



## Anaerobic treatment of sulphate-rich wastewaters

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### Abstract

Until recently, biological treatment of sulphate-rich wastewater was rather unpopular because of the production of H<sub>2</sub>S under anaerobic conditions. Gaseous and dissolved sulphides cause physical-chemical (corrosion, odour, increased effluent chemical oxygen demand) or biological (toxicity) constraints, which may lead to process failure. Anaerobic treatment of sulphate-rich wastewater can nevertheless be applied successfully provided a proper treatment strategy is selected. The strategies currently available are discussed in relation to the aim of the treatment: i) removal of organic matter, ii) removal of sulphate or iii) removal of both. Also a whole spectrum of new biotechnological applications (removal of organic chemical oxygen demand, sulphur, nitrogen and heavy metals), recently developed based on a better insight in sulphur transformations, are discussed.

### Introduction

Sulphate-rich wastewater is generated by many industrial processes that use sulphuric acid or sulphate-rich feed stocks (e.g. fermentation or sea food processing industry). Also the use of reduced sulphur compounds in industrial processes, i.e. sulphide (tanneries, Kraft pulping), sulphite (sulphite pulping), thiosulphate (fixing of photographs) or dithionite (pulp bleaching) contaminates wastewater with sulphate.

Sulphate emissions are not a direct threat for the environment as sulphate is a chemically inert, non volatile and non toxic compound (Shin et al. 1995). However, high sulphate concentrations can cause an unbalance in the natural sulphur cycle. Under anaerobic conditions, dissimilatory sulphate reducing bacteria (SRB) use sulphate as a terminal electron acceptor for the degradation of organic compounds and hydrogen (Oude Elferink et al. 1994). Therefore, the major problem associated with the anaerobic treatment of sulphate-rich wastewater is the production of sulphide. Table 1 lists some important disadvantages as well as potentials of sulphate reduction in anaerobic reactors.

Sulphide will be distributed over the gas phase and the liquid phase according to:

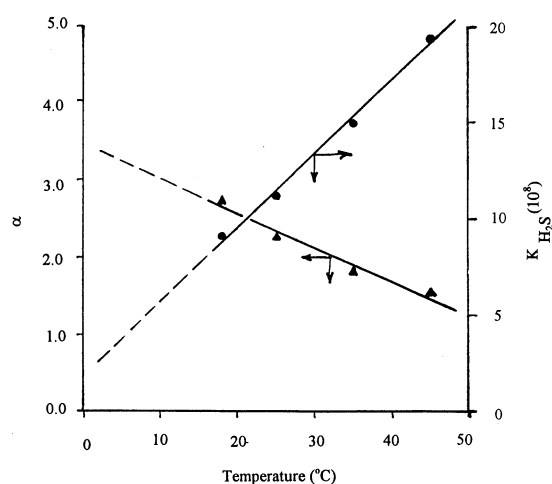
$$[H_2S]_l = \alpha \cdot [H_2S]_g$$

in which  $[H_2S]_l$  and  $[H_2S]_g$  are respectively the concentrations of the H<sub>2</sub>S in the liquid phase and the gas phase (in mol/m<sup>3</sup>) and  $\alpha$  is a dimensionless distribution coefficient. In the liquid phase, the total dissolved sulphide is present in the unionised H<sub>2</sub>S form and as HS<sup>-</sup>. As the pK<sub>a</sub>-value of this acid-base equilibrium is about 7 (Figure 1), small pH-variations in the pH range 6–8 will significantly affect the free (unionised) H<sub>2</sub>S concentration. Figure 1 also points out the dependency of temperature on the free H<sub>2</sub>S concentration.

Table 1 shows that sulphate reduction can cause severe problems when sulphate containing organic wastewater is treated anaerobically. On the other hand, it can also serve as a biological method, together with a sulphide removal step, to remove sulphate (or other oxidised sulphur compounds) and SO<sub>2</sub> from sulphur containing waste streams and off-gasses, respectively.

Table 1. Effects of sulphide formation in anaerobic reactors

DISADVANTAGES	ADVANTAGES
<ul style="list-style-type: none"> <li>– Reduced COD-removal efficiency due to the presence of <math>\text{H}_2\text{S}</math> in the effluent</li> <li>– Corrosion</li> <li>– Accumulation of inert material in the sludge (e.g. metal sulfides)</li> <li>– Less methane formation</li> <li>– Poor biogas quality + need for <math>\text{H}_2\text{S}</math>-removal from the biogas</li> <li>– Malodour</li> <li>– Potential toxicity</li> <li>– Deterioration of aerobic post treatment system (activated sludge bulking; excessive growth of phototrophs)</li> </ul>	<ul style="list-style-type: none"> <li>– Removal of oxidised sulphur compounds (sulphate, sulphite and thiosulphate) from the wastestream</li> <li>– Heavy metal removal</li> <li>– Precipitated metal sulphides (e.g. <math>\text{FeS}</math>) form good precursors for granulation</li> </ul>

Figure 1. Temperature dependence of the absorption coefficient  $\alpha$  (▲) and dissociation constant  $pK_a$  (●) of  $\text{H}_2\text{S}$ .

