# Biotechnological Sulphide Removal with Oxygen

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bijzonder hoogleraar in de anaerobe waterzuivering

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# Biotechnological Sulphide Removal with Oxygen

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*Aan mijn ouders en Rian*

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**Abstract**

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This thesis deals with the development of a new process for biotechnological sulphide removal from wastewater, in which it is attempted to convert sulphide into elemental sulphur by colourless sulphur bacteria. The toxicity, corrosive properties, unpleasant odor and high oxygen demand of sulphide dictate stringent control of its release into the environment.The goals of the research were: development an efficient and reliable high rate treatment system for purification of sulphide containing wastewaters and assessment of the conditions that promote sulphur instead of sulphate formation.

The newly developed biotechnological sulphide removal system accomplishes a removal rate and efficiency which at least is similar to that of other sulphide removal systems, which are based on the oxidation with air. Application of the system is possible in the pH range 6.5 - 9.0 and in a temperature range of approximately 15 - 40 °C.

At least two types of sulphide oxidizing bacteria were found, viz. a sulphate producing (type A) and a sulphur producing bacteria (type B). Type A is inhibited by sulphide and type B by oxygen. As the conversion of sulphide into sulphur is the main aim of the system, growth of type B should be stimulated. This can be accomplished by imposing high sulphide loading rates (>200 mg/l.h) and maintaining low oxygen concentrations (< 4 mg/l). The growth yield of type B (0.3 g DS/mol S) is lower than that of type A (3 g DS/mol S), and therefore little biological sludge will be produced when sulphur is the end-product.

The start-up of the system is fast, viz. 5 days in a CSTR (1% inoculum), when applying a sulphide influent concentration of 100 mg/l and a HRT of 22 minutes. The oxygen concentration should not exceed 4 mg/l, because inhibition of type B should be prevented.

The noncatalyzed chemical sulphide oxidation (at oxygen concentration 4 mg/l) is considerably slower (75 times) than the biological sulphide oxidation at sulphide concentrations below 10 mg/l and about a factor 6 at sulphide concentrations up to 600 mg/l.

In a sulphide reactor merely two groups of unwanted bacteria can develop, viz. bacteria that store the sulphur inside the cell and cause sludge bulking problems, like Thiothrix and bacteria that produce sulphide like Desulfuromonas acetoxidans (sulphur reducer) and Desulfobulbus propionicus (sulphate reducer). Both groups of bacteria can only develop, when organic compounds are present in the wastewater. The problems caused by unwanted bacteria can be minimized by applying high sulphide loading rates (prevention of Thiothrix growth) and a high rotation speed (prevention of the sulphide producing bacteria). The sulphur reducing bacteria, present in the sludge, can use acetate but not propionate, while the sulphate reducing bacteria use propionate but not acetate.

When the newly developed process is applied on anaerobically treated papermill wastewater using a biorotor reactor, a removal rate of 620 mg/l.h at a removal efficiency of 95% is found at a HRT of 13 minutes, while only 8% of the sulphide is converted to sulphate.

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# CHAPTER 1

## General Introduction

### Background of the Study

The emission of sulphide is a major problem associated with anaerobic treatment of sulphate containing waste waters. Sulphate is utilized by sulphur-reducing bacteria as an electron acceptor. Sulphide is the end product of the reduction. Also sulphite and organic sulphur compounds can be converted into sulphide. Release of sulphide into the environment should be controlled because of the following possible problems:

- toxicity; the limit values for $H_2S$ are: TLV (threshold limit value), 10 ppm (14 $mg/m^3$) and STEL (short time exposure limit), 15 ppm (21 $mg/m^3$) (A.C.G.I.H., 1988). At high concentrations (500-1000 ppm) hydrogen sulphide acts primarily as a systemic poison, causing unconsciousness and death through respiratory paralysis. In lower concentrations (50-500 ppm) $H_2S$ acts primarily as a respiratory irritant.
- corrosive properties; when present in the biogas $H_2S$ may cause corrosion problems in boilers and internal combustion engines; when sulphide is present in the effluent it may cause damage to concrete walls of reactors, sewer systems and steel pipelines as well
- unpleasant odor; its characteristic rotten eggs odor is perceptible in fresh air in a concentration of 0.2 ppm
- high oxygen demand; per mole of sulphide two moles of oxygen are required to form sulphate

### Sulphur Chemistry

Out of the thirty-plus ionic and molecular sulphur species that possibly exist, only five are thermodynamically stable in aqueous solutions at room temperature and one atmosphere pressure, namely $HSO_4^-$, $SO_4^{--}$, $S^0$, $H_2S$, $HS^-$. Other sulphur species such as thiosulphate, polysulphides and polythionates appear in the natural environment but are considered to be thermodynamically unstable (Chen, 1974). Figure 1 shows the distribution of the sulphur species as a fuction of the pH and redox potential.

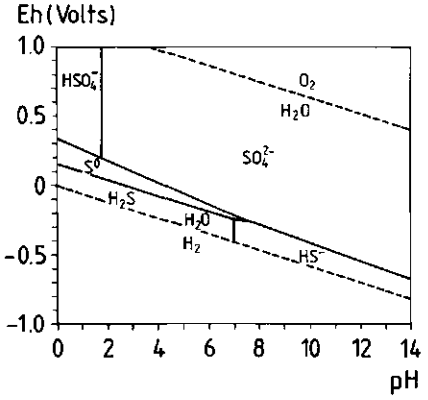


**Figure 1.** pH-Eh predominance diagram of sulphur species

Hydrogen sulphide dissociates in water according to the equations (Garrels & Christ, 1965):

$$H_2S \text{ <===> } H^+ + HS^- \quad (K_1 = 1.0 * 10^{-7})$$

$$HS^- \text{ <===> } H^+ + S^{--} \quad (K_2 = 1.0 * 10^{-14})$$

Consequently, $H_2S$ is the main dissolved component at pH values below 7.0, while $HS^-$ predominates at pH values between neutrality and 14. The concentration of $S^{--}$ is negligible in wastewater. Sulphide can be removed through one of the following methods: a. biological oxidation, b. metal sulphide precipitation, c. volatilization or d. chemical oxidation.

Elemental sulphur exists in nine crystalline forms which are all nearly insoluble in water; maximal 0.16 mg/l (Boulege, 1978). Sulphur may also appear in colloidal form, as a milky-white dispersion in water. The mechanism of sulphur particle formation and particle growth consists of three parts:

1. The homogenous reaction, where molecularly dispersed sulphur is formed immediately upon mixing and results in a supersaturation of the sulphur solution.
2. The condensation stage, where the supersaturated solution begins to nucleate and a slow growth of sulphur micelles starts.
3. The heterogenous stage, where diffusion of the dissolved sulphur to the surface continually increases particle size.

In alkaline solutions sulphur is unstable with respect to the formation of sulphide and thiosulphate. The following reaction equation describes this phenomenon which especially is significant at high pH:

$$4S^0 + 4OH^- \text{ <===> } 2HS^- + S_2O_3^{--} + H_2O$$

It can be concluded from Figure 1 that the formation of sulphur is unlikely at pH exceeding 8 unless excessive amounts of thiosulphate or sulphide are present. Solid elemental sulphur is merely stable in acidic solutions.

The formation of polysulphides ($S_nS^{2-}$ with n=1-5) is the result of the interaction of sulphur with an aqueous solution of sulphide. Upon acidification a whitish colloidal sulphur suspension forms. The general equation of the sulphide-sulphur interaction can be expressed as:

$$HS^- + xS^0 \text{ <===> } S_xS^{2-} + H^+$$

In near-neutral aqueous sulphide solutions, the resulting polysulphide solutions contain approximately equimolar mixtures of tetra- and pentasulphide ions.

**Sulphide removal methods**

Methods for sulphide removal in common use today are physical-chemical processes which involve direct air stripping, chemical precipitation and oxidation. However, the relatively high energy requirements or the high chemical and disposal (chemical sludge like FeS and $MnO_2$) costs constitute important drawbacks of these systems.

Direct air stripping leads to a voluminous air stream contaminated with $H_2S$, which has to be treated. Chemical precipitation generates sludge (e.g. FeS) that must be disposed.

Oxidation processes used for sulphide removal are aeration (catalyzed and uncatalyzed), chlorination, ozonation, potassium permanganate treatment and hydrogen peroxide treatment. In all these oxidation processes sulphur, thiosulphate and sulphate may be the end products.

Uncatalyzed oxidation of sulphide with oxygen is a very slow process (Chen & Morris, 1972). Therefore either pure oxygen without catalysts or air with catalysts are used. Some authors describe catalyzed oxidation of sulphide with air e.g. Martin and Rubin (1987) who used $KMnO_4$ (1 mg-Mn/l) as a catalyst. Lefers et al. (1978) used activated carbon (53 - 530 mg/l) as a catalyst.

Several oxidizing agents can be used to remove sulphide from wastewater (Butler & Nandan, 1981; Candena & Peters, 1988; Chen, 1974). Elemental chlorine is a strong chemical oxidizer that reacts with sulphide in the same manner as sodium hypochlorite according to the equations:

$$HS^- + OCl^- \text{ ---> } S^0 + OH^- + Cl^-$$

$$HS^- + 4OCl^- \text{ ---> } SO_4^{--} + H^+ + 4Cl^-$$

Based on these equations between 2.1 and 8.4 gram of elemental chlorine or 2.2 and 8.8 gram of sodium hypochlorite are required per gram of sulphide. In the presence of organic compounds in the wastewater chlorination is less attractive due to the formation of undesirable chlorinated reaction products.

Ozone is also a very strong oxidizing agent, but also fairly expensive. Due to its high oxidative capacity, ozone converts sulphide into sulphur and sulphate almost instantaneously. Because ozone is very unstable in aqueous solutions, it must be generated on the site. According to Chen (1974) the reaction between sulphide and ozone proceeds according to the equations:

$$HS^- + O_3 \text{ ---> } S^0 + OH^- + O_2$$

$$HS^- + 4O_3 \text{ ---> } SO_4^{--} + 4O_2 + H^+$$

The use of $KMnO_4$ is not attractive primarily because of the relatively high costs and secondarily because the manganese dioxide sludge generated by the reaction must be handled and disposed. The oxidation of sulphide with potassium permagnate is sensitive to the pH and therefore different combinations of sulphate and sulphur may be the end product. Between 3.3 and 13.2 gram of $KMnO_4$ is required per gram of sulphide.

The oxidation rate of sulphide with hydrogen peroxide proceeds relatively slow compared to the other oxidizing agents mentioned above. In the absence of bacterial mass, $H_2O_2$ reacts directly with sulphide according to the reactions:

$$HS^- + H_2O_2 \text{ ---> } S^0 + H_2O + OH^-$$

$$HS^- + 4H_2O_2 \text{ ---> } SO_4^{--} + 4H_2O + H^+$$

The mechanism of the oxidation of sulphide by hydrogen peroxide in the presence of biomass is not well understood, because hydrogen peroxide also reacts with the catalase present in the biomass. Nevertheless, the reaction equations mentioned here are commonly accepted for sulphide oxidation in wastewater.

Hydrogen peroxide is the least expensive oxidizing agent as can be decided from the relative sulphide removal costs between the various chemicals summarized in Table 1.

**Table 1.** Difference in costs of sulphide removal methods as a percentage of the costs of hydrogen peroxide treatment

| | Butler & Nadan (1981) | Tauw (1986) |
|---|---|---|
| $H_2O_2$ | 100 | 100 |
| $KMnO_4$ | 1070* | - |
| Ozone | 1514 | - |
| pure oxygen | - | 65 |
| air with catalyst | - | 47 |
| Fe precipitation | - | 87* |

(* disposal costs of FeS and $MnO_2$ are not considered)

Also some biotechnological sulphide removal processes have been developed. Cork (1985) and Kobayshi et al. (1983) proposed the use of photosynthetic bacteria for sulphide removal with sulphur as the endproduct. However, the requirement for radiant energy is a severe economic disadvantage of this method. Gommers et al. (1988) and Sublette & Sylvester (1987a,b,c) investigated the use of denitrifying bacteria for sulphide oxidation. This system is not widely applicaple because nitrate is needed. These biotechnological sulphide removal systems are all still in the preliminary phase, sothat little if any information is available about the costs of these systems.

**Sulphur Cycle**

The sulphur cycle is schematically presented in Figure 2. Figure 2 shows that the complete cycle can proceed microbiologically, i.e. without the intervention of any macro-organism. Such a situation often occurs in nature and is called a sulfuretum (Postgate, 1968). The three processes sulphate reduction, sulphur reduction and sulphide oxidation, shown in the lower part of Figure 2, are of special interest for the new system which is subject of this study and therefore will be discussed in more detail.

Sulphate reduction in fact is the main cause for the sulphide problems associated with anaerobic waste water treatment. The anaerobic sulphate reducing bacteria are able to convert oxidized sulphur compounds into sulphide. When sulphate containing wastewater is treated in an anaerobic system, sulphide will be produced. This will compells the use of a post-treatment to remove the sulphide. Another reason likely to be of special interest for the development of such a post-treatment system can be found in the fact that it might well be possible that these sulphate reducing bacteria may also grow in the anaerobic part of the biofilms in the sulphide removing reactors. This would mean that in the presence of sulphate and fatty acids, sulphide can be produced, in such a bio-reactor. Research is necessary to prevent this kind of sulphide production.

The developments in the field of microbiology of the sulphate reducing bacetria as far as relevant for anaerobic wastewater treatment, were reviewed recently extensively by Rinzema & Lettinga (1988). Until the end of the 1970s, three genera were isolated, viz. Desulfovibrio sp., Desulfotomaculum sp. and Desulfomonas sp. At present six new genera have been found besides the three that were already known, viz. Desulfobulbus sp., Desulfobacter sp., Desulfococcus sp., Desulfonema sp., Desulfosarcina sp., and Desulfobacterium sp.

As in the proposed method for sulphide removal sulphur should be the end-product of the sulphide oxidation rather than sulphate, the occurrence of sulphur reduction in the sulphide removal reactors is more likely than the occurrence of sulphate reduction, because the sulphur concentration is much higher than the sulphate concentration. The sulphur reducing organisms known are all strictly anaerobic. Since the discovery of Desulfuromas acetoxidans (Pfennig & Biebl 1976),

dissimilatory sulphur reduction in the dark has well been documented for a number of eubacetrial and archaebacterial species. The eubacteria, which can grow anaerobically with elemental sulphur as terminal electron acceptor include besides Desulfuromonas a number of sulphate reducing bacteria (Biebl & Pfennig, 1977), spirillum 5175 (Wolfe & Pfennig, 1977), Wolinella succinogenes (Macy et al., 1986) and certain Campylobacter species (Laanbroek et al.,1978. The anaerobic sulphur-reducing archaebacteria are all thermophilic organisms with temperature optima above 70°C (Widdel 1988). Many of these archaebacteria are also acidophiles, preferring growth at pH well below 6. Many methanogenic bacteria are also able to reduce sulphur to sulphide (Stetter & Gaag, 1983).

Of the sulphur reducing organisms mentioned above, only Desulfuromonas species are able to use acetate and therefore we may expect that only these bacteria might be present in the sulphide removing reactors and not the spirilloid or archaebacterial species. All Desulfuromonas species oxidize acetate with elemental sulphur to carbon dioxide and sulphide.

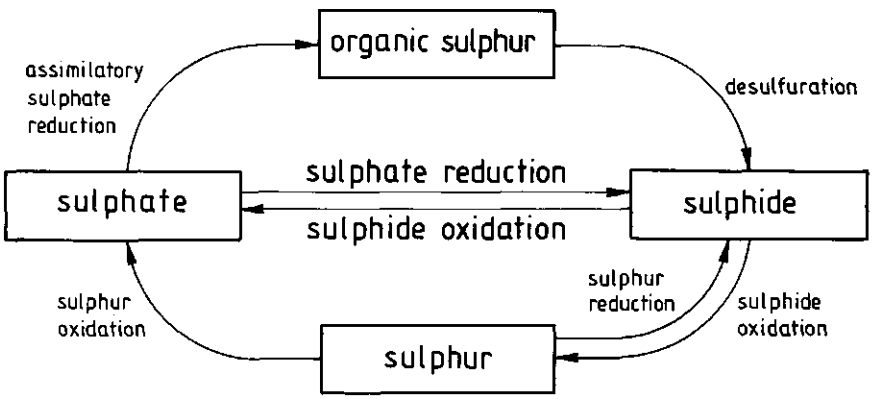


**Figure 2.** schematic representation of the sulphur cycle

In nature sulphide and sulphur can be oxidized biologically in three different ways (6): a. under anaerobic conditions by photosynthetic bacteria, b. by spontaneous chemical oxidation with oxygen and c. oxidation with oxygen or nitrate by the colourless sulphur bacteria.

Most phototrophic bacterial species depend on or are at least capable of utilizing reduced sulphur compounds as photosynthetic electron donors under anaerobic conditions. While sulphide is generally utilized by organisms with this capacity, the ability to use elemental sulphur is typical for the Chromatiaceae and Chlorobiaceae, not for the Rhodospirillaceae and the cyanobacteria (Truper & Fischer, 1982). All species of the family Chlorobiaceae (Green Sulphur Bacteria) oxidize sulphide via elemental sulphur (outside the cell) to sulphate. Elemental sulphur added as sole sulphur source is oxidized to sulphate as well. Thiosulphate is only oxidized by certain strains. The Chromatiaceae (Purple Sulphur Bacteria) - with the exception of the genus Ectothiorhodospira - are capable of intracellular storage of elemental sulphur globules that are formed during the utalization of sulphide and thiosulphate in the light. Ectothiorhodospira species form sulphur globules outside the cell in the medium. The family Rhodospirillaceae (Purple Non-Sulphur Bacteria)are capable of

utilizing sulphide and thiosulphate as electron donors, oxidizing it to sulphate without the production of elemental sulphur globules.

The requirement for radiant energy obviously is a severe economic and technical (sulphur causes a turbid solution) disadvantage for application of the phototrophic bacteria in a biotechnological sulphide removal system.

Whether or not spontaneous chemical oxidation of sulphide plays an important role in the oxidation of sulphide in nature is not clear yet. Jorgensen (1982) describes two experiments, not to be discussed in detail here, which show the complexity of this matter. The first experiment shows that sulphide oxidation is independent of microbial activity in chemocline samples from the Black Sea. In chemocline samples from the Solar Lake, Sinai on the other hand a significant contribution by bacteria to the sulphide oxidation was found.

The uncatalysed chemical oxidation of sulphide with oxygen is relatively slow about 5 mg/l.hr at sulphide and oxygen concentrations not exceeding 10 mg/l (O'Brien & Birkner, 1977; Millero et al., 1987, Wilmot et al., 1988). The spontaneous chemical oxidation rate in nature will depend significantly on the amount of catalysts present and therefore is very difficult to estimate.

**Group of Colourless Sulphur Bacteria**

In the process to be devoloped we have choosen, for a process, based on bio-oxidation with oxygen, because, as is already mentioned above, the two alternatives, oxidation with light or nitrate, seem economically not feasible. Sulphide oxidation with oxygen can be performed by to the group of colourless sulphur bacteria (Kuenen and Beudeker, 1982) and therefore more detailed information about this group is necessary.

To the group of colourless sulphur bacteria belong organisms with widely different types of physiology and morphology, ranging from obligate chemolithothrophs, via facultative chemolithotrophs to heterotrophs which may benefit from sulphide oxidation. The obligate chemolithotrophs belong to the genera Thiobacillus and Thiomicrospira. These organisms are able to generate energy only from the oxidation of inorganic sulphur compounds such as sulphide, thiosulphate and elemental sulphur. Facultative chemolithotrophs are not only able to grow autotrophically with reduced inorganic sulphur compounds as energy source, but are also capable of heterotrophic growth. Such bacteria belong to the genera Thiobacillus, Sulfolobus, Thermothrix and Paracoccus (Table 2). Several Thiobacillus species are able to utilize mixtures of inorganic and organic compounds simultaneously. Heterotrophic bacteria able to oxidize reduced inorganic sulphur compounds may do this to obtain energy. Other reasons might be the removal of the toxic sulphide or for species that lack catalase sulphide oxidation may protect the organism against hydrogen peroxide. This group contains members of the genera Thiobacillus, Pseudomonas and Beggiatoa (Table 2).

**Table 2.** Colourless sulphur bacteria oxidizing inorganic sulphur compounds

| obligate chemolithotrophs | facultative chemolithotrophs | heterotrophs | unclassified |
|---|---|---|---|
| *T.neapolitanus* | *T.intermedius* | *T.perometabolis* | *Thiovulum* |
| *T.denitrificans* | *T. A2* | *Pseudomonas* | *Thiophysa* |
| *T.thiooxidans* | *T.novellus* | *Beggiatoa* | *Thiothrix* |
| *T.thioparus* | *Thermothrix thiopara* | | *Thiospira* |
| *T.ferrooxidans* | *T.acidophilus* | | *Thioploca* |
| *Tms.denitrificans* | *P.denitrificans* | | |
| *T.kabobis* | *S.acidocaldarius* | | |
| *Tms.pelophila* | *S.brierleyi* | | |

These bacteria can derive energy from sulphides, elemental sulphur, thiosulphate, polythionates and sulphite. The final oxidation product is sulphate, but sulphur and polythionates accumulate, sometimes transiently, under certain conditions (Bergey's Manual, 1974).

An important factor for the process to be developed is wether or not these bacteria store the produced sulphur inside the cell. Species from the genera Beggiatoa, Thiothrix and Thiospira accumulate the produced sulphur inside the cell. In this way a lot of cellmass must be produced per unit sulphide removed and the sulphur can not easily be separated from the biomass. These species are also unwanted, because they can cause suldge bulking problems. It is therefore necessary to select bacteria which produce the sulphur extracellulair e.g. the genus Thiobacillus.

Suphide oxidizing bacteria may occur in diverse habitats: in soil,in freshwater and in seawater, in thermal springs and in acid drainage. No special environmental factors are needed for the group of colourless sulphur bacteria. Different species are active in the pH range 0.5 to 10 (Bergey's Manual, 1974) and exhibit temperature optima from about 20 °C to about 75 °C. Sulphide oxidizing bacteria that grow in neutral environments are e.g. T.denitrificans, T.neapolitanus, T.novellus and T.thioparus. No relevant literature is known about the relation between these environmental factors and the sulphide oxidation rate in mixed cultures.

The sulphide oxidation process to be developed, must be controlled in such a way that mainly sulphur will be produced instead of sulphate. It seems most likely that the following pathway exists for inorganic oxidation of sulphur compounds in Thiobacilli (Kelly, 1985; Moriarty & Nicholas ,1970):

$$HS^- \text{ ---> membrane bound } |S^0| \text{ <---> } S^0$$

$$\text{membrane bound } |S^0| \text{ ---> } SO_3^{--} \text{ ---> } SO_4^{--}$$

The biological oxidation of sulphide to sulphate proceeds in two stages. In the first stage which proceeds faster than the second stage, sulphide looses two electrons and membrane bound polymeric sulphur compounds are being formed. In the second step this sulphur is oxidized to sulphite and then to sulphate. Neither Kelly, nor Moriarty investigated the possibilities to prevent the oxidation of sulphur to sulphate.

The following (biological) overall reactions occur in an aerobic sulphide removal system (Kuenen, 1975):

$$2HS^- + O_2 \text{ ---> } 2S^0 + 2OH^-$$

$$2S^0 + 3O_2 \text{ ---> } 2SO_4^{--} + 2H^+$$

So far little relevant information is available about the aerobic oxidation of sulphide into sulphur by the colourless sulphur bacteria. In most investigations thiosulphate was used instead of sulphide while sulphate generally was the end product instead of sulphur.

**Kinetics of Sulphide Oxidation**

Contrary to the biological oxidation of thiosulphate and sulphide into sulphate little if any biological kinetic data are known concerning the oxidation of sulphide into sulphur.

The growth yield of autotrophic sulphide oxidizers is rather low, around 5 - 13 gram dry mass of cell material per mole of substrate, when sulphate is the end-product (Kelly, 1982). Timmer-ten Hoor (1981) found identical chemostat growth yields for sulphide and thiosulphate as substrate, which is consistent with both

oxidation being equivalent to the transfer of eight electrons per molecule oxidized. But Kelly (1982) found that sulphide dependent $CO_2$ fixation by cell suspensions was only about 70% of that with thiosulphate. Possibly in the washed cell suspensions experiments, the autooxidation reduced the amount of sulphide available to the bacteria or possibly sulphide exerted an uncoupling effect as a toxic substrate, thereby lowering the efficiency of the $CO_2$ fixation. Therefore it is not sure whether or not the above mentioned values measured for thiosulphate can be used for sulphide oxidation.

The specific growth rate on a single substrate (thiosulphate to sulphate) is 0.35 1/h for obligate chemolithotrophs like Thiobacillus neapolitanus and 0.10 1/h for facultative chemolithotrophs species like Thiobacillus A2 on a single substrate (thiosulphate) and 0.22 1/h on a mixed substrate (thiosulphate and acetate) (Beudeker et al., 1982).

The review article of Kuhn (et al., 1983) reveals that already extensive studies on the mechanism and the kinetics of the chemical oxidation of sulphide have been made, and that concerning this matter there still does not exist general agreement. In chapter 6 this subject will be discussed in more detail.

**Aim of Investigation**

Based on the information available in the literature and the observations in anaerobic treatment plants showing that elemental sulphur frequently accumulates in pipes and nearby effluent launders, research on a new concept for sulphide removal was started. The process is based on the idea that it should be well possible to convert dissolved sulphide into elemental sulphur using the proper bacteria under conditions of oxygen limitation. Based on this the present investigations were initiated.

The purpose of the research wäs, to develop a high rate, effective and low cost biotechnological process for sulphide removal. The proposed process intends to convert sulphide into elemental sulphur which should be easily removable by sedimentation and -if possible- made suitable for reuse. The advantages of such a process are, a. no catalyst or oxidants (except air) are required, b. no chemical sludge to be disposed, c. low energy consumption, d. possible reuse of sulphur, and e. little if any sulphate or thiosulphate discharge.

As mentioned before, at present still very little useful information is available about the biological oxidation of sulphide, especially for a process where sulphur would be the main end product. Therefore, obviously a considerably research input is necessary to develop such a process and to assess its practical feasability. The fundamental microbial aspects are currently being investigated by Stefess and Kuenen (Department of Microbiology, Technical University Delft). The technological and engineering problems are subject of research at the Agricultural University Wageningen, department of Water Pollution Control. Latter research includes the following research objectives:

- development of a proper, low cost reactor system with high biomass retention and oxidation capacity
- indentification of the control parameters for the sulphur production
- evaluation of the effects of environmental factors on the oxidation rate, i.e. pH, temperature, oxygen concentration, substrate inhibition and the formation of polysulphides
- evaluation of the influence of organic substrates on the sulphide oxidizing bacteria and on the sulphur and sulphate reducing bacteria
- identification of the environmental factors that determine under practical conditions the growth of sulphur bacteria which store the sulphur inside the cell e.g. Thiothrix, in order to assess the possibilities to prevent the growth of these organisms
- assessment of the biological and chemical oxidation rates

**Organisation of this Thesis**

The present study deals with a number of aspects of biotechnological sulphide removal with oxygen mentioned above. The thesis can be divided into three parts: Chapters 2 - 5 describe experiments related to the ecology and fysiology of the bacteria developing in the sulphide removal reactors. Chapter 2 deals with the possible effects of enviromental factors like pH, temperature and oxygen concentration, while Chapter 3 focusses on the optimization of the sulphur production. The development of unwanted bacteria is described in Chapters 4 and 5. Experiments concerning bacteria that store the sulphur intracellulair, like Thiothrix are described in Chapter 4, while experiments concerning sulphur and sulphate reducing bacteria, which can produce sulphide inside the reactor are described in Chapter 5.

The second part includes chapters 6 and 7 which describe the assessment of the biological and chemical kinetics of sulphide oxidation. Chapter 6 deals with the kinetics of the chemical sulphide oxidation and Chapter 7 deals with the biological oxidation.

Chapters 8 - 9 describe the application of the sulphide removal system. In Chapter 8 experimental results will be presented concerning different reactor prototypes using a composite wastewater, while Chapter 9 presents the results obtained in the same prototype reactors but now used for sulphide containing papermill wastewater.

Chapter 10 summarizes the results of the investigations, and provides the general conclusions.

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## CHAPTER 2

# Important Process Conditions for Biotechnological Sulphide Removal with Oxygen

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## ABSTRACT

A new biotechnological process for sulphide removal is proposed. The process is based on the oxidation of sulphide into elemental sulphur, which can be removed by sedimentation. In this study it was found that elemental sulphur and sulphate are the main oxidation products of the biological sulphide oxidation. The sedimentation characteristics become worse as the sulphide concentration increases, due to polysulphide formation. The start-up phase of this biological system is very short; Only four days are needed to reduce the sulphide concentration of 100 to 2 mg/l at a HRT of 22 minutes. Also some environmental factors were evaluated. The optimal pH is situated in the pH-range 8.0-8.5. Significantly lower conversion rates are found at pH = 6.5-7.5 and pH = 9.0, while at pH = 9.5 the sulphide oxidation capacity of the system detoriates. The process temperature was 20 °C, although the optimal temperature is situated in the range 25-35 °C. No substrate inhibition of sulphide was found at sulphide concentrations up to 100 mg/l.

## INTRODUCTION

The emission of sulphide is a major problem associated with anaerobic treatment of sulphate and sulphite containing waste waters. Sulphate is utilized by sulphur-reducing bacteria as an electron acceptor. Sulphide is the end product of the reduction. The toxicity, corrosive properties, unpleasant odor and the high oxygen demand dictate stringent control of its release into the environment. It also has an inhibitory effect on the methanogenesis. According to Anderson (1), sulphide might be ranked as one of the most important inhibitors. The corrosive properties of sulphide are apparent in the damage done to concrete walls of reactors, sewer systems and steel pipelines as well.

Methods for sulphide removal in common use today are physicochemical processes which involve direct air stripping, oxidation and chemical precipitation. However, the relatively high energy requirements or the high chemical and disposal costs are important drawbacks of these systems.

Direct air stripping leads to a voluminous air stream contaminated with $H_2S$, which has to be treated.

Oxidation processes used for sulphide removal are aeration (catalyzed and uncatalyzed) and chemical oxidition (chlorination, ozonation, potassium permanganate treatment and hydrogen peroxide treatment). In all these oxidation processes thiosulphate and sulphate will be the end products. Uncatalyzed oxidation of sulphide is a slow process (27).

