end of the experiment.

Sludge activity tests. The sludge activity in the presence of sulfate at the end of the

experiment was about the same for the sludges from the different systems (Table 5.2).

Table 5.2. The total, methanogenic and sulfidogenic sludge activity (g COD g^{-1} VSS day^{-1}) in the presence and absence of sulfate for sludge samples of the sulfidogenic, methanogenic and mixed methanogenic/sulfidogenic system at the end of the experiment.

system	total	methanogenic	sulfidogenic
sulfidogenic			
+ sulfate	0.95	0.00	0.85
- sulfate	0.00	0.00	
methanogenic			
+ sulfate	1.15	0.85	0.35
- sulfate	1.35	1.15	
mixed			
+ sulfate	1.05	0.25	0.85
- sulfate	0.40	0.32	

In activity assays with sludge samples from the sulfidogenic system in the absence of sulfate, no degradation of the substrate was found. In activity assays with sludge samples from the methanogenic system in the presence of sulfate, with time a decrease in the percentage of organic-COD used by the SRB was found (Fig 5.3a).

This indicates that the number of SRB decreased. However, in all the activity tests it was calculated that the reducing equivalents produced in the degradation of propionate and butyrate to acetate, were completely used by the SRB. Therefore, the decrease in substrate removal by the SRB is a result of a decrease in the acetate consumption by the SRB. In the activity assays at the end of the experiment it was found that all acetate was converted into methane. This indicate that at that time only a low number of acetotrophic SRB (ASRB) were present in the sludge

Activity assays with different sludge fractions showed no significant difference between the fractions (Fig 5.4a). This indicate that the composition of the more granular part resembled the more flocculant part of the sludge of the methanogenic system.

The activity of the sludge of the mixed methanogenic/sulfidogenic system in the presence of sulfate was significantly higher than in the absence of sulfate (Table 5.2). The difference was mainly due to a lower degradation rate of propionate and butyrate in the absence of sulfate. In the activity assays in the presence of sulfate, an increase in the percentage of organic-COD degraded by the SRB with time was found (Fig 5.3b).

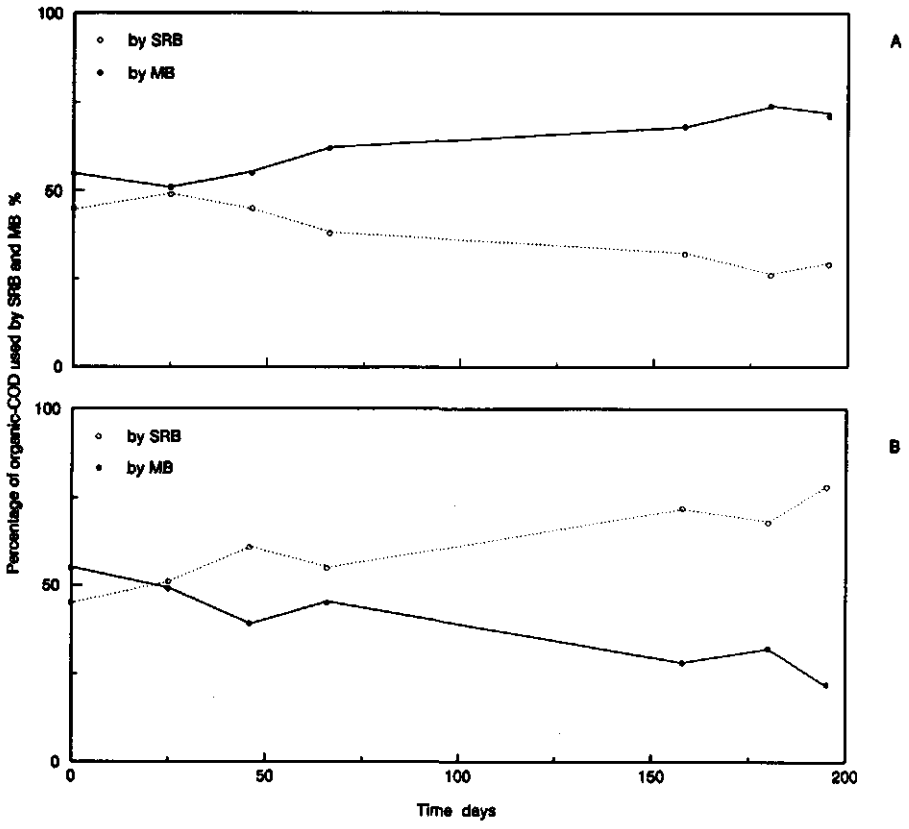


Fig 5.3. The percentage of the degraded organic-COD used by SRB and MB in activity tests with sludge from the methanogenic system (5.3a) and mixed methanogenic/sulfidogenic system (5.3b).

It was calculated that in all the activity tests, the reducing equivalents formed in the degradation of propionate and butyrate to acetate, were completely consumed by the SRB. Therefore, the increase in substrate removal by the SRB is a result of the increase in the acetate consumption by the SRB. In the activity test at the end of the experiment the ratio of acetate used by the SRB relative to the MB was about 2.4. Activity assays with different sludge fractions of sludge samples from the mixed methanogenic/sulfidogenic system showed no significant difference between the fractions (Fig 5.4b). This indicates that the composition of the granular part was similar to that of the flocculant part of the sludge.

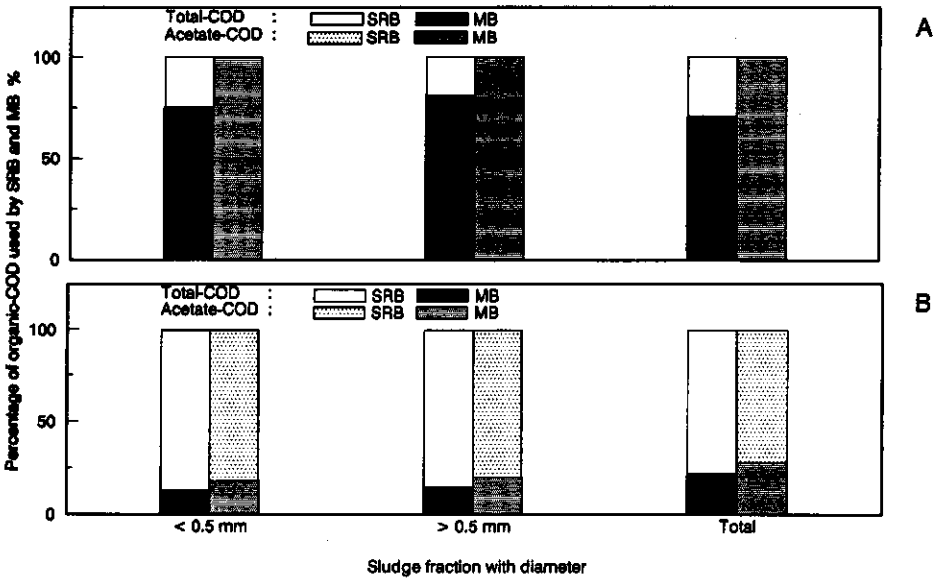


Fig 5.4. The percentage of the degraded organic-COD and acetate used by SRB and MB in activity tests with different sludge fractions for sludges from the methanogenic- (5.4a) and mixed methanogenic/sulfidogenic system (5.4b).

Sludge size distribution. For the sulfidogenic system no significant increase in the sludge diameter was found (Fig 5.5a). This indicates that no granulation in the system occurred. However, at the end of the experiment a tendency towards an increase in the sludge diameter was observed, indicating that in the long term the formation of sulfidogenic sludge granules might be possible.

For the methanogenic and mixed methanogenic/sulfidogenic system, granulation proceeded well (Fig 5.5b,c). No significant difference in the sludge diameter was found. For both systems the average granule diameter increased from about 0.5 mm at the start to 1.5 mm at the end of the experiment.

Granular strength. Since granulation was observed in the methanogenic and mixed methanogenic/sulfidogenic systems only, the granular strength was measured only for sludge samples of these two systems. At the end of the experiment the granular strength of the sludge from the methanogenic and mixed methanogenic/sulfidogenic system was about 6.5 X 10^4 and 2.5 X 10^4 $N.m^{-2}$, respectively. It is clear that the granules formed under pure methanogenic conditions are more stable than the granules formed in the presence of sulfate reduction.

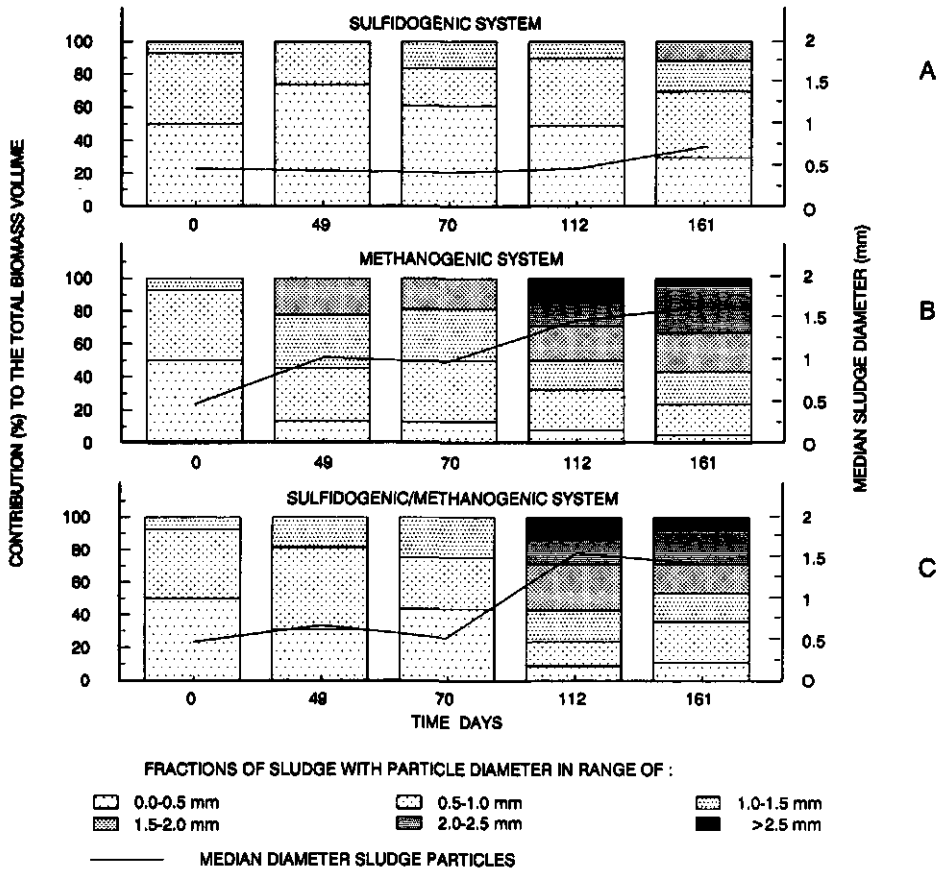


Fig 5.5. The development of the granulation process in the sulfidogenic system (5.5a), the methanogenic system (5.5b) and the mixed methanogenic/sulfidogenic system (5.5c). For each of the systems the percentage of the different sludge fractions based on their contribution to the total biomass volume, and the average granule diameter is shown at different sampling days.

5.3.2 The effect of v_{upw} and HRT on granulation of MB and SRB in mixed methanogenic/sulfidogenic systems

Reactors

The course of the organic loading rate and organic-COD conversion rate in the reactors is shown in Fig 5.6. The average performance of the reactors at the end of the experiment is shown in Table 5.3. In Fig 5.7 the percentage of the organic-COD degraded used by the SRB and MB is shown.

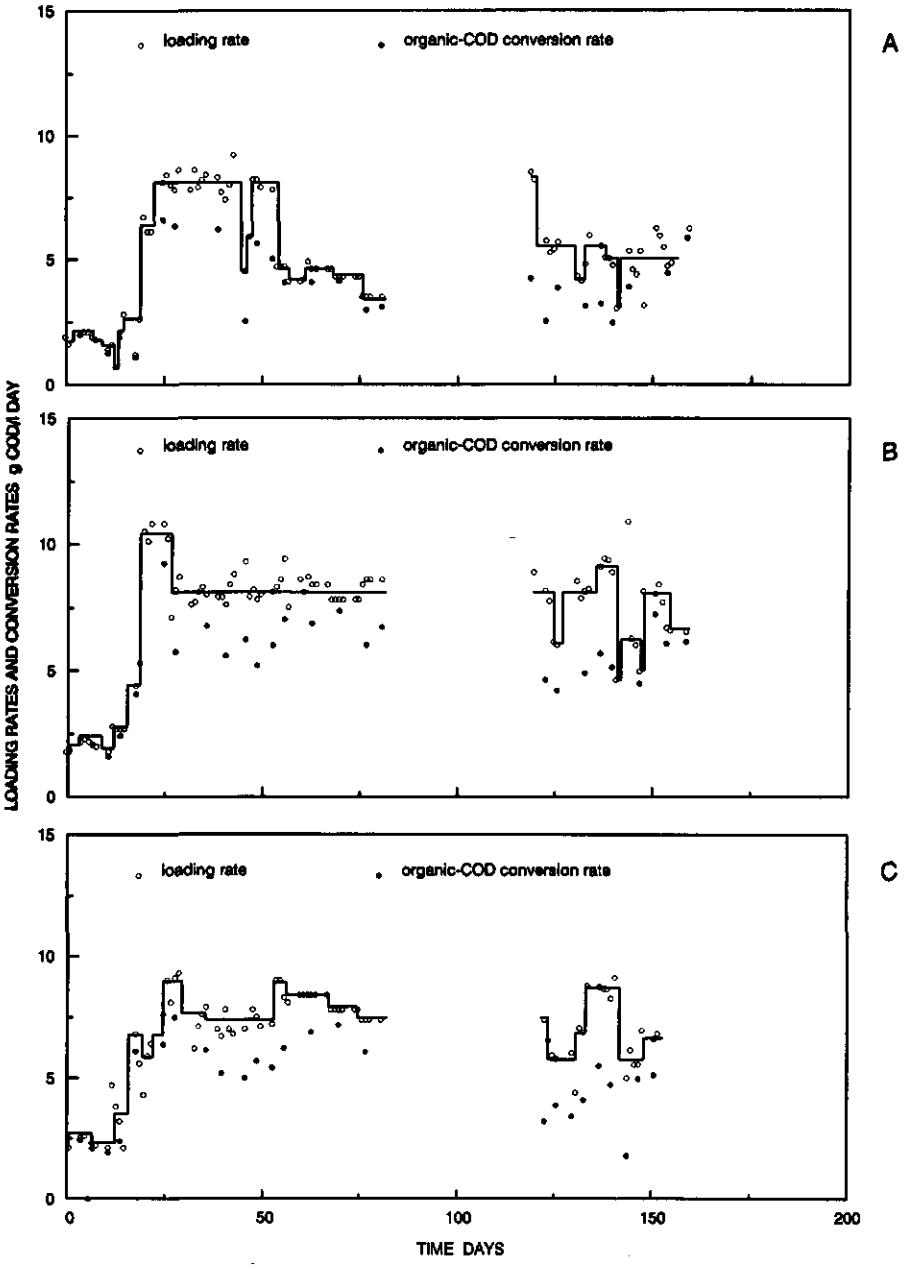


Fig 5.6. The organic loading rate and the organic-COD conversion rate in the non recirculated UASB reactor (5.6a), the recirculated UASB reactor (5.6b) and the UASB/CSTR system (5.6c).

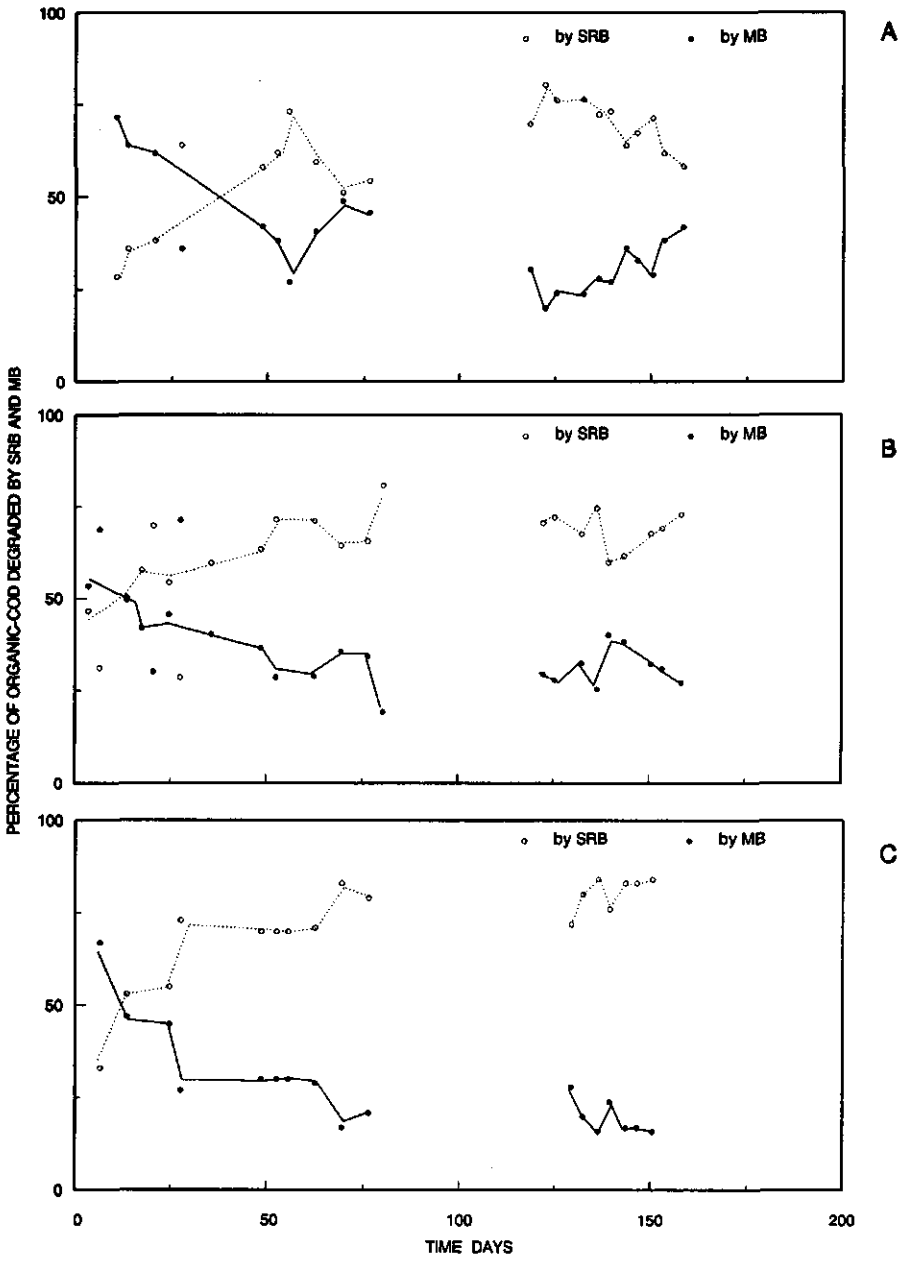


Fig 5.7. The percentage of the degraded organic-COD used by the SRB and the MB in the non recirculated UASB reactor (5.7a), the recirculated UASB reactor (5.7b) and the UASB/CSTR system (5.7c).