### Present status

The presence of the sulphate ion in a wastewater considerably increases the complexity of the biodegradation routes (Widdel 1988). In principle, all process designs applied for the methanogenic treatment of wastewaters can also be applied for sulphate containing wastewaters, provided proper preventive measures against corrosion are made and  $\text{H}_2\text{S}$ -removal from the gas is included (Table 2). The selection of the reactor type depends on the objective of the treatment, which can be either the removal of organic matter, or of sulphate or the removal of both.

### Organic matter removal in the presence of sulphate reduction

When a sulphate-rich wastewater is introduced into an anaerobic bioreactor, organic matter will be removed both via sulphate reduction and methanogenesis (Table 2). In practise, anaerobic treatment always proceeds successfully for wastewaters with chemical oxygen demand (COD) to sulphate ratios exceeding 10 (Figure 2), as for such wastewaters the  $\text{H}_2\text{S}$  concentration in the anaerobic reactor will never exceed the presumed critical value of  $150 \text{ mg.l}^{-1}$  (Rinzema & Lettinga 1988). At COD/sulphate ratios lower than 10, process failures of anaerobic reactors have been reported (Figure 2), while in other cases the process proceeds successfully when precautions are taken to prevent sulphide toxicity (Table 3).

### Organic matter removal by methanogenesis

Considering the potential problems related to the occurrence of sulphate reduction in the anaerobic digestion process (Table 1), a complete suppression of the sulphate reduction and a complete conversion of the organic substrate into methane could be considered as the most optimal option. Therefore, attempts have been made to selectively suppress sulphate reduction by using specific inhibitors, i.e. sulphate analogs (Yadav & Archer 1989), transition elements (Clancy et al. 1992) or antibiotics (Tanimoto et al. 1989). However, so far, no selective inhibitor of SRB has been found that can be used in full scale anaerobic reactors. This

Table 2. Overview of reactor types, sulphate removal efficiencies and extend of sulphate reduction in anaerobic digestion of sulphate-rich wastewaters (Adapted from Lens et al. 1998a)

Reactor type	Influent Type	SO <sub>4</sub> <sup>2-</sup> removal				Reference
		COD (g.l <sup>-1</sup> )	SO <sub>4</sub> <sup>2-</sup> (g.l <sup>-1</sup> )	% SO <sub>4</sub> <sup>2-</sup> reduced	% COD flow by SRB	
UASB	Acetate	1.5–2.1	0.7–3.4	70	50–90	Visser et al. 1993b
EGSB	Acetate/Propionate/Butyrate	0.5–2.5	1.2–4.6	27–68	59–97	Omil et al. 1996
USSB	Acetate/Propionate/Butyrate	0.5–6.0	1.0–12.0	35	67–81	Lens et al. 1998b
MUSB	Acetate	0.2–0.4	0.1–0.2	40–80	100	Arora et al. 1995
AF	Citric acid	25.8	3.4	93	18	Colleran et al. 1994
Bio-electro reactor	Glucose	0.4	0.7–3.0	35–55	NR	Watanabe et al. 1997
HYBRID	Landfill leachate	19.6–42.0	5.9	> 90	NR	Nedwell & Reynolds 1996
CAD	Sea food	10–60	0.6–2.7	96.0	3–12	Omil et al. 1995

Abbreviations: UASB – upflow granular sludge bed reactor; EGSB – expanded granular sludge bed reactor; USSB – upflow staged sludge bed reactor; MUSB – microaerophilic granular sludge bed reactor; AF – anaerobic filter; HYBRID – hybrid reactor; CAD – central activity digester; NR: – Not reported.

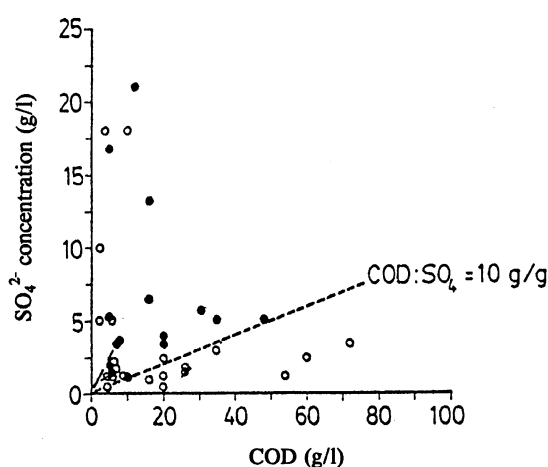


Figure 2. Results presented in the literature on the treatment of sulphate rich wastewater. (○) successful treatment and (●) no success achieved (Adapted from: Rinzema & Lettinga 1988).

implies that sulphate reduction can not be prevented in practice.

### Limitations of anaerobic treatment

#### Substrate utilisation under anaerobic, sulphate-rich conditions

In the presence of sulphate, acidogenic, acetogenic (AB) and methanogenic (MB) bacteria compete with SRB for the available substrates. The outcome of this competition is important, as it will determine to what extend sulphide and methane, the end-products of the anaerobic mineralisation processes, will be produced.