Some authors describe catalyzed oxidation of sulphide with air e.g. Martin and Rubin (3). They found an oxidation rate of 116 mg/l.h when $KMnO_4$ (1 mg-Mn/l) was used as a catalyst. Lefers et al. (4) found oxidation rates of 237 mg/l.h when activated carbon (53 mg/l) was used as a catalyst.

Butler and Nandan (5) describe some oxidizing agents. Chlorination is unwanted due to the formation of undesirable chlorinated reaction products if

organic compounds are present. Ozone on the other hand is the most expensive oxidizing agent mentioned here. The use of $KMnO_4$ is also not attractive primarily because of the high costs and secondarily because the manganese dioxide sludge generated by the reaction must be handled and disposed. Jolley and Forster (2) found a relationship for the oxidation of sulphide with hydrogen peroxide :

$$\text{in sewage:} \qquad R = 9.1(H_2O_2)^{0.99}\,(S^{2-})^{0.99}\ (M/min)$$

Chemical precipitation also generates sludge (e.g. FeS) that must be disposed.

The purpose of the present investigation is, to develop a high rate, effective and low cost biotechnological process for sulphide removal. The principle of the proposed process is to convert sulphide by oxidation into elemental sulphur which can be removed by sedimentation. The advantages of such a process are, a. lower oxygen requirement (less energy consumption), b. the possibility of recovery of elemental sulphur and c. reduction in discharge of sulphate.

In nature sulphide can be oxidized biologically in three different ways (6): a. anaerobic oxidation by photosynthetic bacteria, b. oxidation by denitrifying organisms and c. oxidation with oxygen by the colourless sulphur bacteria.

Cork (7) and Kobayshi et al. (8) proposed the use of photosynthetic bacteria for sulphide removal. The conversion rates reached in these systems are 67 and 54 mg/l.h respectively (sulphur is the endproduct). The requirement for radiant energy however is a severe economic disadvantage.

Gommers et al. (9) and Sublette & Sylvester (10, 11) investigated the use of denitrifying bacteria for sulphide oxidation. The conversion rates reached in these systems are 104 and 74 mg/l.h respectively (sulphur is the endproduct). This system is not widely applicaple because nitrate is needed.

We have choosen, for a process for sulphide removal, based on aerobic oxidation by the group of colourless sulphur bacteria (12). To this group belong organisms with widely different types of physiology and morphology. Genera belonging to the group of colourless sulphur bacteria are: Thiobacillus, Thiomicrospira, Sulfolobus, Thermothrix, Pseudomonas, Thiovulum, Beggiatoa, Thiothrix, Thiospira and Thioploca.

An important factor for the process to be developed is wether or not these bacteria store the produced sulphur inside the cell. Species from the genera Beggiatoa, Thiothrix and Thiospira accumulate the the produced sulphur inside the cell. In this way a lot of cellmass must be produced per unit sulphide removed and the sulphur can not be easily separated from the biomass. For this reason bacteria must be selected which produce the sulphur extracellulair e.g. the genus Thiobacillus.

No special environmental factors are needed for most sulphur bacteria. Different species are active in the pH range 0.5 to 10 (13) and exhibit temperature optima from about 20 °C to about 75 °C. Most species can grow autotrophically, but others can also grow mixotrophically or heterotrophically. They occur in diverse habitats: in soil,in freshwater and in seas, in thermal springs and in acid drainage. These bacteria can derive energy from sulphides, elemental sulphur, thiosulphate, polythionates and sulphite. The final oxidation product is sulphate, but sulphur and polythionates accumulate, sometimes transiently, under certain conditions (13).

An important objective of our process is to convert sulphide into elemental sulphur and not into sulphate. Therefore the oxidation of sulphide must be controlled in order to produce sulphur instead of sulphate. It seems most likely that the following pathway exists for inorganic oxidation of sulphur compounds in Thiobacilli (14):

$HS^- \text{ ---> membrane bound } [S^0] \text{ <---> } S^0$

$\text{membrane bound } [S^0] \text{ ---> } SO_3^{--} \text{ ---> } SO_4^{--}$

The following (biological) overall reactions may occur in a aerobic sulphide removal system (6):

$$2HS^- + O_2 \text{ ---> } 2S^0 + 2OH^-$$

$$2S^0 + 3O_2 \text{ ---> } 2SO_4^{--} + 2H^+$$

The technical feasability of the proposed process depends on the sulphide conversion rate, the percentage of sulphide converted into sulphur and the efficiency of the sulphur sedimentation. The economic feasability depends on the consumption of energy and of chemicals, the complexity of the process and the size of the reactor. The first to be assessed is the principle technical feasability of the system. Very little useful information is available about the biological oxidation of sulphide, especially for a process where sulphur will be the end product.

This first publication presents the results of investigations concerning the effect of environmental factors on the sulphide removal rate. As far as we know no relevant data are available about the influence of the pH, the oxygen concentration and the sulphide concentration on the sulphide oxidation rate in mixed cultures of sulphur bacteria.

At high pH values the formation of sulphur and polysulphide is unlikely. There does not exist general agreement on the pH upperlimit of sulphur and polysulphide formation. In the review article of Kuhn (15) it is mentioned that this upperlimit can vary from pH 8 to pH 9. Since the reactor pH will rise when sulphide is converted into sulphur (see the overall reactions), this upperlimit can be of great importance for the feasability of the process. It will be obvious that the formation of polysulphides can be important for this process, because polysulphides can be toxic, while polysulphide formation also makes the settlement of sulphur impossible. Several authors have found that no stable polysulphides ($S_nS^{2-}$ with n=1-5) are formed above pH 8-9 (4, 16, 17). Only the tetra- and pentasulphide ions are stable polysulphides. Di- and trisulphide ions are unstable and subject to rapid disproportionation e.g.

$$3S_2^{2-} + 2H_2O \text{ ---> } 2HS^- + 2OH^- + S_4^{2-}$$

Giggenbach (17, 18, 19) found that the equilibrium distribution of the polysulphide ions depends on the concentrations of elemental sulphur, sulphide and $OH^-$, as shown by the following equation:

$$B = [SH^-]\,[OH^-] \,/\, [S^0]$$

Log B determines n, the average number of atoms of zero-valent sulphur per polysulphide ion (when log B = -6, n = 3.5; log B = 0, n = 2.8). In near-neutral aqueous sulphide solutions, the resulting polysulphide solutions contain an approximately equimolar mixture of tetra- and pentasulphide ions.

Some relevant experimental results of investigation on the toxicity of polysulphides and the influence of polysulphide formation on sulphur settlement will be reported here. Furthermore the results of investigations concerning the start-up of a sulphide removal reactor will be reported in this article.

In the present study completely mixed and continuously fed reactors were used. Recticulated polyurethane (PUR) foam particles were used to immobilize the biomass. The PUR foam deliverd by Recticel seems to have a low phytotoxicity (20).

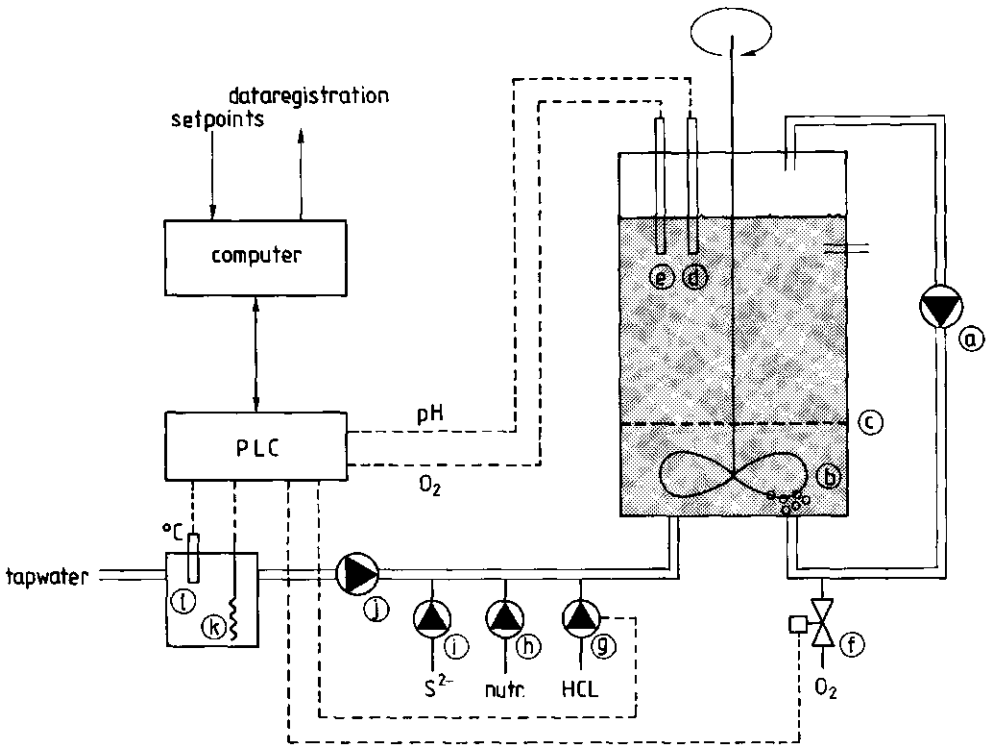


**Figure 1.** laboratory set-up for a mixed continuously fed sulphide oxidizing reactor: a.gasphase recycling pump; b.stirrer; c.separation between stirring compartiment and the reactor (to protect the BSP's); d. pH-electrode; e. oxygen sensor; f.oxygen dosage valve, computer controlled; g. HCl dosage pump, computer controlled; h.nutrient and trace elements solution dosage pump; i. sulphide solution dosage pump; j. tapwater pump; k. heater, computer controlled; l.PT-100 (temperature measurement)

## MATERIALS AND METHODS

### biomass

The first inoculum used was ditch mud. Thereafter all reactors were inoculated with the effluent of one of the reactors under operation.

### experimental solutions and chemicals used

A composite waste water was used as influent in the experiments. This feed solution consisted of nutrients and sodium sulphide in Wageningen tap water. The nutrient solution contained (g/l): $NH_4Cl$ (8), $MgSO_4.7H_2O$ (2), $KH_2PO_4$ (5) and 100 ml trace element solution according to Vishniac & Santer, (1957). All the chemicals used for the nutrient solution were analytical grade supplied by Merck, Darmstadt, F.R.G.. The sulphide solution contained 200 g/l $Na_2S.8H_2O$ of a technical grade supplied by de Vries, Amsterdam, The Netherlands. The nutrient and sulphide solutions were added in proportion of 2 : 1. For pH control 5 M HCl, also of technical grade, was used. Pure oxygen supplied by Hoekloos, Schiedam, The Netherlands was used in the experiments.

### analyses

Sulphide was determined photometrically by the method described by Truper and Schlegel (21). Elemental sulphur was measured after extraction with acetone according to the method described by Bartlett and Skoog (22).

Sulphate was analyzed by liquid chromatography using a Chrompack HPLC column, packed with Ionospher A-5$\mu$(dim: 10 cm x 3 mm; ID), eluent 0.027 M potassiumbiphtalate (flow 0.4 ml/min) and a Knauer differential refractometer as detector. The injection quantity was 20$\mu$l. This method could also detect thiosulphate.

The total sulphur concentration (all sulphur species) was measured by means of inductively-coupled plasma atomic emmision spectrometry as described by Novozamsky (23). This method was combined with the method of Thorpe (24) in order to be able to handle wet samples.

The oxygen concentration was measured with an $O_2$-sensor(WTW; DU 600 201 711). The pH was measured with a pH-electrode (Ingold, 425-60-s7 ing. no. 104253313).

### reactors

Two completely mixed and continuously fed reactors were used. Reactor 1 (5 l volume) contained 300 PUR particles. Reactor 2 (8.3 l volume) contained 400 PUR particles. Except for the volume and amount of PUR particles the reactors were equal in construction (Figure 1).

Recticulated polyurethane (PUR) foam delivered by Recticel, Kesteren, The Netherlands was choosen for biomass support particles (BSP). The dimensions were 1.5x1.5x1.5 cm with 30 pores per inch. The reactor was stirred continuously. The gasphase in the reactors was recycled for purposes of mixing, process control and for preventing escape of $H_2S$ from the system. The oxygen concentration, the temperature and the pH were kept constant using a PI controller. A Tulip computer (PC compact), with a Mitsubishi programmable logic controller (Melsec PLC) was used for data-aquisition and control. The process temperature was kept at 20 °C.

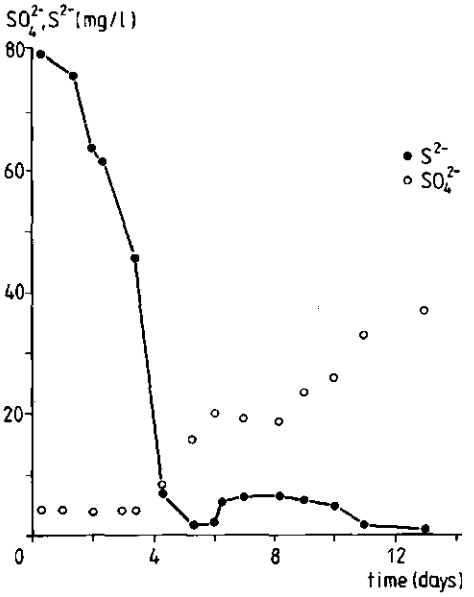


**Figure 2.** the sulphide and sulphate concentrations measured during the start-up phase of a sulphide removing reactor (HRT 22 min; pH 8.0; oxygen concentration maintained at 4 mg/l; 400 BSP's). The sulphide influent concentration: 100 mg/l

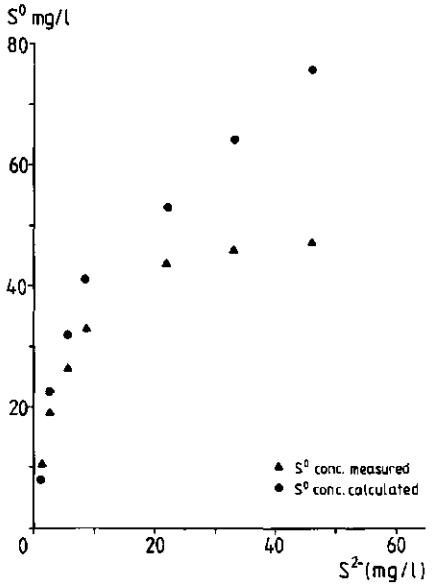


**Figure 3**, the effect of the sulphide concentration on the amount of sulphur that can be settled at pH 8.5 and at an oxygen concentration 1.5 mg/l. The sulphur concentration is calculated using the equation: sulphide concentration influent - sulphide and sulphate concentrations effluent.

**standard activity test**

For the standard activity test a 0.5 l air tight vessel was used to which eight biomass support particles or a cel suspension were added. Next the reaction medium was supplied to the vessel. The medium consisted of a phosphate buffer (50 mM) which was saturated with oxygen. Immediately after closing the vessel the oxygen removal rate was measured, using an oxygen sensor. The oxygen removal rate was directly related to the oxidation rate of sulphur to sulphate. Relative to the amount of oxygen there was a surplus of sulphur present in the reaction vessel because it is impossible to seperate the biomass and the sulphur. Upon adding sulphide to the reaction vessel little if any sulphate was produced anymore and the oxygen removal rate became equivalent to the sulphide oxydation rate (sulphide to sulphur).

## RESULTS

**start up phase**

Figure 2 shows the results of a typical start-up experiment. The reactor in this experiment was inocculated with four PUR particles from another reactor. A green colour appeared, followed by the appearance of a turbid white suspension (elemental sulphur) after four days. In approximately five days the sulphide concentration in the effluent solution went down from 80 to 2 mg/l. As soon as the sulphide concentration dropped below 10 mg/l the sulphate concentration increased.

The predominant bacterium in the PUR particles were small rods (2 m long). These were probably Thiobacillus species, which are known to oxidize sulphide (Kuenen and Beudeker, 1982).

**sulphur balance**

For assessing a reliable sulphur balance the system should be in a steady state situation, which means that the amount of sulphur compounds introduced in the reactor is equal to the amount of sulphur compounds leaving the reactor. The total sulphur concentration (organic, inorganic and all sulphur species) was measured using inductively-coupled plasma atomic emmision spectrometry. In addition the sulphide, elemental sulphur and sulphate concentrations were measured. The results of such a balance test in a typical experiment (mean values) are summarized in Table 1.

**Table 1**, sulphur balance of a reactor in steady state

| | | |
|---|---|---|
| total sulphur concentration influent | | 179.9 mg-S/l |
| total sulphur concentration effluent | | 176.7 mg-S/l |
| sulphide ($S^{2-}$) concentration effluent | 9.3 mg-S/l | |
| sulphur ($S^0$) concentration effluent | 145.9 mg-S/l | |
| sulphate ($SO_4^{--}$) concentration effluent | 22.7 mg-S/l | |
| total $S^{2-}$, $S^0$, $SO_4^{--}$ in effluent | | 177.9 mg-S/l |

Neither thiosulphate nor sulphite were found in the effluent solution. Losses of sulphide from the reactor through stripping of $H_2S$ were not possible because the gas was recycled.
Apparantly apart from elemental sulphur and sulphate little if any other sulphur compounds are being formed.

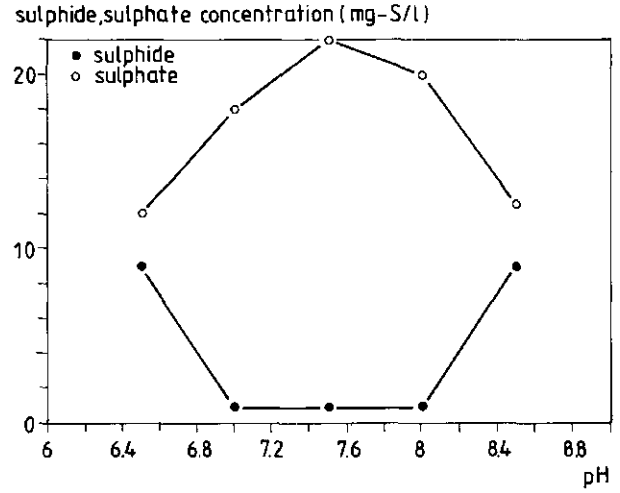


**Figure 4**, the effect of short term pH changes on the sulphide and sulphate concentrations in reactor 1 (HRT 30 min, 300 BSP's), which was adapted to a pH of 7.5. Samples were taken only after four volume changes. The oxygen concentration was maintained at 2 mg/l and the sulphide influent concentration was 80 mg/l

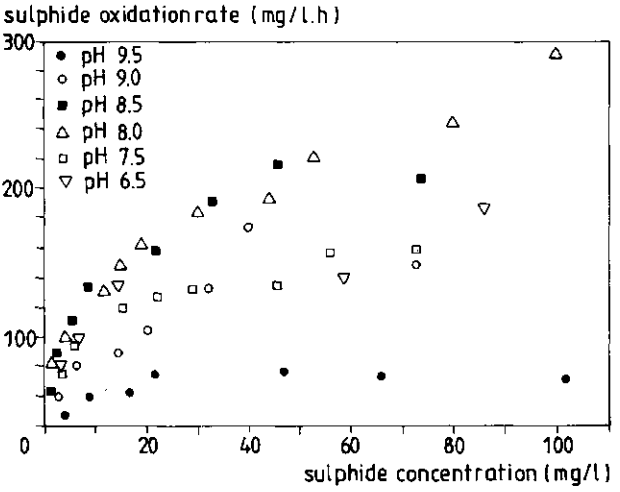


**Figure 5**, the effect of the pH on the sulphide oxidation rate in reactor 2 (HRT 22 min, 400 BSP's). Both the sulphide influent concentration (50 mg/l) and the reactor pH were kept constant for three weeks before the oxidation rate was measured at oxygen concentration 1.5 mg/l. The oxidation rate at a certain pH was measured within one day.

**polysulphide formation**

The yellow greenish colour observed in the reactors at sulphide concentrations exceeding 10 - 30 mg/l suggest polysulphide formation. The effect of polysulphide formation on the settling of elemental sulphur can be estimated by the difference between the measured and calculated (from the sulphur balance) elemental sulphur concentrations. In order to determine the sulphur concentration the samples were centrifuged and then extracted with acetone. Polysulphides are soluble and will not be measured in this sulphur determination. In Figure 3 this is illustrated. The amount of polysulphides increases with increasing sulphide and sulphur concentration. No toxic effects were found from polysulphide and sulphide on the sulphide oxidation rate.

**influence of the pH**

In order to evaluate the influence of the pH on biomass activity, the reactor pH was changed from 7.5 to 8.0 to 8.5 to 7.0 and to 6.5. These pH changes were imposed within one day, so that the biomass, which was cultivated at a pH of 7.5 did not change considerably. Effluent samples were taken after four liquid residence times. The results obtained at the various imposed pH-values are shown in Figure 4, where the effluent sulphide and sulphate concentrations are shown in relation to the reactor pH. The results in Figure 4 show a decreased sulphate formation upon decreasing and increasing the pH within 0.5 - 1 pH-unit relative to the reference pH of 7.5. The sulphide effluent concentration remains unaffected when the imposed pH changes remain within 0.5 pH-unit relative to pH 7.5. However, when imposing pH changes of 1 pH-unit apparantly the sulphide oxidation capacity of the system drops down (11%). Upon returning the reactor pH to 7.5 the system recovered within one hour.

In another experiment, a long term pH-change lasting for three weeks was imposed to the system, so that in principle good adaptation to the new pH could occur. Six pH values were investigated: 6.5, 7.5, 8.0, 8.5, 9.0 and 9.5. During these three weeks the imposed sulphide load remained constant at 1320 mg/h (sulphide influent concentration of 60 mg/l, reactor volume = 8.3 l and HRT = 22 min). Following this three weeks the sulphide load was lowered stepwise and then increased stepwise by decreasing respectively increasing the sulphide influent concentration after four liquid residence times. After every step (four liquid residence times), samples from the effluent were taken. The measurements of the oxidation rates at the imposed different sulphide loading rates at a certain pH-value, were completed within one day. The results obtained allow the assessment how the affinity and maximal oxidation rate of the biomass is affected by the pH. The results of the experiment are presented in Figure 5, where the sulphide oxidation rate is shown in relation to the sulphide concentration in the reactor medium.

The optimal pH apparantly is situated in the pH-range 8.0 - 8.5. Significantly lower conversion rates are found at pH = 6.5 - 7.5 and pH = 9.0, while at pH = 9.5 the sulphide oxidation capacity of the system detoriates.

**influence of the oxygen concentration**

The results of an experiment in which the oxygen concentration in the reactor was varied are presented in Figure 6. In the experiments the sulphide load was increased stepwise. Always after four liquid residence times, samples were taken from the effluent. From the results it is clear that the sulphide oxidation rate increases upon increasing the oxygen concentration at all sulphide levels investigated.

**influence of the temperature**

The effect of temperature on the oxidation rate of the biomass was assessed using the standard activity test. For this reason biomass samples in the form of BSP's were removed from a reactor which was operated at 20°C. For each test at a specified temperature eight BSP's were taken from the reactor. The results of the experiment are shown in Figure 7.

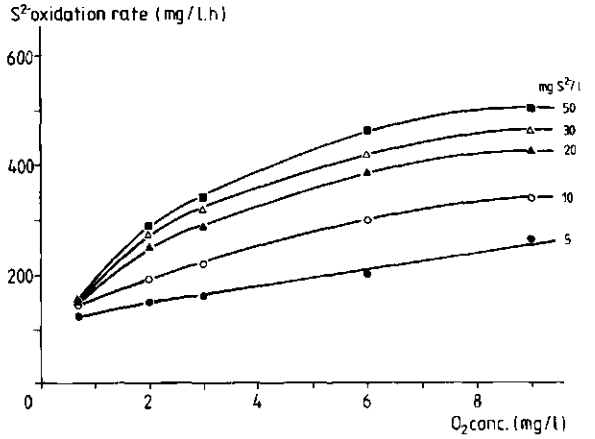


**Figure 6**, the effect of the oxygen concentration and the sulphide concentration in the reactor on the sulphide oxidation rate. The HRT was 30 min with 300 BSP's at pH 8.0.

**influence of the sulphide concentration**

As can be seen in Figure 5 at sulphide concentrations up to 100 mg/l still no clear substrate inhibition effects were found at pH 8.0 in a reactor with biomass support particles. It should be noted that in the presence of sulphur a high percentage of the sulphide will be bound to the sulphur (polysulphides). As BSP's are used in this experiment, it is clear that diffusion limitation effects may significantly affect the process. Therefore the results in Figure 5 may not give the real effect of the sulphide concentration on the oxidation rate. The bacteria inside the BSP's could be protected against sulphide inhibition by the existance of a concentration gradient. In a control experiment also a free cell suspension (HRT 7 h) was exposed to high sulphide concentrations. The results in Figure 8 reveal that even with a free cell suspension there is no substrate inhibition at pH 8.0 and at a sulphide concentration up to 100 mg/l (Figure 8).

## DISCUSSION

The start up of a sulphide removal reactor appears to be easy, under conditions of a short residence time (HRT 22 min) and a inoculum consisting of only four PUR particles in a 8.3 l reactor, a sulphide effluent concentration less then 2 mg/l

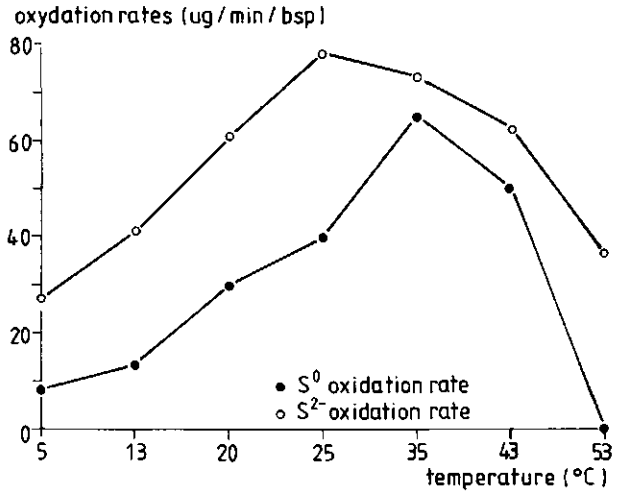


**Figure 7**, the effect of the temperature on the oxidation rate of the BSP's (adapted to 20 °C, pH 8.0 and oxygen concentration 3 mg/l)), measured with the standard activity test at pH 8.0. The initial sulphide concentration (after sulphide injection ) was 2 mg/l.

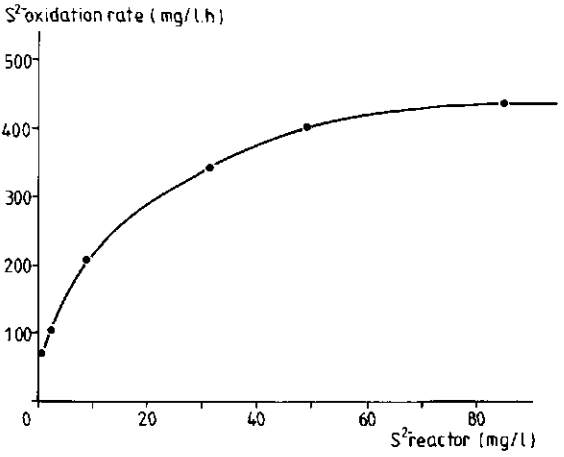


**Figure 8**, the effect of the sulphide concentration on the sulphide oxidation rate of non immobilized biomass in reactor 1 (HRT 7 h) at pH 8.0 and oxygen concentration 9 mg/l

sulphide was found within only five days of operation at an influent concentration of 100 mg/l. The high initial sulphide concentrations in the reactor apparantly do not inhibit the oxidation of sulphide such seriously that dilution of the waste water is nessesary. The green colour of the effluent can be attributed to polysulphide formation. However as soon as the sulphide concentration in the effluent decreases below 20 mg/l the effluent becomes white and turbid (elemental sulphur).

No other end products than elemental sulphur and sulphate were found. This is in good agreement with the findings of Moriarty and Nicholas (26) who also did not observe any intermediates like thiosulphate, in their study of the sulphide oxidation by Thiobacillus concretivoris.

Polysulphide formation was observed (dark green colour) in the whole pH range 6.5-9.5 at sulphide concentrations of 20 - 100 mg/l. The amount of polysulphides has not been determined. However from Figure 3 it can be seen that at pH 8.5 15% of the calculated sulphur was soluble at a sulphide concentration of 10 mg/l. This hinders the settling of sulphur. Also Chen and Morris (27) found that upon increasing the pH beyond 7.5, precipitation of sulphur becomes more and more difficult.

The equation of Giggenbach (see introduction) can be used to calculate factor B and to estimate n, the average number of atoms of zero-valent sulphur per polysulphide ion . At a sulphide concentration of 30 mg/l, the sulphur concentration is 60 mg/l and the $OH^-$concentration is 0.0032 mM (Figure 3). For these values logB = -5.8, which leads to n = 3.4. This value of n suggests that stable tetra- and pentasulphide ions are present in the effluent solutions, which is in good agreement with the dark green colour observed.

The optimal pH range of the biomass (Figure 4), is situated in a relatively narrow pH range of two pH-units, which is quite normal for a bacterial biomass cultivated at a fixed pH. When the biomass is cultivated under conditions of a fixed pH value for a period of three weeks, we find an optimal pH at pH 8.5 and a sharp decrease of the oxidation capacity at pH beyond 9.5. Chen and Morris (27) described a relationship between the pH and rate of chemical oxidation in an aqueous sulphide by oxygen. The sulphide oxidation rate increases from pH 6, reaching a maximum at pH 8.5, declining to a minimum at 9.3. The maximum shifts to lower pH values with increasing in the sulphide concentrations. Their explanation for this complex relationship is that polysulphide acts as a catalyst for the oxygenation of sulphide and they proposed a reaction pathway for the oxygenation of sulphide in which the oxidation of sulphide to sulphur is the rate-determining step. They assume that sulphur bacteria can eliminate this rate-determining step in nature.

The variation of biological oxidation rate with the pH is quite different from that of the chemical oxidation rate. This most likely can be attributed to the fact that sulphur and polysulphides are formed rapidly in the biological oxidation process, while the formation of sulphur is the rate limiting step in the chemical oxidation of sulphide. The sharp decrease in the biological oxidation rate at pH 9.5 can be due to the fact that less polysulphides are formed at pH 9.5 or the formation of polysulphides containing less zero-valent sulphur atoms (polysulphide formation is not measured in our experiments).