Table 5.3. Average performance of the recirculated UASB reactor, the non-recirculated UASB reactor and the UASB/CSTR reactor at the end of the experiment

	UASB	Recirculated UASB	UASB/CSTR
Loading rate			
g $COD.l^{-1}.d^{-1}$	5.3	7.5	7.2
g $COD.g^{-1}$ VSS.d^{-1}	0.9	0.8	1.3
Removal rate			
g $COD.l^{-1}.d^{-1}$	5.1	5.7	4.9
% COD degraded used by :			
SRB Start	48	46	47
end	66	68	82
MB Start	52	54	53
end	34	32	18
% C2COD degraded used by :			
SRB Start	40	42	41
end	58	60	77
MB Start	60	58	59
end	42	40	23

In all reactors an organic-COD removal efficiency of about 75-80 % was obtained. During the course of the experiment a shift in the percentage of organic-COD degraded by SRB and MB was found. At the end of the experiment the ratio of the organic COD removed by SRB relative to MB was about 1.9, 2.1 and 4.6 for the non recirculated UASB, the recirculated UASB, and the UASB/CSTR system, respectively. It is clear that the major shift in substrate utilisation by the SRB and MB was found for the UASB/CSTR system.

This indicates that a longer HRT as was used in the UASB/CSTR system is favourable for the SRB. In all reactors the reducing equivalents generated during the degradation of propionate and sucrose to acetate, was found to be completely oxidised by the SRB during the whole experiment. Therefore, the observed shift in organic-COD removal by SRB and MB in the reactors is a result of a shift in the acetate utilisation by the MB and SRB.

At the end of the experiment the ratio of acetate used by the SRB relative to the MB was about 1.4, 1.5 and 3.5 for the non recirculated UASB, the recirculated UASB, and the UASB/CSTR system, respectively. In all reactors an average sludge yield of about 0.04 g VSS.g^{-1} COD degraded was found.

Sludge characterization

The characteristics of the sludge was followed by means of sludge activity tests and the sludge size distribution.

Sludge activity tests. The sludge activities in the presence of sulfate at the end of the experiment for the sludges from the different systems is listed in Table 5.4.

Table 5.4. The total, methanogenic and sulfidogenic sludge activity (g COD g^{-1} VSS day^{-1}) in the presence of sulfate for sludge samples of the UASB reactor, the recirculated UASB reactor and the UASB/CSTR system at the end of the experiment.

system	total	methanogenic	sulfidogenic
non recirculated UASB reactor	0.9	0.26	0.49
recirculated UASB reactor	0.8	0.24	0.4
UASB/CSTR system	1.0	0.23	0.66

In the UASB/CSTR system the amount of organic-COD and acetate removed by the SRB was significant higher than in the other two systems (Table 5.4). This is in agreement with the observations in the reactors where it was also observed that in the UASB/CSTR system more organic substrate was removed by the SRB than in the two UASB reactors. Apparently, the higher HRT as used in the UASB/CSTR system is favourable for the SRB.
In all reactor a shift in the amount of organic-COD used by the SRB and MB was observed (Fig 5.8). At the end of the experiment the SRB were pre dominant in all reactors.
The sludges from the three systems were separated in a granular and flocculant fractions by means of elutriation. Sludge activity assays with the different fractions showed no significant difference between the sludge fractions (Fig 5.9). No preference of either SRB or MB to the granular or flocculant sludge fraction could be detected.

Sludge size distribution. Fig. 5.10 shows the particle size distribution of the sludges from the different systems at the end of the experiment. It is seen that the granulation process proceeded very well in the recirculated UASB reactor. A clear shift from a suspended sludge towards the formation of sludge granules was observed in this system. In the non-recirculated UASB reactor and the UASB/CSTR system the granulation proceeded less rapidly. These results indicate that a combination of a short HRT and a high liquid upward velocity, as was the case for the recirculated UASB reactor, favours the granulation process. A long HRT or a low upward liquid velocity is less favourable for the granulation process.

5.3.3. The effect of the carrier material on the immobilisation of SRB in sulfidogenic systems

Reactors
In Fig 5.11 the loading and removal rates in the reactors are shown. In Table 5.5 the average reactor performance at the end of the experiment is listed.

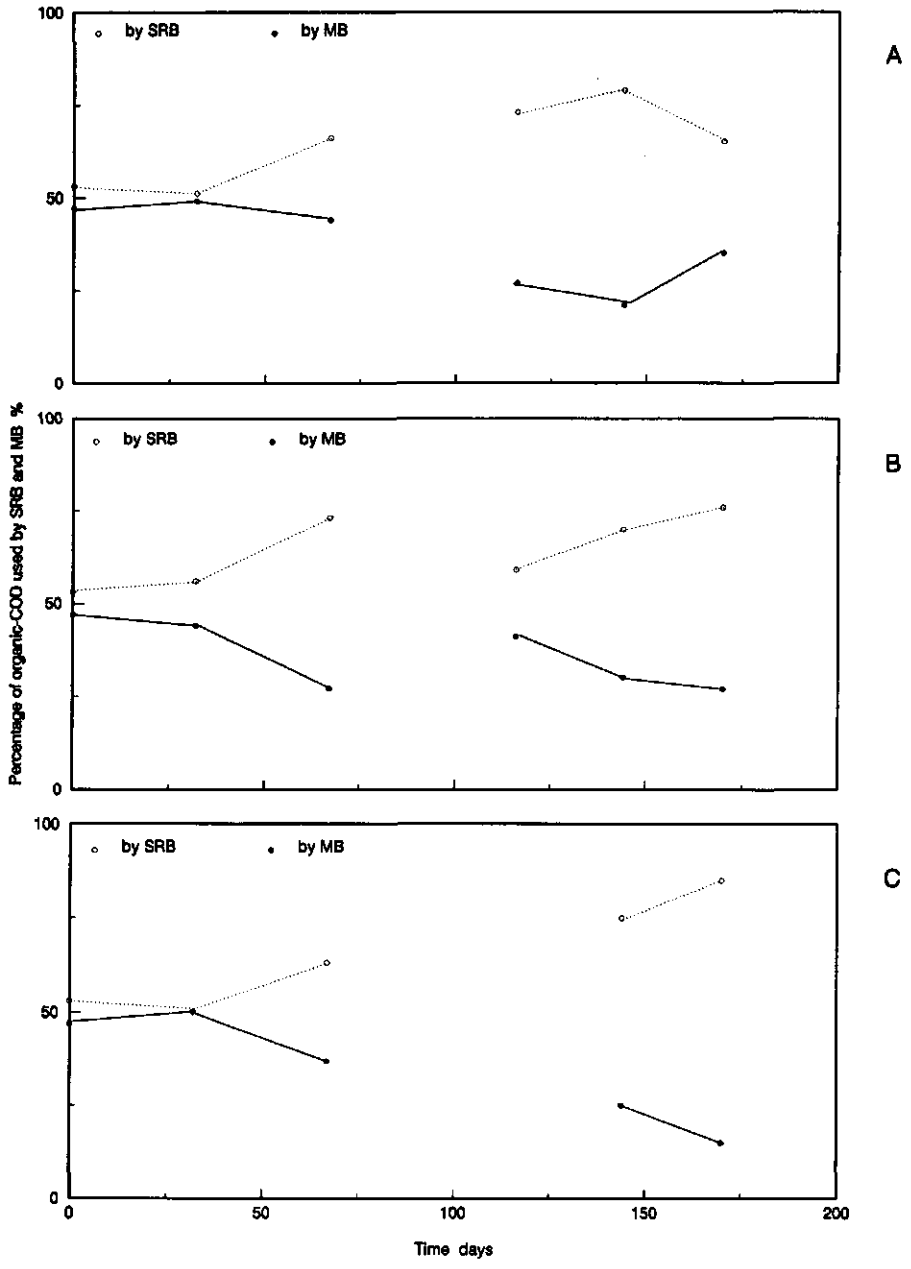


Fig 5.8. The percentage of the degraded organic-COD used by SRB and MB in activity tests with sludge from the non recirculated UASB (5.8a), the recirculated UASB (5.8b) and the UASB/CSTR system (5.8c).

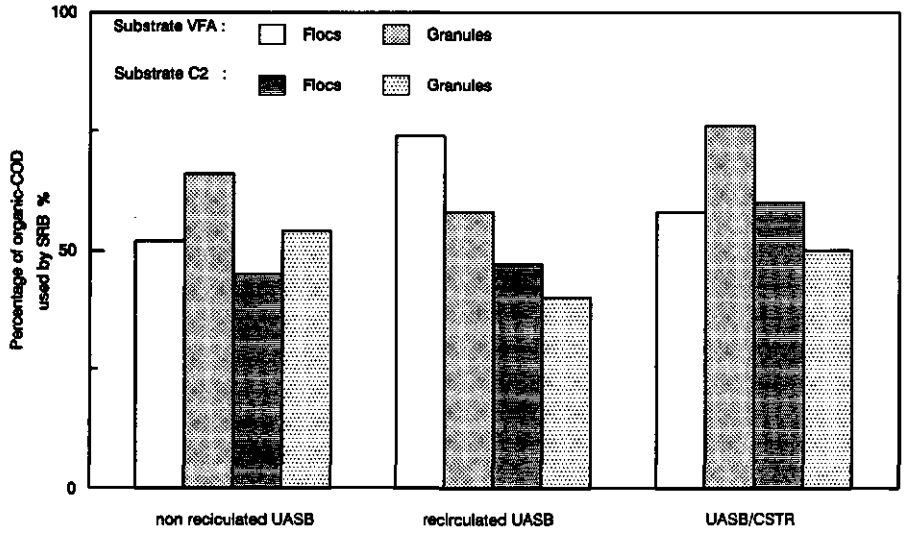


Fig 5.9. The percentage of the organic-COD and acetate used by the SRB and MB in activity test with sludge from the non recirculated UASB, the recirculated UASB and the UASB/CSTR system. The flocs and granules represents sludge fractions with settling velocities < and > 15 $m.h^{-1}$.

Table 5.5. Average performance of the sulfidogenic biofilm reactor, the sulfidogenic UASB reactor and the mixed methanogenic/sulfidogenic UASB reactor at the end of the experiment

		sulfidogenic biofilm reactor	sulfidogenic UASB reactor	mixed UASB reactor
Loading rate	g $COD.l^{-1}.d^{-1}$	13.8	14.5	13.4
	g $COD.g^{-1}$ $VSS.d^{-1}$	1.0	0.9	0.6
Removal rate	g $COD.l^{-1}.d^{-1}$	11.2	8.5	12.7
% COD degraded used by :				
SRB	Start	100	100	70
	end	100	100	70
MB	Start	0	0	30
	end	0	0	30
% C2COD degraded used by :				
SRB	Start	100	100	65
	end	100	100	65
MB	Start	0	0	35
	end	0	0	35

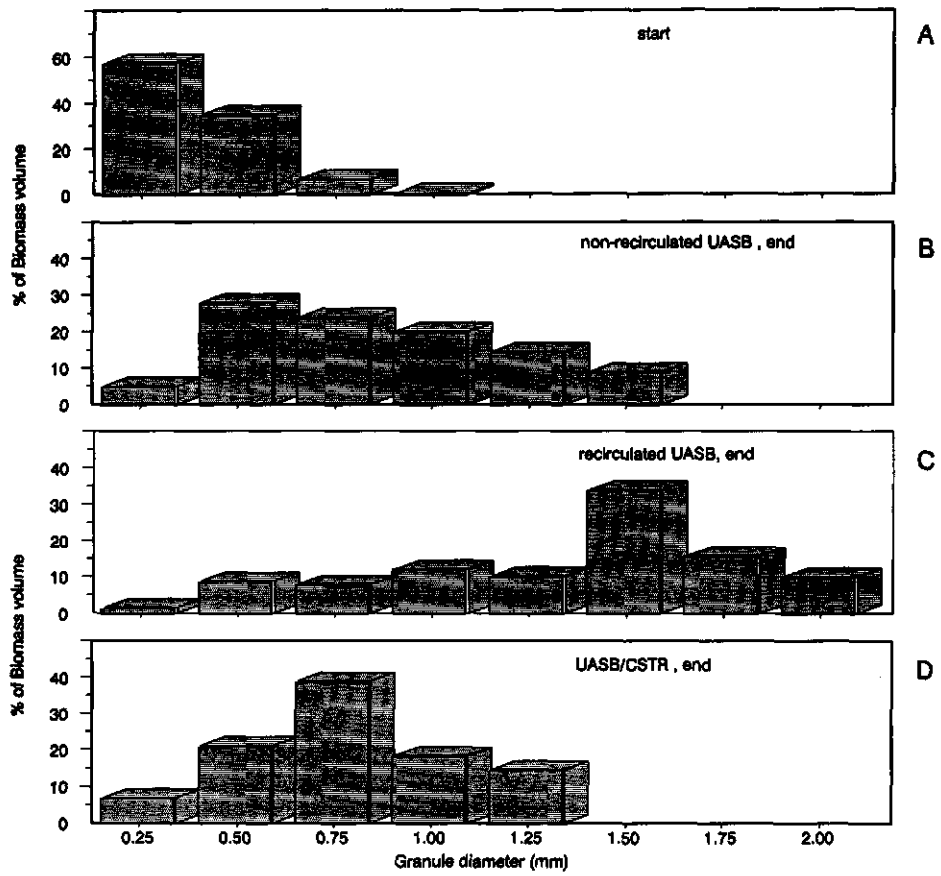


Fig 5.10. The particle size distribution expressed as the contribution in percentage of each sludge fraction to the total biomass volume, at the start of the experiment (5.10a) and after 123 days of operation for sludge of the non recirculated UASB (5.10b), the recirculated UASB (5.10c) and the UASB/CSTR system (5.10c).

The sulfidogenic pumice filled biofilm-reactor was started up applying a batch mode operation to support an initial attachment of the biomass to the pumice. After switching to continuous feeding the suspended non attached biomass was washed out from the reactor. During the whole experiment no measurable amount of methane was produced. The anaerobic degradation of the substrate was completely sulfidogenic in nature.

During days 1-50 an increase in the organic-COD removal rate was observed. This increase was a result of the attachment of biomass to the pumice carrier resulting in a higher sludge concentration in the reactor.

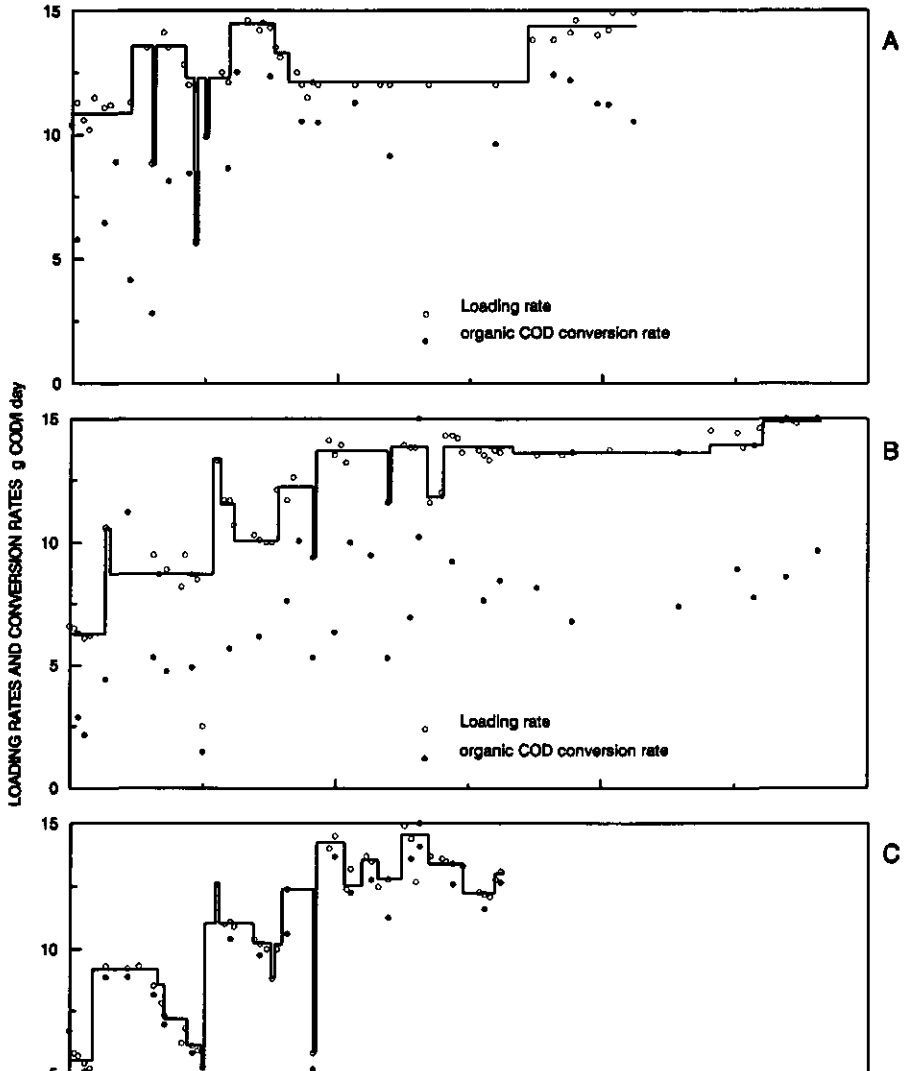


Fig 5.11. The organic loading rate and the organic-COD conversion rate in the sulfidogenic biofilm reactor (5.11a), the sulfidogenic UASB reactor (5.11b) and the mixed methanogenic/sulfidogenic UASB reactor (5.11c).

Due to the biofilm formation on the pumice the density of the particles and, consequently, the settling velocity decreased. In order to prevent a severe biomass washout the recirculation flow had to be decreased from 800 to 400 $l.day^{-1}$. The expanded volume of the pumice covered with biomass increased from 500 ml at the start to about 950 ml at the end of the experiment. At the end of the experiment the removal rate in the reactor was about 11 g $COD.l^{-1}.day^{-1}$, corresponding with 0.8 g $COD.g^{-1}$ $VSS.day^{-1}$.

The sulfidogenic UASB reactor, which was seeded with granular sludge, was started up using 5 $mg.l^{-1}$ chloroform in order to terminate the methanogenic activity in the sludge. During the whole experiment no measurable amount of methane was detected. The anaerobic degradation of the substrate was completely sulfidogenic in nature. During days 1 to 50 an increase in the organic-COD removal rate in the reactor was found. This increase was mainly a result of an increase in the removal of acetate. Propionate and butyrate were very well degraded during the whole experiment. At the end of the experiment the organic-COD removal rate amounted to 9 g $COD.l^{-1}.day^{-1}$, corresponding with 0.55 g $COD.g^{-1}$ $VSS.\ day^{-1}$.In the mixed methanogenic/sulfidogenic UASB reactor, which was seeded with granular sludge, methane production and sulfate reduction occurred simultaneously. During the course of the experiment the amount of organic-COD removed via sulfate reduction and methanogenesis remained fairly constant. The major fraction of the substrate was degraded via sulfate reduction (Fig 5.12). The obtained organic-COD removal rate in the reactor was governed by the imposed organic loading rate (Fig 5.11c).

Sludge characterization

The sludge development in the reactors was followed by means of sludge activities, sludge size distribution, the granular strength of the sludge, and scanning electron microscopy.

sludge activity tests. The assessed sludge activities at the end of the experiment are shown in Table 5.6.

The sludge activities of sludge samples from the sulfidogenic biofilm reactor remained fairly constant during the experiment. At the end of the experiment the activity was about 0.5 g $COD.g^{-1}$ $VSS.day^{-1}$. This was lower than the observed conversion rate in the reactor. In all the activity test no methane production was detected. The degradation of the substrate was completely sulfidogenic in nature.