The importance of this competition increases with a decrease in the COD/sulphate ratio of the wastewater (Figure 3). For waste streams with a COD/sulphate ratio of over 0.67, there is theoretically enough organic matter (COD) available to completely reduce the sulphate present (Rinzema & Lettinga 1988). A complete removal of the organic matter can only be achieved if, in addition to sulphate reduction, methanogenesis occurs. For COD/sulphate ratios lower than 0.67, the amount of organic matter is insufficient for a complete reduction of the sulphate and extra substrate should be added if removal of sulphate is the objective of the treatment.

Both AB and MB can, depending on the conditions, successfully compete with SRB for complex substrates (Colleran et al. 1995). In anaerobic reactors, hydrogenotrophic SRB (HSRB) out-compete hydrogen utilising MB (HMB) provided sufficient sulphate is present (Omil et al. 1996; Rinzema & Lettinga 1988; Visser et al. 1993b). This corroborates with the process fundamentals, as HSRB gain more energy from the consumption of molecular hydrogen and have a higher substrate affinity than HMB, thus decreasing the hydrogen concentration below the threshold value of HMB (Oude Elferink et al. 1994). This explains the rapid inhibition of HMB when sulphate enters an anaerobic bioreactor.

Also acetate utilising SRB (ASRB) have a thermodynamic and kinetic advantage over acetate utilising MB (AMB) in their competition for acetate (Widdel 1988). The expected pre-dominance of ASRB over AMB in excess of sulphate has been confirmed in continuously stirred tank reactors and in the anaero-

Table 3. Measures to reduce the reactor sulphide concentration, thus allowing the integration of methanogenesis and sulphate reduction

Measure	Reference
Dilution of the influent	
Non sulphate containing process water	Rinzema & Lettinga 1988
Recycle of effluent after sulphide removal step by:	
Sulphide stripping	Jensen & Webb 1995
Sulphide precipitation	Särner 1990
Biological sulphide oxidation to elemental sulphur	
<i>Thiobacillus sp.</i> , oxygen	Buisman et al. 1990
<i>Thiobacillus denitrificans</i> , nitrate	Gommers et al. 1988
<i>Chlorobium limicola</i> , sunlight	Kim et al. 1993
Decrease of the unionised sulphide concentration	
Elevation of the reactor pH	Rinzema & Lettinga 1988
Elevation of the reactor temperature	Rintala et al. 1991
Precipitation of sulphide, e.g. with iron salts	McFarland & Jewell 1989
Stripping of the reactor liquid using	Särner 1990
- recirculation of biogas after scrubbing	
- other stripping gas (e.g. N <sub>2</sub> )	
Selective inhibition of SRB	Clancy et al. 1992
Two stage anaerobic digestion	
Separation of sulphide production and methanogenesis	Rinzema & Lettinga 1988

bic contact process (Gupta et al. 1994; Middleton & Lawrence 1977). However, the outcome of the competition is less predictable in modern high-rate anaerobic reactors with sludge retention based on sludge immobilisation. Several studies reported that acetate is completely converted into methane, even in excess of sulphate (Hoeks et al. 1984; Mulder 1984), while others report a predominance of ASRB (Omil et al. 1996). Recently, two new ASRB were isolated from bioreactors (Oude Elferink et al. 1995; 1998a). Their growth kinetic properties are only slightly better than those of *Methanosaeta* sp., the most abundant AMB in bioreactors (Oude Elferink et al. 1998b). Besides growth kinetics, also many other factors influence the competition between ASRB and AMB (Table 4).

### Toxicity

#### Inhibition by sulphide

Sulphide is toxic at higher concentrations for many bacteria. Its accumulation can result in a severe inhibition of the purification process, and can even cause a total process failure. The inhibitory effect of sulphide is presumed to be caused by unionised hydrogen sulphide because only neutral molecules can permeate well through the cell membrane. H<sub>2</sub>S may interfere

with the assimilatory metabolism of sulphur, while it possibly may also affect the intracellular pH (Oude Elferink et al. 1994).

Studies under both mesophilic and thermophilic conditions showed that granular sludge is less inhibited by H<sub>2</sub>S than suspended sludges at low and neutral pH, whereas the inhibition is very similar at high pH values (Visser et al. 1996). In suspended sludges, inhibition is determined by the H<sub>2</sub>S concentration both at low and high pH values (McCartney & Oleszkiewicz 1993) and 50% inhibition was found at H<sub>2</sub>S concentrations ranging from 50 to 130 mg.l<sup>-1</sup>. In sludge granules, inhibition is determined by the total-sulphide (TS) concentration (Visser et al. 1996) and 50% inhibition was found at H<sub>2</sub>S concentrations of 250 and 90 mg.l<sup>-1</sup> at pH values of 6.4–7.2 and 7.8–8.0, respectively. The inhibition of MB is significantly higher than the inhibition of SRB at higher pH values above 7.8. At a lower pH range (pH < 7.0) there is not a distinct difference in the degree of inhibition (Koster et al. 1986).