The results in Figure 6 clearly show that both the oxygen concentration and sulphide concentration affect the sulphide oxidation rate, what could be explained by the equation which describes the chemical oxidation rate (2) and the overall reactions (see intrduction).

The oxidation capacity is highest in the temperature range 25 to 35 °C, although the biomass was cultivated at a temperature of 20 °C. The optimal temperature of the genus Thiobacillus is about 28-30 °C (13). It is possible that the biomass grown in the reactor at 20 °C has an optimimum at 28 °C. It should be noted that the chemical oxidation, which proceeds simultaneously with the biological oxidation has not been measured. However it can be estimated that the chemical oxidation rate will be very low due to the low initial sulphide concentrations (2 mg/l). At higher temperatures the chemical oxidation rate will be higher, so the biological activity at 53°C will be lower as is shown in Figure 7.

The effect of the sulphide concentration on the oxidation rate seems to be a Monod type relationship as can be seen in Figure 8. Up to a sulphide concentration of 100 mg/l no clear inhibitory effects can be observed. Van Gemerden (28) found severe inhibitory effects with phototropic sulphide oxidizing bacteria at sulphide concentration in the range 20 - 60 mg/l.

Sublette and Sylvester (10, 11) found that $H_2S$ is a toxic substrate for Thiobacillus denitrificans. At high sulphide loading $H_2S$ breaktrough occured and elemental sulphur accumulated in the reactor. In terms of sulphide removal rate we found that sulphide is not a toxic substrate when sulphate is not the endproduct. However when sulphate is the endproduct, according to Sublette and Sylvester, sulphide must be considered inhibitory at 10 mg/l.

At an oxygen concentration of 3 mg/l and a pH of 8.5, a sulphide removal capacity of 99 mg/l.h with a removal efficiency of 95% was observed. A removal capacity of 202 mg/l.h can be achieved when a removal capacity of 87% is accepted (effluent concentration 11 mg $S^{2-}$/l). These oxidation rates are relatively high compared to the other biological systems (see introduction). The removal efficiency of the system can be improved by using a plugflow reactor system, due to the fact that sulphide is not inhibitory in high concentrations and the oxidation rate increases at higher sulphide concentrations. The sulphide removal capacity in that case can be increased to 415 mg/l.h with a removal efficiency of 99.5% at a HRT of 13 minutes (29). This high removal capacity can be compared with catalysed air oxidation (see introduction). Therefore, it can be concluded that biological aerobic oxidation of sulphide with sulphur production seems to be a good and presumable very attractive alternative to existing biological and chemical sulphide removal systems.

## ACKNOWLEDGEMENTS

This research was funded by Industriewater Eerbeek B.V., Eerbeek, The Netherlands, Paques B.V., Balk, The Netherlands and the Dutch Gouvernment (VROM), Clean Technology Program. We thank W. M. Wiegant for his valuable suggestions on this article.

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## CHAPTER 3

# Optimization of Sulphur Production in a Biotechnological Sulphide Removing Reactor

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## ABSTRACT

In this article investigations on the optimization of the sulphur production relative to the sulphate production in the biological sulphide removal process will be reported.

It seems that less then 10% sulphate is produced at low oxygen concentration, when the sulphide concentration in the reactor exceeds 10 mg/l. At sulphide concentrations higher than 20 mg/l only 5% of the incoming sulphide is converted to sulphate even at high oxygen concentrations. An immobilized biomass on recticulated polyurethane produced more sulphate than a free cell suspension at the same oxygen and sulphide concentration.

## INTRODUCTION

The emission of sulphide is a major problem associated with anaerobic treatment of sulphate and sulphite containing waste waters. Sulphate is utilized by sulphur-reducing bacteria as an electron acceptor and sulphide is the end product of the reduction. The toxicity, corrosive properties, the unpleasant odor and the high oxygen demand dictate stringent control of its release into the environment. $H_2S$ is one of the more toxic pollutants. It also has an inhibitory effect on methanogenesis. According to Anderson et al. (1), sulphide might be ranked as one of the most important inhibitors. The corrosive properties of sulphide are apparent in the damage done to concrete walls of reactors, sewer systems and steel pipelines. Its characteristic rotten eggs odor is perceptible in fresh air in a dilution of 0.002 mg/l of air.

Methods for sulphide removal in common use today are physicochemical processes which involve direct air stripping, oxidation and chemical precipitation. Important disadvantages of these conventional systems are the relatively high energy requirements or the high chemical and disposal costs (2).

The purpose of our investigations is, to develop an effective, low cost and high rate biotechnological process for sulphide removal in which sulphide is converted into elemental sulphur. This process is based on aerobic oxidation by the group of colourless sulphur bacteria. The advantages of such a process are, a. lower oxygen requirement (less energy consumption), b. the possibility of recovering elemental sulphur and c. reduction in discharge of sulphate.

In our process the oxidation of sulphide must be controlled in such a way that mainly sulphur is produced instead of sulphate. It seems most likely that the following pathway exists for inorganic oxidation of sulphur compounds in Thiobacilli (3, 4):

$$HS^- \text{ ---> membrane bound } [S^0] \text{ <---> } S^0$$

$$\text{membrane bound } [S^0] \text{ ---> } SO_3^{--} \text{ ---> } SO_4^{--}$$

The biological oxidation of sulphide to sulphate proceeds in two stages. In the first stage which proceeds faster than the second stage, sulphide looses two electrons and membrane bound polymeric sulphur compounds are being formed. In the second step this sulphur is oxidized to sulphite and then to sulphate. Neither Kelly (3), nor Moriarty (4) investigated the possibilities to prevent the oxidation of sulphur to sulphate.

The following (biological) overall reactions occur in an aerobic sulphide removal system (5):

$$2HS^- + O_2 \text{ ---> } 2S^0 + 2OH^-$$

$$2S^0 + 3O_2 \text{ ---> } 2SO_4^{--} + 2H^+$$

So far little relevant information is available about the aerobic oxidation of sulphide into sulphur by the colourless sulphur bacteria. In most investigations thiosulphate is used instead of sulphide while sulphate generally was the end product instead of sulphur.

Cork (6) proposed a process for the removal of sulphide based on the use of anaerobic photosynthetic bacteria. Here the sulphide is also converted in elemental sulphur. Hansen et al. (7) found a purple bacterium that oxidizes sulphide to elemental sulphur and sulphate. Sulphide is being converted into elemental sulphur at sulphide concentrations exceeding 2 mg/l; sulphate is formed at lower sulphide concentrations.

Chen and Morris (8) found in the chemical oxidation of sulphide that a high sulphide-to-oxygen ratio results in the precipitation of sulphur, while at a low ratio sulphide is directly oxidized to thiosulphate or to other oxyanions. They also observed that the oxidation of sulphite is inhibited by sulphide.

In a previous communication we described (2) results of investigations dealing with the biological conversion of sulphide into elemental sulphur. It was shown that the products of the sulphide oxidation are elemental sulphur and sulphate and that the formation of these oxidation products proceeds independently of the pH of the reactor solution in the range 6.5-9.0. At high sulphide concentrations the effluent solution became dark green because of polysulphide formation.

It seems that at least three factors are important for the optimization of the sulphur production in our system:

a. the sulphide-to-oxygen ratio (see Chen & Morris (8) for chemical oxidation)
b. the sulphide concentration (see Hansen (7) for purple bacteria and Chen & Morris (8) for chemical oxidation)
c. the sulphide sludge loading (see Sublette and Sylvester (9, 10) for Thiobacillus denitrificans

The effect of these three parameters were investigated in the present study.

## MATERIALS AND METHODS

### biomass

The first reactor was started up using ditch mud as inoculum. Thereafter all reactors were inoculated with the effluent of previously started reactors.

**chemicals**

A composite wastewater was used as influent in the experiments. This feed solution consisted of nutrients and sodium sulphide in tap water, which was not chlorinated. The nutrient solution contained (g/l): $NH_4Cl$ (8), $MgSO_4.7H_2O$ (2), $KH_2PO_4$ (5) and 100 ml trace element solution according to Vishniac & Santer, (11). All the chemicals used for the nutrient solution were analytical grade supplied by Merck, Darmstadt, F.R.G.. The sulphide solution contained 200 g/l $Na_2S.8H_2O$ of a technical grade supplied by de Vries, Amsterdam, The Netherlands. The nutrient and sulphide solutions were added in proportion of 2 : 1. For pH control 5 M HCl, also of technical grade, was used. Pure oxygen supplied by Hoekloos, Schiedam, The Netherlands was used in the experiments.

**analyses**

Sulphide was determined photometrically by the method described by Truper and Schlegel (12).
Sulphate was analyzed by liquid chromatography using a Chrompack HPLC column, packed with Ionospher A-5$\mu$(dim: 10 cm x 3 mm; ID), eluent 0.027 M potassium-biphtalate (flow 0.4 ml/min) and a Knauer differential refractometer as detector. The injection quantity was 20$\mu$l. This method could also detect thiosulphate.
The oxygen concentration was measured with an $O_2$-sensor (WTW; DU 600 201 711).
The pH was measured with a pH-electrode (Ingold, 425-60-s7 ing. no. 104253313).

**reactors**

Two completely mixed and continuously fed reactors (CSTR) were used. Reactor 1 (5 l volume) contained 300 biomass support particles (BSP). Reactor 2 (8.3 l volume) contained 400 biomass support particles (BSP). Except for the volume and the number of biomass support particles the reactors were identical in construction (Figure 1).
Recticulated polyurethane (PUR) foam delivered by Recticel, Kesteren, The Netherlands was chosen as biomass support particles. The dimensions of these BSP cubes were 1.5x1.5x1.5 cm with 30 pores per inch. The gas phase in the reactors was recycled for purposes of mixing, process control and for preventing the escape of $H_2S$ from the system. The oxygen concentration, the temperature and the pH were kept constant using a PI controller. A Tulip computer (PC compact), with a Mitsubishi programmable logic controller (Melsec PLC) was used for data-acquisition and control. The process temperature was kept at 20 °C.

**standard activity test**

For measuring the standard activity a 0.5 l air tight vessel was used to which either biomass support particles or a cell suspension could be added. After adding the biomass the reaction medium was supplied to the vessel. The medium consisted of a phosphate buffer (50 mM; pH=8.0), saturated with oxygen. Immediately after closing the vessel the oxygen removal rate was monitored, using an oxygen sensor. The oxygen removal rate is directly related to the oxidation rate of sulphur to sulphate. Relative to the amount of oxygen generally there is a surplus of sulphur present in the reaction vessel because it is impossible to separate the biomass and the sulphur. When sulphide is added to the reaction vessel little if any sulphate was produced anymore (less then 0.10 mg/min.). In that case the oxygen removal rate becomes equivalent to the sulphide oxidation rate (sulphide to sulphur). A typical standard activity measurement is shown in Figure 2.

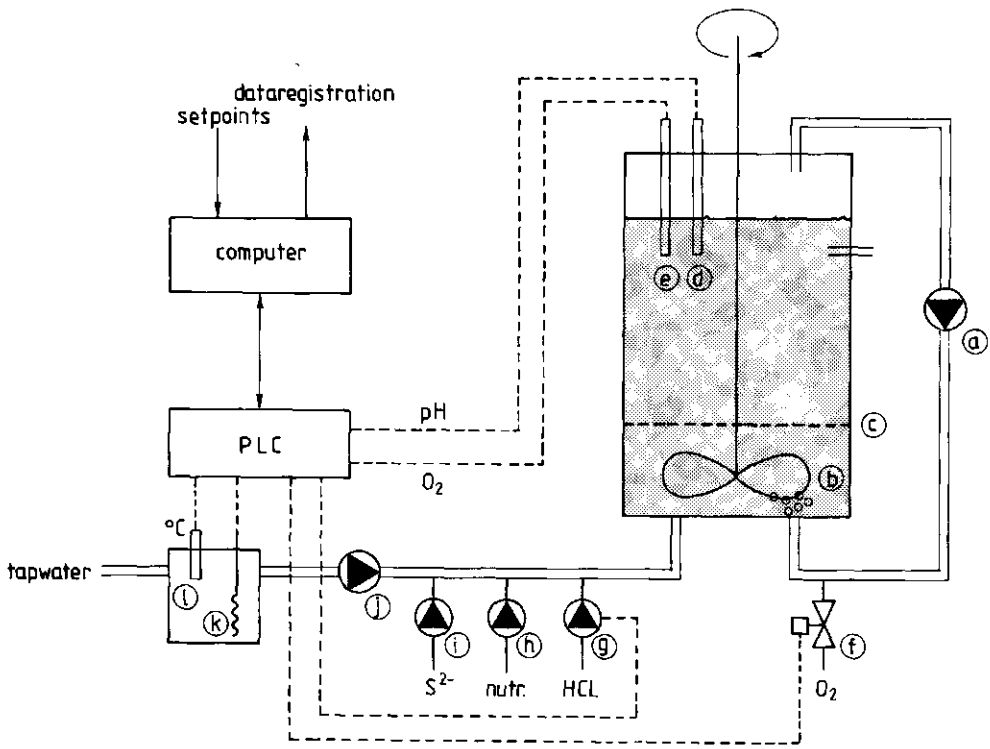


**Figure 1.** Laboratory set-up for a mixed continuously fed sulphide oxidizing reactor: a.gas phase recycling pump; b.stirrer; c.separation between stirring compartment and the reactor (to protect the BSP's); d. pH-electrode; e. oxygen sensor; f.oxygen dosage valve, computer controlled; g. HCl dosage pump, computer controlled; h.nutrient and trace elements solution dosage pump; i. sulphide solution dosage pump; j. tapwater pump; k. heater, computer controlled; l.PT-100 (temperature measurement)

## RESULTS

### effect of sulphide concentration

In the startup of a continuously fed reactor (Figure 3), in which four BSP's of another sulphide removing reactor were used as inoculum was found that only sulphate production occurs, under condition of a low sulphide concentration. An oxygen concentration of 4 mg/l was applied in this experiment.

### effect of oxygen concentration

At low sulphide concentrations, the oxygen concentration has a distinct influence on the amount of sulphate formed. This is illustrated in Figure 4. At a sulphide influent concentration of 90 mg/l and at a hydraulic retention time of 45 minutes, little sulphate is being produced at a oxygen concentration below 1 mg/l, while at oxygen concentrations exceeding 5 mg/l the sulphate production rate does not further increase. It stabilizes at 52% of the sulphide influent concentration. Therefore the sulphur production is maximum at an oxygen concentration of 1 mg/l.

Upon increasing the sulphide load at the same hydraulic retention time also the oxygen concentration at which the sulphur production is maximum increases (Table 1). On the other hand the maximum fractional sulphate production decreases and drops to only 17% at the sulphide influent concentration of 120 mg/l, while it was 76 % at sulphide influent concentration of 46 mg/l.

**Table 1,** the oxygen concentration at which the sulphur production is maximal at different sulphide influent concentrations, at the same hydraulic retention time, in steady state situations

| $S^{2-}$ infl (mg/l) | $O_2$ at max. $S^0$ prod. (mg/l) | max. $SO_4$ prod. at high $O_2$ conc. (%) |
|---|---|---|
| 46 | 0.5 | 76 |
| 90 | 1.1 | 52 |
| 120 | 2.6 | 17 |

In the experiment shown in Figure 4, the sulphide loading rate was kept constant but obviously the sulphide concentration in the reactor solution changed throughout the experiment. Therefore another experiment was conducted in order to assess the influence of the oxygen concentration under conditions of a constant sulphide concentration in the reactor. This experiment was performed at five different sulphide concentrations. The sulphide concentration in the reactor could be kept constant by adjusting the sulphide load. The sulphate production was measured after five liquid retention times. Following this measurement a new oxygen and sulphide concentration was imposed. The results of this experiment are shown in Figure 5.

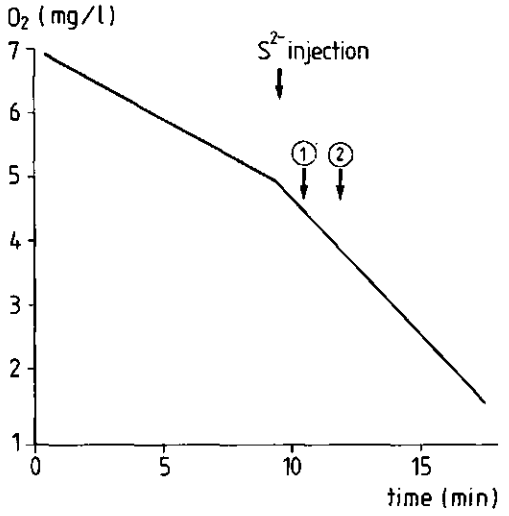


**Figure 2.** A typical activity measurement in a 0.5 l air tight vessel. The medium is phosphate buffer (50 mM), which is saturated with oxygen; at point 1 the sulphate concentration is 10.8 mg-S/l and at point 2 this is 10.9 mg-S/l

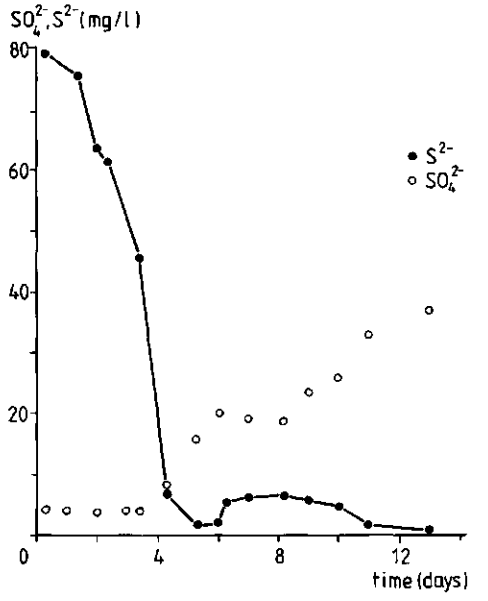


**Figure 3.** The sulphide and sulphate concentrations measured during the start-up phase of a sulphide removing reactor (hydraulic retention time 22 min; pH 8.0; oxygen concentration maintained at 4 mg/l; 400 BSP's). Influent sulphide concentration: 100 mg/l

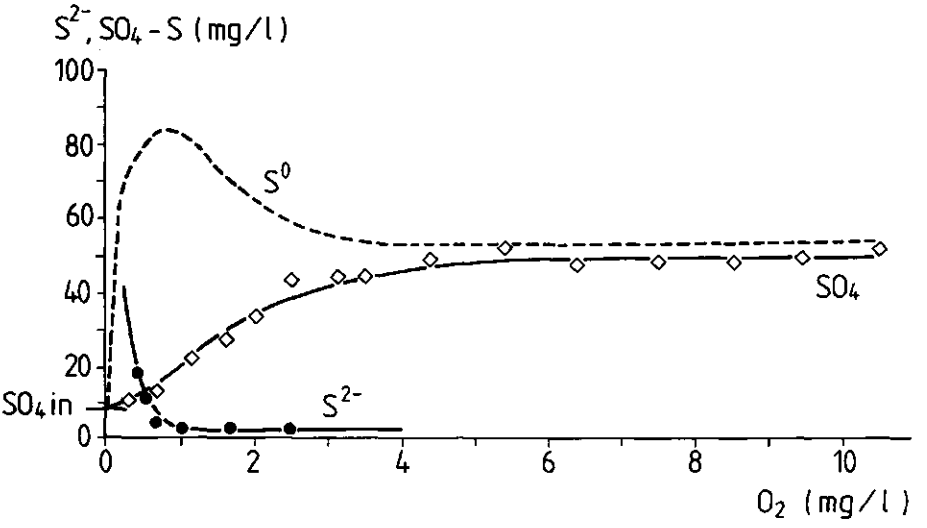


**Figure 4.** The sulphide and sulphate concentration versus the oxygen concentration in reactor 1 (300 PUR particles; hydraulic retention time 45 min.; volume 5.5 l). The sulphide influent concentration was 100 mg/l and the reactor pH = 7.5. Each point represents a steady state

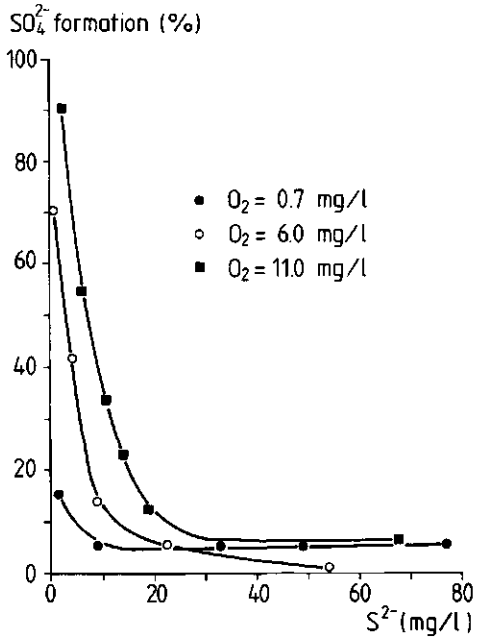


**Figure 5.** The percentage sulphate formation (calculated from the sulphide influent concentration) versus the sulphide concentration at different oxygen concentrations in reactor 1 (300 PUR particles; hydraulic retention time 30 min.; volume 5.5 l). Reactor pH = 8.0.

**biomass sulphide load**

It is obvious that a distinct effect on the oxidation process can be expected from the amount of biomass. This indeed is the case as appears from the results shown in Figure 6. Upon decreasing the amount of biomass in the reactor, accomplished by removing a part of the biomass support particles (BSP), the sulphate production decreases while the sulphide concentration in the effluent only slightly increases (Table 2). The results of the standard activity test (Table 2) show that the sulphide oxidation rate of the BSP's from the reactor remained fairly constant in this experiment, on the other hand the sulphur oxidation rate decreased, which corresponds to the lower sulphate production rate.

**Table 2,** the mean sulphide, sulphur and sulphate effluent concentrations and the activities of the BSP's when the number of BSP's was decreased

| number of BSP's | sulphide effl.con. (mg/l) | sulphate effl.con. (mg/l) | oxidation rate BSP's $S^{2-}$-->$S^0$ (mg/BSP.min) | $S^0$-->$SO_4$ (mg/BSP.min) |
|---|---|---|---|---|
| 300 | 2.8 | 204 | 0.24 | 0.12 |
| 210 | 2.1 | 156 | 0.13 | 0.08 |
| 120 | 6.3 | 107 | 0.14 | 0.07 |
| 60 | 9.1 | 65 | 0.14 | 0.04 |

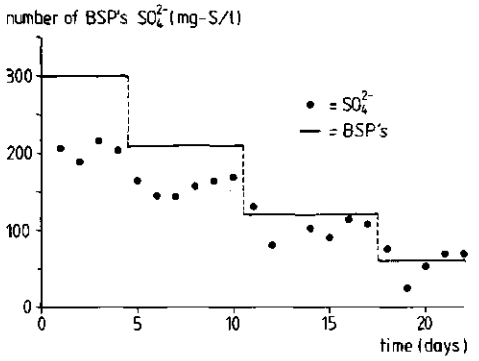


**Figure 6.** The relation between the number of PUR particles and the sulphate concentration in reactor 1 (300 PUR particles at the start of the experiment; hydraulic retention time 30 min.; volume 5.5 l). The number of PUR particles is decreased stepwise. The reactor pH = 8.0, the reactor oxygen concentration = 2 mg/l and the influent sulphide concentration is 330 mg/l.

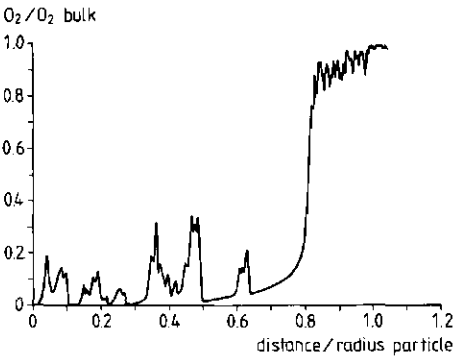


**Figure 7.** The oxygen gradient measured using a micro-electrode in a PUR particle of 15 mm at an oxygen bulk concentration of 7 mg/l and a sulphide concentration of 12 mg/l

**diffusion limitation**

In all experiments described above, PUR sponges were used. Possibly diffusion limitation, occurring in these sponges, could be the main reason for the observed high sulphur production rate under conditions of high oxygen concentrations (Figure 4). The oxygen concentration in a PUR cube of 15 mm was found to be low at a depth exceeding 1.5 mm as was measured with a micro-electrode (Figure 7) at the Technical University of Delft by C.M. Hooijmans (method not yet published).

This steep gradient in the oxygen concentration and probably also in the sulphide concentration could lead to a incorrect interpretation of the results described above. Therefore some additional experiments were conducted using a free cell suspension in order to assess the sulphur production in absence of the diffusion limitation conditions as prevailing in the PUR particles. For this purpose in reactor 1, a free cell suspension was grown at a hydraulic retention time of 7 hours. This experiment was conducted in the same way and under the same conditions as the experiment shown in Figure 5. Figure 8 shows the results.

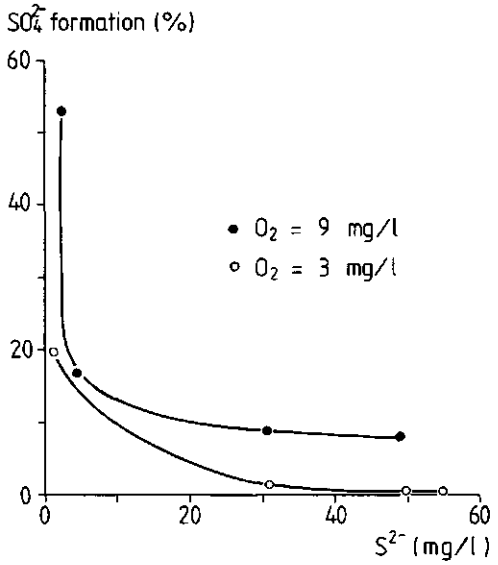


**Figure 8.** The percentage sulphate formation (calculated from the sulphide influent concentration) versus the sulphide concentration in a free cell suspension at different oxygen concentrations in reactor 1 (no PUR particles; hydraulic retention time 7 h.; volume 5.5 l). Reactor pH = 8.0.

## DISCUSSION

The results in Figure 5 with the PUR particle reactor show that in the presence of sulphide concentrations exceeding 10 mg/l less then 10% sulphate is produced when the oxygen concentration remains below 6 mg/l. This relatively low formation of sulphate could mean that sulphide (or polysulphide) is inhibitory or even toxic to the sulphate producing organisms or that sulphide is more preferred as electron donor than elemental sulphur. In denitrifying systems with sulphide as electron donor a similar phenomenon has been observed. Gommers et al. (13) found, in a biological activity measurement of biomass from a sulphide oxidizing denitrifying fluidized bed reactor, that no sulphate was formed in the presence of sulphide. Sublette and Sylvester (9, 10) found elemental sulphur accumulation in a continuously fed, $H_2S$ oxidizing, denitrifying reactor (which produced sulphate) with a Thiobacillus denitrificans culture, when the $H_2S$ feed rate was increased until $H_2S$ breakthrough occurred.

When sulphide indeed is toxic to the biomass in the sulphide removal reactors, these reactors must continually operate in a stressed condition. From Table 2 we learn that despite the stressed conditions in the case of 120 and 60 BSP's in the reactor the oxidation activity per BSP did not decrease compared to the situation of 210 BSP's per reactor. These stressed conditions have been imposed on the reactor system for a sufficient long period of time to be sure that these reactors can be continually operated in a stressed condition. Presently more detailed research is conducted, concerning the influence of sulphide on the activity and viability of organisms in the process culture and the results will be reported in another communication.

In addition to the sulphide concentration, obviously also the sulphide load per unit of biomass is an important factor for the sulphate production. Upon increasing

the sulphide sludge load, the maximum sulphate production (at high oxygen concentrations) rate decreases (Table 1). Accordingly, a lower sulphate production rate is found upon removing biomass support particles from the reactor. Table 2 shows that the sulphide concentration does not increase upon removing 90 BSP's (30%), despite the fact that the sulphate concentration decreased with 25%.

The influence of the oxygen concentration on sulphate production is negligible at sulphide concentrations in the reactor exceeding 20 mg/l (Figure 5). However at sulphide concentrations below 20 mg/l the sulphate production rate increases sharply upon increasing the oxygen concentration.

The results in Figure 5 and Figure 8 show that under conditions of the same oxygen and sulphide bulk concentrations, the sulphate production rate in the reactor with the free cell suspension is lower than in the reactor with PUR foam. The explanation for this difference between both systems presumably can be found in differences in the transport limitation of the reactants and perhaps of the reaction products. Contrary to the free cell suspension system, these transport limitations clearly exist in the PUR particle system. Both oxygen and sulphide, which are the two most important factors that affect the sulphate formation have to diffuse into the PUR particles (Figure 7). In the free cell suspensions the dispersed biomass is exposed to the bulk concentrations, whereas a significant part of the biomass in the PUR particles is exposed to lower concentrations than occur in the bulk solution. Assuming that the biomass in the PUR particles responds in the same manner to the sulphide and oxygen concentrations as the biomass in the free cell suspension , the results obtained in the free cell suspension can serve as the reference situation for the biomass in the PUR particles. From the experiments with the free cell suspension we learn that a significant sulphate formation only proceeds when the sulphide concentration is below 5 mg/l and that the oxygen concentration is of minor importance. For the observed higher sulphate formation in the PUR particle system this means that transport limitation for sulphide very likely is the essential factor rather than the oxygen concentration, which only is of secondary importance.

The diffusion coefficients of the various compounds should be used in order to explain these phenomena. The diffusion coefficients of $O_2$, $HS^-$ and $H_2S$ are 2.81 -, 2.60 - and 2.41 E-09 $m^2$/sec respectively. These values were calculated using the Wilke Chang equation (14), which has an accuracy of about 10%. With this equation the diffusion coefficients can be calculated using the molar volume of the solute.

$$D_{a \text{ in } b} = \frac{7.4 \text{ E-08 } (S_b M_a)^{0.5} T}{\mu (V_a)^{0.6}}$$

D is the diffusion coefficient ($cm^2$/sec) for small concentrations of compound a in solvent b. $V_a$ is the molar volume of solute a ($cm^3$/g-mole) as liquid at its normal boiling point; $\mu$ is the viscosity of the solution in centipoises. $S_b$ is an association parameter for solvent b (is 2.6 for water). T is the absolute temperature (°K) and $M_a$ is the molecular weight of A.The data used for this equation were obtained from Geankoplis (15).