For the sludge of the sulfidogenic UASB reactor the sludge activity increased with time. At the end of the experiment the sludge activity was about 0.5 g $COD.g^{-1}$ $VSS.day^{-1}$. This was about the same as the actual conversion rate observed in the reactor. In all the activity assays no measurable amount of methane could be detected. In the activity assays with the sludge of the mixed methanogenic/sulfidogenic UASB reactor the removal of the organic COD via sulfate reduction and methane production remained fairly constant. The ratio of the substrate removed by SRB and MB was about 70 and 30 %, respectively.

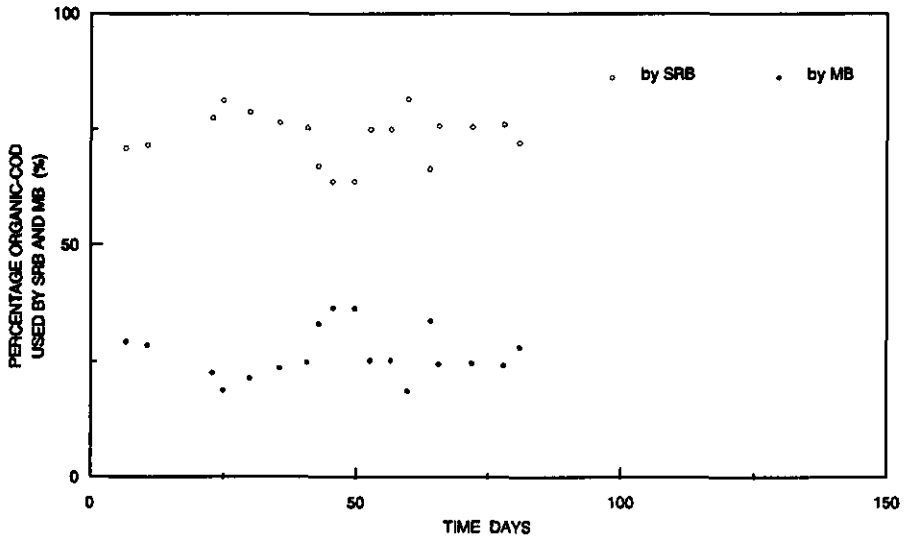


Fig 5.12. The percentage of the degraded organic-COD used by the SRB and the MB in the mixed methanogenic/sulfidogenic UASB reactor.

Table 5.6. The total, methanogenic and sulfidogenic sludge activity (g COD g^{-1} VSS day^{-1}) in the presence of sulfate for sludge samples of the sulfidogenic biofilmreactor, the sulfidogenic UASB reactor and the mixed methanogenic/sulfidogenic UASB reactor at the end of the experiment.

system	total	methanogenic	sulfidogenic
sulfidogenic biofilmreactor	0.5	0	0.45
sulfidogenic UASB reactor	0.5	0	0.48
mixed UASB reactor	0.6	0.19	0.38

Sludge size distribution. At the end of the experiment the median diameter of the pumice particles covered with biomass was about 0.44 mm. The average diameter of the raw pumice was about 0.26 mm. These data illustrates the development of the biofilm on the pumice. In the mixed methanogenic/sulfidogenic and in the sulfidogenic UASB reactor an increase in the median sludge diameter was observed, as is shown in Table 5.7. This show that in the reactors granular growth occurred.

Table 5.7. Average sludge diameter for sludge of the sulfidogenic biofilm reactor, the mixed methanogenic/sulfidogenic UASB reactor and the sulfidogenic UASB reactor

Time days	sulfidogenic biofilm reactor	mixed UASB reactor	sulfidogenic UASB reactor
0	0.26*	1.26	1.24
50	----	1.36	1.28
70	----	1.91	1.50
120	0.44	----	1.98

* average diameter raw pumice

Granular strength. The granular strength of the sludge of the mixed methanogenic-/sulfidogenic UASB reactor increased with time. Contrary, in the sulfidogenic UASB reactor a decrease in the granular strength was found (Fig 5.13). However, despite this decrease in granular strength, no disintegration of the sludge granules in the sulfidogenic UASB reactor was found. The granular structure of the sludge remained intact.

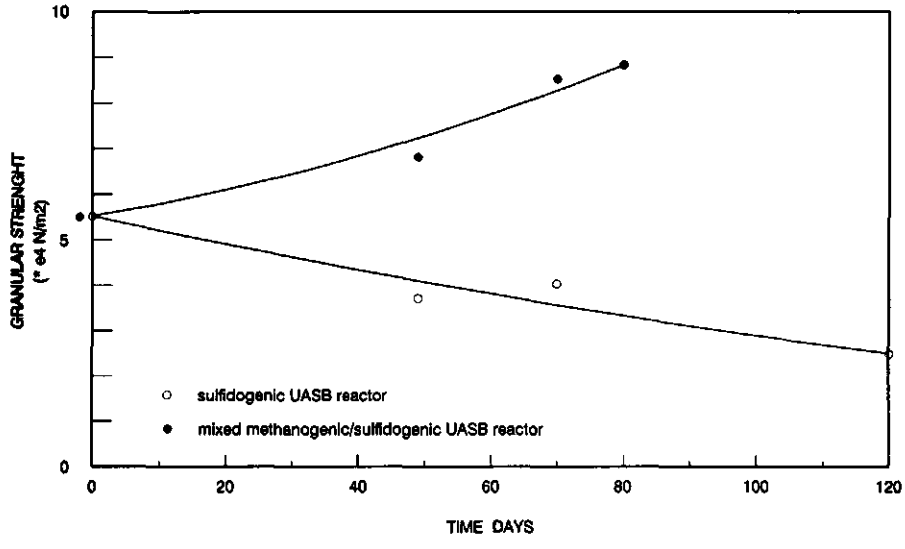


Fig 5.13. The granular strength of the sludge from the sulfidogenic UASB reactor and the mixed methanogenic/sulfidogenic UASB reactor.

Scanning electron microscopy. In the sulfidogenic biofilm reactor the biofilm formation started with the attachment and growth of vibrio shaped bacteria in holes and other disturbances on the pumice surface (Fig 5.14a). Later on, thin (0.2 μm) filamentous bacteria attached over the whole surface of the pumice (Fig 5.14b). The growth of these filamentous bacteria resulted in the final formation of the biofilm as is shown in Fig 5.14c. The thin filamentous bacteria which were found typical for the biofilm reactor were also observed in the granular sludges from the UASB reactors (Fig 5.15). However, the typical filamentous structure as observed in the sulfidogenic biofilm reactor was not found in the sludges from the UASB reactors. For the biomass of all reactors a typical "dome" shaped surface structure was observed (Fig 5.16). These domes probably originate from precipitates on the biofilm and sludge granules. In pure methanogenic reactors such "dome" shape surface structure has not been observed in our laboratory (A. Alphenaar, personal communication).

5.4 Discussion

This study shows that in mixed methanogenic/sulfidogenic systems, the SRB become the pre dominant species and are very efficient in out-competing the MB. Calculations show that the reducing equivalent formed in the oxidation of fatty acids and sucrose to acetate, in the following termed as 'hydrogen', were completely oxidised by the SRB. The pre-domination of the SRB for 'hydrogen' found in this study is in agreement with earlier observations showing that in anaerobic reactors operated with excess sulfate, all 'hydrogen' will be used by the SRB (Mulder 1984, Rinzema and Lettinga 1988). In addition, the calculations show that also the major fraction of the acetate was consumed by the SRB. With respect to the competition between ASRB and acetotrophic MB (AMB) results available in the literature are inconsistent. Both a pre dominance of the ASRB (Rinzema and Schultz 1987, Choi and Rim 1991, Stücki et al. 1993), as also found in this study, and a complete conversion of acetate into methane (Hoeks et al. 1984, Mulder 1984, Rinzema et al. 1986) have been reported. Competition between ASRB and AMB for acetate in anaerobic reactors probably is governed by the growth kinetic and immobilisation properties of the bacteria. A better immobilization capacity of AMB relative to ASRB, resulting in a selective wash-out of SRB, has been mentioned as explanation for the apparent successful competition of AMB against ASRB as observed in the studies of Isa et al. (1986 a,b). However, in the present study we did not observe such a superior immobilisation of the AMB. In the sludges from the mixed methanogenic/sulfidogenic systems any clear significant difference in the amount of substrate used by the SRB and MB in the granular and flocculant part of the sludge was not observed. The ASRB were the pre-dominant species in both sludge fractions. These results strongly indicate that it is more realistic to assume a comparable colonisation capacity for ASRB and AMB, rather than a major difference. Consequently, the kinetic growth properties of ASRB and AMB for acetate most likely are the key factors in the competition.

Fig 5.14.
Scanning electron microscopy pictures of sludge samples from the sulfidogenic biofilm reactor at different stages of the experiment.
Fig 5.14a : the initial biofilm formation, by the attachment of vibrio-like bacteria in the disturbances and holes in the surface.

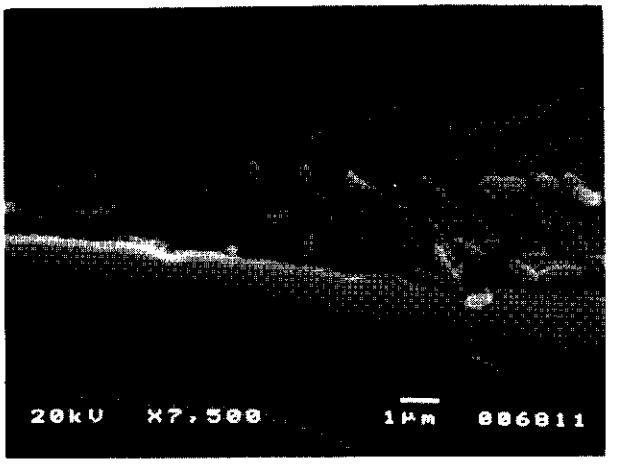


Fig 5.14b : A later stage in the biofilmformation. Attachment of filamentous bacteria to the surface

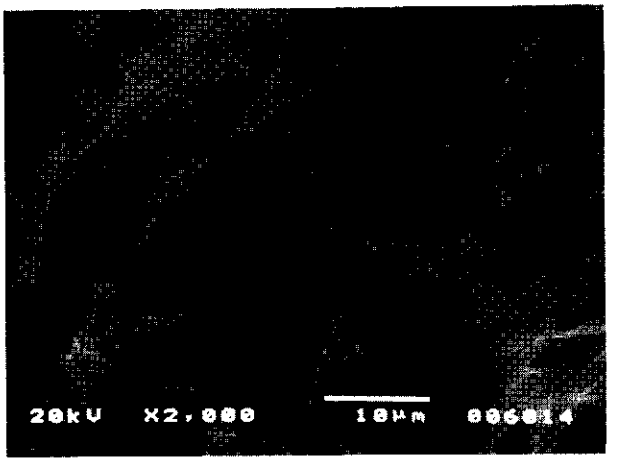


Fig 5.14 c : the final biofilm formed by a matrix of filamentous bacteria.

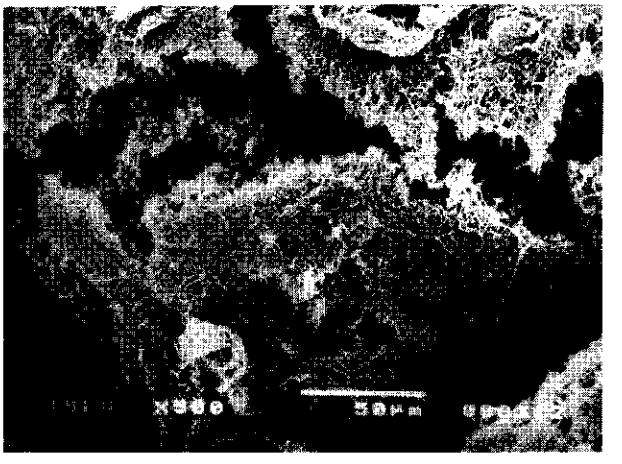


Fig 5.15.
Scanning electron microscopy pictures of a characteristic sludge sample from reactors.
Fig 5.15 a : the sulfidogenic UASB reactor (5.15a)

Fig 5.15b : The mixed methanogenic/sulfidogenic UASB reactor (5.15b).

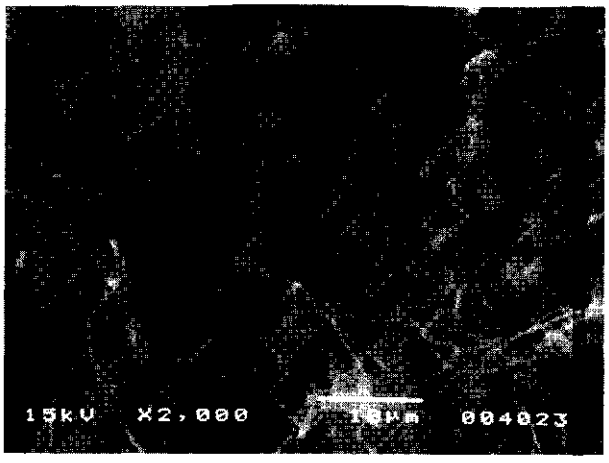
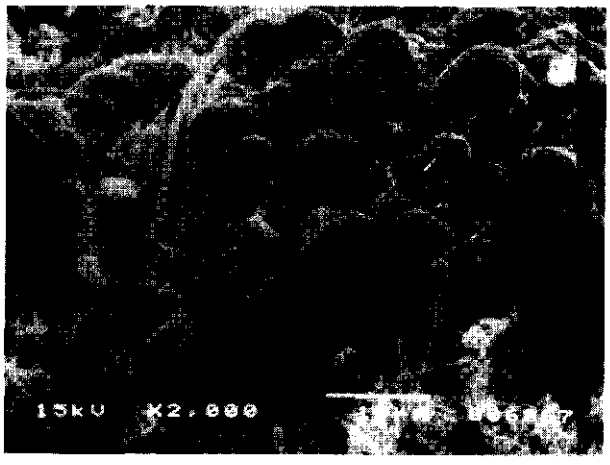


Fig 5.16.
Scanning electron microcopy picture of the surface structure of the sulfidogenic UASB reactor. The typical "dome" shaped structure shown in this figure was also found in the sulfidogenic biofilm reactor and the mixed methanogenic/sulfidogenic UASB reactor.



The competition between ASRB and AMB in relation to their growth properties has already been discussed in chapter 3 and 4. Considering the growth rates of ASRB and AMB at pH 7.5 till 8, which were the average pH values found in this study, a long time can be needed before the ASRB will actually out-compete the AMB from the sludge (see chapter 3,4). In the mixed methanogenic/sulfidogenic reactors used in this study it took about 150 days to increase the percentage of the organic-COD degraded by SRB from about 40-50 % at the start to about 70-80 % at the end of the experiment. Whether the ASRB are able to completely replace the AMB in the sludge can not be derived from the results of this study. However, as was shown in chapter 3, in the long term the degradation of the organic substrate can proceed completely via sulfate reduction as the end step. Although it was observed that the SRB become the pre-dominant species in all mixed methanogenic/sulfidogenic systems investigated, it should be noted that the HRT has an additional effect on the competition between the SRB and MB. The present study shows that in reactors operated at an HRT of about 40 h, the SRB are more competitive than in reactors operated at an HRT of about 7 h. The percentage of organic-COD used by the SRB after 150 days of operation was 82 and 67 % at an HRT of 40 and 7 h, respectively. An explanation for this difference could be that in reactors operated at a long HRT also dispersed growing SRB contribute to the substrate degradation, while in systems operated at short HRT, these dispersed growing SRB have been washed out from the reactor.

In the methanogenic system used in this study a decrease in the number of SRB with time was found. At the start both acetate and 'hydrogen' degrading SRB were present in the sludge. However, the results of the activity assays conducted at the end of the experiment indicate that all acetate was converted into methane whereas all 'hydrogen' was used by the SRB. Apparently, under methanogenic conditions ASRB were expelled, whereas 'hydrogen' degrading SRB remained present in the sludge. This finding can be explained by earlier observations showing that in the absence of sulfate SRB can grow as an acetogen. It has been demonstrated that SRB are capable of a acetogenic oxidation of lactate (Bryant et al. 1977, McInerney and Bryant 1981, Traore et al. 1983) and propionate (Wu et al. 1991, Zellner et al. 1992). The SRB then grow in syntrophy with Hydrogenotrophic MB (HMB).

However, for ASRB such acetogenic properties have not yet been reported. Our results suggest that ASRB do not posses this ability.

With respect to the granulation process the results show that the MB are well capable to form sludge granules at relatively short term. SRB also were found capable of attaching and growing in sludge granules when cultivated simultaneously with the MB. Furthermore, in all mixed methanogenic/sulfidogenic systems, the SRB out-competed the MB in time, resulting in the formation of sulfidogenic granular sludge. However, in the absence of methanogenesis apparently the SRB lacked the ability to form sludge granules at relatively short term, indicating that an active methanogenic bacterial consortium presumably is needed to initiate a rapid formation of sludge granules. Possibly the specific morphology of the filamentous *Methanothrix* (Wiegant 1988), or the specific hydrophobic properties of *Methanothrix* (Van

Loosdrecht et al. 1987, 1990) might be key factors in initiating the granulation process. Several other researches have postulated the important role of *Methanothrix* in the granulation process (Alibhai and Forster 1986 a,b ; Wiegant and de Man 1986 ; Yoda et al. 1989). Another explanation that SRB need MB to achieve a rapid granulation originates from the importance of the sludge selection pressure on the granulation process in anaerobic reactors (Hulshoff Pol 1989). The imposed selection pressure results in the wash-out of dispersed (growing) bacterial matter whereas bacterial (growing) aggregates are retained within the system. The hydraulic loading rate (or upward liquid velocity) and the gas loading rate, originating from the methane production both play an important role in this process (Wiegant and Lettinga 1985, Wiegant 1988). In a pure sulfidogenic system a high gas production lacks, and consequently conditions are less favourable for a rapid formation of sludge granules.

We also investigated the possible role of the sludge selection pressure on the granulation process in mixed sulfidogenic/methanogenic systems. We used the hydraulic retention time and the upward liquid velocity as the sludge selection parameters. The results clearly showed that a combination of a short HRT and a high liquid upward velocity favours the granulation process. Contrary, a long HRT or a low liquid upward velocity was found less favourable for the formation of sludge granules. These findings are in agreement with the observations of Hulshoff Pol (1989). Probably, systems operated at long HRT's allow for growth of dispersed bacterial matter. At low upward liquid velocities flocculant matter can be retained within the reactor, whereas in reactors operated at high upward liquid velocities poorly settling particles presumably are washed out.

Although we observed that in the absence of methanogenesis the SRB are unable to form sludge granules at relatively short term, we also found that they were well able to attach to a carrier like pumice and sludge granules. The SRB were able to form a stable biofilm up to 0.2 mm thickness on the pumice surface. The mechanism of this biofilm formation was related to the attachment of (mainly) vibrio shaped bacteria in holes and other disturbances of the pumice during the initial stage of the film formation. This agrees well with earlier observations showing a similar initial biofilm formation on inert carrier material (Eighmy et al. 1983, Beeftink and Staugaard 1986). The initial stage of the film development was followed by the attachment and growth of filamentous bacteria. The final sulfidogenic biofilm consisted of a matrix formed by the filamentous bacteria in which also other bacteria will be entrapped or attached.

We also observed that granular sludge can represent a suitable carrier for attachment of SRB. Although the stability of the sludge granules decreased with time, no disintegration of the granules was observed.