Methanogens are more sensitive than acidogens and acetogens to H<sub>2</sub>S inhibition both in suspended (Oleszkiewicz et al. 1989) and granular (Shin et al. 1995) sludge, with the exception of syntrophic propionate degrading bacteria, which are more sensitive.

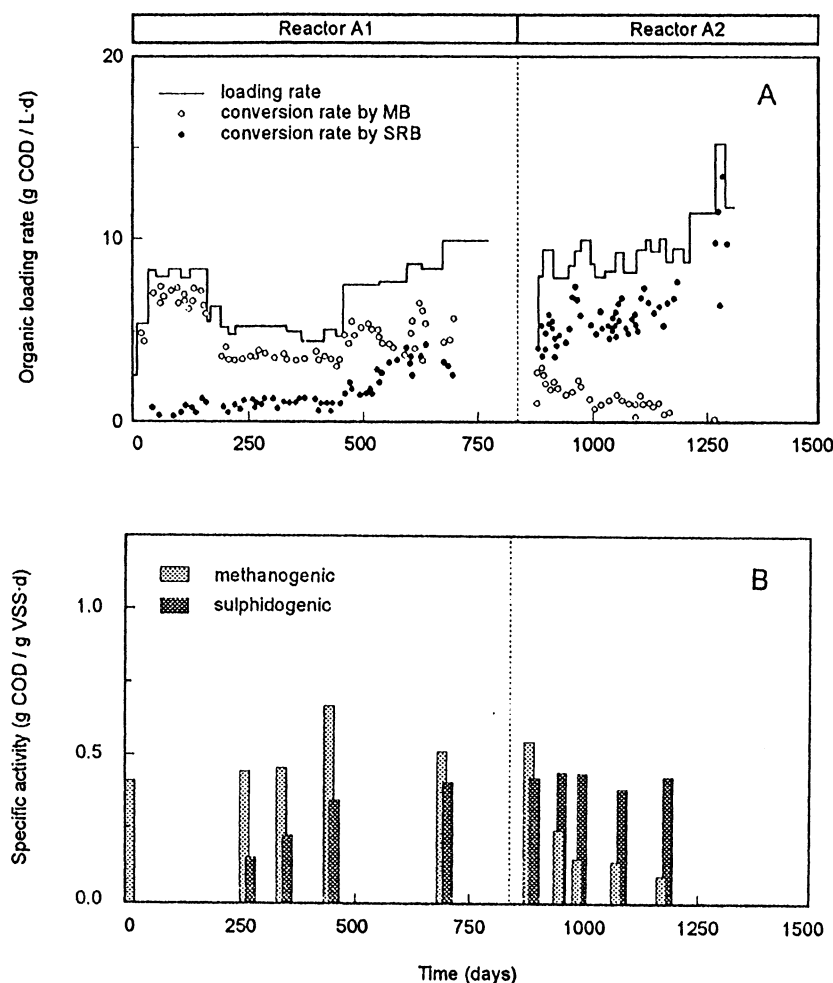


Figure 3. Evolution of the performance and sludge activity of two reactors treating acetate as the sole substrate. In this experiment, the  $\text{COD}/\text{SO}_4^{2-}$  ratio was gradually decreased from 2 to 1 (reactor A1) and finally to 0.5 (reactor A2). (A) Organic loading rate and conversion rates by AMB and ASRB and (B) Specific methanogenic and sulphidogenic activities (Adapted from: Omil et al. 1998).

In a sulphate-reducing fixed bed reactor treating an acetate and sulphate mixture, process failure occurred already at  $\text{H}_2\text{S}$  concentrations above  $50 \text{ mg.l}^{-1}$  (Stucki et al. 1993). This suggests a rather high susceptibility of ASRB. In the pH range of 7.5 till 9, sulphide inhibition of ASRB is determined by the TS concentration rather than the  $\text{H}_2\text{S}$  concentration, both in flocculant (Oleszkiewicz et al. 1989) and granular sludge (Koster et al. 1986; Visser et al. 1996). Besides the pH, also the COD/sulphate ratio influences the susceptibility of a sludge to sulphide toxicity, because of the development of different bacterial associations (McCartney & Oleszkiewicz 1991).

#### *Inhibition by cations*

High cation ( $\text{Na}^+$  and  $\text{Ca}^{2+}$ ) concentrations present in sulphate-rich wastewaters can inhibit anaerobic bacteria. Literature reports on 50% inhibition values of MB by sodium ranging from 6 to  $40 \text{ g.l}^{-1}$ , depending on the history of the sludge, antagonistic and synergistic effects and the test method used (Omil et al. 1995). Although the calcium ion does not exhibit a severe direct toxic effect,  $\text{CaCO}_3$  and/or  $\text{Ca}_3(\text{PO}_4)_2$  precipitates can indirectly upset the reactor performance by scaling (Lettinga 1995), which interferes with a good transport of substrate and reaction products. Serious scaling of biomass by Ca-precipitates may already occur at  $\text{Ca}^{2+}$  concentrations of  $400 \text{ mg.l}^{-1}$ . Also clogging problems can arise from precipitates in the piping system.