In the case sulphur is produced the diffusion coefficient of polysulphide instead of sulphide should be used, because according to Giggenbach (16) in near neutral environments part of the sulphide will be present as an equimolar mixture of tetra- and pentasulphide ions. With this information we estimated the diffusion coefficient of polysulphide at approximately 1,00 E-09 $m^2$/sec, using again the Wilke Chang equation and the data of Geankoplis.

In the case merely sulphate and no sulphur is produced the diffusion coefficient of $HS^-$ and $H_2S$ should be used (no polysulphides will be present, because of the low concentrations of sulphur and sulphide) to estimate the transport rate of sulphide into the particles. The oxygen/sulphide consumption ratio in this case is 2, because sulphate is the end product.

Once higher concentrations of sulphur appear, it will be clear that the transport of sulphide into the PUR particles can proceed relatively slowly (compared to

that of oxygen), because the high concentrations of sulphide and sulphur cause the formation of polysulphides and consequently the diffusion coefficient of polysulphide will govern the transport of sulphide into the particles. Because over 90 % of the sulphide is converted into sulphur the oxygen/sulphide consumption ratio in this case only is 0.5.

The difference in the transport rates and gradients in the particles of the oxygen and sulphide concentrations can be estimated using the differences in diffusion coefficients and the difference in the oxygen/sulphide consumption ratio. Consequently we can calculate what bulk concentration of oxygen is needed for a sufficiently high transport rate to oxidize all sulphide.

When we assume that at a sulphide bulk concentration of 5 mg/l all sulphide is converted to sulphate, then an oxygen bulk concentration of 8.9 mg/l is necessary to oxidize all sulphide inside the PUR particle, because the oxygen concentration in the particle will drop faster than the sulphide concentration, as 2 mole of oxygen are needed to convert 1 mole of sulphide into sulphate and the diffusion coefficients do not differ significantly.

At a sulphide bulk concentration of 20 mg/l the sulphide concentration in the particle will drop faster than the oxygen concentration because little sulphate is produced and thus only 0.5 mole of oxygen is needed to convert 1 mole of sulphide into sulphur. Also the diffusion coefficient of polysulphide should be used, which is significantly lower than the diffusion coefficient of oxygen. Now an oxygen bulk concentration of only 3.6 mg/l is needed to oxidize all sulphide inside the PUR particle.

When the sulphide bulk concentration is between 5 and 20 mg/l and the oxygen bulk concentration is not too low, the sulphide concentration inside the particles will drop faster than the oxygen concentration. This means that at a certain depth in the particle the sulphide concentration is low enough to allow sulphate to be produced and there will still be oxygen present. This explains why sulphate production occurs in the PUR system at higher sulphide concentrations than in the free cell suspension.

From the results presented here it can be concluded that the sulphate production rate can be suppressed by controlling the oxygen concentration. At high sulphide concentrations (exceeding 20 mg/l) in the reactor the oxygen concentration should be increased in order to increase the sulphide oxidation rate. At low sulphide concentration (not exceeding 20 mg/l) the oxygen concentration should be kept low in order to suppress the oxidation of sulphur to sulphate.

This strategy looks similar to that of Cork (6), who used light energy as control parameter with photosynthetic sulphur bacteria.

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## CHAPTER 4

# Growth of Thiothrix in Sulphide Oxidizing Reactors

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## ABSTRACT

In this study the occurence of Thiothrix filaments in sulphide removing waste water treatment systems has been investigated. This research was done with two reactors. A CSTR with a mobile carrier was used with a composite influent (tapwater with sulphide and organic compounds). The influent of the other reactor (a fixed film upflow reactor) consisted of anaerobically treated papermill wastewater, with a sulphide concentration of 140 mg/l.
Thiothrix growth was only found in the pH range 7 - 8.5 and was not found at a medium pH of 9 or higher. At pH=6 abundant fungi growth appeared, but no Thiothrix were found.

Thiothrix did not grow on sulphide in presence of either acetate or glucose, and in absence of a mixture of higher fatty acids. Apparantly Thiothrix needs longer fatty acids for growth.

The sulphide biomass loading rate is the decisive parameter in whether or not Thiothrix will develop in a sulphide removing reactor.
The shearforce, carrier material, sulphide influent concentration and HRT have hardly have any influence on the development of Thiothrix in this reactors.

## INTRODUCTION

Thiothrix spp. are filamentous, colourless, sulphur-oxidizing bacteria which may form rosettes and gonidia and deposit sulphur when grown in the presence of sulphide or thiosulphate (Williams & Unz, 1985a). Thiothrix spp. inhabit sulphide-containing natural waters (Larkin, 1983) and may develop in aerated wasterwaters in which sulphide is present then causing sludge bulking problems (Eikelboom, 1975; Merkel, 1975; Williams & Unz, 1985b). Thiothrix was first described by Winogradsky (1888).

In the biotechnological sulphide removal system developed (Buisman et al., 1989) sulphide is oxidized to elemental sulphur. Growth of Thiothrix in the sulphide removing reactors could represent a serious problem for two main reasons: a. Thiothrix accumulates the sulphur in the cell, which makes the reuse of sulphur more difficult and b. Thiothrix can cause serious sludge bulking problems.

In the case we could accomplish the biotechnological elimination of the sulphide without the development of Thiothrix in the reactor, we have also available an attractive method preventing the growth of Thiothrix in the activated sludge process downstream. Previously Merkel (1975) and Farquhar & Boyle (1972) already found that the proliferation of Thiothrix can be prevented by removing the sulphide beforehand from the influent of the activated sludge process.

In the present study some possibly important growth factors for Thiothrix growth have been investigated. Our experiments were performed with experimental solutions (tapwater with sulphide) and with anaerobically treated wastewater from papermills in Eerbeek, The Netherlands. As in the aerobic activated sludge post treatment system, Thiothrix spp grow abundantly, it can be concluded that the anaerobic effluent in principle is a favourable growth medium for this organism.

In recent literature is reported that Thiothrix requires sulphide or thiosulphate and organic compounds for growth (Larkin, 1983; Williams & Unz, 1985b). Thiothrix strains utilize many organic acids, but generally fail to grow on carbohydrates and amino acids (Williams & Unz, 1985b) and cannot grow on Tween 80 (Williams & Unz, 1985a). Carbondioxide can be fixed by Thiothrix (Larkin, 1983).

Thiothrix strains are found to grow at pH values ranging from 6.5 - 8.5 (Williams & Unz, 1985a). The pH range over which Thiothrix grows in nature has been found to be from 6.7 to 7.3 (Larkin, 1983).
Thiothrix strains grow in a temperature range from 4 °C to 37 °C (Larkin, 1983; Williams & Unz, 1985a)

The amount of reduced sulphur required or tolerated by Thiothrix is not known yet; it has never been point of investigation. From measurements made in sulphur springs containing Thiothrix, it is shown that the sulphide concentration is in the low range of 0.12 to 0.86 mg/liter (Larkin, 1983). Which is similar to that found in Thiothrix-containing activated sludge installations (Farquhar & Boyle, 1971, 1972). On the other hand Merkel (1975) found a sulphide concentration, in the influent of the activated sludge process up to 30 mg/liter. The sulphide influent concentration of the influent of the activated sludge process in Eerbeek, The Netherlands, is 140 mg/l and still Thiothrix grows abundantly here. But the concentration in the aeration tank is less than 1 mg/liter in the Eerbeek installation.

Williams & Unz (1985a) have observed Thiothrix under conditions of both low and high aeration basin dissolved oxygen levels.

Kuenen & Beudeker (1982) proposed a model predicting the occurence of sulphur-oxidizing bacteria as a function of the relative turnover rate of reduced inorganic sulphur compounds and organic substrates during energy-limiting growth conditions in fresh water environments. At a high ratio of reduced inorganic sulphur compounds to organic compounds, obligate chemolithotrophs out-compete mixotrophs and heterotrophs. The addition of organic substrate, seriously lowering the ratio, favoures the mixotrophs. A low ratio favoures heterotrophs which might or might not be able to oxidize sulphide. Thiothrix spp are mixotrophs or heterotrophs able to oxidize sulphide (Larkin, 1983; Williams & Unz, 1985b). When this model is applicable for Thiothrix bacteria, it would be possible to prevent Thiothrix growth by increasing the sulphide concentration relative to the organic substrate concentration.

In this research we investigated the growth of Thiothrix in reactors treating sulphide containing wastewater and we used only mixed cultures.

## MATERIALS AND METHODS

### reactors

A completely mixed and continuously fed 8.3 l reactor was used containing 400 biomass support particles (BSP) of polyurethane or pall rings. Recticulated polyurethane (PUR) foam delivered by Recticel, Kesteren, The Netherlands was used as biomass support particles, with dimensions of 1.5x1.5x1.5 cm, 30 pores per inch and a specific surface of 1375 $m^2/m^3$ (Recticel). The Pall rings used had a diameter of 5 cm and a height of 5 cm. The total surface of the carrier material in the reactor with PUR particles was 1.9 $m^2$ and in the reactor with the pall rings 0.5 $m^2$.

The gas phase in the reactors was recycled for purposes of mixing, process control and for preventing escape of $H_2S$ from the system. The oxygen concentration, the temperature and the pH were kept constant using a PI controller. A Tulip computer (PC compact), with a Mitsubishi programmable logic controller (Melsec PLC) was used for data-aquisition and control. The process temperature was kept at 20 °C.

The 20 liter upflow reactor reactor used for treating the anaerobic effluent of the papermill effluent contained 50 pall rings. The process temperature applied in this reactor was between the 25 °C and 30 °C. The pH varied between 7.5 and 8.0.

**Table 1,** sheme of influents of the different reactors

| reactor | influent |
|---|---|
| 8.3 l CSTR | composite influent |
| 20 l upflow | anaerobicly treated papermill waste water |

**composition of the papermill waste water**

The composition of the anaerobically treated papermill wastewater used in this study is given in Table 2. The papermill wastewater was treated in an UASB reactor (2700 $m^3$). Little variation in the composition of the wastewater occured in time, except for the holidays. In the weekends the sulphide concentration increased slightly.

**Table 2.** composition of the effluent of the anaerobic waste water treatment at the plant Industriewater Eerbeek, The Netherlands

| parameters | | concentration |
|---|---|---|
| COD | (mg/l) | 400 |
| N-kj | (mg/l) | 10 |
| $S^{--}$ | (mg/l) | 140 |
| $SO_4^{--}$ | (mg-S/l) | 20 |
| acetate | (mg/l) | 30 |
| propionate | (mg/l) | 10 |
| alkalinity | (meq) | 18 |
| chloride | (mg/l) | 50 |

**composition of feed solutions**

Composite waste water was used as influent in the experiments with the CSTR. This feed solution consisted of nutrients, acetic acid and sodium sulphide in tap water. In specific experiments we also added a mixture of higher fatty acids in the form of cream (Table 3). The nutrient solution contained (g/l): $NH_4Cl$ (8), $MgSO_4.7H_2O$ (2), $KH_2PO_4$ (5) and 10 ml/l trace element solution according to Vishniac & Santer, (1957). All the chemicals used for the nutrient solution were analytical grade supplied by Merck, Darmstadt, F.R.G.. The sulphide solution contained 200 g/l $Na_2S.8H_2O$ of a technical grade supplied by de Vries, Amsterdam, The Netherlands. The nutrient and sulphide solutions were added in proportion of 2 : 1. For pH control 5 M HCl, also of technical grade, was used. Pure oxygen supplied by Hoekloos, Schiedam, The Netherlands was used in the experiments.

Table 3. the fatty acid composition of cream

| compound | concentratation (g/l) |
|---|---|
| casein | 31 |
| carbohydrates | 35 |
| minerals | 5 |
| milk fat | 200 |
| milk fat contained (weight %): | |
| C-10:0 | 4.6 |
| C-12:0 | 6.6 |
| C-14:0 | 15.3 |
| C-16:0 | 35.1 |
| C-18:0 | 11.3 |
| C-18:1 | 23.5 |
| C-18:2 | 1.0 |

**biomass**

Wether or not Thiothrix growth occured was checked for every week with microscopic research of the biomass.

**analyses**

Sulphide was determined photometrically by the method described by Truper and Schlegel (1964). Elemental sulphur was measured after extraction with acetone according to the method described by Bartlett and Skoog (1954).

Sulphate was analyzed by liquid chromatography using a Chrompack HPLC column, packed with Ionospher A-5$\mu$ (dim: 10 cm x 3 mm; ID), eluent 0.027 M potassiumbiphtalate (flow 0.4 ml/min) and a Knauer differential refractometer as detector. The injection quantity was 20 $\mu$l. This method could also detect thiosulphate.

Acetate and propionate were determined with a gaschromatograph equipped with a 2 m x 4 mm (i.d.) glass column packed with Supelcoport (100-120 mesh) coated with 10% Fluorad FC 431. The temperature of the column, the injection port and the flame ionization detector were 120, 220 and 240 °C, respectively. Nitrogen saturated with formic acid was used as carrier gas at a flow rate of 50 ml/min. The oxygen concentration was measured with an $O_2$-sensor (WTW; DU 600 201 711). The pH was measured with a pH-electrode (Ingold, 425-60-s7 ing. no. 104253313).

## RESULTS

**experiments with CSTR**

The experiments conducted, together with the process conditions applied, are summarized in Table 4a and 4b. All experiments were started using a seed containing Thiothrix. A certain set of process parameters was imposed to the system in the various experiments and after a week it was checked wether or not growth of Thiothrix occured.

Most experiments were conducted at pH=8.0. Upon operating a system at a pH of approximately 6 fungi developed and Thiothrix disappeared completely. This was also the case at pH=9 Thiothrix. In the pH range 7 - 8.5 Thiothrix growth was observed.

Increasing the shearforce by increasing the speed of the stirrer (from 150 rpm to 300 rpm) did not affect the occurence of Thiothrix significantly (Table 4a, number 1 and 3).
Replacing the PUR particles by pall rings (total surface decreased with a factor 4) also hardly influenced the Thiothrix growth (Table 4a, number 1 and 2).

When no organic compounds were added to the influent Thiothrix did not grow. This also is the case when only acetate and glucose, without higher fatty acids, were added as organic substrates. Thiothrix growth proceeds well on acetate when higher fatty acids are present (Table 4a, number 4, 5 and 6).

In an additional experiment we ceased the addition of the higher fatty acids. In accordance with the observations made the experiment mentioned in Tabel 4a, it indeed was found that the amount of Thiothrix dropped down distinctly within a few days. After two weeks only very few Thiothrix filaments were left. The sulphide influent concentration in this experiment was 60 mg/l, the HRT was 1 hour, pH=8.0, oxygen concentration was 3 mg/l and the sulphide effluent concentration was 2 mg/l. All these parameters were kept constant during the experiment.

**Table 4a.** effect of the biomass support particles (BSP), stirrer speed and organic compounds on the growth of Thiothrix

| experiment number | HRT (h) | $S^{2-}$ infl (mg/l) | $S^{2-}$ effl (mg/l) | BSP | acetate infl (mg/l) | fatty acids (mg/l) | observed Thiothrix growth |
|---|---|---|---|---|---|---|---|
| 1 | .7 | 50 | 3 | pur | 45 | 5 | ++ |
| 2 | .7 | 50 | 3 | pall | 45 | 5 | ++ |
| 3 | .7 | 50 | 3 | pur | 45 | 5 | + |
| 4 | .7 | 100 | 3 | pall | 45 | 5 | + |
| 5 | .3 | 50 | 3 | pur | 45 | 0 | - |
| 6 | .3 | 50 | 3 | pur | 0 | 0 | - |

**notes:**
1 the stirrer speed was 150 rpm
3 the stirrer speed was increased from 150 rpm to 300 rpm
5 10 mg/l glucose was present in the influent
++ abundant Thiothrix growth
\+ Thiothrix growth appears but is not abundant
\- no Thiothirx growth

**Table 4b.** effect of the sulphide loading rate on the growth of Thiothrix; in all experiments the influent concentrations of acetate and higher fatty acids was respectively 45 and 5 mg/l; the pH was 8 and oxygen concentration was 3 mg/l.

| experiment number | HRT (h) | $S^{2-}$ infl (mg/l) | $S^{2-}$ effl (mg/l) | BSP | $S^{2-}$ loading rate (mg/l.h) | observed Thiothrix growth |
|---|---|---|---|---|---|---|
| 1 | .7 | 30 | 2 | pall | 43 | ++ |
| 2 | .7 | 50 | 3 | pall | 71 | ++ |
| 3 | .7 | 100 | 5 | pall | 143 | + |
| 4 | .3 | 60 | 30 | pall | 180 | + |
| 5 | .3 | 100 | 7 | pur | 300 | - |

The effect of the sulphide loading rate on the growth of Thiothrix is shown in Table 4b. Upon increasing the sulphide loading rate Thiothrix becomes less dominant. At a sulphide loading rate of 300 mg/l.h no Thiothrix bacteria could be found in the reactor.

**experiments with the upflow reactor**

Different liquid hydraulic retention times (HRT) were imposed to the upflow reactor, using the anaerobically treated papermill wastewater as feed. The influent sulphide concentration was approximately 140 mg/l sulphide. In July this concentration dropped to 20 mg/l, due to the holydays. The reactor pH varied between 7.5 and 8.0, and the oxygen concentration was always maintained below 4 mg/l. The results of the various experimental runs are reported in Table 5. In one of the runs the influent was diluted with tapwater, in order to impose a low HRT at a low sulphide loading rate.

**Table 5.** The effect of different HRT's and different sulphide influent concentrations on the occurence of Thiothrix.

| flow rate (l/h) | | HRT (h) | $S^{2-}$ in (mg/l) | $S^{2-}$ load (mg/l.h) | $S^{2-}$ effl (mg/l) | Thiothrix growth |
|---|---|---|---|---|---|---|
| 75 | | 0.3 | 140 | 525 | 14 | - |
| 15 | | 1.3 | 140 | 105 | 25 | - |
| 1.5 | | 13.3 | 140 | 10.5 | 0.5 | ++ |
| 15 | (1) | 1.3 | 25 | 18.8 | 2.0 | ++ |
| 71 | (2) | 0.3 | 3.0 | 10.5 | 0.0 | ++ |

**notes:**

1. lower influent concentration because of holydays
2. lower influent concentration because of dilution with tapwater

## DISCUSSION

It seems that the sulphide biomass loading rate is one of the main decisive parameters that determines wether or not Thiothrix will grow in the sulphide removing reactors. Table 5 shows that at a flow rate of 15 l/h Thiothrix does not grow when the influent sulphide concentration was 140 mg/l but growth occured when the sulphide influent concentration became 20 mg/l. However growth of Thiothrix also occured at an influent concentration of 140 mg/l when the flow rate was decreased to 1.5 l/h. So neither the HRT nor the sulphide influent concentration, but the sulphide loading rate is an important factor determining whether or not Thiothrix will grow in a sulphide removing reactor. We can conclude that Thiothrix growth is possible up to a sulphide concentration of 30 mg/l.

The results shown in Table 4b support this conclusion, but here we find Thiothrix growth at a sulphide loading rate of 180 mg/l.h, while in the experiment with the upflow reactor no growth was found at a loading rate of 105 mg/l.h. This difference can be due to different reactor configuration, i.e. the upflow reactor is more plug flow operated. Also a different biomass concentration or the difference in temperature in the CSTR and upflow reactor might be the reason.

Sofar it can not be explained why Thiothrix does not grow at high sulphide loading rates, because hardly anything is known about the sulphide affinity and sulphide inhibition of Thiothrix. We presume that at higher sulphide loading rates Thiothrix is unable to compete against the sulphur producing organisms.

According to Nielsen (1984) who investigated a sulphur storage test, up to a sulphide concentration of 30 mg/l sulphur storage occured in the Thiothrix bacteria, but not beyond 30 mg/l sulphide. Therefore sulphide concentrations exceeding 30 mg/l might be toxic for Thiothrix.

In our reactor systems the sulphide concentration in the reactor can not be changed without changing the sulphide loading rate. For this reason it is very difficult to assess the effect of the sulphide concentration in the present experiments. Only in experiment 4 (Table 4b) the sulphide concentration was 30 mg/l. It turned out that under these conditions Thiothrix could be well maintained in the bacterial culture, which is in accordance with the results of Nielsen. Becausew the sulphide effluent concentration should be much lower than 30 mg/l, it seems not possible to use the sulphide concentration for prevention of Thiothrix growth prevention.

In the present investigation sulphide influent concentrations up to 100 mg/l in the CSTR have been applied and in the upflow reactor up to 140 mg/l. It is obvious that the sulphide influent concentration is only an important factor with respect to the sulphide loading rate.

In the present investigations Thiothrix growth is found in the pH range 7 - 8.5, which is in accordance with the results of Williams and Unz (1985a). The pH can not be used to prevent Thiothrix growth, because the pH of anaerobic effluent is in the same range.

The experimental data presented here clearly show that little if any Thiothrix growth was found in absence of higher fatty acids (C-10 to C-18). This could mean that Thiothrix is not able to use acetate to assimilate celmaterial like Microthrix parvicella (Slijkhuis, 1983). This bacterium needs long fatty acids to grow. It is impossible to use the presence or absence of fatty acids to prevent Thiothrix growth, because of the nature of wastewater.

The data in Table 5 show that the ratio sulphide/organic compounds is not decisive for Thiothrix growth. The ratio stays the same at all experimental runs but and the occurence of Thiothrix does not stay the same. Therefore it is obvious that the model of Kuenen & Beudeker (see introduction) is not applicable to Thiothrix and it is still unclear whether or not Thiothrix is mixotrophic or heterotrophic.

## CONCLUSION

Growth of Thiothrix in sulphide removing reactors can be prevented by applying high volumetric loading rates. These high loading rates were already necessary in order to produce sulphur instead of sulphate. Therefore we conclude that growth of Thiothrix bacteria will not be a problem in sulphide removal reactors, with sulphur production.

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## CHAPTER 5

# Sulphur and Sulphate Reduction with Acetate and Propionate in an Aerobic Biotechnological Process for Sulphide Removal

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## ABSTRACT

The maximal sulphide production rate of sulphur- and sulphate-reducing bacteria was 50 mg sulphide per biomass support particle per day in an aerobic sulphide removal reactor with polyurethane (PUR) foam as carrier material. The optimal pH and temperature for the sulphide producing bacteria was 8.0 and 30°C respectively. Different dimensions of PUR particles and Rasschig rings were tested as carrier material. When using PUR particles, the sulphide production rate amounted always 4% of the sulphide removing rate, independent of the dimensions and pore size of the polyurethane support particles. With the Rassich rings this was only 2% and for reactors in which no carrier material was present even lower (0.6%).

Anaerobic media with different mixtures of acetate, propionate, sulphur and sulphate inoculated with sludge from the reactor showed the presence of acetate-degrading sulphur-reducing but not of acetate-degrading sulphate-reducing bacteria. With propionate as sole electron donor no degradation occurred in the presence of sulphur within two weeks, whereas sulphate-dependent propionate oxidation started after five to six days of incubation

Bacteria were isolated and resembled morphologically and physiologically Desulfuromonas acetoxidans and Desulfobulbus propionicus.

## INTRODUCTION

Under anaerobic conditions sulphate, sulphite, and organic sulphur compounds present in wastewater are reduced by bacterial activity to sulphide. The odor, toxicity, oxygen demand and corrosion problems caused by sulphide in wastewater frequently necessitate a post-treatment, e.g. by the oxidation of sulphide to a less harmfull form.

At our university we are developing a biotechnological sulphide removal process, based on the microbial oxidation of sulphide with oxygen to elemental sulphur. We found that conversion of sulphide into sulphur rather than into sulphate is greatly enhanced when high sulphide loading rates are imposed to the system (Buisman et al., 1989a,b)

Aerobic microbial sulphide oxidation is mainly carried out by chemolitho-autotrophic bacteria, belonging to the genus Thiobacillus.
In aerobic sulphide oxidizing reactors anaerobic conditions may occur within the polyurethane foam particles due to high sulphide loading rates or due to insufficient oxygen supply. In the presence of organic compounds, anaerobiosis may result in a reduction of the earlier formed sulphur and sulphate to sulphide.
Dissimilatory sulphur reduction in the dark is well documented for a number of eubacterial and archaebacterial species. The eubacteria able to grow anaerobically with elemental sulphur as terminal electron acceptor include Desulfuromonas (Pfennig & Biebl, 1976) a number of sulphate reducing bacteria (Biebl & Pfennig, 1977), spirillum 5175 (Wolfe & Pfennig, 1977), Wolinella succinogenes (Macy et al., 1986) and certain Campylobacter species (Laanbroek et al.,1978. Also many methanogenic bacteria are able to reduce sulphur to sulphide (Stetter & Gaag, 1983).

Only Desulfuromonas species are able to oxidize acetate with elemental sulphur to carbon dioxide:

$$CH_3COO^- + 4S + 4H_2O \text{ ---> } 2HCO_3^- + 4H_2S + H^+$$

It has been found that propionate is also removed under anaerobic conditions in sludge from the sulphide removing reactor. So far no fresh water sulphur-reducing bacteria are known which are able to use propionate (Widdel 1988). Therefore, it may be possible that a sulphate reducing bacterium like e.g. Desulfobulbus propionicus converts propionate to acetate:

$$4CH_3CH_2COO^- + 3SO_4^{--} \text{ ===> } 4CH_3COO^- + 3HS^- + H^+ + 4HCO_3^-$$

The aim of the present study was to investigate the relative importance of sulphur and sulphate reduction in the anaerobic zones of the aerobic sulphide oxidation reactor.

## MATERIALS AND METHODS

### biomass, influent, reactors

The composition of the influent used in the experiments has been described in previous communications (Buisman et al., 1989a,b).

A 5 liter completely mixed and continuously fed reactor was used, which contained 240 recticulated polyurethane (PUR) foam particles (Recticel). This reactor has also been described before (Buisman et al., 1989a,b).

### sulphide removal activity test

The sulphide removal activity of either biomass support particles or suspended cells was measured in 0.5 l air tight vessels. After adding the biomass, the reaction medium was supplied to the vessel. The medium consisted of a phosphate buffer (50 mM; pH=8) which was saturated with oxygen. Immediately after closing the vessel the oxygen removal rate was followed, using an oxygen sensor. Because an excess of sulphur present in the biomass sample, the oxygen removal rate is directly related to the oxidation rate of sulphur to sulphate. When adding also sulphide to the reaction vessel little if any sulphate was produced and consequently the oxygen removal rate then becomes equivalent to the sulphide oxydation rate (sulphide to sulphur). The temperature in the vessel was maintained at the same level as in the reactor from which the biomass was taken.

### sulphur reduction activity test

In this test a 0.569 liter serum flask was used. The medium consisted once again of a phosphate buffer (50 mM; pH=8; 30°C), which was flushed with nitrogen gas to remove oxygen. Biomass, acetate and propionate were added to the flasks and the sulphide and fatty acids were measured in time. The increase in the sulphide concentration is equivalent to the sulphur reduction rate.

### enrichment of sulphur and sulphate reducing bacteria

Anaerobic enrichments were done in a bicarbonate-buffered medium under a gas phase of N2/CO2 (80/20). For the preparation of the medium 900 ml demineralized water was boiled and cooled under oxygen-free N2. Na2HPO4.2$H_2O$, 0.53 g, KH2PO4 0.41 g, 1 ml trace elements solution, 1 ml 0.05 % resazurin and substrate (20 mM sodium propionate or 20 mM sodium acetate) were added and portions of 45 ml were

distributed into 120 ml bottles, which contained already elemental sulfur or sodium-sulfate to give final amounts of 80 and 20 mmoles per liter, respectively. After sealing with butyl rubber stoppers and aluminum caps, 0.5 atmosphere N2/CO2 was placed above the media. A mineral salt solution and a bicarbonate solution were added in a volume of 2.5 ml each. The nutrient solution contained (g/l) $MgCl2.6H_2O$, 2.0; $CaCl2.2H_2O$, 2.2; NH4Cl, 6.0; NaCl, 6.0; and 20 ml of a vitamin solution. The bicarbonate solution consisted of NaHCO3, 80 (g/l) and $Na2S.8H_2O$ 4.8 (g/l). The trace elements solution was a tenfold concentrated trace element solution described by Pfennig and Lippert (1966) and the vitamin solution as given by Stams et al. (1983). Under these conditions the pH of the medium was about 7.2. 5 ml reactor fluid was added with a syringe and bottles were incubated at 30 °C in the dark. After various periods of time samples of 2.5 ml were taken with a syringe. Part of the solution was immediately added to 4 % zincacetate for the determination of sulfide. The remainder was centrifuged and the supernatent was used for the analysis of fatty acids and sulfate. For further cultivation of the anaerobic bacteria sterile media were used.

**analyses**

Analyses of sulphide, elemental sulphur and sulphate were carried out as described elsewhere (Buisman et al., 1989a,b) and acetate and propionate were determined as described by Koster et al., 1986)

## RESULTS

**start up at 30°C**

A typical example of a start-up phase at 30°C of a 5 liter sulphide removal reactor is shown in Figure 1. During the start up phase almost every day four PUR particles were removed in order to measure the sulphur/sulphate reduction activity.

**pH and temperature**

In order to assess the effect of the pH and temperature on the sulphide producing bacteria, PUR particles were taken from the reactor and their activity was measured at different pH and temperature values. The effect of the pH was investigated at 30°C and the effect of the temperature at pH 8.0. The optimal pH and temperature were 8.0 and 30°C, respectively (Figure 2 and 3).

**fatty acid concentration**

In order to assess the relative importance of acetate and propionate for the sulphide production rate, batch experiments were conducted, using different ratios of acetate and propionate. The experiment was conducted at two different temperatures. The reactor, from which the bsp's were taken, was operated at a sulphide influent concentration of 110 mg/l, acetate and propionate influent concentrations of 52 and 8 mg/l, respectively, and a HRT of 24 minutes. The pH was maintained at 8.0 and the oxygen concentration at 4.0 mg/l. Under these operational conditions the sulphide effluent concentration amounted to only 2 mg/l, while the acetate and propionate effluent concentrations were 0 mg/l. The results of this experiment are summarized in Table 1.