With respect to the stability of granular sludge this study clearly shows that granules formed under methanogenic conditions exert a higher strength than granules from a mixed methanogenic/sulfidogenic- or from a pure sulfidogenic culture. However, this does not automatically mean that the granules in the sulfidogenic and mixed methanogenic/sulfidogenic reactor are less stable than the granules in methanogenic systems. In anaerobic reactors, the

production of biogas is the major cause for shear forces on the sludge granules (Christensen et al. 1989). Also biogas accumulate in the interior of the granule, causing an internal force which could result in a detoriation of the granule (Liu and Pfeffer 1991, Kosaric et al. 1990). In order to remain a stable aggregate a methanogenic granule must posses enough strength. In mixed methanogenic/sulfidogenic and especially pure sulfidogenic systems there is less gas production. Therefore, sulfidogenic sludge granules can remain stable aggregates at much lower granular strength than methanogenic granules.

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CHAPTER 6

Anaerobic degradation of volatile fatty acids at different sulfate concentrations in UASB reactors

6.1 Introduction

Sofar, little is known about the competition between acetogenic bacteria (AB) and sulfate reducing bacteria (SRB) for propionate and butyrate in anaerobic digesters. For wastewater, containing an excess of sulfate it is assumed that SRB will out-compete AB, because of their better growth kinetics properties. At low sulfate concentrations, only part of the fatty acids can be oxidized by the SRB, whereas the remainder is oxidized by syntrophic associations of AB and methanogenic bacteria (MB). It is also possible that AB are involved in the fatty acid oxidation to acetate and H_2, where the H_2 is partly used by SRB and partly by MB, whereas acetate is degraded by MB. It is also possible that fatty acid oxidizing SRB can act as an acetogen. SRB can grow syntrophically in the absence of sulfate with lactate (Bryant et al. 1977, Mc Inerney and Bryant 1981, Traore et al. 1983) and propionate (Wu et al. 1991). Sofar, for butyrate a syntrophic oxidation by SRB has not been reported, but there exist indications that SRB can grow on butyrate as an acetogen (Heppner et al. 1992). At low sulfate concentrations competition between different types of SRB may occur as well. It has been shown that the affinity for sulfate decreases in the following order : *Desulfovibrio*, *desulfobulbus*, *Desulfobacter*. These bacteria are specialized on H_2 and lactate, propionate, and acetate, respectively (Laanbroek et al. 1984). Therefore, at low sulfate concentrations a syntrophic oxidation of propionate and butyrate by AB coupled with H_2 consumption by SRB or MB very likely is important.

With respect to the degradation of H_2 and acetate in anaerobic reactors, according to several authors the SRB out-compete the MB for H_2 (Mulder 1984, Rinzema et al. 1986, Rinzema and Lettinga 1988). For acetate both a pre dominance of the MB (Hoeks et al. 1984, Mulder 1984), and SRB (Rinzema and Schultz 1987, Stucki et al. 1993) has been reported. Competition between the SRB and MB has also been discussed in chapters 3, 4 and 5.

In this research we studied the role of the SRB in the degradation of volatile fatty acids in UASB reactors operated under conditions ranging from sulfate limitation to an excess of sulfate.

6.2 Materials and methods

General

Three UASB reactors operated at different COD/sulfate ratios were used. The performance

of the reactors and the development of the sludge characteristics were followed with time.

Operation of the UASB reactors

Three 1.7 l UASB reactors as described in chapter 2 were used. The reactors were seeded with 32 g volatile suspended solids (VSS) of granular sludge from the UASB-reactor at the Aviko potato processing factory at Steenderen, The Netherlands. Before seeding, the sludge was crushed under anaerobic conditions at room temperature and pH 7 by passing it several times through a syringe needle (Microlance 21G1½ 0.8 X 40).

The organic substrate consisted of acetate, propionate and butyrate (1:1:1, based on COD values). The COD to SO_4 ratios used, with COD and SO_4 both expressed in g/l, were 10.39, 3.62 and 0.57.

Sludge characterization

The sludges of the UASB reactors were characterized by means of sludge activity measurements and most probable number (MPN) counting.

The activity assay used to asses the total, methanogenic and sulfidogenic sludge activity was according to the method described in chapter 2.4. Acetate, propionate or butyrate were used as substrates in the absence and in the presence of sulfate.

The MPN counts were performed as described in chapter 2.4. The highest dilutions of the different MPN tests where growth occurred, were subcultured five times in 125 ml serum vials containing 40 ml medium. Each transfer was carried out using an inoculum size of 5 %. The cultures obtained were then used for the determination of substrate removal rates and growth rates. Cultures obtained with acetate, propionate or butyrate in the presence and absence of sulfate were tested.

6.3 Results

Performance of the UASB reactors

The UASB reactors were started at a loading rate of 2.5 g $COD.l^{-1}.day^{-1}$ and at different concentrations of sulfate. The loading rate was increased stepwise at days 7, 14 and 40 to about 15-20 g $COD.l^{-1}.day^{-1}$, which corresponds to about 2 g $COD.g^{-1}$ $VSS.day^{-1}$. The loading rates were elevated by increasing the COD and sulfate concentration of the influent, keeping the COD to sulfate ratio of the influent constant. The last increment in loading rate on day 40 resulted in a high wash-out of biomass, probably due to the increased methane production, which resulted in flotation of the sludge. From day 50 onwards, the sludge concentration in the reactors and the applied loading rate remained fairly constant.

The results in Figs. 6.1 and 6.2 show the loading and conversion rates, the percentage of the degraded substrate used by the SRB and MB, as found in reactors 1,2 and 3. It is clear that from day 50 onwards the reactor performance remained fairly constant.

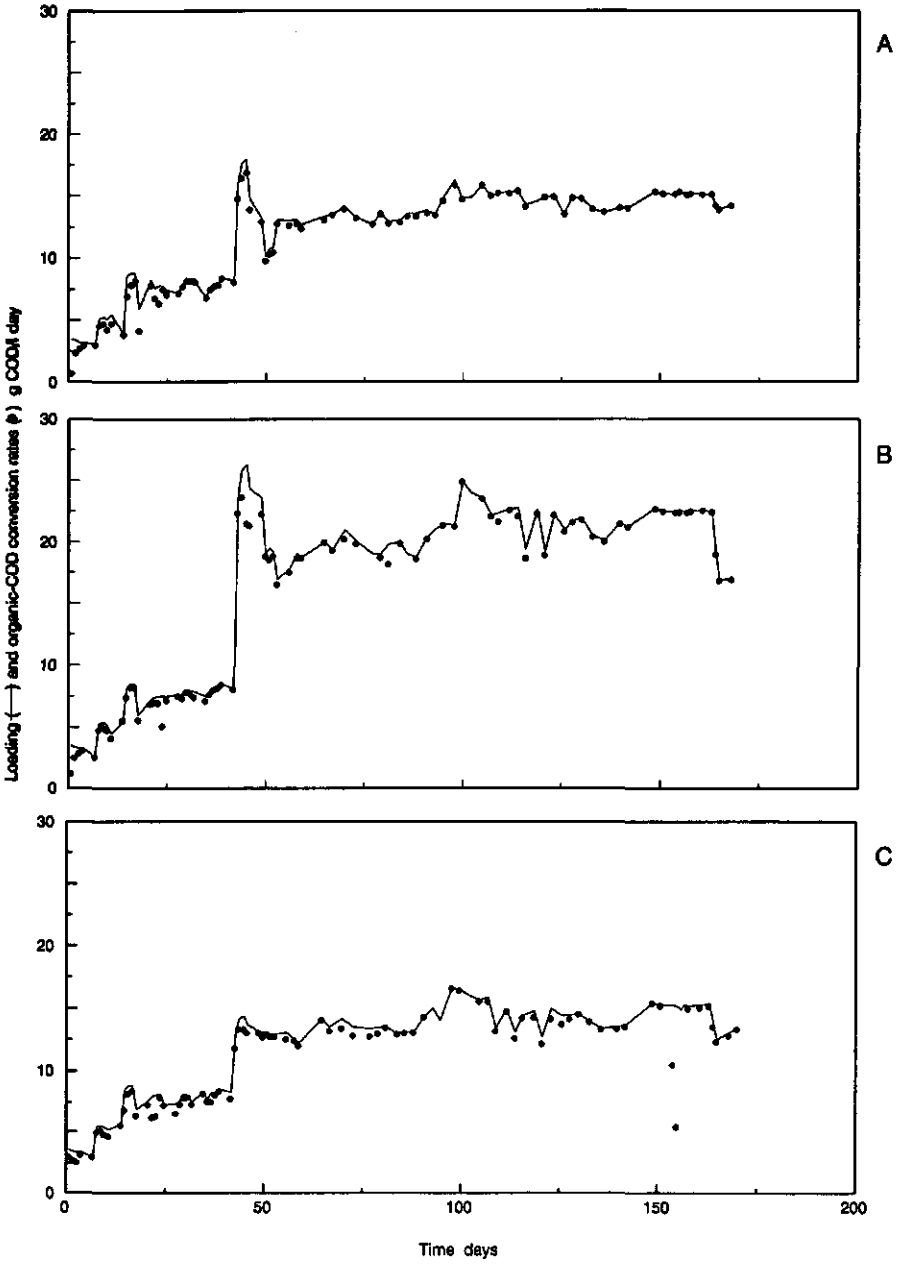

Fig 6.1. Organic loading rate and organic-COD conversion rate in reactor 1 (A), 2 (B) and 3 (C). The COD/sulfate ratio of the influent of the reactor was 10.4, 3.6 and 0.6, with COD and sulfate both expressed in $g.l^{-1}$, for reactor 1, 2 and 3, respectively.

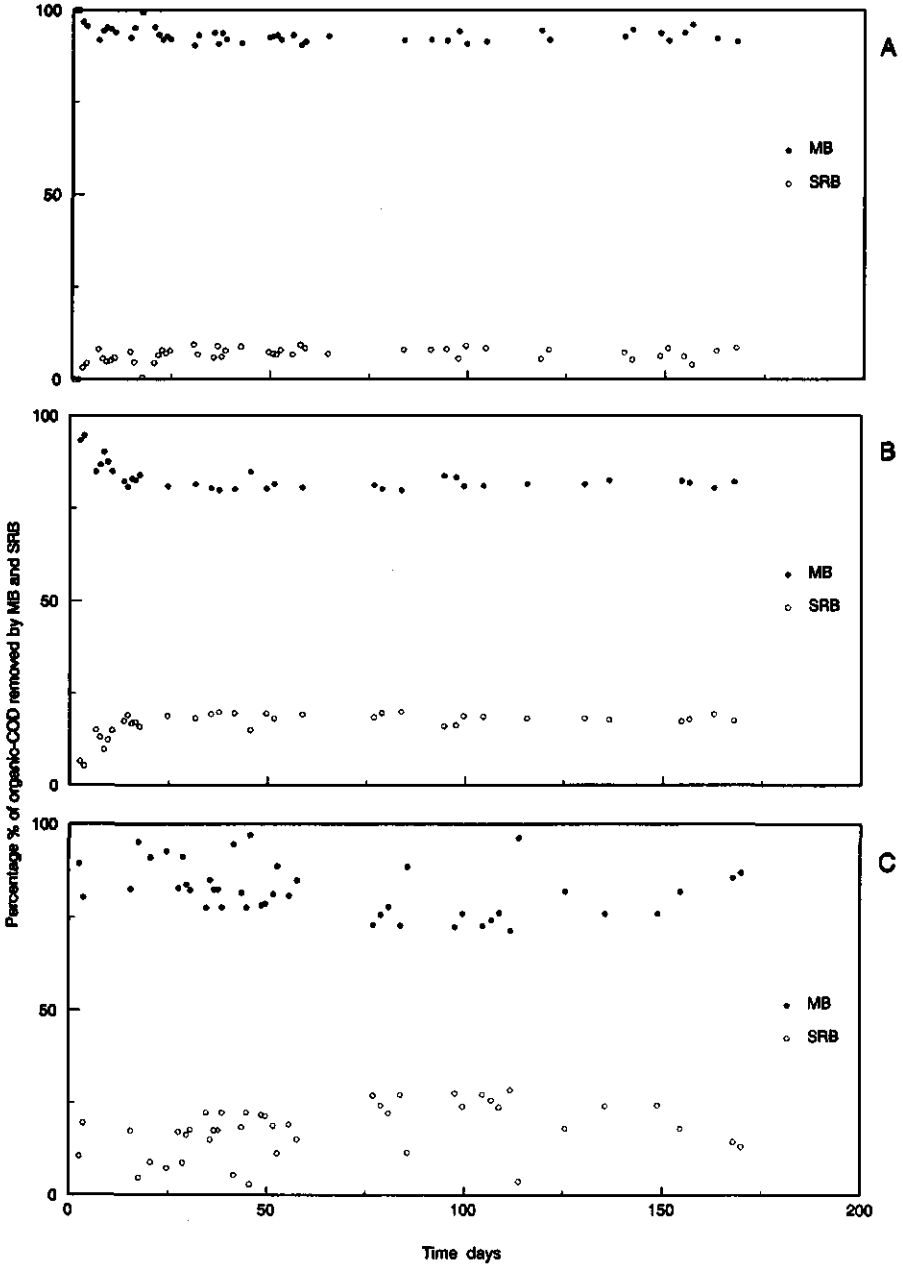

Fig 6.2. Percentage of organic-COD degraded used by SRB and MB in reactor 1 (A), 2 (B) and 3 (C). The COD/sulfate ratio of the influent of the reactor was 10.4, 3.6 and 0.6, with COD and sulfate both expressed in $g.l^{-1}$, for reactor 1, 2 and 3, respectively.

The average reactor performance over the last 100 days is shown in Table 6.1.

Table 6.1. The average performance of reactor 1, 2 and 3 during the last 120 days

	Reactor 1	Reactor 2	Reactor 3
Organic Loading rate			
g COD.l^{-1} day	14	20	13
Sludge loading rate			
g COD.g^{-1} VSS.day^{-1}	2.3	1.8	2.0
COD/sulfate-influent	10.39	3.62	0.57
organic-COD removal (%)	99	99	99
Percentage (%) of COD removed used by			
SRB	7.5	18.5	20.5
MB	92.5	81.5	79.5

All reactors gave a good removal (>98 %) of the organic COD. In reactors 1 and 2, operated at a COD to sulfate ratio of 10.39 and 3.62, a sulfate removal of about 82 and 90 % was obtained. The amount of organic-COD removed via sulfate reduction amounted to 7 and 18 % in reactors 1 and 2, respectively. Major part of the organic COD was removed via methanogenesis.

Even in reactor 3, operated under conditions of excess sulfate, the amount of organic COD removed via sulfate reduction remained low, i.e. only 20 %, and therefore also here the major part of the organic COD was removed via methanogenesis.

The reactors have been operated for a period of 170 days. The calculated sludge age in the reactors amounted to 50-60 days. A net sludge yield of 0.015-0.020 g VSS.g^{-1} COD degraded was found for all reactors.

Sludge characterization

To improve the insight of bacterial groups involved in the degradation of the fatty acids at the different sulfate concentrations, three sets of experiments were performed. Activity tests carried out with single fatty acids in the presence and absence of sulfate (see table 6.2). Bacteria were enumerated with the MPN technique using media with fatty acids in the presence and absence of sulfate (see Fig 6.3), and the different physiological groups of bacteria were enriched and their growth rates measured (see table 6.3).

Activity tests. The assessed specific activities for the sludges of the UASB reactors at the end of the experiment are summarized in Table 6.2.

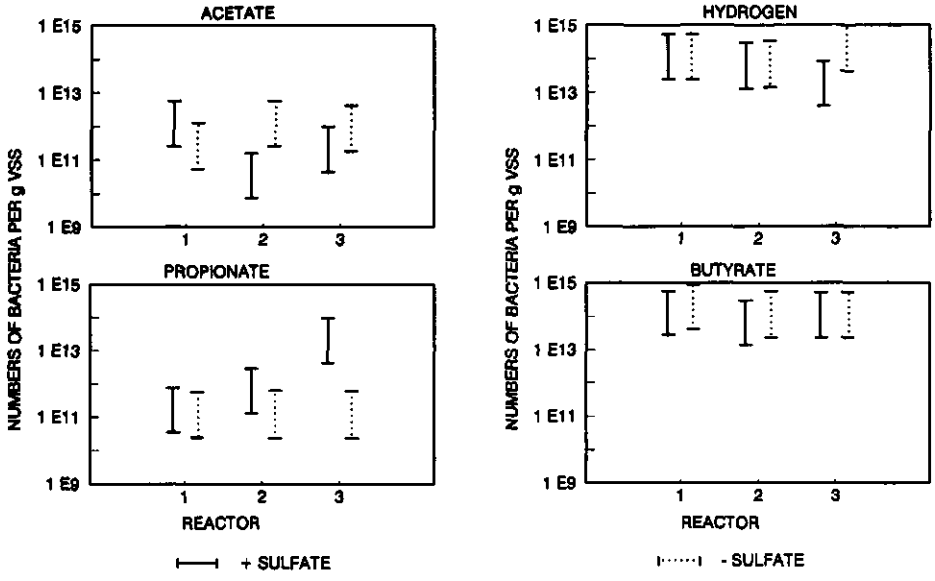

Fig 6.3. Numbers of bacteria (95 % confidence-interval) per gram VSS obtained at the end of the experiment for different substrates in the presence and absence of sulfate for sludge from reactor 1,2 and 3. The organic-COD/sulfate ratio of the feed of reactor 1, 2 and 3, was 10.4, 3.6 and 0.6 respectively, with COD and sulfate both expressed in $g.l^{-1}$.

Table 6.2. Sludge activities (g $COD.g^{-1}$ $VSS.day^{-1}$) obtained at the end of the experiment for different substrates in the presence and absence of sulfate for sludge from reactor 1,2 and 3.

	Sludge from reactor		
Substrate	1	2	3
Acetate	1.8	1.3	1.3
Acetate, sulfate	1.6	1.4	1.4
propionate	0.7	0.2	0.3
propionate, sulfate	1.7	1.5	2.1
butyrate	2.5	2.0	1.6
butyrate, sulfate	5.5	4.4	5.7

Table 6.3. Substrate removal rates and doubling times (T_d) of different enrichments obtained from the highest positive MPN-tubes.

Substrate	removal rate μ mole.(mg protein.day)$^{-1}$	Td Days
Acetate	7.4	3.5
Acetate + sulfate	7.7	3.5
Propionate	4.2	5.3
Propionate + M.f.[1]	5.2	4.4
Propionate + sulfate	29.4	1.8
Butyrate + M.f.[1]	13.4	3.0
Butyrate + sulfate	10.7	4.2

[1] M.f. is Methanobacterium formicicum.

With acetate as the substrate, the potential activity of the three sludges was in the same range, irrespective of the presence or the absence of sulfate. In the activity test the potential activity for acetate was about the same as the acetate conversion rate measured in the UASB reactors, which was about 1.5 gCOD.g^{-1} VSS.day^{-1}. In all the activity test, acetate was converted into methane exclusively, indicating that acetate degrading SRB are of little importance.

In the presence of sulfate, the activity with propionate was significantly higher than in the absence of sulfate. In the sludges from the reactors operated at a high or moderately high sulfate concentration (reactors 2 and 3), the specific activity for propionate assessed in the absence of sulfate (see table 6.2) was far lower than the conversion rate of propionate measured in the UASB reactors during operation, which was about 0.7 g COD.g^{-1} VSS.day^{-1}. When sulfate was present in the activity test, the assessed specific activity for propionate (see Table 6.2) became significantly higher than the conversion rate of propionate measured in the reactors. In the activity tests up to 3/4 mole sulfide.mole^{-1} degraded propionate was formed.