Table 4. Factors determining the outcome of the competition between SRB and MB in high-rate anaerobic reactors

Factor	Reference
Inoculum composition	
Type of seed sludge	McCartney & Oleszkiewicz 1991
Bacterial composition	Harada et al. 1994; Omil et al. 1998
Attachment properties of bacteria	Isa et al. 1986a; 1986b
Experimental run time	Harada et al. 1994; Omil et al. 1998
Inoculation with new bacterial species	Omil et al. 1997a
Influent composition	
Type of COD	Polprasert & Haas 1995
Acetate concentration	Yoda et al. 1987
Sulphate concentration	Overmeire et al. 1994
Sulphide concentration	Omil et al. 1996
Operational conditions	
pH	Visser et al. 1996
Temperature	Visser et al. 1992

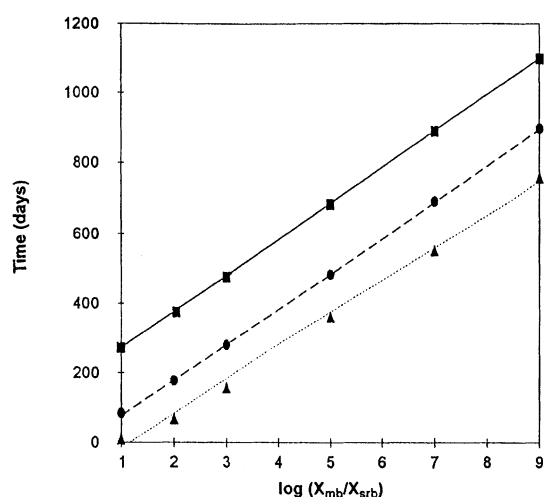


Figure 4. Effect of the SRB/MB ratio of the inoculum on the time required for SRB to consume 10% (▲), 50% (●) and 90% (■) of the substrate acetate (After: Omil et al. 1998). X = Biomass concentration.

Moreover, calcium-phosphate precipitation can cause phosphate deficiency (Lettinga 1995).

## Future perspectives

### Anaerobic digestion technology

#### Steering of the competition between SRB-MB

Methods that influence the outcome of the competition between SRB and MB would be useful to develop fully methanogenic or sulphate-reducing sludges. Moreover, they can prevent potential process failures due to sulphide inhibition. Thus far however, adequate practical methods to steer the competition between ASRB and AMB are not available (Lens et al. 1998a; van Houten et al. 1997). The best way to steer a reactor towards the predominance of one population over the other involves the manipulation of the inoculum size (Figure 4). The initial MB/SRB ratio of a sludge can be manipulated by adding new bacteria or to create unfavourable conditions for the undesired population during a short time interval (Table 5). Successful methods that enhance the development of a SRB population after selective inhibition by MB include shocks of high sulphide concentrations (Omil et al. 1996) or high (65 °C) temperatures (Visser et al. 1993a).

#### Operation temperature

Because of the biological nature of the anaerobic process, there is a strong dependency on the temperature. There is, however, a continuous extension of the temperature limits for anaerobic digestion of sulphate-rich wastewaters. Rebac et al. (1996) showed

Table 5. Effect of changes in process conditions on the competition between SRB-MB

Measure	Increase of the share of SRB to the total COD removal (%) <sup>*</sup>	Reference
Manipulation of the influent composition		
Increase of acetate concentration	-15	Omil et al. 1996
Addition of iron (2 g/l)	0	Isa et al. 1986a; 1986b
Addition of transition elements	0	Clancy et al. 1992
Manipulation of the biomass composition		
Addition of <i>Desulforhabdus amnigenes</i>	0	Omil et al. 1997a
Exposure to oxygen	35	Omil et al. 1997a
Manipulation of the operational conditions		
Alteration of pH	41	Omil et al. 1996
Shock treatment		
Temperature decrease to 15 °C	0	Omil et al. 1997b
Temperature increase to 65 °C	30	Visser et al. 1993b
Manipulation of the reactor design		
EGSB (upflow velocity 4–6 m/h)	-30	Omil et al. 1996
Staged sludge bed reactor	10	Lens et al. 1998b

\* Negative value means an increase in the share of the MB to the total COD removal.

that at temperatures as low as 10–12 °C, organic loading rates of 10–12 kg COD.m<sup>-3</sup>.day<sup>-1</sup> can be achieved at hydraulic retention times of 1.6–2.5 h in expanded granular sludge bed reactors (upward velocity 10 m.h<sup>-1</sup>).

For hot and concentrated industrial wastewaters, thermophilic conditions may be an attractive alternative for mesophilic treatment (Rintala et al. 1991). Increased process temperatures decrease the fraction of unionised H<sub>2</sub>S in the liquid and shift the gas-water partitioning towards the gas phase due to the lower solubility of H<sub>2</sub>S. The H<sub>2</sub>S stripping effect resulting from the high biogas production rates in thermophilic processes further decreases liquid H<sub>2</sub>S concentrations. This allows the successful treatment of sulphate-rich wastewaters at 55 °C (van Houten et al. 1997; Visser et al. 1992) or even at 70 °C (Rintala & Lepistö 1992).