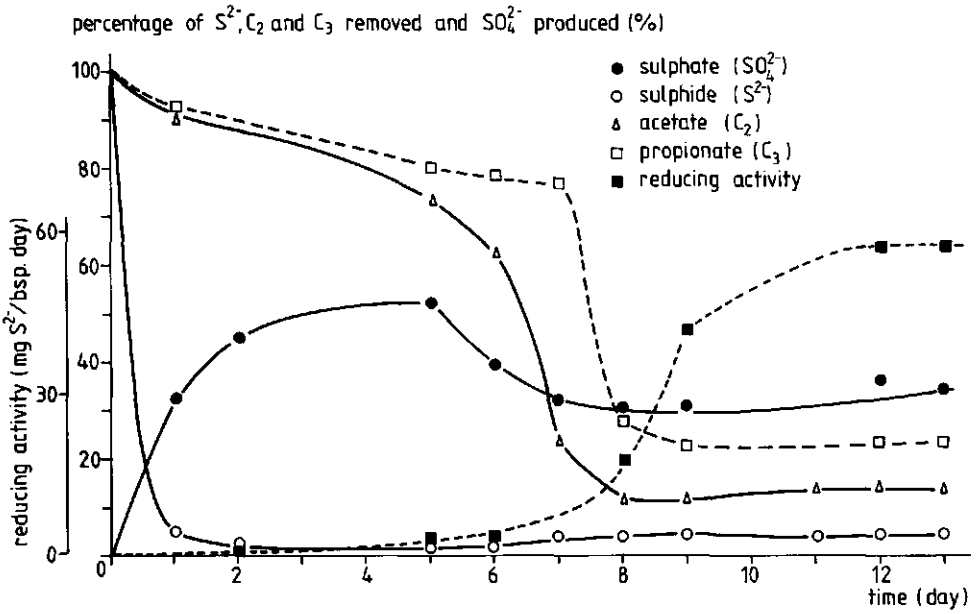


**Figure 1.** The start up fase of a sulphide removing reactor at 30 °C and a pH of 8.0; the decrease of the concentrations of sulphide, acetate and propionate is shown as a percentage of their influent concentration which were respectively 125, 65 and 13 mg/l. The increase of the sulphate concentration is given as a percentage of the sulphide influent concentration. Sulphide producing activity was measured with an anaerobic test assay.

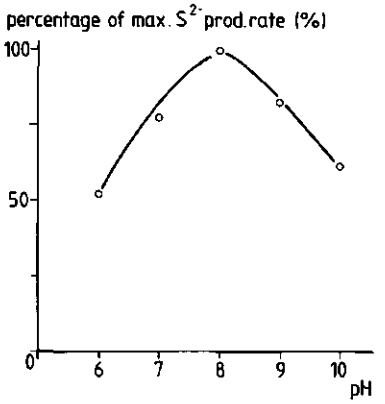


**Figure 2.** Effect of the pH on the sulphide producing activity. The activity at pH 8.0 was 15 mg/bsp.day. The conditions of the reactor from which the bsp's were taken are sulphide influent and effluent concentrations 100 and 2, acetate influent and effluent concentration 84 and 0, propionate influent and effluent concentrations 16 and 0, HRT 22 minutes, pH 8.0 and the oxygen concentration was 5.0 mg/l

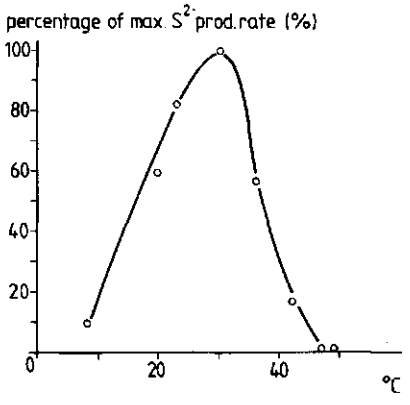


**Figure 3.** Effect of the temperature on the sulphide producing activty. The activity at 30 °C was 15 mg/bsp.day. The conditions of the reactor from which the biomass was taken were the same as mentioned in Figure 2.

**Table 1.** effect of different ratios of acetate and propionate on the sulphide production rate

| experiment number | acetate conc. (mg/l) | propionate conc. (mg/l) | sulphide production rate (mg/bsp.day) at 20°C | at 30°C |
|---|---|---|---|---|
| 1 | 200 | 0 | 22 | 39 |
| 2 | 100 | 100 | 23 | 49 |
| 3 | 0 | 200 | 4 | 16 |

Table 1 shows that acetate is more important than propionate for the sulphide production. An additional experiment with different acetate concentrations was conducted. The results of this experiment are shown in Figure 4.

**bsp dimensions**

The sulphate- and sulphur-reducing bacteria are strictly anaerobic. Therefore it is possible that the dimensions of the carrier material have a significant influence on the sulphide production rate, as these dimensions are also a factor dictating the anaerobic space available for the sulphide producing bacteria. To demonstrate this possible effect of the dimensions of the carrier material, we conducted an experiment in which Rasschig rings and four different types of PUR particles were present in the reactor. The Rasschig rings used have a cilindrical shape with a diameter of 5 cm and a height of 3 cm with a total surface of 180 $cm^2$. After an adaption period of two weeks this carrier material was removed from the reactor and the anaerobic sulphide producing and aerobic sulphide removing activities were measured. The reactor conditions were the same as for the experiments shown in Figure 2. The results of this experiment are summarized in Table 2.

**Table 2.** The effect of the BSP dimensions on the anaerobic sulphide producing and aerobic sulphide removing activities

| BSP (cm) | $S2^-$ production rate (mg/bsp.day) | $S^{2-}$ removal rate (mg/bsp.day) |
|---|---|---|
| PUR 1.5x1.5x1.5, 30 ppi | 10.3 | 260 |
| PUR 1.5x1.5x1.5, 10 ppi | 4.8 | 140 |
| PUR 1.0x1.0x1.0, 30 ppi | 2.5 | 60 |
| PUR .75x.75x.75, 30 ppi | 2.0 | 60 |
| Rasschig ring, 3x5 | 9.9 | 430 |

In an additional experiment the bioreactor was started up and operated without carrier material at a HRT of 14 hour, an oxygen concentration of 4.0 mg/l and pH=8.0. After an adaption period of two weeks the suspended cells, developed in the biorotor, were also tested for the anaerobic sulphide production and aerobic sulphide oxidation rates. The sulphide influent concentration was 6000 mg/l, in order to achieve more or less the same sulphide loading rate as mentioned above. The acetate and propionate influent concentrations were 3000 and 500 mg/l, respectively. Under these operational conditions the sulphide effluent concentration amounted to 41 mg/l, while the acetate and propionate effluent concentrations were 1008 and 220 mg/l. The sulphate effluent concentration was 44 mg/l. The assessed sulphide production rate of the cell suspension was 60 mg $S^{2-}$/l.day and the sulphide oxidation rate 9300 mg/l.day.

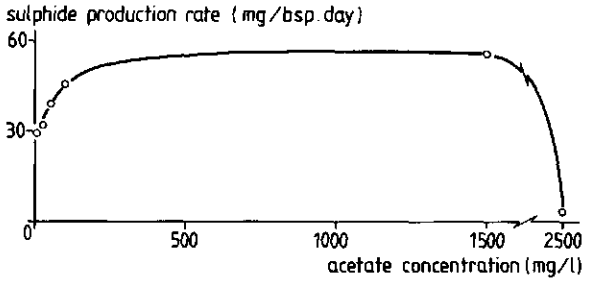


**Figure 4.** Effect of the acetate concentration on the sulphide production rate measured in anaerobic serum bottles. The reactor conditions of the reactor from which the biomass was taken were: acetate influent 50 mg/l, effluent 1 mg/l; propionate influent 10 mg/l, effluent 0,3 mg/l; sulfide influent 119 mg/l, effluent 2 mg/l.

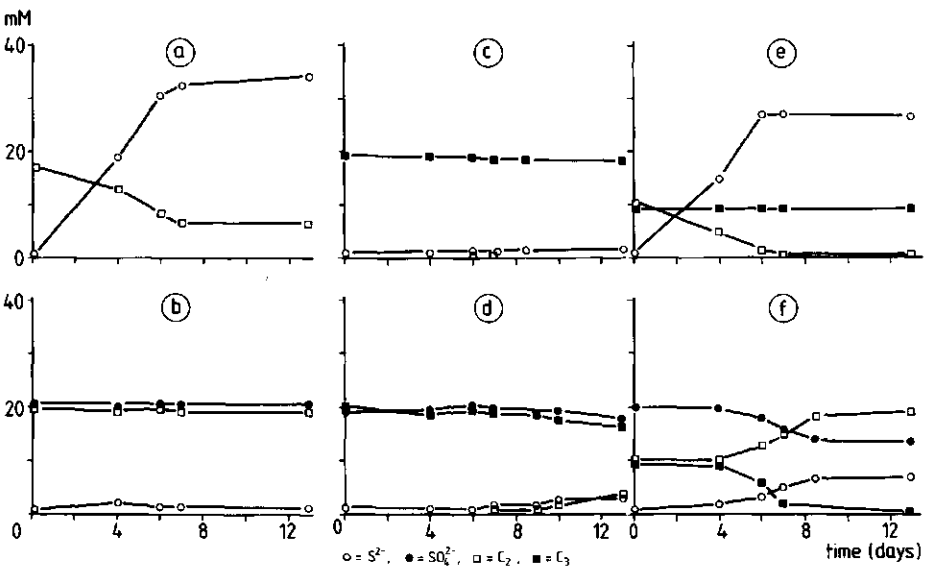


**Figure 5.** The fate of acetate and propionate in the presence of sulphate and sulphur after inoculation with sludge from a sulphide removing reactor incubated at 30°C. a. 20 mM acetate, 80 mM sulphur, b. 20 mM acetate, 20 mM sulphate, c. 20 mM propionate, 80 mM sulphur, d. 20 mM propionate, 20 mM sulphate, e. 10 mM acetate, 10 mM propionate, 80 mM sulphur, f. 10 mM acetate, 10 mM propionate, 20 mM sulphate.

**enrichment experiment**

In order to assess the ratio of sulphide production and acetate and propionate removal a growth experiment was conducted. Anaerobic serum bottles were inoculated with only a few procent seed material, sothat this seed material could not disturb the COD balance. The results in Figure 4 showed already that this was necessary, because without adding acetate 30 mg/bsp.day sulphide was produced. This experiment was also needed to assess the physiological features of the biomass. Therefore bottles with different concentrations of sulphate, sulphur, acetate and propionate were inoculated with sludge from a sulphide removing reactor. No sulphide production was observed in case either fatty acids or sulphur and sulphate were omitted. The results of this experiment are shown in Figures 5a - 5f. Bacteria were isolated and resembled morphologically and physiologically Desulfuromonas acetoxidans (Figure 5a) and Desulfobulbus propionicus (Figure 5d).

An additional experiment was conducted to make a mass balance. Anaerobic bottles with the same media and enrichment cultures were used as inoculum instead of the sludge. The total removal of acetate, propionate, sulphate and the production of sulphide were measured after 7 days. Sulphur was not measured because it was not possible to take homogenuous sulphur samples. The results of this experiment are shown in Table 3.

**Table 3.** balance study of sulphate and sulphur reducing bacteria appearent in sulphide removing reactors

| medium | $C_2$ removal (mM) | $C_3$ removal (mM) | $SO_4^{--}$ removal (mM) | $S^{2-}$ production (mM) |
|---|---|---|---|---|
| 20 mM $C_2$, 80 mM $S^0$ | 13.0 | | | 36.2 |
| 20 mM $C_2$, 20 mM $SO_4^{--}$ | 0.0 | | 0.0 | 0.0 |
| 20 mM $C_3$, 80 mM $S^0$ | | 0.0 | | 0.0 |
| 20 mM $C_3$, 20 mM $SO_4^{--}$ | -18.6 | 19.6 | 13.3 | 11.7 |

## DISCUSSION

**Effect of pH and temperature**

The temperature and pH were maintained at their optimal values of 30°C and 8.0 in the reactor. Increasing the temperature with 10 °C led to an activity drop of 70%, while decreasing the temperature with 10°C led to an activity drop of 40% (Figure 3). Pfennig & Biebl (1976) found for Desulfuromonas acetoxidans a temperature optimum at 30°C and no growth at all at 40°C. For Desulfobulbus the temperature range for growth is 10 - 43°C and the optimum growth temperature range is 28 - 39°C (Widdel & Pfennig, Bergy's Manual, 1984). Upon increasing or decreasing the pH with one unit the sulphide producing activity decreases 20% (Figure 2). Pfennig & Biebl found for Desulfuromonas acetoxidans an optimal initial pH of 7.8-8.0. For Desulfobulbus the pH range for growth is 6.0 - 8.6 with an optium pH of 7.2.

**Effect of volatile fatty acids**

The results in Table 3 show that acetate is a far more important substrate than propionate concerning the sulphide production rate at 20°C. The sulphide production with only propionate is 16% of that with solely acetate at 20°C, but at 30°C this ratio is increased up to 41%. It can be concluded therefore that the bacteria using acetate are less temperature sensitive than the bacteria using propionate. In

presence of both acetate and propionate the sulphide production rate does not exceed the sum of those found with merely one of these fatty acids as substrate. Sulphide is already produced in the absence of acetate (Figure 4), showing the presence of degradable organic material in the PUR particles. The sulphide production rate is almost constant between 100 and 1500 mg/l acetate and at 2500 mg/l acetate is inhibitory.

Figure 5 shows the fate of acetate and propionate in the presence of sulphur and/or sulphate as electron acceptors. A rapid and complete degradation of acetate was observed in bottles where elemental sulphur was added. In bottles with sulphate no degradation of acetate occurred. This shows that acetate-degrading sulphur-reducing but no acetate-degrading sulphate-reducing bacteria were present in the sludge from the sulphide-oxidation reactor. With propionate as electron donor no degradation occurred in the presence of sulphur; However, sulphate-dependent propionate oxidation started after five to six days of incubation (Figure 5f) or after 9 days (Figure 5d). The difference between these two experiments likely to be be due to little variation in the inoculum. No incubations were made with both sulphur and sulphate as electron acceptors. From the above, however, it is evident that then propionate would have been degraded completely by sequentially propionate-degrading sulphate reducers and acetate-degrading sulphur-reducing bacteria.
It can be concluded from results in Figure 5 and in Table 1 that sulphide production in a sulphide removing reactor mainly must be attributed to the sulphur reducing bacteria and not to the sulphate reducing bacteria, because the sulphur reducing bacteria grow much faster and they can produce 4 mole sulphide from 1 mole of acetate, while the sulphate reducers produce only 0.75 mole sulphide from 1 mole propionate.

The balances presented in Table 3 do not fit completely. The ratio acetate sulphide should be 1 : 4, but we found 1 : 2.8. According to Biebl & Pfennig this ratio should practically be 1 : 3.65 because about 9% acetate is required for the formation of cell material. In the growth experiment shown in Figure 5a the molar ratio sulphide acetate decreases during the experiment. The ratio was for day 4, 6, 7 and 13 was 4.3, 3.5, 3.3 and 3.3, respectively. This decreasing ratio can be explained by sulphide losses through sampling, because sulphide is a very reactive compound and at higher sulphide concentrations the losses are bigger. From Table 3 we learn that 12% of the sulphate removed was not found as sulphide. From the results of Figure 5a it can be concluded that the sulphur reducing bacteria are not inhibited up to very high sulphide concentrations of at least 1000 mg/l. It seems that the bacteria stop growing at a sulphide concentration around 1100 mg/l. This is in accordance with the results of Pfennig & Biebl (1976), who found no inhibition up to a sulphide concentration of 900 mg/l.

**dimensions of carrier material**

In a free cell suspension a sulphide production rate of only 60 mg/l.day has been found which is only 0.6% of the sulphide removal rate, which would mean a sulphide production rate of 300 mg/day for the entire reactor. In the reactor containing 240 PUR particles the sulphide production rate would amount to 2500 - 5000 mg/reactor.day. From this it is obvious that the observed difference in sulphide production rates between reactors with and without PUR particles must be attributed mainly to the presence of anaerobic space inside the PUR particles or in the biofilm. This conclusion is supported further by the results in Table 2. When using smaller PUR particles the anaerobic space inside the particles decreases and therefore the sulphide production rate per $cm^3$ should decrease. The sulphide production rate decreases indeed with decreasing diameter of the PUR particle, but at the same time the sulphide removing capacity also dercreases in the same order of magnitude. The sulphide production rate remains at more or less 4% of the sulphide removal rate. This is also the case when the pore size is increased. Therefore it looks obvious that not the anaerobic space inside the PUR particle determines the sulphide production but probably the anaerobic space inside the biofilm, which is independent of diameter and pore size of the PUR particle. When

using Rasschig rings the sulphide producing activity is only 2% of the aerobic sulphide removing capacity. Therefore it can be concluded that PUR particles are a less suitable carrier material for sulphide removal systems where the influent contains also volatile fatty acids besides sulphide.

**start up phase**

Using the information given above, the behaviour of the reactor (Figure 1) during the start up phase can be explained. One day after starting the reactor sulphide is removed for more than 90 %, but acetate and propionate removal is negligible. After 6 days the degradation of these compounds starts. At the same time the sulphur/sulphate reduction activity increases sharply. After 6 days of operation an apparent anaerobiosis occurred as is indicated by the fast increase in the sulphur/sulphate reduction activity. The sulphate concentration decreases during the start up of the reducing bacteria. This could be due to the use of sulphate by the sulphate-reducing bacteria. An additional explanation is that because of the sulphur/sulphate reducing activity the sulphide sludge loading is increased, and this will lead to a lower sulphate production rate (Buisman et al., 1989b).

**sulphur cycle in the reactor**

From the above results a concept for the sulphur cycle inside the sulphide removing reactors can be made. The four most important reactions in which sulphur or sulphide are involved are:

1. $2HS^- + O_2 \dashrightarrow 2S^0 + 2OH^-$
2. $2HS^- + 4O_2 \dashrightarrow 2SO_4^{--} + 2H^+$
3. $CH_3COO^- + 4S + 4H_2O \dashrightarrow 2HCO_3^- + 4H_2S + H^+$
4. $4CH_3CH_2COO^- + 3SO_4^{--} \dashrightarrow 4CH_3COO^- + 3HS^- + H^+ + 4HCO_3^-$

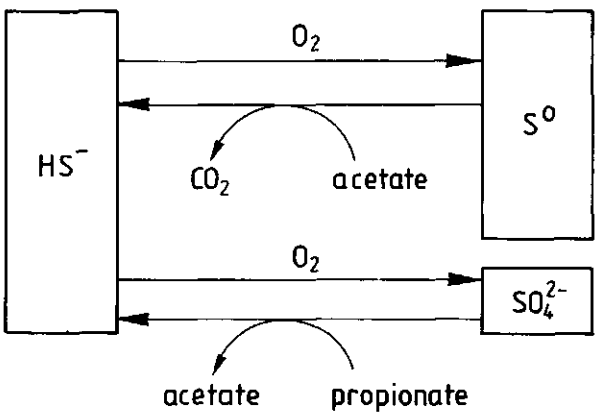


**Figure 6.** Schematic representation of the sulphur cycle in the sulphide removing reactors

Reactions 1 and 2 are performed by sulphide oxidizing bacteria like Thiobacillus (Kuenen, 1975). Desulfuromonas can oxidize acetate (reaction 3) and Desulfobulbus can oxidize propionate (reaction 4). Figure 6 shows schematically how this sulphur cycle takes place in the sulphide removing reactor when acetate and propionate are present in the influent.

Pfennig & Biebl describe a sulphur cycle in the anaerobic environment, in which the sulphur respiration of Desulfuromonas is the counterpart of the sunlight-driven anoxygenic photosynthesis of the green sulphur bacteria with sulphide. The present study shows that not only in anaerobic environments such a sulphur cycle may exist. Also in aerobic environments with biofilms a sulphur cycle can exist, which is carried out by Thiobacilli and Desulfuromonas.

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## CHAPTER 6

# Kinetics of Chemical and Biological Sulphide Oxidation in Aquous Solutions

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## ABSTRACT

A new equation for the non catalyzed chemical oxidation rate of sulphide in a phosphate buffered system at pH 8.0 and 25 °C is found. Our experiments show that the reaction order with respect to the oxygen concentration dependents on the sulphide concentration. The following equation is proposed:

$$R_i = k\,[S]^m[O]^{n\log[S]} \qquad (mg/l.h)$$

The values for the constants m, n, k were found to be 0.408, 0.391 and 0.566 respectively.

The biological oxidation rate was found to be a factor 75 faster than the chemical noncatalyzed oxidation rate at sulphide concentrations around 10 mg/l. At higher sulphide concentrations this difference becomes less, e.g. at 100 mg/l the biological oxidation rate is only 7 times faster than the chemical oxidation rate.

The two cell suspensions used in the experiments behave quite differently towards the sulphide concentration. Cell suspension 1 (taken from a reactor operated at a sulphide concentration of 7 mg/l) exerts its maximal oxidation rate (230 mg/l.h) at a sulphide concentration of 10 mg/l. Cell suspension 2 (taken from a reactor operated at a sulphide concentration of 95 mg/l) exerts its maximal biological oxidation capacity (120 mg/l.h) at a sulphide concentration of 150 mg/l. The total oxidation rate (chemical and biological) of cell suspension 2 at 150 mg/l is 210 mg/l.h (of which only 5% chemical). Cell suspension 1 shows severe substrate inhibition at sulphide concentrations exceeding 10 mg/l, while cell suspension 2 shows no sulphide inhibition up to a sulphide concentration of 600 mg/l.

## SYMBOLS

| | |
|---|---|
| S | total sulphide concentration (mol/l) or (mg/l) |
| S | $= [\ H_2S + HS^- + S^{2-}\ ]$ |
| O | oxygen concentration (mol/l) or (mg/l) |
| $R_i$ | initial oxidation rate (mol/l.h) or (mg/l.h) |
| $R_i$ | $= (dS/dt)_{t=0}$ |
| k | rate constant ($mol^p/l^q$) or ($mg^p/l^q$) |
| I | ionic strenght (mol/l) |
| m | reaction order with respect to sulphide |
| n | reaction order with respect to oxygen |

## INTRODUCTION

Under anaerobic conditions sulphate, sulphite, and organic sulphur compounds present in wastewater are reduced by bacterial activity to sulphide. The odor, toxicity, oxygen demand and corrosion problems caused by sulphide in wastewater frequently necessitate wastewater treatment e.g. oxidation of sulphide to a less harmful form. In the case an oxidation process is applied it is necessary to know whether or not a chemical, a biological or perhaps even a combined process should be employed for sulphide removal.

The review article of Kuhn (et al., 1983) reveals that already extensive studies on the mechanism and the kinetics of the chemical oxidation of sulphide have been made, and that there still does not exist general agreement concerning this matter. Jolley & Forster (1985) found the following relation for the chemical oxidation of sulphide, i.e.

$$R_i = k\,[S]^{1.15}[O]^{0.69} \quad (M/min)$$

for initial sulphide concentrations of 0.05 mM to 0.2 mM. The oxygen concentration was 0.6 mM, the pH was 7 but the temperature was not noted. The value of k (for constant oxygen concentration) amounted to 44.3 for a phosphate buffered system and to 67.6 for sewage.
Millero (et al., 1987) found a quite different relation for sulphide oxygenation at pH 8.0, i.e.

$$R = k\,[S][O] \quad (M/h)$$

with

$$\log k = 11.78 - 3000/T + 0.44\,I^{0.5}$$

The experiments were conducted under conditions of air-saturation, in the temperature range 5 - 65 °C and at an ionic strength range of 0 - 6 M. They used an 0.02 M borate buffer. The initial sulphide concentration was 0.025 mM.
Wilmot (et al., 1988) measured the sulphide oxidation rate in three types of wastewater. They found that at least a part of the sulphide is oxidized biologically. For the wastewater with the lowest biological component (15% of the total oxidation rate appeared to be biological) they found the following relation:

$$R_i = k\,[S]^{0.38}[O]^{0.21} \quad (mg/l.min)$$

The range of sulphide concentrations investigated was between 0.09 - 0.3 mM and for oxygen between 0.16 - 0.62 mM. The pH was 7.0 and the temperature was 20 °C. The value of the rate constant k amounted 0.055.
Finally O'Brien & Birkner presented the following relation:

$$R_i = k\,[S]^{1.02}[O]^{0.80} \quad (M/min)$$

for sulphide concentrations in the range 0.022 - 1.21 mM and for oxygen concentrations in the range 0.21 - 1.1 mM. The pH was 7.55, the temperature was 25 °C and the ionic strength was 0.155 M. For the value of k they found 1.44 in a tris(2-amino-2-(hydroxymethyl)-1,3-propanediol) buffer.

As far as we know no relevant literature is available about the relation of the biological sulphide oxidation rate in relation to the sulphide concentration. According to Jorgensen (1982) it is not clear wether or not sulphur-bacteria can compete with spontaneous chemical oxidation of sulphide.

In this publication we will present results of the measurement of the chemical and biological oxidation rates of the sulphide oxidation in a phosphate buffered system in the sulphide concentration range 0.15 - 28 mM at oxygen concentration 0.25 mM, and at pH 8.0 and a temperature of 25°C.

For the chemical sulphide oxidation we also attempted to assess the reaction order with respect to the oxygen concentration, at the same conditions as mentioned above and in the oxygen concentration range of 0.016 - 0.3 mM. We found that this reaction order with respect to the oxygen concentration (n) is dependent of the sulphide concentration. The relations mentioned above will be compared to the chemical oxidation rate we found.

## MATERIALS AND METHODS

**analyses**
The total sulphide concentration was determined photometrically by the method described by Truper and Schlegel (1964). The oxygen concentration was measured by a WTW oxygen sensor (oxi 219/90). The pH was measured with a pH-electrode (Ingold, 425-60-s7 ing. no. 104253313).

**buffer solutions**
Experiments were conducted at pH 8.0, using phosphate buffered sytems. The chemicals used for this buffer were: $KH_2PO_4$ (20 g/l) and NaOH (5 g/l). When the sulphide concentration in the reactor exceeded 100 mg/l a HCl solution was also injected to keep the pH at 8.

**reactor**
A 0.5 liter air tight and temperature controlled vessel was used, see Figure 1. A magnetic stirrer was used for mixing.

**experimental procedures for chemical oxidation**
The oxygenation of sulphide is affected by small quantities of transition metals and organic compounds (O'Brien & Birkner, 1977), therefore all experimental equipment was rinsed thoroughly with water in order to avoid catalytic effects.

The buffer solutions were oxygenated to near saturation with air, after which the reactor was sealed. The oxygen concentration was monitored over a period of 15 minutes to ensure that it had stabilized at a constant value.

The reaction was initiated by injecting an aliquot of a fresh sulphide stock solution into the reaction vessel. The stock sulphide solution was prepared by dissolving $Na_2S.9H_2O$ in deoxygenated distilled water. The sulphide concentration in the stock solutions was determined at the moment the experiment was conducted. The reaction rate was determined by monitoring the oxygen concentration.

**experimental procedures for biological oxidation**
For the assessement of the biological oxidation rate, cell suspensions were taken from two reactors, which were oxidizing sulphide to sulphur and/or to sulphate under different conditions. The biological sulphide elimination process and the reactors used for that purpose are described in detail in previous communications (Buisman et al., 1989a,b; Chapter 2 and 3). Six samples of 200 ml suspension were taken from these reactors and centrifuged (17000 x g). Hereafter the pellet was washed two times in posphate buffer.

The pellet (from a 200 ml sample) was resuspended in the same posphate buffer as used for determining the chemical oxidation rate. After sealing of the reactor, the endogeneous activity could be measured in this system when no sulphide was present yet. Then the same procedure was repeated, but now in the presence of sulphide as was applied for determining the chemical oxidation rate.

**calculation of sulphide oxidation rate**
The sulphide oxidation rate was calculated using the oxygen removal rate data. This procedure was choossen to prevent disturbance of the oxidation process due to sampling. In the calculation it was assumed that a 0.5 mol oxygen is required for the oxidation of 1 mol sulphide to sulphur. This assumption may not be true for the

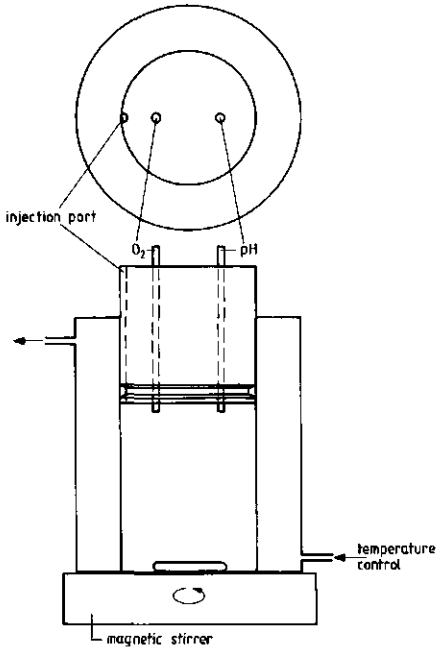


**Figure 1.** Reactor used for sulphide oxidation rate measurements

lower sulphide concentrations, because a bigger fraction of the sulphide may be converted to sulphate or sulphite in that case.

In a previous publication (Buisman et al., 1989a; Chapter 2) we reported that the main oxidation products of biological sulphide oxidation are elemental sulphur and sulphate. We found also (Buisman et al., 1989b; Chapter 3) that in a cell suspension practically no sulphate is formed, if the sulphide concentration exceeds 5 mg/l, at oxygen concentrations up to 9 mg/l and at pH 8. The main reaction product under these circumstances must be sulphur. It is therefore justified to use the factor of 0.5 in the case of the biological oxidation.

For the chemical oxidation of sulphide O'Brien & Birkner (1977) found that a high sulphide/oxygen ratio, along with a total sulphur concentration exceeding 32 mg/l, leads to the formation of sulphur. A low ratio favors the production of sulphite, thiosulphate, and sulphate. The distribution of these reaction products is not affected by the pH of the solution over the range 7.5 - 11. O'Brien and Birkner also found at a low sulphide oxygen ratio that the oxidation of sulphide yields equimolar concentrations of sulphite, thiosulphate (as S), and sulphate. In this case per mol of sulphide oxidized 1.38 mol of oxygen is required. Therefore the chemical oxidation rates at sulphide concentrations below 30 mg/l shown in Figure 2 are slightly too high, because the sulphide/oxygen factor should be between 0.5 and 1.38 in stead of 0.5 as was assumed in our calculations.

## RESULTS

Four experiments were conducted in order to assess the biological and noncatalyzed chemical sulphide oxidation rates, with respect to the sulphide concentration. First of all the auto-oxidation rate (chemical) was measured. Then the biological oxidation rates of the two different cell suspensions were measured. The differences between the chemical and biological oxidation rates might be due to the presence of elemental sulphur and/or the presence of some trace elements in the cell suspensions, which both have a catalytic effect with respect to the sulphide oxidation reaction (Chen & Morris, 1972). For this reason we also conducted an experiment with a boiled (3 minutes) cell suspension. In this way it was possible to estimate the effect of sulphur and trace elements possibly present in the cell suspension.