Butyrate degradation rates in the presence of sulfate always exceeded those found in the absence of sulfate (Table 6.2). However, for each of the sludges the specific activity in the absence of sulfate (see table 6.2) always exceed the butyrate conversion rate measured in the UASB reactors, which amounted to 0.7 g COD.g^{-1} VSS.day^{-1}. In the activity test with sulfate, about 0.5 mole sulfide.mole^{-1} degraded butyrate was formed.

Bacterial countings. In case sulfate reduction occurs in the activity tests, no distinction can be made between a syntrophic type of degradation coupled to sulfate reduction or a direct oxidation of propionate and butyrate by the SRB. MPN countings can provide insight in the

role of AB and SRB in the presence of sulfate. The assessed number of bacteria per gram VSS in the sludges of the reactors at the end of the experiment are shown in Fig 6.3.

MPN enumerations done with acetate as the substrate confirmed the findings made with the activity tests. In each of the three sludges about equal numbers were counted irrespective of the presence or the absence of sulfate, and in all tubes methane was the only end-product. This shows that only low numbers of acetate degrading SRB could have been present in these sludges.

Extremely high numbers of both hydrogenotrophic MB (HMB) and hydrogenotrophic SRB (HSRB) were present in each of the sludge types. No difference in the number of HMB and HSRB in the sludges of the three reactors was found. However, with propionate as substrate, large differences in numbers of bacteria were found when counted in the presence or absence of sulfate, especially in the sludge from reactor 3, which had been grown with an excess of sulfate. Fig. 6.3 clearly shoes that compared to reactor 1, operated at low sulfate concentrations, in the reactor 3, operated under conditions of excess sulfate, an increase in the number of propionate degrading SRB relative to syntrophic propionate degraders occurred. This difference was due to increased numbers of propionate degrading sulfate reducers rather than to decrease in the number of syntrophic bacteria.

In the MPN tubes with butyrate as substrate, no difference was found in numbers counted with or without sulfate.

Growth kinetic properties. Bacteria obtained from the highest dilutions of the MPN countings were enriched further by repeated transfer in the same media. From the enrichment cultures obtained, the activity per cell and the specific growth rates were measured, as shown in Table 6.3. With acetate, only *Methanothrix*-like bacteria were enriched both in the presence and absence of sulfate. Table 6.3 shows clearly that there exist no difference in the acetate removal rate and the doubling time of the enrichment culture in the presence or absence of sulfate. The propionate-degrading SRB show both a higher activity per cell and a higher specific growth rate than the syntrophic bacteria grown in the presence of *M. formicicum*, which had been added in order to prevent diffusion distances becoming limiting for growth. Remarkably, syntrophic butyrate-degrading consortia had comparable substrate conversion rates and specific growth rates to the enriched butyrate-degrading SRB.

6.4 Discussion

From the results of the activity assays and MPN counts, it is evident that in the UASB reactors acetate is mainly consumed by MB, even in the presence of excess sulfate. Reducing equivalents formed in the oxidation of propionate and butyrate to acetate, in the following indicated as "hydrogen", were oxidized by the SRB. An increased sulfate reduction at higher sulfate concentrations in the reactors was only found in case sufficient "hydrogen" was available.

In our experiments we found that acetotrophic MB (AMB) apparently can effectively compete with acetotrophic SRB (ASRB), even in the presence of an excess of sulfate. Competition between SRB and MB for acetate was already discussed extensively in chapters 3, 4 and 5. In the seed sludge used in the present experiment the number of ASRB was extremely low, because there exist a complete lack of sulfidogenic activity with acetate and little if any acetate degrading SRB were counted in the MPN counts. As shown in chapter 4, in that case a long time might be needed before the ASRB will develop in the sludge or can out compete the MB. Probably, the reactor was not sufficient long in operation for the SRB to develop in the sludge in significant numbers.

The assessed growth rates for propionate as substrate clearly reveal the better growth kinetic properties of propionate-degrading SRB as compared to syntrophic propionate-degrading bacteria, provided that sufficient sulfate is present. The doubling time for the syntrophic enrichment culture in this study corresponded well with the doubling time found for *Syntrophobacter wolinii*, growing in co-culture with *Methanospirillum hungatei* (Boone and Bryant 1980). The doubling time for the syntrophic enrichment culture, however, was higher than that found for *Desulfobulbus propionicus* (Stams et al. 1984). Our results show that the outcome of the competition between SRB and AB depends strongly on the sulfate to organic-COD ratio. For reactors operated at low and moderate sulfate to organic-COD ratios, the decreased sulfate availability could limit the growth of the propionate-degrading SRB. This will benefit the propionate-degrading AB over the SRB. In addition, at low sulfate concentrations, besides competition between propionate-degrading SRB and AB, different groups of SRB will also compete for the available sulfate. Under sulfate limiting conditions, propionate degrading SRB are not very effective competitors for H_2-consuming SRB. This in turn can enable propionate degrading AB to compete with propionate degrading SRB, as was observed in this study. These findings are in agreement with earlier observations of Laanbroek et al. (1984) showing that propionate-degrading *Desulfobulbus* species have a lower sulfate affinity than H_2-oxidizing *Desulfovibrio* species.

Under conditions of excess sulfate, sulfate limitation and sulfate competition between different groups of SRB becomes unimportant. Consequently, a predominance of propionate-degrading SRB over AB is to be expected. This indeed was observed in this study. These findings are in agreement with other studies performed in sediments, where it was found that propionate was directly degraded by SRB (Banat and Nedwell 1983, Sörensen et al. 1981). However, the SRB were unable to expel syntrophic propionate oxidizers from the sludge. The number of syntrophic oxidizers was roughly the same for the sludges operated at different sulfate concentrations, whereas the number of propionate-degrading SRB increased for sludges of reactors operated at higher sulfate concentrations. A possible explanation for this is that part of the syntrophic propionate-degrading bacteria that are counted during the MPN test are SRB capable of acetogenic growth in the absence of sulfate.

Although *Desulfobulbus propionicus* and *D. elongatus* are unable to grow as acetogens (Samain et al. 1984, Widdel and Pfennig 1982), *Desulfobulbus*-like bacteria have been

observed in granular sludge that grew syntrophically on propionate (Wu et al. 1991). In the presence of sulfate the bacteria then again act as true sulfate reducers. According to Wu and Hickey (1992), the acetogenic oxidation of propionate in sludge granules cultivated at very low sulfate concentrations in brewery wastewater or a fatty acid mixture, is mainly performed by propionate-degrading SRB capable of acetogenic growth on propionate.
In contrast to propionate-degrading AB, syntrophic butyrate oxidizers were found to be effective competitors of SRB, even under conditions of excess sulfate. The growth rates of enrichment cultures of butyrate-degrading AB and SRB were very similar. The doubling time of the syntrophic enrichment culture was lower than reported for *Syntrophomonas wolfei* (McInerney et al. 1981). The doubling time of the sulfidogenic enrichment culture was higher than that observed for a butyrate-degrading sulfate reducer isolated by Nanninga and Gottschal (1987). Contrary to our findings with granular sludges it was shown for sediments that the major fraction of butyrate is directly oxidized by SRB (Banat and Nedwell 1983). Unfortunately, very little is known about the competition for butyrate between AB and SRB in anaerobic reactors. Reports only mention that the reducing equivalents produced during the oxidation of fermentation products such as propionate and butyrate to acetate, are completely used by the SRB (Mulder 1984, Rinzema et al. 1986, Rinzema and Lettinga 1988). In these studies no distinction was made between a syntrophic degradation coupled to HSRB, and a direct incomplete oxidation by SRB. Our results show clearly that butyrate-degrading AB can compete with SRB.

With respect to the competition between SRB and MB for H_2, the results of the MPN counts show that SRB are unable to replace the MB completely in the sludge of the reactors. These findings seem to be in disagreement with the observations in the reactors where we found that at an increasing sulfate to organic-COD ratio an increase in "hydrogen" oxidation by SRB occurred. In the case of an excess of sulfate even a complete consumption of "hydrogen" by the SRB was observed. A similar disagreement was found in the activity assays. In the absence of sulfate, butyrate and propionate were degraded,showing the presence of HMB. However, in the activity test in the presence of sulfate a complete oxidation of "hydrogen" by SRB was observed. This can be explained when HSRB keep the H_2 concentration below the threshold value for MB, as was suggested by Lovley et al. (1982) and Lovley (1985). The observations that HMB persist in the sludge in the presence of excess sulfate might be explained by mixotrophic growth of MB. For example, *Methanosarcina* species are capable to consume both H_2 and acetate (Vogels et al. 1988).

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CHAPTER 7

The degradation of fatty acids and sulfate in thermophilic UASB reactors at 55 °C

7.1 Introduction

Application of anaerobic digestion under thermophilic conditions may become an attractive alternative for mesophilic treatment, especially for the treatment of hot and concentrated wastewaters (Wiegant 1986, Zinder 1988). Laboratory studies demonstrated the feasibility of high rate thermophilic anaerobic treatment systems for the treatment of industrial wastewaters (Schraa and Jewell 1984, Wiegant 1986).

Some of the potential attractive wastewater streams for thermophilic treatment have high concentrations of sulfate or sulfite, such as wastewaters originating from the pulp and paper manufacturing. In the anaerobic treatment of such sulfate rich wastewaters in addition to methanogenesis, sulfate reduction will occur as the end-step of the anaerobic mineralisation process. As already discussed in detail in chapter 1, the occurrence of sulfate reduction can cause serious problems in the anaerobic treatment. On the other hand, the sulfate reduction process can be used to remove oxidised sulfur compounds and/or heavy metals from wastewater. Sofar, most research concerning anaerobic treatment of sulfate containing wastewater dealt with mesophilic treatment systems. Very little is known sofar about the treatment of these wastewaters under thermophilic conditions.

The goal of this research therefore was to asses the feasibility of treating sulfate containing wastewaters under thermophilic conditions, and to study the competition between the thermophilic methanogenic bacteria (MB), sulfate reducing bacteria (SRB) and acetogenic bacteria (AB) during the degradation of fatty acids.

7.2 Materials and Methods

General

Two types of experiment were performed :

1 A start up experiment of a thermophilic (55 °C) UASB reactor using mesophilic granular sludge as the inoculum.
2 Experiments conducted at different pH values in order to asses the effect of the pH on the sulfate reduction and methanogenesis in thermophilic (55 °C) UASB reactors

Operation of the UASB reactors

1 The start up of a thermophilic (55 °C) UASB reactor using mesophilic granular sludge as the inoculum.

A 5.75 l UASB reactor (see chapter 2 for description) was equipped with a stirring blade which was used intermittently (5 seconds every 30 minutes at 100 rpm).

The reactor was seeded with 2.5 l mesophilic granular sludge obtained from the Aviko potato processing factory at Steenderen, the Netherlands. The reactor was started up using a solution of acetate and sulfate as the feed. During the second part of the experiment a substrate solution consisting of a mixture of acetate, propionate, butyrate (C2:C3:C4 = 1:1:1, based on COD values), and sulfate was used.

2 The effect of the pH value on the sulfate reduction and methanogenesis in thermophilic (55 °C) UASB reactors

Three 0.15 l UASB reactors as described in chapter 2 were used. The reactors were seeded with 50 g granular sludge obtained from the UASB reactor in experiment 1 after completion of that experiment. The reactors were fed with a solution consisting of acetate, propionate, butyrate (C2:C3:C4 = 1:1:1, based on COD values), and sulfate (COD/sulfate = 0.57 with COD and sulfate both expressed as $g.l^{-1}$).

Sludge characterization

The sludge in the reactors used in the two experiments was characterized by means of the methanogenic activity as a function of the pH. The activity was assessed as described in chapter 2.4 for the specific methanogenic sludge activity.

7.3 Results

7.3.1 The start-up of a thermophilic (55 °C) UASB-reactor using mesophilic granular sludge

Performance of the UASB reactor

The general performance of the reactor throughout the experiment is shown in Fig. 7.1, while, table 7.1 summarizes the average performance achieved during the different stages of the experiment.

The reactor was started at 30 °C with acetate and sulfate as the feed. During this stage the acetate removal was accomplished completely by the MB.

After three weeks of operation at 30 °C the temperature was increased to 55 °C, resulting in an almost immediate and complete cessation of the methane production and acetate removal. After about 1 week of operation under these conditions the acetate removal and methane production recovered, but at the same time also sulfate reduction was observed. Along with time, the acetate removal and sulfate reduction gradually improved further, but the contrary occurred with the methane production.

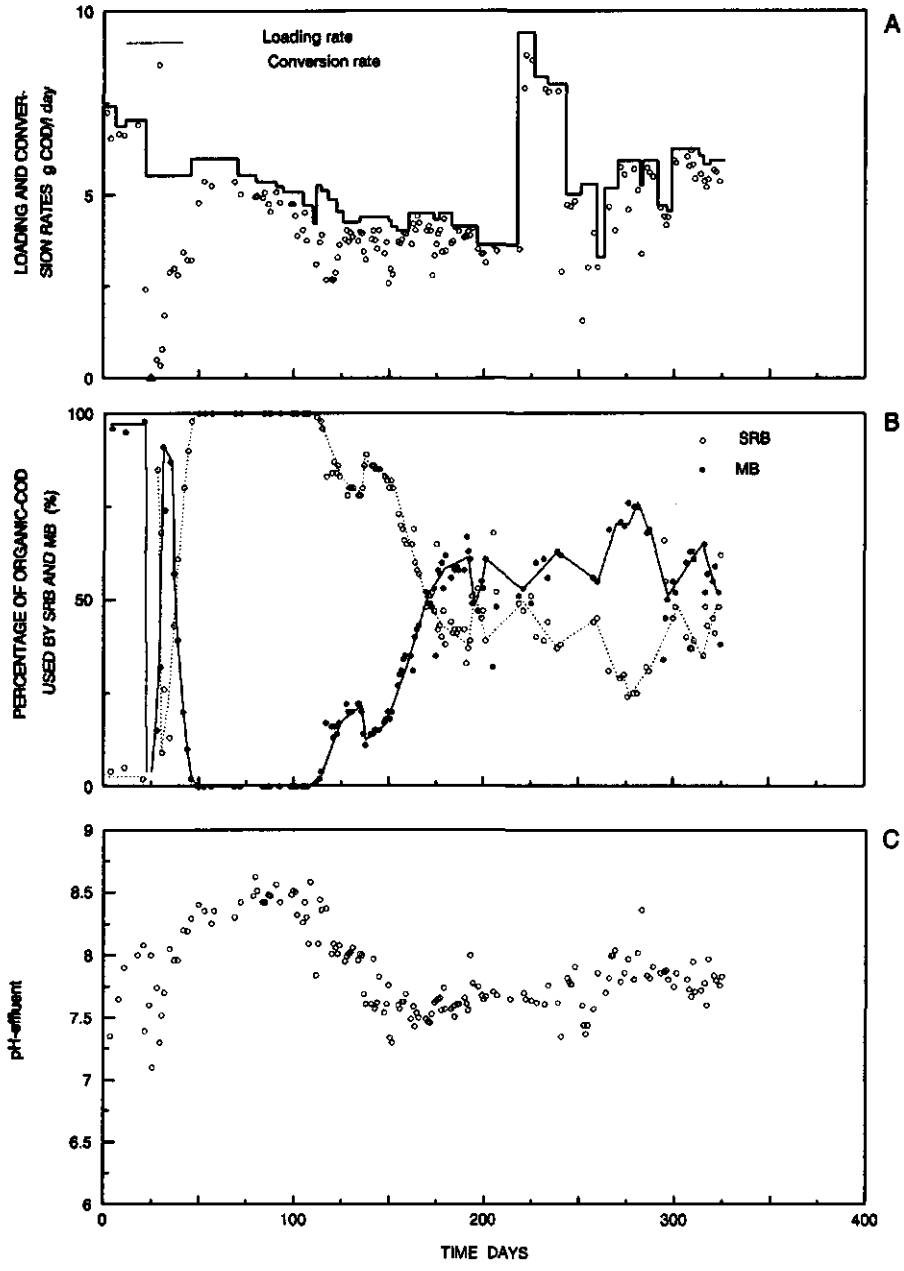

Fig 7.1. Organic loading rate and organic-COD conversion rate (a), the percentage of the organic-COD degraded used by the SRB and MB (b), and the effluent pH (c) in the UASB reactor.

Table 7.1. Average influent values and reactor performance in the UASB reactor.

		Period		
		days 0-21	days 50-115	days 175-350
C2-influent	g COD.l^{-1}	2.5	2.0	0.7
C3-influent	g COD.l^{-1}	0	0	0.7
C4-influent	g COD.l^{-1}	0	0	0.7
SO_4-influent	g.l^{-1}	1.2	4	4
Loading rate	g COD.l^{-1}.day^{-1}	7.2	5.1	5.2
Conversion rate	g COD.l^{-1}.day^{-1}	7.0	4.4	4.8
pH-effluent		7.8	8.4	7.7
Temperature	°C	30	55	55
Percentage COD degraded used by :				
	SRB	3	100	44
	MB	97	0	55

Methanogenesis even ceased completely at day 45. From then onwards the acetate degradation was accomplished completely by the SRB. After 115 days of continuous operation the organic substrate was changed from acetate to a mixture of acetate, propionate and butyrate. This resulted in a decrease of the effluent pH from 8.3-8.5 to 7.6-7.8. The decrease in pH was accompanied by a recovery of the methane production. Moreover, it turned out that the methane production gradually increased further in the course of the experiment. From days 200 onwards a kind of equilibrium between the amount of organic-COD removed via methanogenesis and sulfate reduction was established. In the remainder part of the experiment about 56 and 44 % of the total-organic COD, and about 64 and 36 % of the acetate was removed by the MB and SRB, respectively.

Sludge characterization

The methanogenic characteristics of sludge of the UASB reactor were determined in relation to the pH. The results are depicted in Fig 7.2.

The optimal pH with respect to the specific methanogenic activity was in the range 7.1-7.3. At high pH values the specific activity drops down rapidly, viz only 10 % of the activity is left at pH 8. At low pH-values the activity drops down relatively slow, ie still 80 % of the activity is left at pH 6.2-6.4.

7.3.2 The effect of the pH on sulfate reduction and methanogenesis under thermophilic (55 °C) conditions

Performance of the UASB Reactors

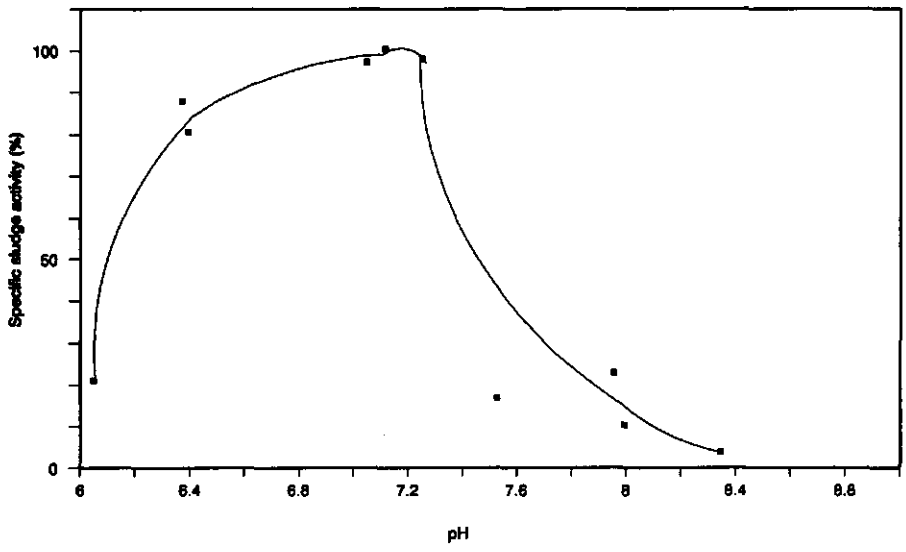

Fig 7.2. Specific methanogenic activity at different pH values for sludge of the UASB reactor.