#### *Organic matter removal by sulphate-reducing bacteria*

SRB are present in anaerobic and even aerobic wastewater treatment sludges (Lens et al. 1995a). The development of treatment processes using their degradative capacity to degrade a wide range of organic compounds (Widdel 1988) opens promising perspectives

Table 6. Biotechnological applications using sulphate reducing bacteria

Application	Reference
Biological sulphate removal	
Industrial wastewaters	Särner 1990
Acid mine drainage	Maree et al. 1991
Spent sulphuric acid	Stucki et al. 1993
Scrubbing waters SO <sub>2</sub> -rich gasses	Kaufman et al. 1996
Heavy metal removal	
Extensive treatment (wetlands)	Hao et al. 1996
High rate reactors	Tichy et al. 1998
Process water	
Acid mine drainage	Barnes et al. 1991
Ground water	Scheeren et al. 1991
Microaerobic treatment	
Treatment domestic sewage	Takahashi & Kyosai 1991
Reduction waste sludge production	Lens et al. 1995b
Solid waste treatment	
Gypsum processes	Deswaef et al. 1996

for environmental biotechnology (Table 6). SRB do not require balanced growth with acetogens, which implies less sensitivity to organic overloads. Moreover, SRB are less sensitive to toxicants. Heavy metals are precipitated by sulphide, thus reducing their potential toxic effects (Hao et al. 1996). Moreover, SRB can metabolise organic inhibitors such as aromatics (toluene, ethylbenzene), alkanes, chlorinated compounds (chloroform) and long chain fatty acids (Widdel 1988). On the other hand, reactors based on organic matter removal by sulphate reduction lack one of the major advantages of methanogenic treatment: the recovery of methane from organic compounds.

#### *Sulphate-reducing reactors*

Anaerobic reactors in which organic matter is completely degraded by SRB might be an elegant alternative for methanogenic wastewater treatment. Sulphate-reducing Upflow Anaerobic Sludge Bed (UASB) reactors can be operated at sludge loading rates of 0.9 to 1.0 g COD per g volatile suspended solids per day (Visser et al. 1993b). Nedwell & Reynolds (1996) reported that sulphate-reducing and methanogenic hybrid reactors treating landfill leachates offered equal organic removal efficiencies at low organic loading rates ( $< 1 \text{ kg COD} \cdot \text{m}^{-3} \cdot \text{day}^{-1}$ ). However, the sulphate-reducing reactor was less effective in COD removal at higher loading rates. In contrast, mesophilic (30 °C) volatile fatty acid fed sulphate-reducing UASB reactors (liquid upward velocity  $0.65 \text{ m} \cdot \text{h}^{-1}$ ) had a COD removal capacity comparable to methanogenic systems at volumetric loading rates of  $7.5 \text{ kg COD} \cdot \text{m}^{-3} \cdot \text{day}^{-1}$  (Alphenaar et al. 1993).

Sulphate-reducing granular sludge can be obtained by feeding a reactor, inoculated with methanogenic sludge, a wastewater with a COD/sulphate ratio of 0.5. The population shift between MB and SRB proceeds relatively slow (about 100 days) and after the start-up phase, MB still consume 5% to 20% of the organic material (Omil et al. 1996). By treating the sludge with  $\text{CHCl}_3$  ( $5 \text{ mg} \cdot \text{l}^{-1}$ ) for 1–5 days during the start-up, MB were completely eliminated in the inoculum, thus creating a sulphate-reducing sludge already from the start-up (Visser et al. 1993b).

Elimination of substrates by syntrophic associations in methanogenic reactors requires a low  $\text{H}_2$  partial pressure. In contrast, their degradation by sulphate reducers does not depend on a low  $\text{H}_2$  partial pressure (Widdel 1988). Hence, high-rate sulphate-reducing removal systems for substrates as propionate and butyrate can be applied. The major draw-back of

sulphate-reducing reactor systems is their inefficient acetate removal, as this fatty acid generally accounts for the majority of the residual COD in their effluents (Lens et al. 1998b; Nedwell & Reynolds 1996; Omil et al. 1996). There are indications that ASRB have a poor affinity for sulphate. Hence, HSRB out-compete ASRB for the available sulphate. Optimisation of the performance of sulphate-reducing reactors can be done by methods that integrate acetate scavenging processes, i.e. denitrification (Lens et al. 1998a) or the use of staged reactors, which allows the development of primarily acetotrophic biomass in the last stages (Lens et al. 1998b).

#### *Micro-aerobic treatment*

Another way to utilise the capacities of SRB to degrade organic matter, is the use of reactors with combined aerobic/anaerobic conditions. These conditions are needed for a complete mineralisation of certain xenobiotics (Field et al. 1995), where (facultative) aerobic bacteria utilise the degradation products of the SRB.