**Table 1.** the difference in operational conditions between the two reactors from which the cell suspensions were taken

| | $S^{2-}$ loading rate (mg/l.h) | $S^{2-}$ removal rate (mg/l.h) | $S^{2-}$ concentration in reactor (mg/l) |
|---|---|---|---|
| reactor 1 | 146 | 144 | 7 |
| reactor 2 | 417 | 402 | 95 |

The results of these experiments are shown in Figure 2.
Table 1 provides the main differences in operational conditions between the two reactors with the cell suspensions.

In order to be able to compare the biological and chemical oxidation rates, all four experiments were conducted in the same way. The pellet from a 200 ml cell suspension was resuspended each time in 500 ml phosphate buffer (dilution factor 2.5) for each measurement. Polysulphide formation became visible from the green

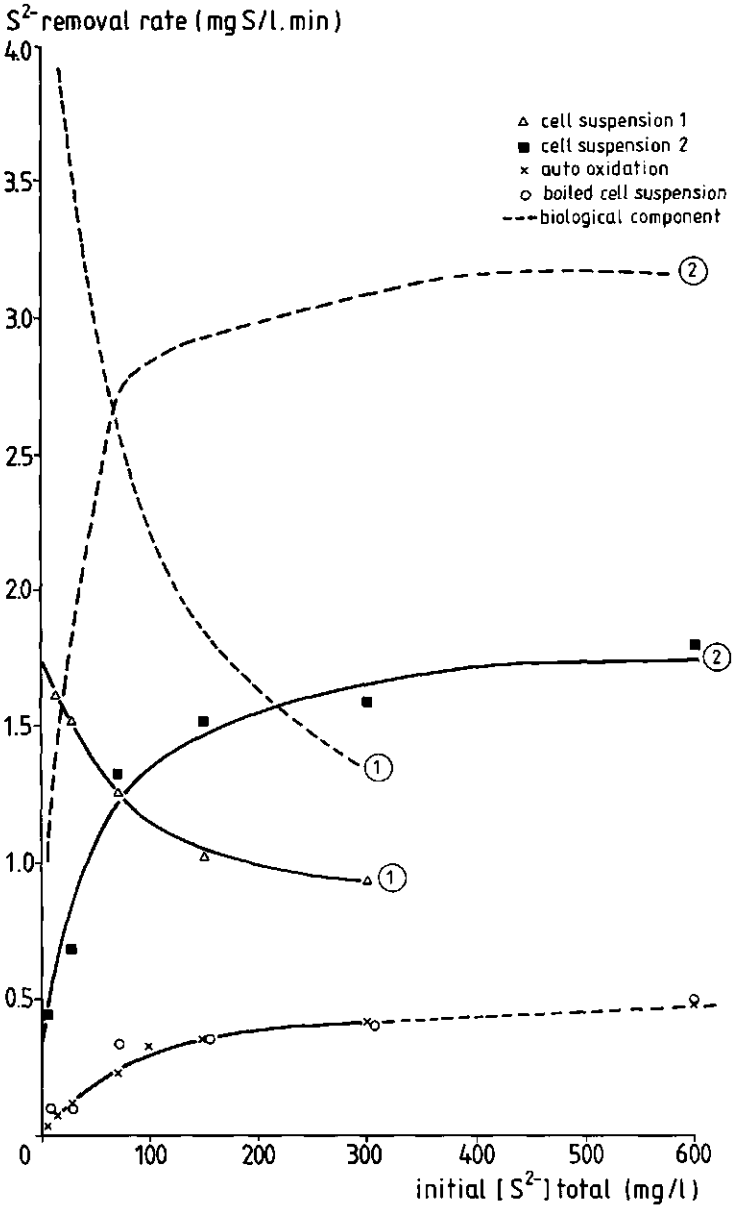


**Figure 2.** Sulphide removal rates after injection of a sulphide solution in a phosphate buffer, a phosphate buffer with boiled cell suspension and in phosphate buffer with two different cell suspensions (described in Table 1), at pH 8, 25°C and oxygen concentration of 8 mg/l. The dotted lines show the calculated biological oxidation rate corrected for the chemical oxidation and dilution

colour appearing in the medium when the sulphide concentration in the reactor exceeded 75 mg/l.

For the noncatalyzed chemical oxidation only, the reaction order with respect to the oxygen concentration (n) was assessed. In order to assess the reaction order n, the oxygen concentration was variated from 0.1 to 8.5 mg/l and the sulphide concentration from 5 to 300 mg/l. The results of these experiments are summarized in Table 2 (total number of data is 88).

**Table 2.** Chemical oxidation rate (mg/l.min) at different sulphide and oxygen concentrations (mg/l)

| $HS^-$ conc | 5 | 10 | 20 | 50 | 100 | 300 |
|---|---|---|---|---|---|---|
| $O_2$ conc | | | | | | |
| 8.5 | 0.0317 | 0.0746 | 0.0910 | 0.222 | 0.35 | |
| 8 | | | 0.0514 | | | 0.82 |
| 7 | 0.0365 | 0.0523 | | 0.220 | 0.43 | 1.00 |
| 6 | | 0.0640 | | 0.210 | 0.28 | 0.92 |
| 5 | | 0.0424 | | | | |
| 4 | 0.0367 | 0.0336 | 0.0666 | 0.127 | 0.24 | 0.63 |
| 3 | 0.0317 | 0.0386 | | 0.115 | 0.16 | |
| 2 | 0.0283 | 0.0288 | 0.0450 | | 0.092 | 0.39 |
| 1 | 0.0180 | 0.0246 | 0.0241 | 0.0488 | | 0.24 |
| 0.5 | 0.0170 | 0.0160 | 0.0198 | 0.0258 | 0.042 | 0.13 |
| 0.1 | | | 0.0080 | 0.0103 | | |

## DISCUSSION

In order to be able to describe the chemical oxidation of sulphide with oxygen we tried out five models. The constants in each model are calculated from the data in Table 2. Also the error ΣR was calculated, which can be determined according to the following equation (Ordinary Least-square Estimate; Norton, 1986):

$$\Sigma R = \sum_{\text{all exp.}} (\text{measured values} - \text{calculated values})^2$$

The variance of the oxidation rate should be:

$$\text{var}(R_i) = \frac{\Sigma R}{(\text{number of exp.} - 1)}$$

Model I assumes that the exponents m and n are both constant and independent of the sulphide and oxygen concentration. Model II suggests that a releation between n and the sulphide concentration exists and also a relation between m and the oxygen concentration. In model III m is constant and n is dependent on the sulphide concentration and in model IV n is constant and m is dependent on the oxygen concentration. Model V is similar as model III but now n is dependent on the sulphide concentration according to a logorithmic function. The equations and calculated constants are:

**Table 3.** Values of the noncatalyzed chemical oxidation rates on the basis of literature data, calculated for three sulphide concentrations and those of the biological oxidation as measured in the present study (oxygen concentration 8 mg/l, temperature 25 °C, pH 8.0)

| number | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| $S^{2-}$ conc. (mg/l) | [ sulphide oxidation rates mg/l.h) ] | | | | | |
| 10 | 3.0 | 0.97 | 26 | 0.16 | 12 | 230 |
| 100 | 18.6 | 10 | 370 | 1.6 | 29 | 120 |
| 1000 | 108.6 | 106 | 5200 | 16 | 71 | - |

1. chemical oxidation rate, this study
2. O'Brien & Birkner (1977)
3. Jolley & Forster (1985)
4. Millero (et al. 1987) we used I=0.043M, k=63.3
5. Wilmot (et al., 1988)
6. maximal biological rate, this study

Despite the equations proposed by the various authors differ significantly, it can be inferred from Table 3 that the reaction rates are in the same order of magnitude. The reason why so different equations were found is hard to give. All the researchers mentioned above used a different analysis method to determine sulphide and the sulphide oxidation rate, while most of them used also a different buffer solution. Moreover in all cases the investigators conducted their experiments at distinctly lower sulphide concentrations, ranging from 0.8 - 39 mg/l, as compared to our investigations, i.e. 10 - 600 mg/l. As is shown in the present study the reaction order of oxygen is very likely dependent on the sulphide concentration, which could mean that the range of the sulphide concentrations determines the reaction order with respect to the oxygen concentration in the different investigations. The results of Jolley & Forster deviate very significantly. Very likely uncorrect units were presented, they should not be in M/min but M/h. In the latter case the calculated oxidation rates become a factor 60 lower and then become of the same order of magnitude as those in our experiments and of the other researchers.

From the results in Figure 2 it also can be inferred that biological oxidation proceeds distinctly faster than the noncatalyzed chemical oxidation. At sulphide concentrations around 150 mg/l the biological oxidation rate still 7 times faster than the chemical. The biological oxidation rate below 10 mg/l is a factor 75 faster than the chemical oxidation rate. At higher sulphide concentrations a larger part of the oxidation rate measured in the cell suspensions must be due to chemical oxidation. To find the biological component of the total oxidation rate, the chemical activity is subtracted from the total oxidation rate, see the dotted lines in Figure 2.

The oxidation rates in the reactors with the cell suspensions (Table 1) as well as the measurements made on the cell suspensions (Figure 2; dotted lines) can also be compared. Reactor 1 exerts a total oxidation capacity of 144 mg/l.h at a sulphide concentration of 7 mg/l (Table 1), as was calculated from the difference of the sulphide influent and effluent concentrations and the reciproke of the recidence time. With the cell suspension of reactor 1 an biological oxidation capacity of 230 mg/l.h is found at 10 mg/l (Figure 2). At 10 mg/l sulphide the chemical oxidation is below 2 % of the biological oxidation.

Reactor 2 exerts a total oxidation capacity of 400 mg/l.h at a sulphide concentration of 95 mg/l (Table 1). With the cell suspension of reactor 2 an biological oxidation capacity of 118 mg/l.h at 100 mg/l is found (Figure 2). In this

**model I:** $R_i = k[S]^m[O]^n$ (mg/l.h)

$k = 0.2874$
$n = 0.642$
$m = 0.597$
$\Sigma R = 0.932$
$var(R_i) = 0.0108$

**model II:** $R_i = K[S]^{m[O]}[O]^{n[S]}$ (mg/l.h)

$k = 1.65$
$n = 0.00095$
$m = 0.0632$
$\Sigma R = 0.999$
$var(R_i) = 0.0116$

**model III:** $R_i = k[S]^m[O]^{n[S]}$ (mg/l.h)

$k = 0.810$
$n = 0.0030$
$m = 0.402$
$\Sigma R = 0.845$
$var(R_i) = 0.0098$

**model IV:** $R_i = k[S]^{m[O]}[O]^n$ (mg/l.h)

$k = 15.36$
$n = 0.0622$
$m = 0.0707$
$\Sigma R = 1.241$
$var(R_i) = 0.0143$

**model V:** $R_i = k[S]^m[O]^{n\log[S]}$ (mg/l.h)

$k = 0.566$
$n = 0.391$
$m = 0.408$
$\Sigma R = 0.642$
$var(R_i) = 0.00738$

Model V fits the experimental values the best, as can be seen from the low value for $var(R_i)$.

That the reaction order with respect to the oxygen concentration n, increases with increasing sulphide concentrations can be explained from the difference in product formation at different sulphide concentrations. At high sulphide concentrations only sulphur is produced and at lower sulphide concentrations also sulphate, sulphite and thiosulphate are produced (see Materials and methods section) and therefore less oxygen is needed at high sulphide concentrations which very likely influences n.

The data in table 3 allow a comparison of the results obtained in the present study with those in other investigations in which more or less the same circumstances were applied, e.g. a constant pH value around 8 and a temperature around 25 °C. The chemical oxidation rate for the cases shown in Table 3 were calculated for three sulphide concentrations e.g. 10, 100 and 1000 mg/l and for an oxygen concentration of 8 mg/l.

case (sulphide concentration of 100 mg/l), the chemical oxidation obviously is no longer negligible, but is already 15 %. The maximal biological oxidation rate (corrected for the chemical activity) of cell suspension 2 is 120 mg/l.h and is found at a sulphide concentration of 150 mg/l. The biological oxidation rate calculated with the BOM experiment is a factor 3.4 lower than the total oxidation rate found in the reactor, while the noncatalysed chemical oxidation rate amounts to only 16.8 mg/l.h. An explanation for this difference could be the presence trace elements in the reactor but not in the experiments shown in Figure 2. These trace elements catalyse both the chemical and biological oxidation. Also oxygen could be inhibitory for the bacteria present in high loaded reactors. The activity measurements are conducted at an oxygen concentration of 8 mg/l, while the oxygen concentration in the reactor only amounts to 4 mg/l.

The cell suspension taken from reactor 2 is less inhibited by a high sulphide concentrations, than cell suspension 1. For all organisms, sulphide is a toxic compound, with inhibition constants (the concentration at which the specific growth rate is reduced to half its maximal value) of 60 -120 mg/l (van Gemerden 1984). It is obvious that the bacteria grown in a sulphide removing reactor at 94 mg/l have a very high resistance against sulphide inhibition. The inhibition constant for the cell suspension 1 is around 100 mg/l, while with cell suspension 2 no inhibition can be found up to a sulphide concentration of 600 mg/l.

At this stage of our investigations we cannot explain the significant differences between the two cell suspensions. More research is required for that purpose.

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## CHAPTER 7

# Kinetic Parameters of a Mixed Culture oxidizing Sulphide and Sulphur with Oxygen

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## ABSTRACT

Kinetic parameters of the biological sulphide oxidation are described. The influence of the sulphide loading rate on the growth yield and on the specific oxidation rate were investigated with free cell suspensions. It is concluded that at least two types of bacteria were present, viz. sulphate producers (type A) that grow at sulphide loading rates up to 200 mg/l.h and sulphur producers (type B) that grow at higher loading rates. Type A bacteria have a growth yield of 3 g DS/mol S, while type B bacteria have a growth yield of 0.3 g DS/mol S. Type A has a high affinity for sulphide and is inhibited by sulphide at sulphide concentrations exceeding 10 mg/l. Type B has a low affinity for sulphide and is not inhibited by sulphide, but by oxygen.

## INTRODUCTION

Under anaerobic conditions sulphate, sulphite, and organic sulphur compounds present in wastewater are reduced by bacterial activity to sulphide. The odour, toxicity, oxygen demand and corrosion problems caused by sulphide in wastewater frequently necessiate wastewater treatment, e.g. oxidation of sulphide to a less harmful form. In case an oxidation process is applied it is necessary to know whether or not a chemical or biological process should be employed for sulphide removal. In a previous communication (Buisman et al., 1989a) it was shown that the biological oxidation of sulphide with oxygen is significantly faster than the chemical non-catalyzed oxidation of sulphide with oxygen. We found that the chemical oxidation of sulphide with oxygen in a phosphate buffered system at pH=8 and at 20°C can be described with the following equation:

$$R_i = k\,[S]^m[O]^{n\log[S]} \qquad (mg/l.h)$$

in which: $R_i$ = initial oxidation rate (mg/l.h)
S = total sulphide concentration (mg/l)
O = oxygen concentration (mg/l)
k = rate constant
m = reaction order with respect to sulphide
n = reaction order with respect to oxygen

The values for the constants m, n, k were found to be 0.408, 0.391 and 0.566 respectively (Buisman et al., 1989a).

The principle of the biotechnological sulphide removal process is that sulphide is microbiologically converted to elemental sulphur with oxygen. The process has already been described previously (Buisman et al., 1989b)

The advantages of this process are: a. no catalyst or oxidants necessary other than air, b. no chemical sludge to be disposed, c. little biological sludge is produced, d. low energy consumption, e. possible reuse of sulphur, f. little if any sulphate or thiosulphate discharge

The micro-organisms present in the sulphide removal reactors are most likely obligate and facultative chemolithotrophic colourless sulphur bacteria like

Thiobacillus (Kuenen & Beudeker, 1982). The end-products of the biological sulphide oxidation are elemental sulphur and sulphate. The biological overall reactions in an aerobic sulphide removal system are (Kuenen, 1975):

$$2HS^- + O_2 \text{ ---> } 2S^0 + 2OH^-$$

$$2S^0 + 3O_2 \text{ ---> } 2SO_4^{--} + 2H^+$$

As far as we know hardly any relevant literature is available about kinetic parameters of microbial cultures that convert sulphide into elemental sulphur. More information is available about the biological oxidation of thiosulphate and sulphide into sulphate. The growth yield of autotrophic sulphide oxidizers is rather low, around 5 - 13 gram dry mass of cell material per mole of substrate, when sulphate is the end-product (Kelly, 1982). Timmer-ten Hoor (1981) found identical growth yields with Thiobacillus denitrificans grown in a chemostat for sulphide and thiosulphate as substrate (using nitrate instead of oxygen as electron acceptor). This is consistent with the view that in both oxidations eight electrons per molecule oxidized are transferred. Beudeker (et al., 1982) found also identical growth yields on thiosulphate and sulphide with Thiobacillus neapolitanus, using oxygen and very low sulphide concentrations (< 0.02 mg/l). On the other hand Kelly (1982) found that sulphide dependent $CO_2$ fixation by cell suspensions was only about 70% of that with thiosulphate. A possible reason for this could be that less sulphide was available for bacterial growth due to chemical oxidation of sulphide or possibly sulphide exerted an uncoupling effect as a toxic substrate, thereby lowering the efficiency of the $CO_2$ fixation. Therefore it is not sure whether or not the above mentioned values found for thiosulphate can be used for sulphide oxidation.

Growth yield is defined as: $\frac{dX}{dt} = -Y\frac{dS}{dt}$

In a steady state situation ($dX/dt = 0$) in a completely mixed reactor, a material balance over the reactor will give:

$$Y = X/(S_i - S_e)$$

Part of the sulfide will be oxidized chemically and therefore less sulphide will be available for the biomass. The above mentioned equation needs a correction term to account for that, viz.:

$$Y_c = X/(S_i - S_e - S_c)$$

in which:
$Y$ = growth yield (mg-N/mg-S)
$Y_c$ = growth yield corrected for the chemical oxidation (mg-N/mg-S)
$X$ = biomass concentration (mg-N/l)
$S_i$ = sulphide influent concentration (mg/l)
$S_e$ = sulphide effluent concentration (mg/l)
$S_c$ = part of influent concentration that is oxidized chemically (mg/l)

The specific growth rate of bacteria oxidizing thiosulphate to sulphate is 0.35 1/h for obligate chemolithotrophs like Thiobacillus neapolitanus on a single substrate and 0.10 1/h for facultative chemolithotrophs species like Thiobacillus A2 on a single substrate (thiosulphate) and 0.22 1/h on a mixed substrate (thiosulphate and acetate) (Beudeker et al., 1982). In mixed cultures also mixotrophic organisms may be present in the reactor, even when no organic compounds are present in the influent (Sublette & Sylvester, 1987).

The aim of the present study is to assess kinetic data for a mixed culture that converts sulphide into elemental sulphur and to compare these results with a culture producing merely. The kinetic parameters studied are growth yield, substrate

inhibition and specific activity. Growth rates were measured using wash-out experiments (Wiegant, 1986).

## MATERIALS AND METHODS

### analyses

The total sulphide concentration was determined photometrically by the method described by Truper and Schlegel (1964). The sulphate concentration was measured using a HPLC as described previously (Buisman et al., 1989b; Chapter 2).

The biomass concentration was measured as the total nitrogen concentration according to the method developed by Novozamsky et al. (1983). We used this method because it is not disturbed by the high sulphur concentrations present in the biomass samples.

The oxygen concentration was measured by a WTW oxygen sensor (oxi 219/90) and the pH with a pH-electrode (Ingold, 425-60-s7 ing. no. 104253313).

### activity measurements

The chemical and biological sulphide oxidation rates were measured in a 0.5 liter biological oxygen monitor using a phosphate buffered system as described previously (Buisman et al., 1989a; Chapter 6).

### reactors

Two completely mixed 8.3 liter reactors without carrier material were used, which were described in detail previously (Buisman et al., 1989b; Chapter 2). The process temperature was maintained constant at 23°C in all experiments.

## RESULTS

### growth yields

The biomass concentration at different volumetric sulphide loading rates was measured in order to assess the growth yield of the mixed cultures. For this purpose, at each loading rate steady states were established at different liquid retention times. The results of the experiments are summarized in Table 1. Chemical oxidation will remove a significant part of the sulphide available to the biomass. In order to be able to estimate the contribution of the chemical oxidation, we measured the sulphide oxidation rate of the supernatant after centrifugation of the reactor medium, using the activity measurement test, assuming biological activity is negligible in the supernatant. At sulphide effluent concentrations below 2 mg/l the chemical oxidation was found to be less than 1% of the total oxidation. The percentage of the chemical oxidation of the total oxidation is also shown in Table 1. The relation between the imposed sludge loading rate and the growth yield is shown in Figure 1.

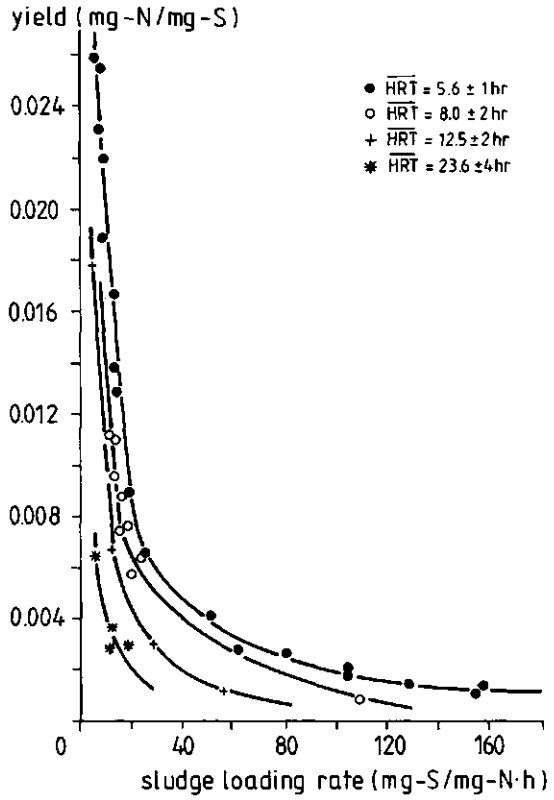


**Figure 1.** Growth yields as a function of the sludge loading rate at four different retention times, measured at the steady state situations mentioned in Table 1; the growth yields are corrected for the chemical oxidation; the oxygen concentration varied between 3.0 and 8.0 for the different steady states

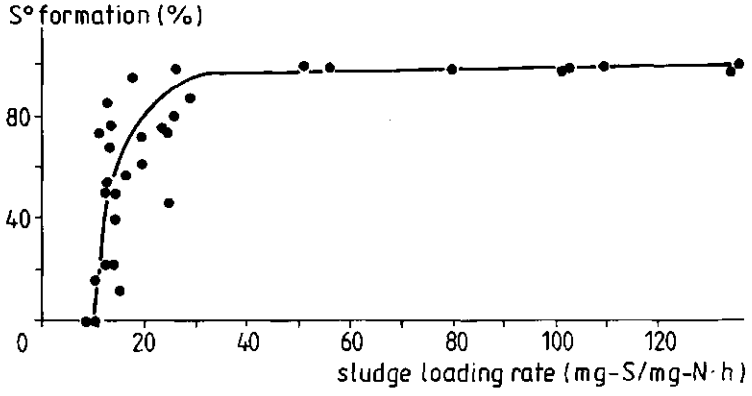


**Figure 2.** Percentage of sulphide converted to sulphur as a function of the sludge loading rates at steady states situations mentioned in Figure 1

**Table 1.** Steady state situations at different hydraulic retention times (HRT) and volumetric sulphide loading rates; also the correction for chemical oxidation and the sulphide concentration are shown.

| HRT (h) | volumetric load (mg $S^{2-}$/l.hr) | biomass effl. conc (mg-N/l) | sulphide effl. conc. (mg/l) | correction for chemical oxidation (%) |
|---|---|---|---|---|
| 4.2 | 91 | 6.2 | 1.8 | <1 |
| 4.8 | 202 | 4.0 | 2.0 | <1 |
| 5.0 | 50 | 5.5 | <0.2 | <1 |
| 5.0 | 140 | 5.5 | 0.9 | <1 |
| 5.4 | 742 | 4.3 | 52 | 5.1 |
| 5.4 | 55 | 4.2 | 0.6 | <1 |
| 5.5 | 76 | 5.7 | 0.5 | <1 |
| 5.5 | 50 | 5.2 | 0.7 | <1 |
| 5.7 | 55 | 2.6 | 0.8 | <1 |
| 6.0 | 123 | 4.8 | 1.2 | <1 |
| 6.0 | 240 | 3.0 | 32 | 10.9 |
| 6.0 | 404 | 3.9 | 55 | 9.9 |
| 6.0 | 477 | 3.0 | 73 | 10.5 |
| 6.0 | 81 | 4.4 | 0.5 | <1 |
| 6.0 | 123 | 4.9 | 2.5 | 3.0 |
| 6.0 | 240 | 3.9 | 4.5 | 3.2 |
| 6.0 | 404 | 3.2 | 31 | 6.4 |
| 6.0 | 477 | 4.6 | 49 | 7.7 |
| 6.0 | 550 | 5.3 | 85 | 10.2 |
| 6.5 | 140 | 6.0 | <0.2 | <1 |
| 6.8 | 76 | 5.8 | 0.5 | <1 |
| 6.9 | 90 | 4.3 | 8.9 | 10.7 |
| 7.0 | 77 | 4.7 | <0.2 | <1 |
| 7.8 | 125 | 10.9 | 0.8 | <1 |
| 8.2 | 77 | 6.0 | 0.5 | <1 |
| 9.0 | 140 | 9.4 | 2.3 | 2.4 |
| 9.0 | 140 | 7.2 | 3.5 | 3.3 |
| 10.5 | 235 | 2.1 | 136 | 35.4 |
| 11.3 | 91 | 16.9 | 5.9 | 7.7 |
| 11.7 | 287 | 10.0 | <0.2 | <1 |
| 12.0 | 140 | 11.3 | <0.2 | <1 |
| 15.0 | 235 | 4.3 | 162 | 40.1 |
| 19.0 | 235 | 13.6 | 90 | 24.8 |
| 20.0 | 140 | 10.4 | 0.4 | <1 |
| 20.6 | 79 | 5.7 | 0.5 | <1 |
| 25.7 | 92 | 14.3 | 3.8 | 5.5 |
| 28.0 | 140 | 11.4 | 2.0 | 2.2 |

**sulphur formation**

In order to assess the relation between the sulphur and/or sulphate production and the sulphide sludge loading rate, the sulphur formation was measured in the steady states mentioned in Table 1. The results are shown in Figure 2. At sulphide loads below 10 mg-S/mg-N.hr merely sulphate is formed, while at sulphide loads exceeding 43 mg-S/mg-N.hr merey sulphur is produced. The oxygen concentration was between 3 and 7 mg/l in all the steady states shown in Figure 2.

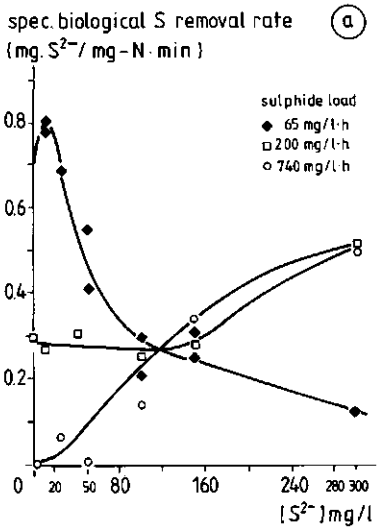


**Figure 3a.** The effect of the sulphide concentration at oxygen concentration of 7 mg/l on the specific activity of three cel suspensions grown at different sulphide loading rates, viz. 65, 200 and 740 mg/l.h at an oxygen concentration of 3 mg/l

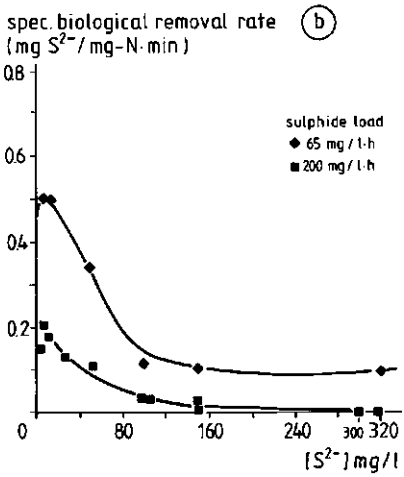


**Figure 3b.** The effect of the sulphide concentration at oxygen concentration 7 mg/l on the specific activity of two cel suspensions grown at different sulphide loading rates, viz. 65 and 200 mg/l.h at an oxygen concentration of 7 mg/l

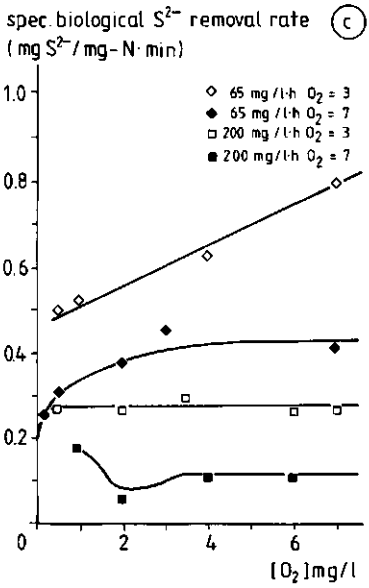


**Figure 3c.** The effect of the oxygen concentration at sulphide concentration 10 mg/l on the specific activity of four cel suspensions grown at different sulphide loading rates, viz. 65 and 200 mg/l.h and different oxygen concentrations, viz. 3 and 7 mg/

**specific biological sulphide oxidation rate**

In order to improve our understanding of the bacterial behaviour in the sulphide oxidizing reactors, the specific biological activity under different conditions was measured. The influence of the oxygen concentration and the sulphide loading rate on the specific sulphide oxidation rate were assessed at five steady states, differing in both the sulphide loading rate and the oxygen concentration (Table 2). The pH was maintained constant at pH=8.