The results of the UASB reactors 1, 2 and 3, operated at 55 °C at an organic loading rate of 6.5-7 g COD.l^{-1}.day^{-1} are shown Figs 7.3, 7.4 and 7.5. The calculated average values of the COD fraction (in %) removed by SRB and MB, together with imposed loading rates, the amount of COD converted and the influent- and effluent-pH values are listed in Table 7.2. Reactor 1 was started at an influent-pH of approximately 5.5. and during period day 1-15, the influent-pH was gradually decreased from 5.5 to 2.1. This resulted in a drop of the effluent-pH from about 7.9 to 6.7. During the remainder part of the experiment the reactor was operated at a pH-influent 2.0 during period day 15-50 and a pH-influent 3.6 during period day 51-120.

This resulted in effluent-pH values of about 6.7 and 7.3, respectively. At both pH levels a high organic-COD removal (> 90 %) was achieved. About 51 and 49 % of the removed organic-COD was degraded via methane production and sulfate reduction, respectively. In order to asses the effects of short term (8 h) pH variations, the influent-pH was increased to 9.9, 11.5 and 13 at days 64, 68 and 96. Following each pH-shock the influent-pH was brought back to 3.6. A pH-shock of 9.9 and 11.5 did not have any severe effect on the reactor performance, but the pH shock of 13 caused a sharp decrease in the organic-COD removal efficiency.

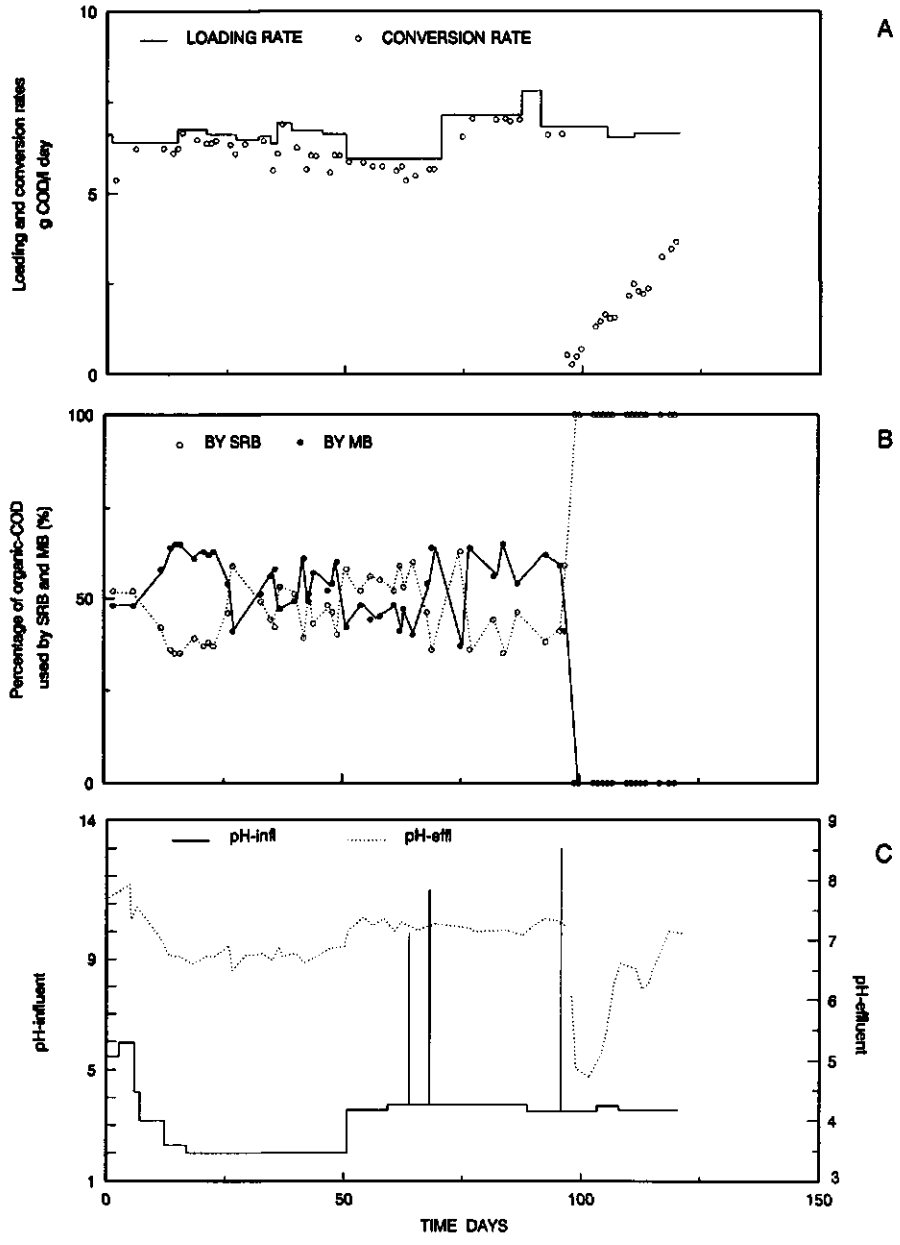

Fig 7.3. Organic loading rate and organic-COD conversion rate (a), the percentage of the organic-COD degraded used by the SRB and MB (b), and the effluent pH (c) in the reactor 1.

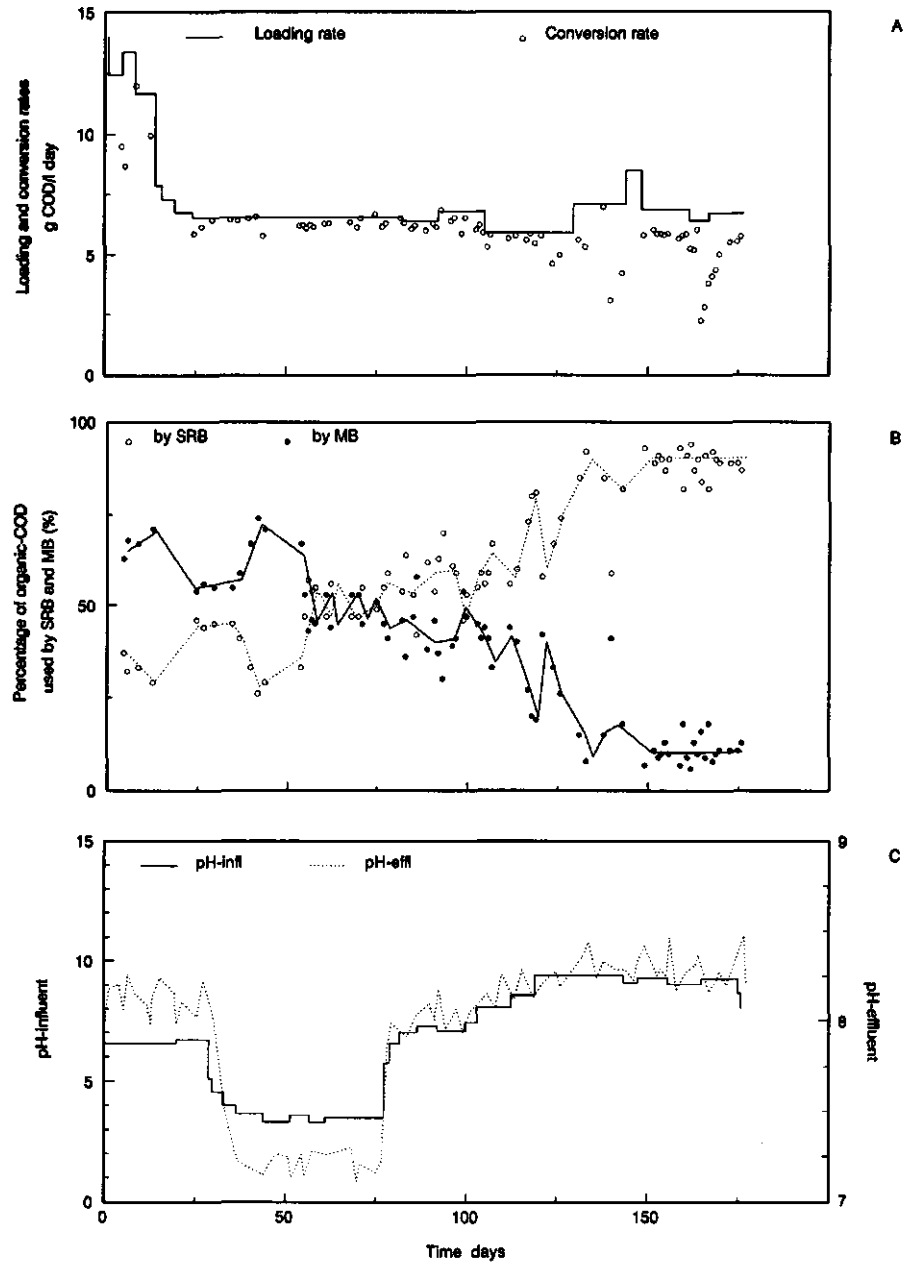

Fig 7.4. Organic loading rate and organic-COD conversion rate (a), the percentage of the organic-COD degraded used by the SRB and MB (b), and the effluent pH (c) in the reactor 2.

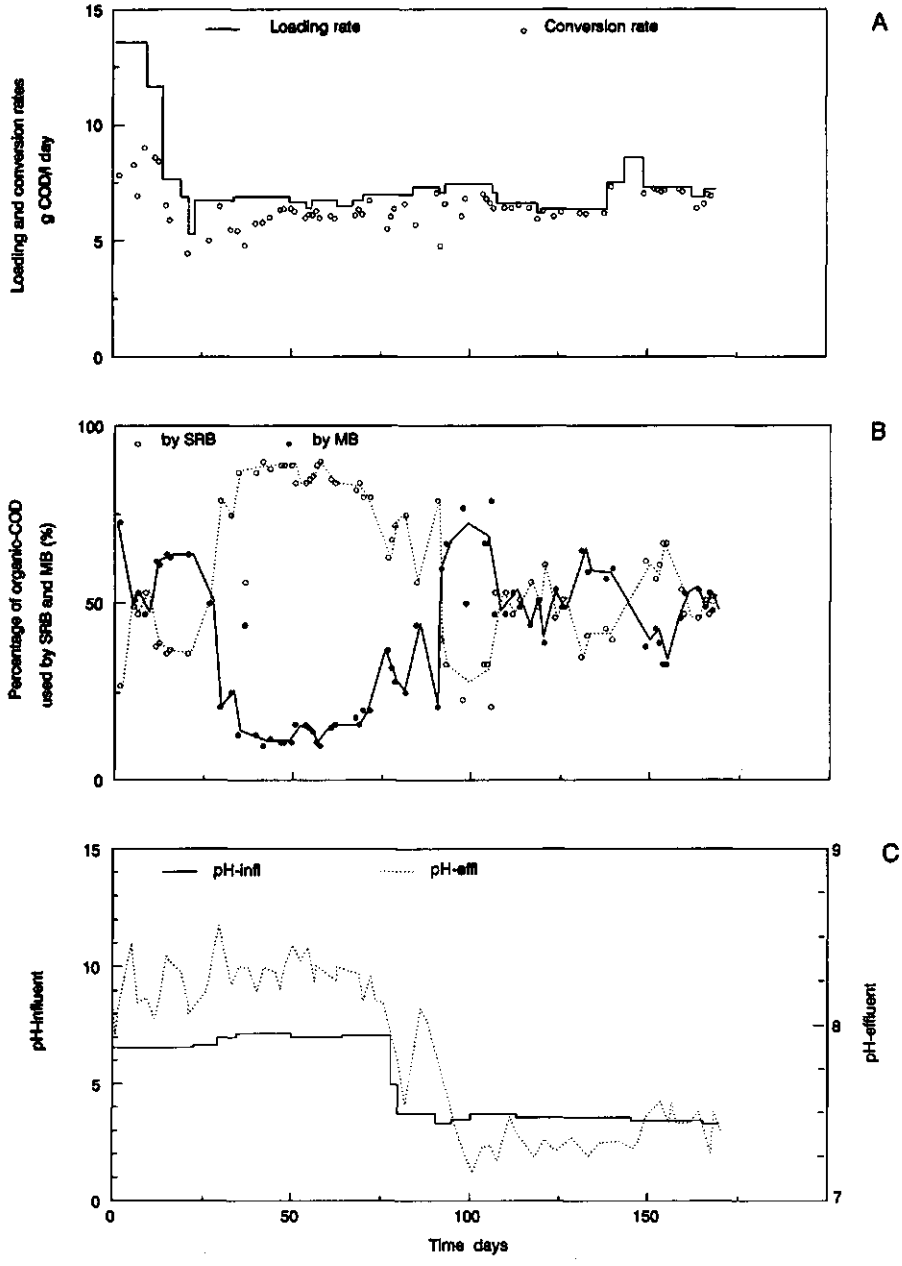

Fig 7.5. Organic loading rate and organic-COD conversion rate (a), the percentage of the organic-COD degraded used by the SRB and MB (b), and the effluent pH (c) in the reactor 3.

Table 7.2. Average performance thermophilic UASB reactors at 55 °C.

				Loading and Conversion rates		Percentage of COD degraded used by SRB and MB			
		pH		load	conversion	Total COD		Acetate	
Reactor	Days	influent	effluent	g COD.l^{-1}.day^{-1}		SRB	MB	SRB	MB
1[a]	20-50	2.1	6.75	6.6	6.2	45	55	31	69
	50-95	3.6	7.25	6.6	6.5	49	51	36	64
2[b]	35-75	3.6	7.2	6.5	6.4	45	55	28	72
	120-180	9.2	8.3	6.8	5.2	85	15	80	20
3[c]	35-75	6.9	8.3	6.7	6.1	84	16	78	22
	80-180	3.5	7.4	7.1	6.7	50	50	35	65

[a]Days 0-20, start-up period, influent pH decreased from 6.1 to 2.1. On days 64, 68 and 96 influent-pH values of 9.9, 11.5 and 13 were imposed. Each pH variation lasted 8 h.

[b]Days 0-20, start-up period at influent-pH 6.5. Days 20-35, influent-pH decreased form 6.5 to 3.5. days 76-120, influent-pH increased from 3.5 to 9.2.

[c]days 0-20, start-up period at influent-pH 6.5. Days 75-80, influent-pH decreased from 6.9 to 3.5.

After the influent-pH was brought back from 13 to 3.6, the decreased organic-COD removal capacity of the reactor initiated a serious drop of the effluent-pH, viz from 7.3 to 4.7. Despite this low pH value, the sulfate reduction process recovered relatively rapidly, and as a result the effluent-pH raised from 4.7 on day 98 to about 6.9 on day 120. Contrary to the sulfate reduction, methanogenesis did not recover within a 30 day period following the pH shock.

Reactor 2 was started using an influent-pH of 6.5. From days 25 onwards the influent-pH was lowered stepwise to 3.5 at day 35, resulting in a drop of the effluent-pH from 7.1 to 7.2. During the remaining part of the experiment the reactor was operated at an influent-pH level of 3.5 during period day 35-75, and a pH-influent of 9.2 during period day 115-175. The effluent-pH values during these periods were 7.2 and 8.3 respectively. At the effluent-pH of 7.2 up to 55 and 45 % of the removed organic-COD was degraded via methane production and sulfate reduction, respectively.

Between period day 75 to 115 the influent pH was increased stepwise from 3.5 to 9.3, which resulted in an increase of the effluent-pH from 7.2 to 8.3. During this period the fraction of the removed organic-COD degraded via methane production decreased from about 55 to 15 %. From day 115 onwards, when the reactor was operated at an effluent-pH of 8.3, the methane production remained low. During this period the organic-COD removal via methanogenesis and sulfate reduction amounted to 15 and 85 % respectively. Reactor 3 was started at an influent-pH of 6.5. During the experiment the reactor was operated at an

influent-pH 6.95 during period day 20-75, and an influent pH 3.5 during period day 76-184. This resulted in effluent-pH values of 8.3 and 7.4 respectively. When operated at an effluent pH of 8.3, the organic-COD removal via methanogenesis and sulfate reduction were 22 and 78 %, respectively. At day 75 the influent-pH was decreased from 6.9 to 3.5, resulting in a decrease of the effluent-pH from 8.3 to 7.4. The lowering of the influent pH to 3.5 was accompanied by an increase of the methane production. From day 110 onwards about 50 and 50 % of the organic-COD was removed via methanogenesis and sulfate reduction, respectively.

Table 7.3 summarise the calculated average amount of organic COD and acetate used by the SRB and MB.

Table 7.3 Percentage of Total-COD and acetate degraded, used by the SRB and MB in the UASB reactors at different pH values.

	Percentage of substrate degraded used by SRB and MB			
	total COD		acetate	
pH	SRB	MB	SRB	MB
6.5-7.5				
Avg[a]	48	52	33	67
Std[b]	8.82	8.82	11.92	11.92
n[c]	73	73	73	73
8.0-8.5				
Avg[a]	85	15	79	21
Std[b]	8.52	8.52	11.2	11.2
n[c]	51	51	51	51

[a]Avg = average, [b]Std = standard deviation, [c]n = number of observations
The data of the reactors were collected after the establishment of a pseudo steady state with respect to the removal of the substrate via methanogenesis and/or sulfate reduction at the imposed pH values. The data collected are days 20-96 (reactor 1), days 35-75, 120-180 (reactor 2) and days 30-75, 110-180 (reactor 3).

Sludge characterization

The methanogenic activity of the sludge of the UASB reactor was assessed in relation to the pH. The Results are shown in Fig 7.6.

The optimal pH with respect to the specific methanogenic activity was found at about pH 7. At high pH values the activity dropped rapidly, viz at pH 8 only about 5 % of the activity was left. At low pH values the drop in activity was relatively slow, ie at pH values 6.2 till 6.4 still about 80 % of the activity was present. For the sludge cultivated to pH values 7.3 and 8.3 a pH dependency was found.

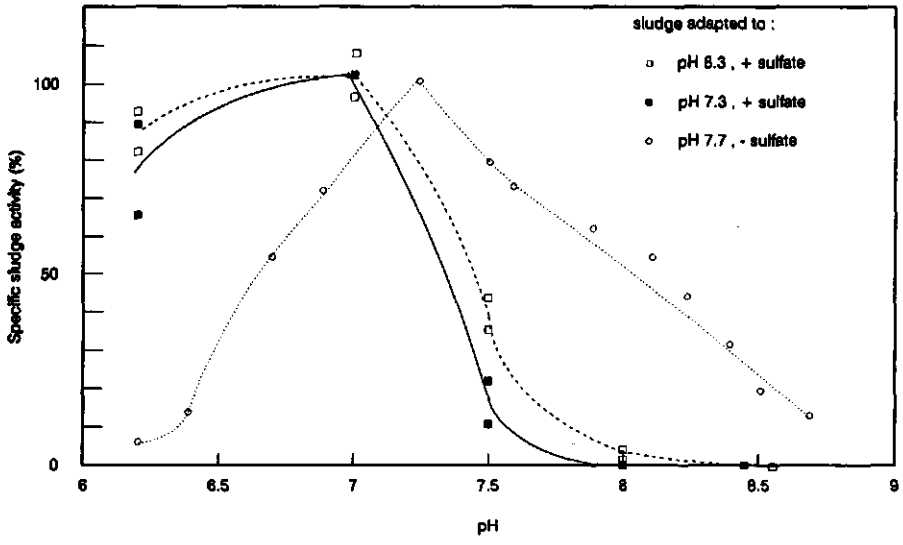

Fig 7.6. Specific methanogenic activity at different pH values for granular sludge obtained from the UASB reactors and adapted to a pH value of about 7.3 and 8.3. In addition the specific methanogenic activity of sludge adapted to pH 7.7 in the absence of sulfate as measured by van Lier (1994) is shown. The maximal activities (100 %) were 1.1, 2.4 and 3.7 g $CH_4COD.g^{-1}$ VSS day^{-1} for sludge adapted to pH 8.3, 7.3 and 7.7, respectively.