Micro-aerophilic conditions can be created in UASB reactors by dosing controlled amounts of oxygen to the sulphate-rich wastewaters. In hybrid reactors, the upper reactor part with the biofilm can be aerated and spatially separated from the anaerobic granular sludge section (Tilche et al. 1994). Alternatively, a single (Arora et al. 1995) or multiple (Takahashi & Kyosai 1991) sequence of aerated down-flow and non aerated upflow ( $6 \text{ m} \cdot \text{h}^{-1}$ ) reactors can be applied. In the latter reactor type, a special type of granular sludge develops, composed of SRB in the inner anaerobic layers and sulphide-oxidising (*Beggiatoa sp.*) bacteria in the oxygenated periphery of the granules (Takahashi & Kyosai 1991).

The close interaction between aerobic and anaerobic conditions also prevails in highly loaded aerobic fixed film reactors (Kuenen et al. 1986). Thus, a sulphur cycle at micro-scale can develop in aerobic rotating biological contactor (Alleman et al. 1982) or trickling filter (Lens et al. 1995a) biofilms. Stimulation of the activity of SRB by decreasing the COD/sulphate ratio of the wastewater from 20.0 to 0.5 resulted in a 50% reduction of the waste sludge production (Lens et al. 1995b). Practical applications to reduce the waste sludge production of aerobic bioreactors are, however, limited by the lack of sulphate removal by these aerobic fixed film reactors.



### Biological sulphate removal

Biological sulphate removal is a cost-effective alternative for costly and sometimes complex physico-chemical sulphate removal methods (Maree et al. 1991). Biological sulphate removal consists of two steps with (dissimilatory) sulphate reduction to sulphide as the first one. The sulphide produced in the first stage is then biologically oxidised to elemental sulphur ( $S^0$ ).

In the sulphate-reducing stage, a complete reduction of sulphate to sulphide is desired. Channelling of reducing equivalents towards the SRB is enhanced by the ability of the SRB to effectively compete with other anaerobic bacteria for the available organic substrate and the sensitivity of the other bacteria for sulphide. For wastewaters that contains no or insufficient electron donor and carbon source for a complete sulphate reduction, addition of an appropriate electron donor is required. The selection of the electron donor depends on i) the costs of the added electron donor per unit reduced sulphate and ii) the rest pollution of the additive in the wastestream, which should be low or easily removable. Based on the last criterion, simple organic compounds (ethanol, methanol) or synthesis gas (a mixture of  $H_2$ , CO and  $CO_2$ ) are preferred above complex wastes (e.g. molasses). Hydrogenotrophic methanogenesis is under mesophilic conditions minimal, as SRB out-compete other anaerobic bacteria more effectively with  $H_2$  than with simple organic compounds as electron donor. Using a mixture of  $H_2$  and  $CO_2$  (80%:20%), sulphate loading rates of  $30 \text{ g SO}_4^{2-} \cdot \text{l}^{-1} \cdot \text{day}^{-1}$  can be achieved at  $30^\circ\text{C}$  within 10 days of operation (Figure 5) in gas-lift reactors (which provide good mass transfer rates) with pumice as carrier material (to immobilise SRB) when the free  $H_2S$  concentration is kept below  $450 \text{ mg} \cdot \text{l}^{-1}$  (van Houten et al. 1994). These experiments revealed that the HSRB were not autotrophic and needed acetate as carbon source. Acetate is formed by homoacetogens. Due to the low affinity of the homoacetogens for  $H_2$ , it is possible that under conditions of  $H_2$ -limitation insufficient amounts of acetate come available for the HSRB, which may result in a predominance of the HMB.  $H_2$ -gas is, however, too expensive to be used in its pure form, but addition of synthesis gas (a mixture of  $H_2$ , CO and  $CO_2$ ) is an elegant and economic alternative (van Houten et al. 1996). It appeared that CO is not used as electron donor by SRB, and it exerts a toxic effect on SRB and thus limits the sulphate loading rate to  $10 \text{ g SO}_4^{2-} \cdot \text{l}^{-1} \cdot \text{day}^{-1}$  at CO concen-

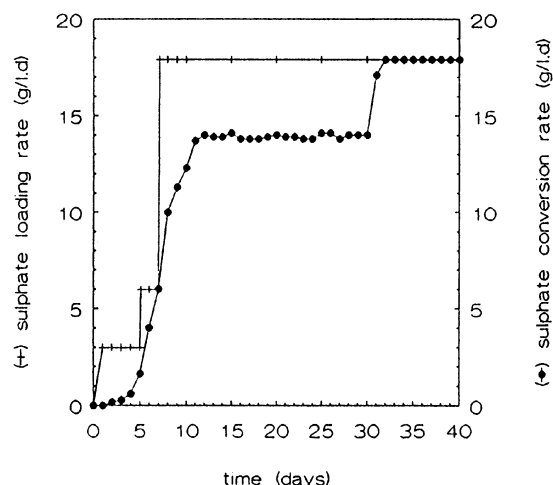


Figure 5. Sulphate loading and conversion rate with a  $H_2:CO_2$  (80%:20%) mixture in a sulphate reducing gas-lift reactor with pumice as carrier material (After: van Houten et al. 1994).

trations in the gas phase concentrations between 5% and 20% (van Houten et al. 1996). With CO, layered biomass particles developed. Homoacetogenic *Aceetobacterium* sp. are mainly located in the periphery, whereas *Desulphovibrio* sp. were located inside the aggregate (van Houten et al. 1996).