**Table 2.** Process conditions at the five steady states at which the specific oxidation rates were measured.

| $S^{2-}$ load (mg/l.h) | $O_2$ conc (mg/l) | $S^{2-}$effl.conc (mg/l) | $SO_4$ formation (%) | biomass effl. conc (mg-N/l) |
|---|---|---|---|---|
| 65 | 7.0 | 0.6 | 100 | 4.2 |
| 65 | 3.0 | 0.8 | 100 | 2.6 |
| 200 | 7.0 | 2.0 | 5 | 4.0 |
| 200 | 3.0 | 0.0 | 18 | 7.6 |
| 740 | 4.0 | 52.1 | 5 | 4.3 |

The specific activity of the biomass, grown at the different steady states, was measured in the activity assay, at different sulphide and oxygen concentrations, in order to assess the affinity (relation between the concentration and oxidation rate) of the biomass for sulphide and oxygen. Sulphide affinity was measured at an initial oxygen concentration of 7.0 mg/l and oxygen affinity at an initial sulphide concentration of 10 mg/l. The specific biological sulphide oxidation rate was measured in a phosphate buffered system at pH=8. A correction for the chemical oxidation proceeding in phosphate buffered systems was applied, using the equation mentioned in the introduction.

The measured specific oxidation rate is shown as a function of the initial sulphide concentration in Figures 3a and 3b. Figure 3a shows the specific oxidation rate of biomass grown at an oxygen concentration of 3 mg/l, while Figure 3b refers to biomass grown at an oxygen concentration of 7 mg/l. The specific oxidation rate as a function of the initial oxygen concentration is shown in Figure 3c.

In the activity measurements, the oxidation process was found to proceed in two stages, when the initial sulphide concentration was below 10 mg/l. The first stage proceeded faster than the second stage, and the amount of oxygen consumed here corresponds exactly to that necessary to oxidize all sulphide to sulphur. Therefore we assume that merely sulphur is formed in the first stage. The measurements shown in Figures 3a, 3b and 3c all refer to the oxidation rate of this first stage. At sulphide concentrations exceeding 10 mg/l the second stage was not found because the initial oxygen concentration did not exceed 7 mg/l, making a distinction between oxygen limitation and a second stage impossible.

In an additional experiment the specific oxidation capacity of a cell suspension grown at a HRT of 10 hour and at a sulphide loading rate of 240 mg/l.h was found to be 0.004 mg$S^{2-}$/mg-N.min. This indicates that a similar affinity is found with biomass grown at 240 mg/l.h as with a biomass grown at 740 mg/l.h.

**wash-out experiments**

In order to assess the specific growth rate of the sulphide oxidizing biomass, wash-out experiments were conducted as described by Wiegant (1986), at two different sulphide loading rates, viz 50 and 145 mg/l.h. The principle of the method is that, at a constant influent concentration, the hydraulic retention time (HRT) is decreased in such a way that the biomass will wash-out. From the decreasing biomass concentration the growth rate can be calculated.

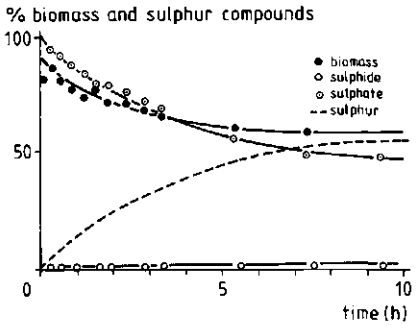


**Figure 4a.** Wash-out experiment an influent concentration of 275 mg/l; The HRT is decreased form 5.5 hour to a HRT of 2.5 hour. The pH and oxygen concentration were maintained constant during the experiment and amounted to 8 and to 6 mg/l respectively.

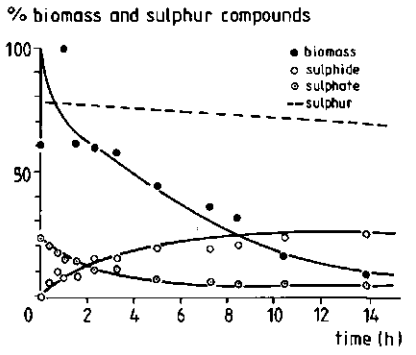


**Figure 4b.** Wash-out experiment at an influent concentration of 725 mg/l. The HRT is decreased form 5.0 hour to a HRT of 2.5 hour. The pH and oxygen concentration were maintained constant during the experiment and amounted to 8 and to 6 mg/l respectively.

In the first experiment a steady state was achieved at a HRT of 5.5 hour and a sulphide influent concentration of 275 mg/l, with a biomass concentration of 5.2 mg-N/l. The HRT then was decreased to 2.5 hour (Figure 4a). The results show that the sulphide concentration did not increase, but that the end-product changed from sulphate to sulphur. Complete wash-out of the culture did not occur, but a new steady state established, implying a maximum specific growth rate of at least 0.4 1/h.

The second experiment was started at a HRT of 5 hour and a sulphide influent concentration of 725 mg/l, with an initial biomass concentration of 5.5 mg-N/l. The HRT was decreased to 2.5 hour (Figure 4b). The results indicate that sulphide inhibition might occur, because the sulphide concentration increased up to 191 mg/l. The growth rate that can be calculated from Figure 4b amounted to 0.26 1/h.

## DISCUSSION

### sulphur formation

The relation between the sulphide sludge loading rate and the percentage of sulphur formation follows from Figure 2. Stefess and Kuenen (1989) found the same kind of relationship for a pure culture of Thiobacillus neapolitanus, but they found sulphur formation at loads exceeding approximately 0.8 mg-S/mg-N.h, whereas we find sulphur formation at sulphide loading rates exceeding 10 mg-S/mg-N.h. This might be due to the different kind of biomass that they used.

### substrate inhibition

The results shown in Figure 3a (biomass grown at an oxygen concentration of 3 mg/l) indicate that biomass, cultivated at a volumetric loading rate of 65 mg/l.h, is inhibited by sulphide (at least 50 % inhibition at 60 mg/l sulphide), while biomass grown at a sulphide loading rate of 740 mg/l.h apparently is not inhibited by sulphide. At a sulphide loading rate of 200 mg/l.h no inhibition was found either. However, the results in Figure 3b (biomass grown at an oxygen concentration of 7 mg/l) show that in this case substrate inhibition is found with biomass cultivated at 200 mg/l.h. Therefore we can conclude that biomass (cultivated at 200 mg/l.h) grown at a steady state oxygen concentration of 3 $mgO_2/l$ shows no sulphide inhibition, while biomass grown at a steady state oxygen concentration of 7 $mgO_2/l$ shows severe inhibition (Figure 3a and 3b).

The results presented in Figure 3a indicate that at volumetric loading rates exceeding 200 mg/l.h a biomass develops that is not inhibited by sulphide up to sulphide concentrations of 300 mg/l. In a previous research (Buisman et al., 1989a; Chapter 6) we found very similar results. Biomass grown at a volumetric sulphide loading of 420 mg/l.h shows no inhibition up to a sulphide concentration of 600 mg/l, while a cell suspension grown at a volumetric sulphide loading of 145 mg/l.h shows substrate inhibition at sulphide concentrations exceeding 10 mg/l. Apparently a high volumetric sulphide loading rate selects a biomass that is not sensitive for sulphide inhibition. These bacteria will develop in the reactor at a sulphide loading rates exceeding 150-200 mg/l.h and at a low oxygen concentration (around 3 mg/l). At higher oxygen concentrations substrate inhibition is still found up to a sulphide loading rate of 200 mg/l.h.

### specific biological sulphide oxidation

Apparently there are at least two different types of biomass present in the mixed culture, one developing at low sulphide loading rates and one at high sulphide loading rates. We conclude this from the following observations:

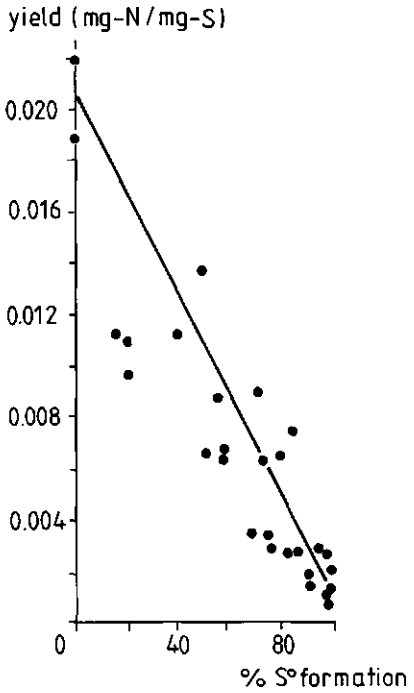


**Figure 5.** The relation between the growth yield and the percentage of sulphur formation calculated for the steady states mentioned in Table 1 and Figure 1. The values for the growth yield are corrected for the chemical oxidation.

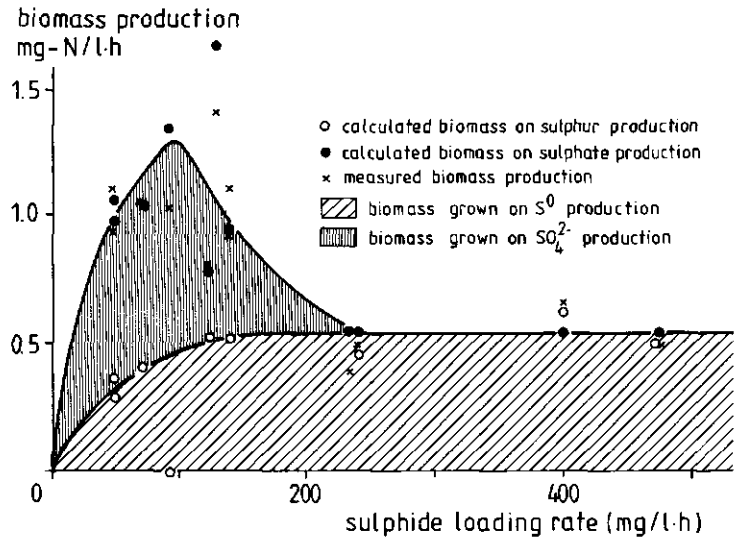


**Figure 6.** The biomass production as a function of the sulphide volumetric loading rate. Besides the measured values also the calculated values based on the sulphate and sulphur formation and on the values for the growth yields (0.011 and 0.0012 respectively) are shown. The calculated values for biomass production based on sulphate formation are added to the calculated values based on sulphur prodcution, sothat two areas are found, viz. biomass production on sulphate and on sulphur. The measured values refer to steady state situations mentioned in Table 1, at oxygen concentrations below 6 mg/l and a HRT between 5 and 7 hour.

a. significantly different sulphide affinities and maximal oxidation capacities are found for biomass grown at low and high loading rates. From the results in Figure 3a it can be estimated that the overall maximum oxidation rate for biomass grown at an oxygen concentration of 3 mg/l and a sulphide loading rate of 65 mg/l.h amounts to 0.8 $mgS^{2-}$/mg-N.min at a sulphide concentration of 10 mg/l, while biomass grown at a sulphide loading rate of 740 mg/l.h has a maximum oxidation rate of 0.5 $mgS^{2-}$/mg-N.min at a sulphide concentration of 300 mg/l.
b. no sulphide inhibition is found for biomass grown at high sulphide loading rates, while severe sulphide inhibition is found for biomass grown at low loading rates.

Therefore we define a type A that grows up to a sulphide loading rate of 200-240 mg/l.h, which can be inferred from the fact that at 240 mg/l.h a low activity was measured (0.004 $mgS^{2-}$/mg-N.min), indicating that type A is not present at this loading rate, while type A still was present at a loading rate of 200 mg/l.h. Type A has a high affinity for sulphide and a high overall maximum oxidation rate at a sulphide concentration of 10 mg/l, while type B grows at sulphide loading rates exceeding approximately 200 mg/l.h and is dominant at loading rates exceeding 240 mg/l.h. Type B has a low affinity for sulphide and a maximum oxidation rate which is only 60% of that of type A. At a loading rate of 200 mg/l.h probably both types of biomass develop in the reactor.

Apart from the sulphide loading rate also the oxygen concentration is important in selecting the type of biomass. Figure 3b shows the specific oxidation rates of biomass grown at an oxygen concentration of 7 mg/l. At both loading rates (65 and 200 mg/l.h) much lower maximum oxidation rate values are found as compared to the values found at an oxygen concentration of 3 mg/l. From the shape of the curve representing 200 mg/l.h (Figure 3b) it can be concluded that type B can hardly grow at high oxygen concentrations. This important conclusion is supported by the results shown in Figure 3c. Biomass grown at a sulphide loading rate of 65 mg/l.h (type A) shows an increasing activity at higher oxygen concentrations, while with a biomass grown at a loading rate of 200 mg/l.h (combination of type A and B) no effect of the oxygen concentration is found. Therefore we assume that type B has its maximum oxidation capacity at low oxygen concentration and is inhibited at higher oxygen concentrations.

**growth yields**

From Figures 1 and 2 it can be inferred that the growth yield decreases when the end-product of the sulphide oxidation changes from sulphate to sulphur, because at higher sludge loading rates both the growth yield and the percentage sulphate formation decrease sharply. The relation between the percentage sulphur formation and the yield is shown in Figure 5. It is obvious that the growth yield decreases when the percentage of sulphur formation increases, because the bacteria can derive less energy from the oxidation of sulphide to sulphur than from the oxidation of sulphide to sulphate. An important question therefore is, why the bacteria form sulphur at a higher sludge load instead of sulphate. An explanation could be the toxic effect of the sulphide on the biomass. It is also possible that not the sulphide itself is toxic but the formed polysulphides (Beudeker et al.,1982). These polysulphides also may catalyse the chemical oxidation (Kuenen, 1975; Chen & Morris, 1972) which leads to a lower bacterial growth yield, because then less sulphide will be available for the bacteria. But it is also possible and in fact quite likely, that the biological oxidation of sulphide to sulphur proceeds much faster than the oxidation of sulphide to sulphate.

As mentioned above very likely - at least - two types of organisms are active in the reactors. These two types of organisms have quite different growth yields on sulphide. We calculated the growth yields at those process conditions, where only one of the types is dominant. For type A this will be at high oxygen concentrations and sulphide loading rates below 200 mg/l.h, while type B will be dominant at

sulphide loading rates exceeding 240 mg/l.h and at low oxygen concentrations. Organisms defined as type A can produce sulphur and sulphate (Figure 4a). With the values from Table 1 the yield for the sulphur and sulphate producing reactions can be estimated. However, it is not clear whether or not the bacteria belonging to type A can use the sulphur formation for growth. We calculated for the sulphur formation and the sulphate formation a yield (type A) of 0.0039 and 0.011 mg-N/mg-S respectively. At high sulphide loading rates only type B can grow and merely sulphur is produced. The groth yield for the sulphur forming biomass (type B) amounted to 0.0012 mg-N/mg-S. Assuming a N-content of the dry biomass of 12 %, it can be estimated that the growth yield of the mixed biomass will vary from 2.9 - 0.32 g DS/mol S. These values are significantly lower than those found by Kelly (1982), who reported a range of 5-13 g DS/mol S for autotrophic sulphide oxidizers. The values mentioned by Kelly all relate to situations where sulphide is merely converted to sulphate. Therefore the lower values found in our investigations correspond reasonably well to the data of Kelly.

Using the values for the sulphur and sulphate growth yield (0.0012 and 0.011 respectively) and the sulphur and sulphate production, the biomass production can be estimated. Figure 6 shows these estimated values, together with the measured biomass production as a function of the volumetric sulphide loading rate. It appears that biomass production is maximal at a sulphide loading rate of 100-150 mg/l.h and approaches a constant value above a sulphide loading rate of 240 mg/l.h, when the sulphate production becomes zero.

**Wash-out experiments**

From the results in Figure 4a and 4b it can be concluded that it is very difficult to determine the maximal specific growth rate from wash-out experiments, because:

- during wash-out conditions at low sulphide loading rates the end-product of the oxidation shifts from sulphate to sulphur.
- at higher sulphide loading rates the sulphide concentration during wash-out increases up to 190 mg/l and therefore inhibition might occur.
- very likely at least two very different types of biomass are present in the mixed culture.

Despite these difficulties in determining growth rates, the following conclusions can be drawn from the wash-out experiments. As type A will dominate at a sulphide loading rate of 50 mg/l, the results in Figure 4a clearly demonstrate that type A must be capable to produce sulphur instead of sulphate. A second observation is that the biomass shown in Figure 4a does not wash out, while the biomass in Figure 4b does. Therefore it can be concluded that the biomass cultivated at lower sulphide loading rates grows faster than the biomass cultivated at higher sulphide loading rates.

**ecological situation**

The natural ecological niche for type B bacteria, which have not been described before, might be a situation in which high concentrations of sulphide are present but little oxygen, dictating sulphur production. In the reactor situation, type A can grow much faster than type B at low sulphide loading rates and high oxygen concentrations. At moderate sulphide loadings type A needs type B, in order to enable its growth, because the sulphide concentration in the reactor should be kept low to prevent inhibition. At higher sulphide loading rates, when the sulphide concentration increases, type A will wash out due to substrate inhibition. At high sulphide loadings and high oxygen concentrations the sulphide removal will be merely chemical, because type A is inhibited by the high sulphide concentration and type B by the high oxygen concentration.

## CONCLUSIONS

It was found that in free cell suspensions in sulphide removing reactors at least two types of organisms are present. Table 3 summarizes the characteristics of these two types.

**Table 3.** Characteristics of the two types of biomass

| | A | B |
|---|---|---|
| sulphide affinity | high | low |
| max. oxidation capacity ($mgS^{2-}$/mg-N.min) | 0.8 | 0.5 |
| yield on sulphate (mg-N/mg-S) | 0.011 | - |
| yield on sulphur (mg-N/mg-S) | 0.0039 | 0.0012 |
| substrate inhibition with sulphide | yes | no |
| substrate inhibition with oxygen | no | yes |


## ACKNOWLEDGEMENTS

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## CHAPTER 8

# Biotechnological Sulphide Removal in three Polyurethane Carrier Reactors: Stirred Reactor, Biorotor Reactor and Upflow Reactor

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## ABSTRACT

Three reactor systems were compared in order to assess their suitibility for a new biotechnological sulphide removal process. This process is based on the conversion of sulphide to sulphur. The sulphide removal rates under conditions where the sulphide effluent concentration did not exceed 2 mg/l was for the CSTR, biorotor and upflow reactor 2.4, 10, 11 kg-S/$m^3$.day respectively. When using Rasschig rings instead of PUR particles the capacity for the biorotor system decreased to 5.0 kg-S/$m^3$.day. The applicable hydraulic retention times at a sulphide influent concentration of 100 mg/l for sulphide effluent concentrations below 2 mg/l was for the CSTR, biorotor and upflow reactor 35, 10 and 13 minutes respectively.

Under identical operational conditions the sulphate production in the biorotor and upstream reactor remains lower than in the CSTR. The sulphate production rate in the biorotor can be controlled by changing the oxygen concentration in the gasphase and in the other two reactor systems by changing the dissolved oxygen concentration.

## INTRODUCTION

The emission of sulphide is a major problem associated with anaerobic treatment of sulphate and sulphite containing waste waters. Sulphate is utilized by sulphur-reducing bacteria as an electron acceptor and sulphide is the end product of the reduction. The toxicity, corrosive properties, the unpleasant odor and the high oxygen demand dictate stringent control of its release into the environment. $H_2S$ is one of the more toxic pollutants. It also has an inhibitory effect on methanogenesis. According to Anderson (et al., 1982), sulphide might be ranked as one of the most important inhibitors. The corrosive properties of sulphide are apparent in the damage done to concrete walls of reactors, sewer systems and steel pipelines.

Methods for sulphide removal in common use today are physicochemical processes which involve direct air stripping, oxidation and chemical precipitation. Important disadvantages of these conventional systems are the relatively high energy requirements or the high chemical and disposal costs (Buisman et al., 1989a).

In this publication we report about three biotechnological sulphide removal systems, based on a principle which was already described in detail in previous communications (Buisman et al., 1989a). This new biotechnological sulphide removal system, is based on the oxidation of sulphide to sulphur. The removal rate of a prototype biorotor reactor was found to be more than 400 mg/l.h sulphide at a removal efficiency of 99.5%. The advantages of this new process are: a. no catalyst and no oxidants necessary (except for air), b. no chemical sludge to be disposed, c. little biological sludge is produced (0.75 g biomass/mol sulphur), d. low energy consumption, e. possible reuse of sulphur, f. little if any sulphate or thiosulphate discharge and g. fast process with high removal efficiency.

Table 1 compares some other sulphide removing sytems with the new system on basis of their sulphide removal rates. As the systems have not been tested yet at the comparable conditions like temperature and pH, and the new process sofar is still in the preliminary phase, Table 1 obviously provides only a rough comparison. Nervertheless it can be concluded that the removal rate of the new system competes quite well with sulphide removing systems based on the biological and/or chemical catalyzed oxidation of sulphide with air.

**Table 1.** comparison with other systems

| system description (*= end product is sulphur) | | sulphide removal rate (mg/l.h) | HRT (h) | removal efficiency (%) |
|---|---|---|---|---|
| **BIOLOGICAL** | | | | |
| with $NO_3^-$ | (1) | 104 | 0.09 | >99 |
| with light | (2)* | 15 | 24 | 95 |
| with $O_2$ | (3)* | 415 | 0.22 | >99 |
| **($H_2S$ removal from gas)** | | | | |
| with $NO_3^-$ | (4) | 74 | 34 | >99 |
| with light | (5)* | 67 | fed batch | - |
| ***CHEMICAL* with air** | | | | |
| catalyst $KMnO_4$ (1mg-Mn/l) | (6) | 116 | batch | 90 |
| catalyst activated carbon (53 mg/l) | (7) | 237 | batch | 74 |

**notes:**
1. denitrifying fluidized bed (Gommers et al., 1987)
2. Photosynthetic bacteria (Kobayashi et al., 1983)
3. colourless sulphur bacteria (this study)
4. T.denitrificans (Sublette and Sylvester, 1987a,b)
5. Chlorobium thiosulfatophilum (Cork, 1985)
6. (Martin and Rubin, 1978)
7. (Lefers et al., 1978)

Important criteria for the optimization of the newly developed biotechnological sulphide removal process are:
- minimize the unwanted sulphate production
- minimize the aeration costs
- simple reactor configuration
- minimize the reactor volume
- minimize chemicals usage

In addition to the CSTR system which was already described in a previous communication (Buisman et al., 1989a), we will evaluate in this publication the first results of two other reactor systems, e.g. a fixed film upflow reactor and a biorotor reactor. In all reactors recticulated polyurethane was used as carrier material.

## MATERIALS AND METHODS

### chemicals

A composite waste water was used as influent in the experiments. This feed solution consisted of nutrients and sodium sulphide in tap water. The nutrient solution contained (g/l): $NH_4Cl$ (8), $MgSO_4.7H_2O$ (2), $KH_2PO_4$ (5) and 100 ml trace element solution according to Vishniac & Santer, (1957). All the chemicals used for the nutrient solution were analytical grade supplied by Merck, Darmstadt, F.R.G.. The sulphide solution contained 200 g/l $Na_2S.8H_2O$ of a technical grade supplied by de Vries, Amsterdam, The Netherlands. The nutrient and sulphide solutions were added in proportion of 2 : 1. For pH control 5 M HCl, also of technical grade, was used. Pure oxygen supplied by Hoekloos, Schiedam, The Netherlands was used in the experiments.

### analyses

Sulphide was determined photometrically by the method described by Truper and Schlegel (1964).

Sulphate was analyzed by liquid chromatography using a Chrompack HPLC column, packed with Ionospher A-5$\mu$ (dim: 10 cm x 3 mm; ID), eluent 0.027 M potassiumbiphtalate (flow 0.4 ml/min) and a Knauer differential refractometer as detector. The injection quantity was 20 $\mu$l. This method could also detect thiosulphate.

The oxygen concentration was measured with an $O_2$-sensor (WTW; DU 600 201 711). The liquid oxygen concentration in the biorotor was to low to measure. Therefore the oxygen concentration in the gasphase was measured. The oxygen concentration in the gasphase was measured with with a Servomex $O_2$ gas-analysator and the pH with a pH-electrode (Ingold, 425-60-s7 ing. no. 104253313).

### reactors

Recticulated polyurethane (PUR) foam delivered by Recticel, Kesteren, The Netherlands was choosen as biomass support particles. The dimensions were 1.5x1.5x1.5 cm with 30 pores per inch and a specific surface of 1375 $m^2/m^3$ (according to Recticel). Also Rasschig rings were used in one experiment. This carrier material has a cilindrical shape with a diameter of 5 cm and a height of 3 cm. The total surface of one ring is approximately 180 $cm^2$. The oxygen concentration, the temperature and the pH were kept constant using a PI controller. A Tulip computer (PC compact), with a Mitsubishi programmable logic controller (Melsec PLC), was used for data-aquisition and control. The process temperature was kept at 20 °C. The reactors were continuously operated at a constant pH of 8.5.

**Reactor 1:** was a 8.3 l CSTR with 400 PUR particles, which was already described in a previous communication (Buisman et al., 1989; Chapter 2).

**Reactor 2:** was a 6.0 l biorotor reactor (with a 3 l wet volume) containing 640 PUR particles. The PUR particles were put in a rotating cage of steel. The diameter of the reactor was 13.5 cm and its length was 44 cm. The diameter of the cage was 13.3 cm, with a length of 30 cm. The cage was submerged 50% and the rotation speed was 14 rpm (Figure 1).

**Reactor 3:** was a 20 l fixed film submerged upflow reactor with 2600 PUR particles. The diameter and the height of this reactor were 20 cm and 87 cm respectively (Figure 2).

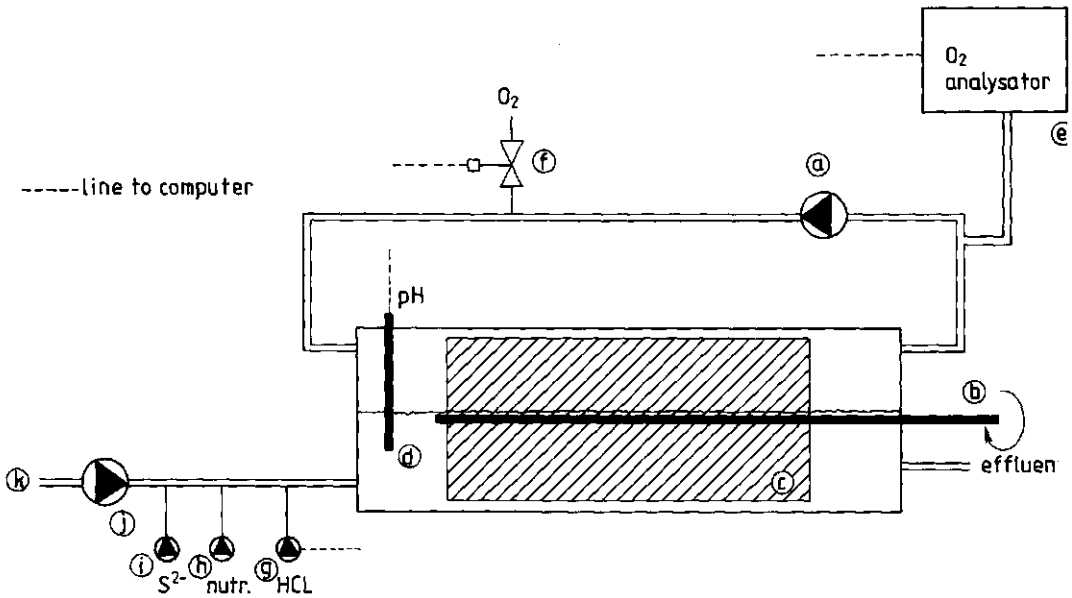


**Figure 1.** laboratory set-up for a biorotor continuously fed sulphide oxidizing reactor: a.gasphase recycling pump; b.rotation engine; c.cage around the BSP's; d. pH-electrode; e. oxygen analysator for gasphase; f.oxygen dosage valve, computer controlled; g. HCl dosage pump, computer controlled; h.nutrient and trace elements solution dosage pump; i. sulphide solution dosage pump; j. tapwater pump; k. temperature controlled tapwater

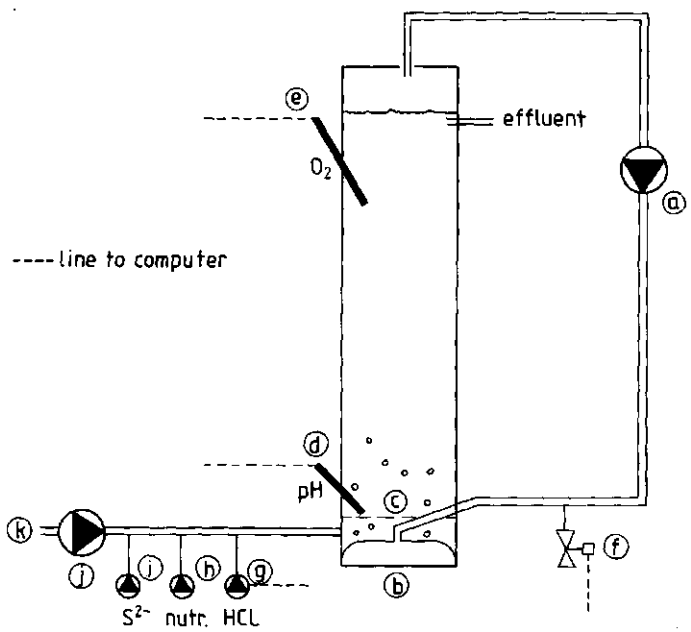


**Figure 2.** laboratory set-up for a upstream continuously fed sulphide oxidizing reactor: a.gasphase recycling pump; b.aeration stone; c.separation between aeration stone and the reactor; d. pH-electrode; e. oxygen sensor; f.oxygen dosage valve, computer controlled; g. HCl dosage pump, computer controlled; h.nutrient and trace elements solution dosage pump; i. sulphide solution dosage pump; j. tapwater pump; k. temperature controlled tapwater

**oxygen concentration control:** In reactor 1 and 3 the oxygen concentration in the liquid was controlled, while in reactor 2 the oxygen concentration in the gasphase was controlled.

### residence time distribution measurement

We used a one-shot input of NaCl as tracer to determine the residence time distribution. The effluent NaCl concentrations were measured using a conductivity sensor. The HRT of the reactors during the experiment was 13 minutes. The flow character in the reactors can be expressed by a number that stands for the number of CSTR's in a serie. We found for the upflow reactor, the biorotor and the biorotor with Rasschig rings: 2.1, 3.7, 2.5 respectively.