The results in Fig 7.6 also reveal that in sludge adapted to 55 °C in the absence of sulfate, the MB are relatively more sensitive to lower pH values, but less to high pH values.

7.4 Discussion

The results of this study clearly reveals that under thermophilic conditions SRB can compete with the MB.

The results show that in fact all reducing equivalents formed in the oxidation of propionate and butyrate to acetate, in the following termed 'hydrogen' were used by the SRB, which also was found for mesophilic (Mulder 1984, Rinzema et al. 1986) and thermophilic systems (Rintala et al 1991, Rintala and Lettinga 1992). The degradation of 'hydrogen' by the SRB can be accomplished either via the oxidation of molecular hydrogen produced by acetogens in the oxidation of propionate and butyrate, or via a direct oxidation of these fatty acids to acetate. However, the results of our experiments do not allow to draw a conclusion which one of these routes prevails. In the discussion in chapter 6 we showed that under mesophilic

conditions, and under conditions of sufficient sulfate the direct oxidation of propionate by the SRB is the predominant route, whereas for butyrate also the AB involved in the degradation. With respect to the competition between SRB and MB for acetate apparently the pH exerts a strong effect (see table 7.3). At pH values ranging from 6.5 till 7.5 the MB are capable to compete with the SRB, which in a UASB-system then will result in the establishment of a kind of equilibrium for acetate degradation via methanogenesis and sulfate reduction. However, at pH values in the range 8-8.5, the SRB will out-compete the MB. Results of the specific methanogenic activity assays show that for MB cultivated at pH values 7.3 and 8.3, high pH values in the range of 8-8.5 are quite unfavourable. This in turn gives the SRB the opportunity to pre-dominate. The optimal pH for methanogens was found at pH about 7. Under these conditions the MB indeed are found more competitive with the SRB. As shown in chapter 4, under mesophilic conditions high pH values also are favourable for the acetotrophic SRB (ASRB) relative to the acetotrophic MB (AMB).

According to the results obtained by van Lier (1994), in anaerobic sludge cultivated at 55 °C with a fatty acid mixture without sulfate, the MB are less sensitive for high pH values than the MB cultivated in the sludge in the present study. Since van Lier used the same seed sludge as in our experiments, the differences found in sludge characteristics indicate that in another more pH sensitive methanogenic consortium might develop in the presence of sulfate. In addition to the pH, very likely also the sulfide concentration in the digester can influence the activity of MB and SRB, and consequently the competition between these organisms. Considering the fact that we assessed the methanogenic activities in the absence of sulfide, and that at pH values of about 8 the specific methanogenic activity will already be strongly inhibited, it can be assumed that sulfide toxicity at these pH values will not play an important role. However, at pH values around 7 we observed a strong competition between the SRB and MB. In this pH range the sulfide concentration could become an important factor in the competition between the SRB and MB. However, sofar too little information is available with respect to the sulfide sensitivity of thermophilic SRB and MB, to allow a proper evaluation of its role. In chapter 4 it was shown that the sulfide sensitivity of ASRB and AMB under mesophilic conditions is very similar at pH values of 7 and 7.5. If this would be also true for thermophilic systems, an important additional effect of the sulfide concentration on the competition is very unlikely.

Sofar, only little reliable data is available for the thermophilic treatment of wastewaters with high levels of sulfate. Although a number of thermophilic methanogens have been isolated and described, only few pure cultures of thermophilic SRB are known sofar, and only very recently a thermophilic sulfate reducer capable to use acetate was described (Min and Zinder 1990). In studies dealing with the thermophilic treatment of clarified white water from a thermomechanical pulping process, it was found that sulfate reduction could contribute 20 till 60 % of the total COD removal at 55 °C. (Rintala et al. 1991). They also studied degradation of sulfate rich acidified wastewater (Rintala and Lettinga 1992). The results obtained reveal that at 37 °C all acetate was used by the MB, whereas it was used by

the SRB after the temperature was shifted to 55 °C. Like found in our experiments, the results reveal that thermophilic ASRB can out-compete AMB at 55 °C. In addition, experimental results obtained at our lab also showed that SRB could become the pre-dominant organisms at 65 °C (A. Visser, unpublished results).

Contrarily to the results obtained at 30 °C (see chapter 3), which indicate that a very long period of time might be involved before the ASRB become the pre-dominant organisms, the results of the present study show that after switching the temperature from the mesophilic to the thermophilic range, a complete removal of the COD via sulfate reduction can be accomplished within a relatively short period of time.

With respect to the process stability the results of the pH shock experiments clearly reveal that sulfate reduction is less sensible to process disturbances than methanogenesis. In mesophilic systems it was also found that SRB are less sensitive to process disturbances than MB (Visser et al. 1993). Apparently, the anaerobic degradation of organic compounds in the presence of sulfate is more stable than the degradation process under pure methanogenic conditions.

8.5 References

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CHAPTER 8

Summary

Anaerobic wastewater treatment is presently accepted as an attractive alternative for aerobic treatment. Of the existing high-rate anaerobic treatment systems the Upflow Anaerobic Sludge Bed (UASB) reactor is the system with the widest application. More than 300 full scale UASB reactors are now in operation or under construction. In most of these reactors easily biodegradable wastewaters with no or only low concentrations of inhibiting compounds are treated. Several types of industrial wastewater that can be treated anaerobically, have high concentrations of sulfate or sulfite. During the anaerobic treatment of these wastewaters, in addition to methanogenesis, sulfate-reduction will occur as the end step of the degradation process. Sulfate reduction can cause several problems in the anaerobic digestion process, i.e. a decrease in COD removal efficiency due to the presence of sulfide in the effluent, an inhibition of the anaerobic bacteria by (hydrogen)sulfide, a reduction of the amount of the methane production, a reduction of the quality of the biogas, corrosion and malodour problems. On the other hand, sulfate reduction can be beneficial, for instance for the removal of heavy metals due to precipitation of poorly soluble metalsulfides and for the removal of oxidised sulfur compounds. For this application the sulfate reduction process should be combined with a sulfide removal step.

It is important to get a better understanding of the sulfate reduction process as it occurs in anaerobic reactors, in order to achieve of an optimal process performance and control.

The goals of this thesis were to gain a better understanding of :

* the role of sulfate reduction in anaerobic degradation of organic compounds, such as volatile fatty acids.
* the substrate competition between the sulfate-reducing, methanogenic and acetogenic bacteria, with special attention to the effect of the kinetic growth properties, the immobilisation properties, pH, temperature and the sulfide concentration.

Chapter 1 reviews the present knowledge of the sulfate reduction process , methanogenesis and of the treatment of sulfate containing wastewaters in anaerobic reactors.

Based on thermodynamic and kinetic data, it is expected that sulfate reducing bacteria (SRB) will out-compete methanogens (MB) for the hydrogen generated in the anaerobic degradation process. This has been confirmed by studies in sediments and anaerobic reactors. These studies show that the hydrogenotrophic SRB (HSRB) out-compete the hydrogenotrophic MB (HMB). This means that for a complex wastewater with sufficient sulfate, at least 30 % of the organic-COD will be removed via sulfate reduction. For acetate contradicting results have been reported. On the one hand a complete utilization of acetate by acetotrophic MB (AMB), and on the other hand a predominance of acetotrophic SRB (ASRB) has been observed . Many factors might affect the competition for acetate, including the kinetic growth

properties of the bacteria, the acetate concentration, the sulfate concentration, the immobilisation properties of the bacteria, the type of substrate used, the type of seed sludge used and the effect of environmental conditions such as the temperature, the pH and the presence of toxic compounds (sulfide). An important aspect in the treatment of sulfate containing wastewaters is the toxicity of sulfide, sulfite and high concentrations of cations such as sodium or calcium. Sulfite, although very toxic for anaerobic bacteria, normally does not cause serious problems in continuous reactors, because it is rapidly reduced in the less toxic sulfide. The effect of sulfide on methanogenesis has been investigated extensively. H_2S concentrations causing a 50 % inhibition of the MB ranging from 50 up to 250 mg H_2S-S.l^{-1} are reported. In general, immobilized biomass is less sulfide sensitive than suspended sludge. To guarantee a successful methanogenic anaerobic treatment in UASB reactors, a maximal allowable H_2S concentration of 150 mg.l^{-1} has been proposed. Contrary to the MB, little is known about the toxic effect of sulfide on the AB and the SRB.

There is evidence that for sludge adapted to high COD/sulfate ratios, the degradation of propionate is the most sulfide sensitive step in the anaerobic degradation process. Propionate degradation seems to be severely inhibited by sulfide levels exceeding 120 mg H_2S-S.l^{-1}. An uncontrolled accumulation of sulfide in anaerobic reactors can cause complete process failure. Frequently, additional measures are needed to prevent H_2S accumulation, such as dilution of the wastewater and the elevation of the pH. The sulfide concentration in the anaerobic reactor can also be reduced by extending the anaerobic treatment step with a sulfide removal step. There exist several options for integrating the anaerobic treatment with a sulfide removal step:

- sulfide-precipitation in the anaerobic reactor
- sulfide-stripping with the biogas combined with gasscrubbing and gasrecirculation
- sulfide removal from the effluent combined with effluent recirculation.

Chapter 3, 4 and 5 describe the research on the competition between the MB and SRB. In chapter 3 the anaerobic degradation of acetate plus sulfate, and a mixture acetate, propionate and butyrate plus sulfate in UASB reactors is described. The results obtained reveal that the reducing equivalents which are generated during the oxidation of butyrate and propionate to acetate, termed 'hydrogen', are effectively and completely scavenged by SRB. However, from these experiments it can not be concluded whether the oxidation of 'hydrogen' by the SRB is a conversion of molecular hydrogen by HSRB during a syntrophic conversion of fatty acids coupled to sulfate reduction, or a direct incomplete oxidation of these fatty acids by SRB. The ability of the SRB to out-compete the HMB or AB for 'hydrogen' was also observed in other experiments described in this thesis (Chapters 5,6,7,8). For the sludge adapted to a mixture of acetate, propionate, butyrate and sulfate, it was observed that propionate and butyrate were not degraded in the absence of sulfate. This illustrates the crucial role of the SRB in the anaerobic degradation of these fatty acids in sulfate adapted sludge. It was further observed that after prolonged operation of the UASB reactors ASRB were able to out-compete AMB for acetate. It took 250 days in one UASB

reactor and 400 days in the other to observe an increase in the amount of acetate used by the ASRB from 50 till 90 %. With a simple growth model, based on the kinetic properties of the ASRB and AMB as measured in our laboratory (chapter 4), it can be shown that the ASRB can eventually expel the AMB from the sludge after a long period of operation.

Chapter 4 presents the experimental results of the investigations dealing with the effect of the pH and sulfide concentration on the kinetic growth properties of ASRB and AMB. These results reveal that at lower pH values (< 6.9) the growth properties of AMB are better than those of the ASRB. Consequently, a pre-dominance of the AMB can be expected under these conditions. However, at higher pH values (> about 7.7) the reverse is true, and it is very likely that under these conditions the ASRB become the pre-dominant organisms. Within the pH range 6.9-7.7 the AMB and ASRB are competable. The maximal growth rates and the acetate affinities of both organisms are in the same range and AMB and ASRB are equally inhibited by the toxic sulfide. The outcome of the competition under these conditions therefore depend on the sulfate concentration in the digester. At sufficiently high sulfate concentrations ASRB can out-compete AMB, whereas at low sulfate levels AMB have higher growth rates than ASRB. The inhibition of AMB and ASRB by sulfide at pH values in the range 7-9 is dictated by the total-sulfide concentration rather than by the H_2S concentration. At higher pH levels, the inhibitory effect of H_2S becomes more severe. A 50 % inhibition of the growth rates and of the acetate degradation rates of the AMB and ASRB was found at about 500 $mg.l^{-1}$ total-sulfide at pH 7-7.5. At pH values ranging from 8 to 9 the growth rates are inhibited more seriously than the degradation rates. At pH 8 a 50 % inhibition of the growth and the degradation rates of AMB was found at concentrations of 250 and 500 $mg.l^{-1}$ total-sulfide, respectively. For the ASRB these levels are 900 and 1500 $mg.l^{-1}$ of total-sulfide, respectively.

In addition to the kinetic characteristics of the bacteria, the competition between the SRB and MB in anaerobic reactors also depends on biomass retention through bacterial immobilisation. Bacteria that do not sufficiently attach to sludge granules or biofilms will be washed out of the reactor. Such organisms therefore will be poor competitors for bacteria with good adhering properties. In the experiments discussed in chapter 5 the immobilisation capacity of MB and SRB through granulation in UASB reactors was assessed under different conditions. The results obtained reveal that there doesn't exist any clear difference in attachment of SRB and MB. Apparently, immobilisation is not a important factor in the competition between SRB and MB. The competition therefore is mainly determined by the kinetic growth properties of the bacteria. With the mixed methanogenic/sulfidogenic culture used in the granulation experiments, the SRB became the predominant organisms, resulting in the formation of a sulfidogenic granular sludge. In the UASB reactor fed without sulfate, a large population of 'hydrogen' degrading SRB remained present in the sludge, whereas ASRB were expelled. This indicates that sludge, cultivated on a substrate with low sulfate levels, contains a large population of 'hydrogen' degrading sulfate-reducers, whereas ASRB will be absent or only present in low quantities. This phenomenon can be explained by

reports in the literature stating that 'hydrogen' degrading SRB can grow by means of an acetogenic oxidation of lactate, ethanol and propionate in the absence of sulfate. Sofar there are no reports dealing with acetogenic growth of ASRB. Our observations also indicate that the ASRB do not posses this ability.

Chapter 6 describes the results of experiments concerning the anaerobic degradation of propionate and butyrate at different COD/sulfate ratios in UASB reactors. At COD/sulfate ratios of 10, the syntrophic degradation of propionate is coupled with the consumption of the generated hydrogen by both HSRB and HMB. At this ratio there exist a sulfate limitation. Propionate degrading SRB are poor competitors for the HSRB for the available sulfate. Also the growth of the propionate degrading sulfate reducers will be reduced by sulfate limitation. Syntrophic propionate oxidizers can therefore effectively compete with SRB.
At low COD/sulfate ratios 0.5 a direct incomplete oxidation of propionate by the SRB is the most important route. The higher growth rates of the SRB as well as the unlimited availability of sulfate allow SRB to out-compete the syntrophic propionate oxidizers. In contrast with the propionate utilization it was found that for butyrate the AB are competitive with SRB at both low and high COD/sulfate ratios. It was also observed that if the reactor is operated at under conditions of excess sulfate, even though no hydrogen was used by HMB, they still remain present in the sludge in large number.

Chapter 7 describes experiments concerning the possible use of anaerobic treatment of sulfate containing wastewater at 55 °C. The results show that SRB out-compete HMB under thermophilic conditions . Also ASRB can compete with AMB for acetate. One month after the start-up a complete conversion of acetate via sulfate reduction was observed. The competition between ASRB and AMB appears to be pH dependent. At pH values exceeding 7.7, acetate is mainly converted by the ASRB. In the pH range of 6.7 to 7.3 a kind of equilibrium between the AMB and ASRB was established. SRB are less sensitive toward pH shocks than MB. The methane production did not recover within one month following a pH shock, whereas the sulfate reduction process completely recovered within this time.

Based on the results of the investigations the following general conclusions can be drawn with respect to the competition between SRB, MB and AB, the immobilisation of SRB and MB, and the toxicity of sulfide on ASRB and AMB.

1. Competition between SRB, MB and AB

1.1 Competition for hydrogen

- HSRB can effectively out-compete HMB under mesophilic as well as thermophilic (55 °C) conditions. If sufficient sulfate is available hydrogen will be completely oxidized by HSRB within a relatively short period of time ; even with seed sludges adapted to very low sulfate concentrations this will be the case.
- In general, for wastewaters with a COD/sulfate ratio exceeding 2.2, sufficient hydrogen will be generated for the reduction of all the sulfate. Consequently, a good sulfate reduction is to be expected for these wastewaters.

1.2 competition for acetate

- The competition between the ASRB and AMB in anaerobic reactors for acetate depends on the pH, the sulfate concentration and the sulfide concentration.
- At pH values below 6.9 and under mesophilic conditions AMB grow faster than ASRB and therefore may out-compete ASRB. At pH values exceeding 7.7 ASRB out-compete AMB. ASRB are capable to grow and degrade acetate up to a pH value of 10. In the pH range of 6.9 to 7.7 AMB are compatible with ASRB at sufficient low sulfate concentrations. At higher sulfate concentrations ASRB will become predominant.
- Under thermophilic conditions and under conditions of excess sulfate and at pH values higher than 7.7 the sulfidogenic bacteria will prevail. At pH values 6.7-7.3 degradation via methanogenesis and sulfate reduction will proceed.

1.3 competition for propionate

- Competition between the SRB and AB for propionate depends on the COD/sulfate ratio. At COD/sulfate ratios around 10 the predominant route is a syntrophic oxidation of propionate by acetogens coupled to sulfate reduction of the generated hydrogen. Under conditions of excess sulfate (COD/sulfate 0.5) propionate is degraded mainly by a direct oxidation of SRB.

1.4 competition for butyrate

- Syntrophic butyrate oxidizers can compete well with SRB at both low and high sulfate concentrations.

2. Immobilisation/granulation of SRB and MB in anaerobic reactors.

- SRB and MB both are well capable to attach and grow in sludge granules during the formation of granular sludge from a suspended seed sludge. The bacteria possess a similar capacity for attachment. The formation of a sulfidogenic granular sludge therefore is well possible.
- A combination of a high liquid upward velocity (0.35 $m.h^{-1}$) and a short hydraulic retention time (7 h) is beneficial for the rapid formation of sludge granules from a suspended sludge when treating wastewater with high levels of sulfate. At a low liquid upward velocity (0.05 $m.h^{-1}$) or a long hydraulic retention time (45 h) the formation of sludge granules proceeds slower.

3. Toxicity of sulfide on ASRB and AMB

- ASRB and AMB are equally inhibited by sulfide at pH values ranging from 7 to 7.5. A 50 % inhibition of the acetate degradation rates as well as the growth rates was obtained at about 500 $mg.l^{-1}$ total-sulfide. At pH values exceeding 8 the AMB are significantly more inhibited by sulfide than ASRB. 50 % inhibition concentrations found for the acetate degradation rates and the growth rates of AMB are about 500 and 250 $mg.l^{-1}$ total-sulfide, respectively. For the ASRB these data are 1500 and 900

$mg.l^{-1}$, respectively.