In the second step, sulphide removal can be done by precipitation of sulphide, sulphur removal from  $H_2S$  enriched stripping gas or partial oxidation of  $H_2S$  to  $S^0$ . The relatively high energy requirements for stripping or the high chemical and disposal (chemical sludge like  $FeS$  or  $MnO_2$ ) costs constitute important draw-backs of physical-chemical methods. Partial biological oxidation of sulphide to  $S^0$  (Buisman et al. 1990) is a cheap alternative which also allows sulphur reclamation, since  $S^0$  is non-soluble and thus can be removed from the wastewater (Jensen & Webb 1995). Under oxygen limited conditions, i.e. dissolved oxygen concentrations below  $0.1 \text{ mg} \cdot \text{l}^{-1}$ ,  $S^0$  is the major end product of the sulphide oxidation, while sulphate is formed under sulphide-limiting conditions.  $S^0$  formation requires four times less oxygen compared to complete oxidation and, consequently, a lower energy consumption for aeration. In reactors with low shear forces, i.e. when the reactor liquid is aerated in a spatially separated aeration unit, well settling  $S^0$  particles (mean diameter 3 mm, settling velocity  $> 25 \text{ m} \cdot \text{h}^{-1}$  for 90% of the sludge) are formed under autotrophic conditions (Janssen et al. 1997).

### Removal of heavy metals

Several sulphate-rich wastewaters, e.g. acid mine drainage, spoil leachates and landfill percolates often are also contaminated with heavy metals (Tichy et al. 1998). The extreme low solubility of metal sulphides that are formed in sulfidogenic bioreactors, allows the removal of heavy metals from the water stream by precipitation (Gadd & White 1993). Hence, maximisation of sulphide production in sulphate-reducing reactor units is an attractive way to remove both sulphate and heavy metals from a wastewater (Hao et al. 1996). As these types of wastewater usually contain little or no organic matter, an alternative electron donor (e.g. synthesis gas) has to be added (see above). Full scale processes are currently applied for heavy metal removal from a Zn and Cd (effluent concentrations below the ppb range) contaminated groundwater at a long-standing smelter site (Scheeren et al. 1991).

### Nitrogen removal

The nitrogen and sulphur metabolism interact at various levels of the wastewater treatment process. Sulphide is toxic for nitrifiers (Hooper & Terry 1973) and thus can upset nitrification in the aerobic post treatment. Besides, SRB can readily utilise most of the carbon sources added to denitrify a wastewater (e.g. methanol and lower volatile fatty acids), thus leading to loss of carbon source for the denitrification step. However, methanol utilisation by SRB can be avoided by proper selection of the hydraulic residence time (van der Hoek et al. 1988). On the other hand, sulphide can also contribute to nitrogen removal. As electron donor it can be re-oxidised to  $S^0$  or sulphate by *Thiobacillus denitrificans* using nitrate as electron acceptor. This type of denitrification reduces the overall requirements for carbon at a nutrient removal plant (Garuti et al. 1992). Also SRB can be involved in alternative denitrification routes, as some SRB can use nitrate, instead of sulphate, as terminal electron acceptor (Widdel 1988). However, SRB convert nitrate to ammonium, and thus other treatment steps are needed if a complete nitrogen removal is required. Hence, the sulphur cycle offers possibilities to integrate nitrogen removal in the treatment process, which need to be further optimised by appropriate design of the reactor configuration.

### Conclusions

1. The presence of sulphate (or sulphur compounds) in a wastewater has to be considered when designing an anaerobic treatment system. It requires precautions to prevent sulphide toxicity in methanogenic reactors, but also allows unique process applications.
2. Methanogenic treatment of sulphate-rich wastewaters results in the competition between SRB and MB for the available electron donor. To date, limited practical methods exist to steer the competition between these two bacterial populations. This is, however, of utmost importance, as free  $H_2S$  concentrations higher than  $150 \text{ mg.l}^{-1}$  can cause process failure due to toxicity. In general, wastewaters with a COD/sulphate ratio higher than 10 can be treated in a methanogenic system without any problem. For wastewaters with a lower COD/sulphate ratio, methods to prevent the sulphide toxicity can be required.
3. The application of sulphate reduction in combination with sulphide removal techniques, and in particular partial sulphide oxidation to elemental sulphur, can be used for the removal of sulphate from wastewaters. In order to obtain complete sulphate reduction, a wastewater COD/sulphate ratio of 0.67 is required. If the ratio is lower, extra electron donor (i.e. methanol, ethanol,  $H_2$  or synthesis gas) has to be dosed. Partial oxidation of sulphide to elemental sulphur requires dissolved oxygen concentrations below  $0.1 \text{ mg.l}^{-1}$  in the sulphide-oxidising reactor.

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