- In the pH range of 7 to 9 the toxicity of sulfide is dictated by the total-sulfide concentration rather than the undissociated H_2S concentrations. For AMB, an increase of the pH from 7 to a level of 7.5 to 8 does not reduce the inhibition by sulfide, as has been suggested in literature. At pH 8 AMB are even more inhibited than in a pH range from 7 to 7.5. In contrast, such an increase in the pH reduces the sulfide toxicity of the ASRB. This can be contributed to a decreased sensitivity of the ASRB for the total-sulfide concentration. As far as the inhibition by the undissociated H_2S is concerned, it was found that at increasing pH values the inhibition also increase.

Samenvatting

Anaërobe afvalwaterzuivering wordt momenteel gezien als een aantrekkelijk alternatief voor aërobe zuivering. Het meest toegepaste systeem is de Upflow Anaerobic Sludge Bed (UASB) reactor. Meer dan 200 anaërobe reactoren zijn momenteel in gebruik of in aanbouw. In de meeste anaërobe installaties wordt goed afbreekbaar afvalwater met geen of slecht zeer geringe concentraties aan toxische verbindingen behandeld. Verschillende industriële afvalwater stromen bevatten hoge concentraties aan sulfaat en/of sulfiet. Tijdens de anaërobe zuivering van deze afvalwaters zal naast methaangisting ook sulfaatreductie als de terminale stap van het zuiveringsproces optreden. Sulfaatreductie kan verschillende problemen veroorzaken zoals een verminderde CZV verwijdering t.g.v. de aanwezigheid van sulfide in het effluent, remming van anaërobe bacteriën door (waterstof)sulfide, een verminderde methaanopbrengst, een daling van de kwaliteit van het biogas door aanwezigheid van H_2S, corrosie- en stankproblemen. Hier staat tegenover dat sulfaatreductie ook enkele voordelen kent zoals de verwijdering van zware metalen t.g.v. slecht oplosbare metaalsulfiden neerslagen en de verwijdering van geoxydeerde zwavelverbindingen uit afvalwaters. Voor deze laatst toepassing moet sulfaatreductie gecombineerd worden met een sulfideverwijderingsstap.

Een beter inzicht in sulfaatreductie is belangrijk voor zowel de beheersing als de toepassing van het sulfaatreductie proces.

De doelstellingen van dit proefschrift waren het verkrijgen van een beter inzicht in :

* De rol van sulfaatreductie in de anaërobe afbraak van azijnzuur, propionzuur en boterzuur.
* De substraatcompetitie tussen sulfaatreduceerders (SRB), methanogene- (MB) en acetogene bacteriën (AB), met name wat betreft het effect van de kinetische groei-eigenschappen, de immobilisatie-eigenschappen, pH, temperatuur en de sulfideconcentratie.

In Hoofdstuk 1 wordt een overzicht gegeven van de huidige kennis omtrent de sulfaatreductie, de methanogenese en de anaërobe zuivering van sulfaathoudend afvalwater in anaërobe reactoren.

Op basis van thermodynamische en kinetische gegevens verwachten we dat de hydrogenotrofe SRB (HSRB) in staat zijn hydrogenotrofe MB (HMB) te verdrijven. In studies verricht aan sedimenten en anaërobe reactoren wordt dit bevestigd. Hier is aangetoond dat alle waterstof door de HSRB wordt omgezet. Dit betekent dat voor een complexe afvalstroom met voldoende sulfaat, minstens 30 % van de organische-CZV via sulfaatreductie wordt omgezet. Voor azijnzuur zijn er tegenstrijdige resultaten gevonden waaronder enerzijds een volledige omzetting van acetaat door acetotrofe MB (AMB) en anderzijds een dominantie van acetotrofe SRB (ASRB). Diverse factoren kunnen van belang zijn in de competitie om azijnzuur waaronder de groei-eigenschappen van de bacteriën, de acetaatconcentratie, de sulfaatconcentratie, de hechtings-eigenschappen van de bacteriën, de aard van het substraat

en/of entslib en het effect van milieucondities zoals de temperatuur, pH en de aanwezigheid van toxische verbindingen (sulfide). Belangrijk aspecten van de anaërobe zuivering van sulfaathoudend afvalwater zijn de toxiciteitsproblemen met sulfide, sulfiet en hoge concentraties aan kationen zoals Na^+ en Ca^{++}. Het zeer toxische sulfiet veroorzaakt normaal geen problemen, omdat sulfiet in anaërobe reactoren snel gereduceerd wordt tot het minder giftige sulfide. Het effect van sulfide op MB is veelvuldig onderzocht. H_2S concentraties die een remming van 50 % veroorzaken variëren van 50 tot 250 $mg.l^{-1}$. In het algemeen geldt dat een geïmmobiliseerde biomassa minder sulfide-gevoelig is dan een gesuspendeerde biomassa. Om een succesvolle (methanogene) anaërobe zuivering in UASB reactoren te garanderen, is een maximaal toelaatbare H_2S concentratie van 150 $mg.l^{-1}$ voorgesteld. Wat betreft de sulfide gevoeligheid van AB en SRB is slecht weinig bekend. Er zijn aanwijzingen gevonden dat, voor slib geadapteerd aan relatief hoge CZV/sulfaat verhoudingen, de omzetting van propionzuur de meest sulfide gevoelige stap is. Literatuur gegevens geven aan dat Propionzuur afbraak sterk geremd wordt als de H_2S concentratie hoger wordt dan 120 $mg.l^{-1}$. Een onbeheerste accumulatie van sulfide in anaërobe reactoren kan leiden tot een volledige proces verstoring. In veel gevallen zijn extra maatregelen nodig om een accumulatie van sulfide (H_2S) te voorkomen, zoals de verdunning van het afvalwater of de verhoging van de reactor-pH. Een alternatief is om de anaërobe zuivering te integreren met een sulfideverwijderingsstap. Mogelijke procesconfiguraties zijn :

- sulfide precipitatie in de anaërobe reactor,
- strippen van sulfide met het biogas gecombineerd met gaswassen en gasrecirculatie, en
- sulfide verwijdering uit het effluent gecombineerd met effluentrecirculatie.

In de hoofdstukken 3,4 en 5 wordt het onderzoek gericht op de competitie tussen SRB en MB beschreven. In hoofdstuk 3 wordt de anaërobe afbraak van azijnzuur plus sulfaat, en een mengsel van azijnzuur, propionzuur, boterzuur en sulfaat beschreven. Dit onderzoek toont aan dat de reductie equivalenten gevormd tijdens de oxydatie van propionzuur en boterzuur tot azijnzuur, aangeduid als 'waterstof', effectief en volledig door de SRB wordt omgezet. Echter, uit de experimenten kan niet geconcludeerd worden of de oxydatie van 'waterstof' betrekking had op de omzetting van moleculaire waterstof door HSRB tijdens een syntrofe omzetting van vetzuren gekoppeld aan sulfaatreductie, of dat deze betrekking had op de directe onvolledige oxydatie van propionzuur en boterzuur door de SRB. De succesvolle competitie van de SRB om 'waterstof' is in alle experimenten die in dit proefschrift beschreven staan gevonden (zie Hoofdstukken 5,6,7,8). Voor het slib gekweekt op een mengsel van acetaat, propionaat, butyraat en sulfaat, toonden we aan dat in afwezigheid van sulfaat propionzuur en boterzuur niet worden afgebroken. Deze waarnemingen illustreren de cruciale rol van de SRB in de afbraak van deze vetzuren in aan-sulfaat geadapteerd slib.

Verder bleek dat ASRB na lange cultivatie ook in staat waren AMB te verdrijven. In de verschillende UASB reactoren duurde het 250 tot 400 dagen voordat een verhoging van 50 tot 90 % in het percentage azijnzuur omgezet door ASRB gerealiseerd was. Berekeningen uitgevoerd met een eenvoudig groei-model tonen aan dat uitgaande van de groei-

eigenschappen van ASRB en AMB zoals gemeten in ons laboratorium (zie hoofdstuk 4), ASRB de AMB na een lange tijd uit het slib kunnen verdrijven.

In hoofdstuk 4 worden de resultaten van de experimenten mbt het effect van de pH en de sulfideconcentratie op de kinetische groei-eigenschappen van AMB en ASRB beschreven. Deze experimenten tonen aan dat bij lage pH's ($< \pm 6.9$) AMB een hogere groeisnelheid hebben dan ASRB. Dit betekent dat onder deze condities een dominantie van AMB te verwachten is. Bij pH waarden $> \pm 7.7$ is de groeisnelheid van ASRB hoger dan van AMB. Onder deze pH condities zullen de ASRB waarschijnlijk dominant worden. In de pH range van 6.9 tot 7.7 is er een sterke competitie tussen de bacteriën. De maximale groeisnelheden en de substraat affiniteiten liggen voor beide bacteriën in dezelfde orde van grootte. Bovendien worden de ASRB en AMB in gelijke mate geremd door het toxische sulfide. Onder deze condities wordt de uitslag van de competitie bepaald door de sulfaatconcentratie in de reactor. Bij voldoende hoge sulfaatconcentraties worden de ASRB dominant, terwijl bij lagere sulfaatconcentraties de AMB in staat zijn om met ASRB te concurreren. Wat betreft sulfide toxiciteit wordt de remming van de AMB en de ASRB in het pH gebied van 7 tot 9 bepaald door de totaal-sulfide concentratie en niet door de ongedissocieerde H_2S concentratie. Bij hogere pH's bleek eenzelfde H_2S concentratie een sterker remmend effect te hebben. Een 50 % remming van de groeisnelheid en de substraatomzettingssnelheid van de AMB en ASRB werd voor pH 7 en 7.5 gevonden bij $\pm$ 500 mg.l^{-1} totaal-sulfide. Bij pH 8 en 9 bleek de groeisnelheid sterker geremd te worden dan de substraatomzettingssnelheid. Een 50 % remming van de acetaatomzettingssnelheid en groeisnelheid van de AMB bij pH 8 werd gevonden bij respectievelijk 500 en 250 mg.l^{-1} totaal-sulfide. Voor de ASRB was dat bij respectievelijk $\pm$ 1500 en 900 mg.l^{-1} totaal-sulfide.

Naast de groei-eigenschappen van de bacteriën wordt de competitie tussen de MB en SRB ook bepaald door de hechtings-eigenschappen. Micro-organismen die niet of in onvoldoende mate in staat zijn om te hechten aan slibkorrels of een biofilm zullen uit de reactor spoelen. Deze bacteriën zijn dan ook niet in staat om te concurreren met bacteriën die zich wel goed kunnen immobiliseren. In de experimenten beschreven in hoofdstuk 5 werden de hechtingseigenschappen van de SRB en MB onderzocht tijdens het korrelvormingsproces in UASB reactoren onder verschillende proces-condities. Deze experimenten tonen aan dat het dat SRB en MB een gelijke potentie tot hechting hebben. Dit betekent dat de competitie tussen de bacteriën met name bepaald wordt door de groei-eigenschappen van de organismen. In de reactoren gevoed met sulfaathoudend afvalwater werden de SRB de dominante organismen, hetgeen leidde tot de vorming van een sulfidogeen korrelslib. In de reactor die niet gevoed werd met sulfaat bleek dat 'waterstof' consumerende SRB in het slib aanwezig bleven terwijl ASRB uit het slib verdwenen. Dit betekent dat in slib geadapteerd aan afvalwater met geringe hoeveelheden sulfaat, 'waterstof' consumerende SRB aanwezig zijn in grote getale, terwijl ASRB slecht in zeer geringe mate aanwezig zullen zijn. Dit verschijnsel kan verklaard worden door waarnemingen uit de literatuur waarin is aangetoond dat 'waterstof' oxyderende SRB in staat zijn om, in afwezigheid van sulfaat, te groeien als

een acetogeen met lactaat, ethanol en/of propionaat. Voor ASRB zijn dergelijke eigenschappen nog niet waargenomen. Onze resultaten suggereren dat deze bacteriën die eigenschap niet bezitten.

Hoofdstuk 6 beschrijft de resultaten van de experimenten waarin de anaërobe afbraak van propionzuur en boterzuur bij verschillen CZV/sulfaat verhouding werd onderzocht. Bij een CZV/sulfaat verhouding van 10 bleek een syntrofe omzetting van propionzuur door een acetogeen, gekoppeld aan waterstof consumptie door HSRB en HMB de dominante afbraak route. Bij deze CZV/sulfaat ratio is er sprake van sulfaat limitatie. Propionzuur afbrekende SRB zijn slecht in staat om te concurreren met de waterstof consumerende HSRB om het aanwezige sulfaat. Hiernaast zal de groei van de propionaat oxyderende SRB sulfaat gelimiteerd zijn. Hierdoor zijn syntrofe propionzuur oxydeerders in staat om met de SRB te concurreren. Bij lage CZV/sulfaat verhoudingen van 0.5 was een directe onvolledige oxydatie van propionzuur via sulfaatreductie de dominante afbraakroute. De betere groei-eigenschappen van de SRB en de voldoende beschikbaarheid van sulfaat stellen de SRB dan in staat om te concurreren met acetogenen. Mbt de afbraak van boterzuur bleek dat de syntrofe bacteriën goed in staat waren te concurreren met de SRB bij lage en hoge sulfaatconcentraties. Verder werd waargenomen dat, hoewel in de reactor die bedreven werd met een overmaat aan sulfaat geen waterstof consumptie door de HMB werd gevonden, HMB in het slib aanwezig bleven.

In hoofdstuk 7 worden de experiment uitgevoerd onder thermofiele condities (55 °) beschreven. Dit onderzoek toont aan dat in thermofiele systemen de HSRB de HMB verdrijven. Hiernaast waren de ASRB ook in staat om te concurreren met de AMB. In de reactor werd waargenomen dat 1 maand na de opstart een volledige oxydatie van acetaat door de ASRB optrad. De competitie tussen de ASRB en de AMB was afhankelijk van de pH. Bij pH's hoger dan $\pm$ 7.7 was de omzetting van acetaat via sulfaatreductie de dominante route. Bij pH waarden van 6.7 tot 7.3 was er een pseudo evenwicht tussen de omzetting van azijnzuur via sulfaatreductie en methaanproduktie. pH-schokken toonden aan dat SRB minder gevoelig zijn voor kort durende pH variaties dan MB. In tegenstelling tot het sulfaatreductie proces trad er geen herstel op in de methaanproduktie binnen 1 maand na de pH schok.

Uit de resultaten verkregen uit de diverse experimenten kunnen de volgende algemene conclusies worden getrokken :

1. Competitie tussen SRB, MB en AB

1.1 Competitie om waterstof

- HSRB zijn in staat om de HMB weg te concurreren onder zowel mesofiele als thermofiele (55 °C) condities. Indien voldoende sulfaat aanwezig is zal alle waterstof door de HSRB in relatieve korte tijd omgezet worden ; zelfs in slib geadapteerd aan zeer lage sulfaat concentraties.
- Voor complexe afvalwaters met een CZV/sulfaat verhouding $\geq$ 2.2 wordt er voldoende waterstof gegenereerd om alle sulfaat te reduceren. Voor deze afvalwater stromen is

een goede verregaande sulfaatreductie te verwachten.

1.2 Competitie om azijnzuur

- De competitie tussen ASRB en AMB in anaërobe reactoren wordt beïnvloed door de pH, de sulfaatconcentratie en de sulfideconcentratie.
- Onder mesofiele condities en pH waarden lager dan ± 6.9 groeien de AMB sneller dan de ASRB, waardoor de AMB waarschijnlijk dominant zullen worden. Bij pH waarden > ± 7.7 worden de ASRB dominant. Groei en acetaat afbraak door ASRB is mogelijk tot pH 10. In het pH gebied 6.9-7.7 kunnen de AMB goed concurreren met ASRB bij voldoende lage sulfaatconcentraties. Bij hogere sulfaatconcentraties worden ASRB dominant.
- Onder thermofiele (55 °C) condities en in de aanwezigheid van voldoende sulfaat wordt bij pH > ± 7.7 de CZV voornamelijk via sulfaatreductie afgebroken. In het pH gebied 6.7-7.3 vindt de omzetting van CZV zowel via sulfaatreductie als methaangisting plaats.

1.3 Competitie om propionzuur

- De competitie tussen SRB en AB voor propionzuur is afhankelijk van de CZV/sulfaat verhouding. Bij CZV/sulfaat verhoudingen ± 10 is een syntrofe omzetting door een acetogeen, gekoppeld aan waterstof consumptie door HSRB en HMB, de belangrijkste afbraakroute. In de aanwezigheid van een overmaat sulfaat (CZV/sulfaat 0.5) is een directe oxydatie van propionzuur door de SRB de voornaamste afbraakroute.

1.4 Competitie om boterzuur

- Syntrofe boterzuur oxydeerders zijn goed in staat om te concurreren met SRB bij lage en hoge sulfaatconcentraties.

2. Immobilisatie/granulatie van SRB en MB in anaërobe reactoren

- SRB en MB zijn beide goed in staat om zich te hechten aan, en te groeien in korrelslib tijdens de vorming van slibkorrels vanuit een slibsuspensie. De bacteriën hebben een gelijke vermogen tot hechting. De vorming van een sulfidogeen korrelslib is goed mogelijk.
- De combinatie van een hoge opwaartse vloeistof snelheid (0.65 $m.h^{-1}$) en een korte hydraulische verblijftijd (7 uur) is gunstig voor de vorming van slibkorrels vanuit een slibsuspensie. Een lage opwaartse vloeistofsnelheid (0.05 $m.h^{-1}$) of een lange hydraulische verblijftijd (45 uur) is minder gunstige om te komen tot een snelle korrelvorming.

3. Toxiciteit van sulfide voor AMB en ASRB

- In het pH gebied 7-7.5 worden ASRB en AMB in ongeveer gelijke mate door sulfide geremd. Een 50 % remming van de groeisnelheid en de omzettingssnelheid van acetaat treedt op bij ± 500 $mg.l^{-1}$ totaal-sulfide. Bij pH's hoger dan 8 worden AMB veel sterker geremd dan ASRB. 50 % inhibitie concentraties van de groei en omzettingssnelheid van

acetaat zijn gevonden bij respectievelijk 250 en 500 voor de AMB, en 900 en 1500 $mg.l^{-1}$ totaal-sulfide voor de ASRB.

- In het pH gebied 7-9 wordt de remming van AMB en ASRB bepaald door de totaal-sulfide en niet door de ongedissocieerde H_2S concentratie. Voor de AMB leidt een verhoging van de pH van 7 tot 7.5 of 8 niet tot een reductie van de remming door sulfide. Bij pH 8 waren de AMB zelfs gevoeliger voor sulfide dan bij een pH 7 tot 7.5. Voor de ASRB werd wel een reductie in de remming door sulfide gevonden indien de pH van 7-7.5 tot 8-9 verhoogd wordt. Dit was een gevolg van een verminderde gevoeligheid voor totaal-sulfide. In termen van ongedissocieerd H_2S, was de inhibitie van eenzelfde H_2S concentratie bij pH 8-9 sterker dan bij pH 7-7.5.

Curriculum vitae

André Visser werd op 30 augustus 1961 geboren in Breda. In 1979 behaalde hij het Atheneum-B diploma aan de rijksscholengemeendschap te breda en startte hij met studeren aan de Landbouwhogeschool te Wageningen. Hier werd in 1988 de studie milieuhygiëne, specialisatie waterzuivering voltooid. Van 1988 tot 1994 was hij verbonden aan de vakgroep milieutechnologie van de Landbouwuniversiteit. Hij werkte hier eerst als assistent in opleiding, later als tijdelijk wetenschappelijk medewerker.
Sinds mei 1994 werkt hij bij SEGHERSengineeringWATER n.v. Van mei 1994 tot januari 1995 was hij verbonden aan de proces afdeling. Sinds januari 1995 werkt hij bij de afdeling sales & marketing.