## RESULTS

### capacity

The sulphide removal capacity for the three reactors was measured by increasing the sulphide volumetric loading rate using higher sulphide influent concentrations at the same HRT. After each change in the sulphide influent concentration the sulphide and sulphate concentrations in the effluent were measured after a sufficiently long adaptation period passed, viz. six days. The pH was maintained at 8.5 in all reactors and a HRT of 13 minutes was applied in the biorotor and upstream reactor and of 22 minutes in the CSTR. The dissolved oxygen concentration in the CSTR and upflow reactor was maintained at 3 mg/l. The oxygen concentration in the gasphase of the biorotor reactor was kept at 20%. The experimental results are shown in Figures 3 to 5. Figure 3 shows the relation between the sulphide loading rate and the amount of sulphide converted into sulphur or sulphate, while Figure 4 shows the relation between the sulphide removal efficiency and the imposed sulphide load for the three reactors. Table 2 gives the results on basis of the amount of biomass support particles (BSP) present per liter reactor contents. The data shown in Figure 5 refer to the calculated percentage of the sulphide converted to sulphate in relation to the effluent sulphide concentration.

**Table 2.** The sulphide removal rate per BSP and per liter reactor contents, by a sulphide loading rate sothat the sulphide effluent concentration is approximately 9 mg/l; the removal efficiency is also shown

| reactortype | $S^{2-}$ effl conc. (mg/l) | removal rate per BSP (mg-S/BSP.h) | removal rate per liter (mg-S/l.h) | removal efficiency (%) | number BSP's (BSP/l) |
|---|---|---|---|---|---|
| CSTR | 11 | 4.2 | 203 | 87 | 48 |
| biorotor | 9 | 4.6 | 492 | 96 | 107 |
| upflow | 8 | 5.1 | 662 | 95 | 130 |

### sulphide gradient

In a CSTR system obviously no gradients can be measured. The sulphide gradients measured in the biorotor reactor and the upflow reactor are shown in Figure 6, for an influent sulphide concentration of 100 mg/l, a HRT of 13 minutes and a pH of 8.5. The oxygen concentration in the upflow reactor is 3 mg/l in the upper part of

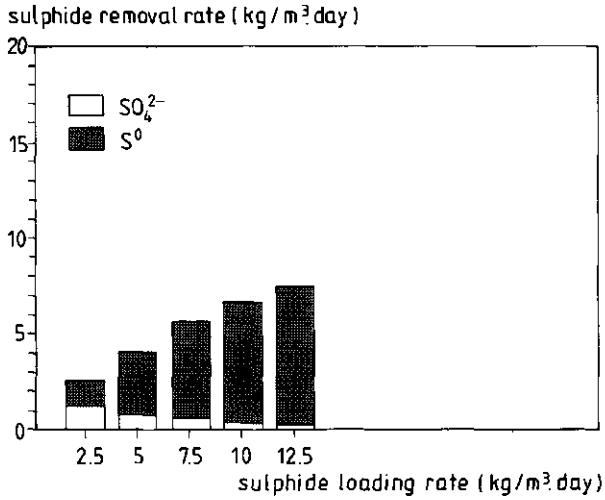


**Figure 3a.** Capacity of the stirred reactor shown as the sulphate and sulphur production rates at pH=8.5, oxygen concentration of 3.0 mg/l, and HRT of 20 minutes

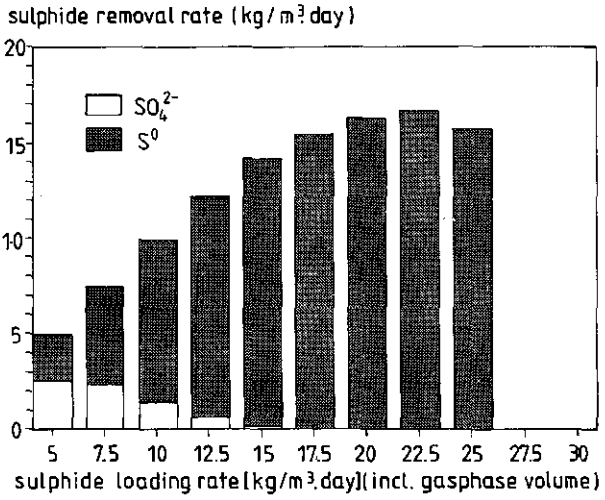


**Figure 3b.** Capacity of the biorotor reactor shown as the sulphate and sulphur production rates at pH=8.5, oxygen concentration in the gasphase of 20 % and HRT of 13 minutes

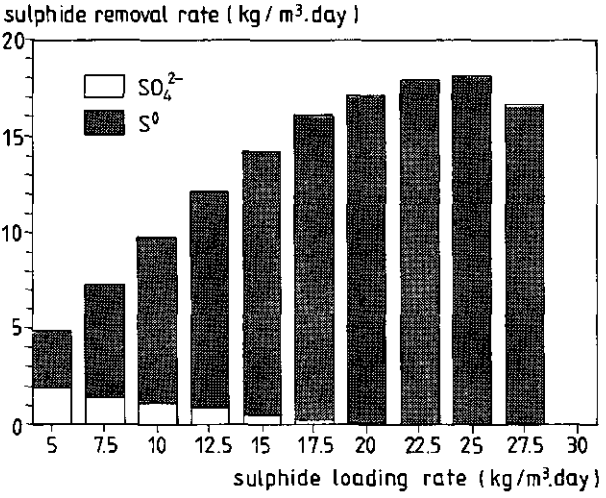


**Figure 3c.** Capacity of the upflow reactor shown as the sulphate and sulphur production rates at pH=8.5, oxygen concentration of 3.0 mg/l, and HRT of 13 minutes

the reactor; in the lower part of the reactor the oxygen concentration was not measured. The oxygen concentration in the gasphase of the biorotor is 20%.

**effect of oxygen concentration**

The oxygen concentration in the upflow reactor was measured merely in the upper part. Upon changing the oxygen supply, also the oxygen concentration in the reactor will change. The effect of the oxygen concentration on the effluent sulphide and sulphate concentrations is the same as for the CSTR, the results of which were published somewhere else (Buisman et al., 1989a,b)
The oxygen liquid concentration in the biorotor reactor is often too low to be measured accurately. Therefore the oxygen concentration in the gasphase was measured. Figure 7 shows how a change in the oxygen gasphase concentration affects the sulphide and sulphate gradients in the biorotor reactor. Two different oxygen concentrations are used (15% and 40%) at a HRT of 13 minutes and at a sulphide influent concentration of 150 mg/l.

**effect of HRT**

The effect of the HRT was also measured for the three reactors. The sulphide concentrations were measured after an adaptation period of six days following a change in the HRT. The sulphide influent concentration was kept constant at 100 mg/l in this experiment. The oxygen concentrations were the same as in the capacity experiment. The results of this experiment are shown in Figure 8.

**effect of carrier material in biorotor**

In addition to PUR particles also Rasschig rings were tested in the biorotor reactor. For this purpose the 640 PUR particles were replaced by 35 Rasschig rings, which filled the cage completely. In order to be able to compare the two types of carrier material adequately, the same experiment was conducted with the rings as with the PUR particles. The sulphide loading rate was increased after an adaptation period of six days by increasing the sulphide influent concentration. The sulphide and sulphate concentrations in the effluent are shown in Figure 9.

## DISCUSSION

The observed differences in the sulphide removal rate, among the three reactors can mainly be attributed to the different number of biomass support particles (BSP's) present in the reactors. This can be inferred from Table 2. The volumetric removal rate of the biorotor is a factor 2 faster and that of the upflow reactor a factor 3 faster as compared to the CSTR, while the calculated removal rate per BSP is only 10% and 20% faster than those from the CSTR respectively. These slightly higher specific activity of the BSP's in the biorotor and upflow reactor can be explained by the fact that the flow dynamics in these two reactor types deviate more from a complete mixed system. This means that the sulphide concentration in a significant part of the reactor volume in these systems is higher than in the CSTR system and thus a higher specific activity per BSP will prevail, because at higher sulphide concentrations the sulphide oxidation rate is faster. Also the fact that less turbulence prevails in the upflow reactor can be a factor which increases the removal rate per BSP, because of less attrition the BSP's will very likely contain more attached active biomass than in the CSTR system.

In a previous communication (Buisman et al., 1989b) we already reported that the sulphate production rate is inhibited by higher sulphide concentrations in the reactor. Because of the concentration gradients in the biorotor and upflow reactors

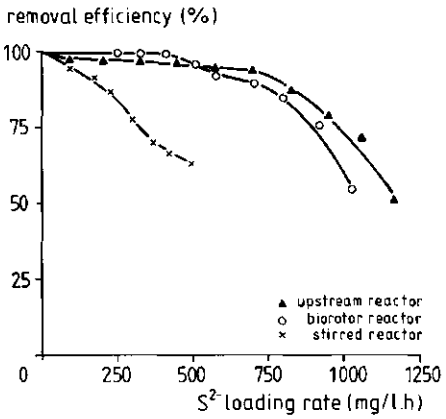


**Figure 4.** The removal efficiency for the three reactor types at the same conditions as in Figure 3

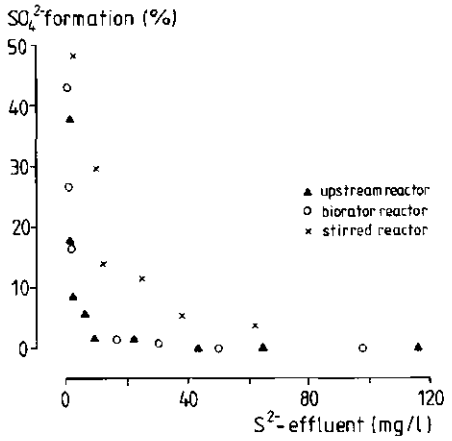


**Figure 5.** Percentage of sulphate formation from the total sulphide influent concentration; the conditions are the same as in Figure 3

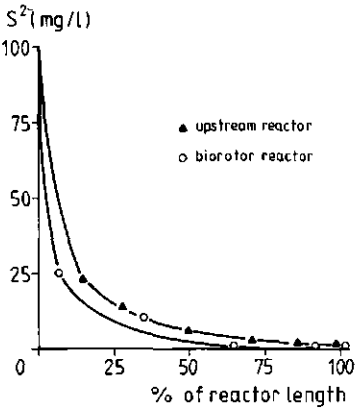


**Figure 6.** The sulphide gradient in the biorotor reactor and in the upstream reactor;the influent concentration is 100 mg/l, pH=8.5 and the HRT is 13 minutes

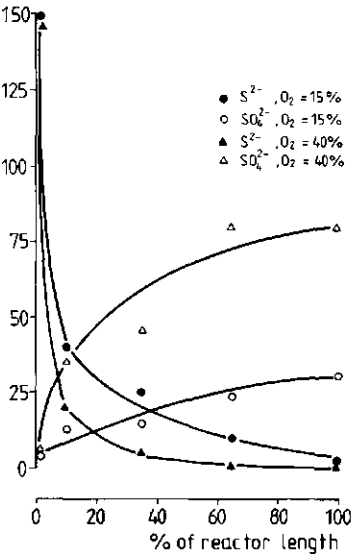


**Figure 7.** The sulphide and sulphate concentrations in the biorotor reactor at two different oxygen concentrations, 15 % and 40 % respectively; The pH=8.5, the HRT is 13 minutes and the sulphide influent concentration is 150 mg/l

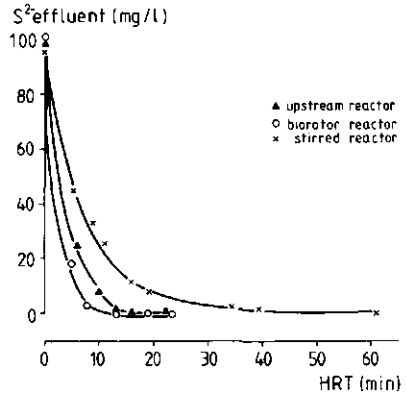


**Figure 8.** The effect of the HRT on the sulphide effluent concentrations for the three reactor types; the conditions are the same as in Figure 3 except for the HRT

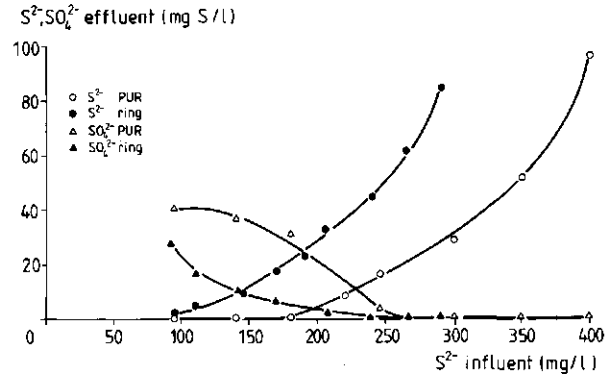


**Figure 9.** The sulphide and sulphate effluent concentrations in the biorotor reactor with a. PUR particles and b. rasschi rings as biomass support material as a function of the sulphide influent concentration; the pH=8.5, HRT is 13 minutes and the oxygen gasphase concentration is 20 %

resulting form the plugflow character of the liquid, there will prevail a relatively higher sulphide concentration in the upstream parts of these reactors. This explains why the sulphate production in the biorotor and upflow reactor remains lower than in the CSTR at comparable sulphide effluent concentrations. This would mean that the liquid flow dynamics are an important factor in reducing the sulphate production and therefore flow dynamic criteria can be used for optimizing the sulphur production in the sulphide removing process.

The effect of the oxygen concentration is the same as found previously (Buisman et al., 1989a,b), i.e. the higher the oxygen concentration the higher the sulphate production. Figure 7 shows that it is possible to control the sulphate production in the biorotor reactor with the oxygen concentration in the gasphase.

Upon imposing a higher sulphide loading rate on the system either by lowering the hydraulic retention time (Figure 8) or by increasing the sulphide influent concentration (Figure 3), less sulphate is produced and the volumetric sulphide conversion rate increases.

When using Rasschig rings the oxidation capacity drops down significantly, but this is also the case for the sulphate production rate. These poor capacities could be expected, because the surface of the Rasschig rings is only 20 % of that of the PUR particles, consequently the biomass concentration in the reactor will be accordingly lower. The reason why the oxidation capacity of the reactor packed with Rasschig rings is still more than 20% of the reactor packed with PUR particles, probably can be attributed to the fact that only the outer 1 to 1.5 mm of the PUR particles, comprising 40 - 20% of the available surface area (Buisman et al., 1989) is used for bacterial attachment and or is assesseble for substrate supply.

## CONCLUSIONS

The newly developed biotechnological sulphide removal system exerts a removal rate and removal efficiency which at least is similar to that of other sulphide removing systems.

The sulphide removal capacity of the three investigated reactor systems depends mainly on the number of BSP's per liter reactor contents. The results indicate that flow dynamics of the reactor systems is an important factor for the sulphate production rate at a specific sulphide load. The more the flow deviates from a complete mixed system the less sulphate is being produced.
Upon increasing the sulphide loading rate by decreasing the HRT or increasing the sulphide influent concentration, the sulphate production rate will decrease. The sulphate production rate will also decrease upon lowering the oxygen concentration.

## ACKNOWLEDGEMENTS

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## CHAPTER 9

# Sulphide Removal from Anaerobic Waste Treatment Effluent of a Papermill

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## ABSTRACT

An upflow and a biorotor reactor, which have been previously tested using composite waste waters, were investigated for their suitibility for sulphide removal from anaerobically treated papermill waste water. Upon treating this waste water, sulphur reduction and clogging problems occurred. These problems did not occur in lab experiments using the same reactors. Due to these problems we concluded that the upflow reactor, at least in the configuration investigated, was not suitable for this type of waste water.

In the biorotor reactor sulphur reduction and clogging could be prevented by using Rasschig rings as carrier material at a rotation speed of 46 rpm. In this configuration of the biorotor reactor a sulphide removal rate of 620 mg/l.h was found at a HRT of 13 minutes with a removal efficiency of 95%. At lower rotation speeds the removal efficiency deteriorates seriously. Using polyurethane sheets or particles instead of the Rasschig ring the removal effciency also decreased in the biorotor reactor.

## INTRODUCTION

In papermills often alum (aluminium sulphate) and sulphuric acid are used. In an anaerobic waste water treatment system this sulphate frequently is being converted into sulphide by sulphate-reducing bacteria. The toxicity, corrosive properties, the unpleasant odor and the high oxygen demand of sulphide dictate stringent control of its release into the environment. Sulphide also disturbs the aerobic activated sludge post-treatment system. In the presence of sulphide bacteria like Thiothrix develop and bulking sludge problems will occur. Therefore a cheap and reliable sulphide removal system is needed to improve the treatment of papermill waste water. In previous communications a new biotechnological sulphide removing system has been proposed, which was also compared with other sulphide removing systems (Buisman et al., 1989 a,b,c). In these communications we showed that at the sulphide sludge loading rates applied in the system, little if any growth of Thiothrix bacteria occurs in the reactors (Buisman et al., 1989d).

The principle of the new system is based on the conversion of sulphide into elemental sulphur using colourless sulphur bacteria and a controlled oxygen supply. The advantages of such a biotechnological process with sulphur production are: a. no catalyst or oxidants (except air) are required, b. no chemical sludge to be disposed, c. little biological sludge is produced (0.75 g biomass/mol sulphur), d. low energy consumption, e. possible reuse of sulphur, f. little if any sulphate or thiosulphate discharge and g. the process proceeds fast at a high removal efficiency.

In the present study this new system was investigated at labscale to assess its feasibility for removing sulphide from anaerobically treated papermill waste water (6 - 20 liter reactors). The major differences with the previous investigations are the presence of organic compounds, like acetate and propionate and a significant concentration of bicarbonate in the sulphide containing solution.

Moreover the temperature of the solution was 27°C instead of 20 °C. The same reactors and carrier materials are used in this study as in the previous lab study (Buisman et al., 1989c).

## MATERIALS AND METHODS

### composition of the papermill waste water

The composition of the waste water used in this study is shown in Table 1. There was little variation in the composition of the waste water in time (except at holidays). During the weekends the sulphide concentration was increase slightly higher.

**Table 1.** composition of the effluent of the anaerobic waste water treatment at the plant Industriewater Eerbeek, The Netherlands

| parameters | | concentration |
|---|---|---|
| COD | (mg/l) | 400 |
| N-kj | (mg/l) | 10 |
| $S^{--}$ | (mg/l) | 120 - 180 |
| $SO_4^{--}$ | (mg-S/l) | 0 - 20 |
| acetate | (mg/l) | 30 - 80 |
| propionate | (mg/l) | 2 - 15 |
| alkalinity | (meq) | 18 |
| chloride | (mg/l) | 50 |

### analyses

Sulphide was determined photometrically by the method described by Truper and Schlegel (1964). Elemental sulphur was measured after extraction with acetone according to the method described by Bartlett and Skoog (1954).

Sulphate was analyzed by liquid chromatography using a Chrompack HPLC column, packed with Ionospher A-5$\mu$ (dim: 10 cm x 3 mm; ID), eluent 0.027 M potassiumbiphtalate (flow 0.4 ml/min) and a Knauer differential refractometer as detector. The injection quantity was 20 $\mu$l. Thiosulphate could also be detected using this method.

Acetate and propionate were determined using a gaschromatograph equipped with a 2 m x 4 mm (i.d.) glass column packed with Supelcoport (100-120 mesh) coated with 10% Fluorad FC 431. The temperature of the column, the injection port and the flame ionization detector were 120, 220 and 240 °C, respectively. Nitrogen saturated with formic acid was used as carrier gas at a flow rate of 50 ml/min.

The oxygen concentration was measured with an $O_2$-sensor (WTW; DU 600 201 711) and the pH with a pH-electrode (Ingold, 425-60-s7 ing. no. 104253313).

### reactors

Two different biomass support particles (BSP's) were used in the experiments e.g. recticulated polyurethane (PUR) foam delivered by Recticel, Kesteren, The Netherlands and Rasschig rings. The dimensions of the PUR particles were 1.5x1.5x1.5 cm with 30 pores per inch and a specific surface of 1375 $m^2/m^3$ (according to Recticel). In addition one experiment was conducted using polyurethane sheets, with dimensions as shown in Figure 1. The diameter of the

Rasschig rings was 5 cm at a height of 3 cm. The surface of one ring is approximately 180 $cm^2$.

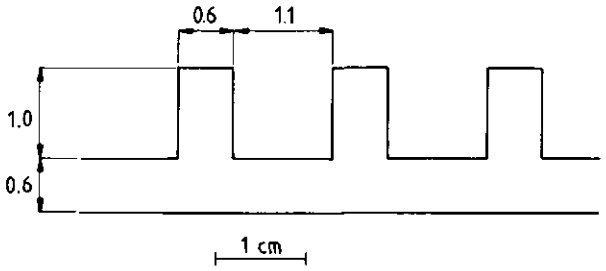


**Figure 1.** The dimensions and shape of the Polyurethane sheets used as carrier material

The experiments were conducted at a process temperature of approximately 27 °C and the pH in the reactor remained between 7.7 and 8.2. The oxygen concentration in the upflow reactor was about 5 mg/l and the oxygen concentration in the gasphase of the biorotor 20% (air) at an air flow of 22 liter/minute through the reactor.

**Reactor 1:** consisted of a 6.0 l biorotor reactor (inclusive the gas phase) containing 640 PUR particles or 35 Rasschig rings. The BSP's were kept in a rotating cage of steel. The diameter of the reactor was 13.5 cm and its length 44 cm. The diameter of the cage amounted to 13.3 cm, at a length of 30 cm. The cage was submerged 50% and the system was operated at a rotation speed of 14 or 46 rpm (Figure 2).

**Reactor 2:** consisted of a 20 l fixed film upflow reactor containing 2600 PUR particles or 78 Rasschig rings. The diameter and the height of this reactor was 20 cm and 87 cm respectively (Figure 3).

## RESULTS

### start up

Both reactors were started using polyurethane particles. Starting from the third day after inoculation the sulphate, sulphide and acetate concentrations of the influent and the effluent of the reactors were measured daily (Figure 4 and 5). During the first week very good sulphide removal efficiencies were achieved, i.e. 96% for the upflow reactor at a HRT of 16 minutes and 97% for the biorotor reactor at a HRT of 13 minutes. Moreover less than 3% of the sulphide was converted into sulphate.

The results in Figure 5 show that during the first week little if any acetate was removed. However, in the second week acetate removal became apparent in the upflow reactor and at the same time the sulphide removal efficiency dropped from 96 to 85%.

In the biorotor reactor the hydraulic retention time was decreased in the second week, to 7.4 minutes and it was increased again in the third week to 13 minutes. In this reactor acetate removal became apparent in the third week and as in the upflow reactor the sulphide removal efficiency dropped down.

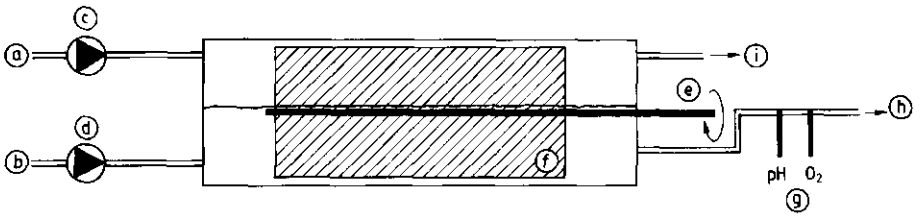


**Figure 2.** laboratory set-up for a biorotor continuously fed sulphide oxidizing reactor: a.air; b.anaerobically treated wastewater; c.gasphase recycling pump; d.influent pump; e.rotation engine; f.cage around the BSP's; g. pH and oxygen electrodes; h. effluent; i. effluent gas phase

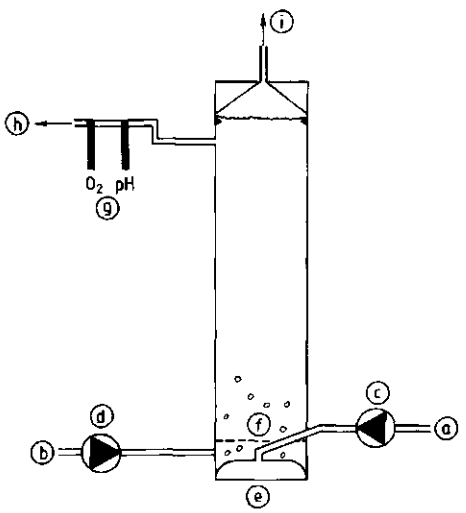


**Figure 3.** laboratory set-up for a upflow continuously fed sulphide oxidizing reactor: a.air; b. anaerobically treated wastewater; c.gasphase recycling pump; d.influent pump e.aeration stone; f.separation between aeration stone and the reactor; g.pH-electrode and oxygen sensor; h.efflunt; i.gas phase effluent

**sulphur reduction**

The results above indicate the existence of a relation between the removal of acetate and the deterioration of the sulphide removal are related. Besides the oxidation of sulphide to sulphur and to sulphate, the occurrence of sulphur or sulphate reduction inside the reactors looks possible. In order to get more information about this matter, we conducted an experiment in which both the aeration and the supply of sulphide containing influent was interrupted. Instead of a sulphide containing influent the system was fed with a solution, consisting of tapwater with acetate, sulphate and nutrients at the same flow rate as before. The experiment therefore enables the measurement of the sulphate or sulphur reduction under conditions of the same pH, HRT and temperature as before. The sulphate, acetate and sulphide concentrations were measured in the effluent of the upflow reactor. The results are summarized in Table 2. It was not possible to conduct the same experiment in the biorotor, because oxygen is continuously transferred from the gas compartment to the liquid.

Table 2. sulphide production in the upflow reactor

| compound (mg/l) | influent tapwater | effluent upflow |
|---|---|---|
| oxygen | 7 | <1 |
| acetate | 135 | 130 |
| sulphate | 40 | 42 |
| sulphide | 0 | 16 |

In an additional experiment we attempted to confirm that little if any sulphate is used for the production of sulphide. The experiment was conducted using biomass removed from two rings of the upflow reactor. This biomass was placed in a 0.569 liter serum flask, containig a medium consisting of a phosphate buffer with 100 mg/l acetate and 100 mg/l propionate. The medium was flushed with nitrogen gas in the serum bottle to remove oxygen. The initial sulphate concentration was 55 mg/l. Five hours after the start of the experiment the sulphate concentration was 56 mg/l, while during this five hours the sulphide concentration increased from 0 mg/l to 16 mg/l.

An additional anaerobic batch experiment was conducted in order to assess the sulphur reduction rate. For this purpose three Rasschig rings from the upflow reactor were put in a 5.5 liter vessel, which contained an oxygen free medium consisting of a posphate buffer (50 mM). Acetate (60 mg/l) and propionate (10 mg/l) were added. The sulphide concentration and fatty acids concentration were followed. Any increase in the sulphide concentration very likely will originate from the sulphur present. After 2 days the sulphide concentration reached 173 mg/l, after 8 days even 345 mg/l. The results obtained over the first 48 hours of the experiment are shown in Figure 6.

**clogging**

Besides sulphur reduction, the lower removal efficiencies can also be attributed to the occurence of clogging in the upflow reactor. Clogging manifested both with the PUR particles as well as with the PUR sheets and Rasschig rings. Therefore we concluded that the upflow reactor, at least in the configuration investigated, was not suitable as sulphide removal system for this type of waste water. The experiments with this reactor were terminated for this reason.
In the biorotor clogging manifested merely with the PUR sheets, not with the Rasschig rings and PUR particles.

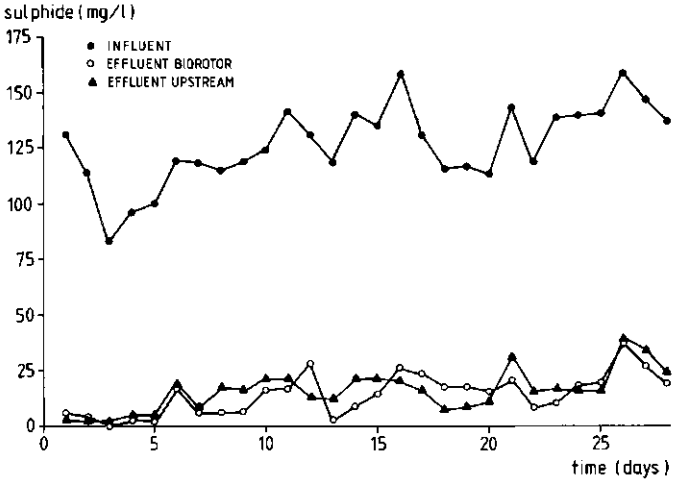


**Figure 4.** The influent and effluent sulphide concentrations of the biorotor (HRT 13 minutes, rotation speed 14 rpm) and the upflow reactor (HRT 16 minutes) during the start-up phase; temperature is about 27°C pH is about 8 and as carrier material PUR particles; the weekends are not shown in this figure because no samples were taken then

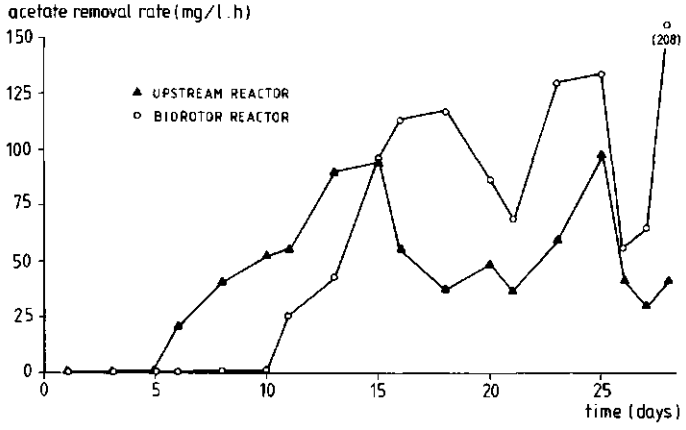


**Figure 5.** The acetate removal rate of the upflow and the biorotor reactors at the same time interval as Figure 4

**carrier material in the biorotor**

In order to assess the possibilities to improve the performance of the biorotor, different carrier materials were investigated. The type of carrier material might be an important factor in surpressing the sulphur reduction. The results are shown in Table 3. The data presented in Table 3 concern the average values of at least five days continuous operation at steady state conditions.

**effect of rotation speed of the biorotor**

In addition to the type of carrier material also the rotation speed of the biorotor could be an important means in lowering the extent of sulphur reduction. Upon increasing the speed, the biofilm becomes thinner, which will reduce the anaerobic conditions and consequently a higher speed might lead to a decreased sulphur reduction. The results (average values obtained after at least five days of steady state operation) are shown in Table 3. The PUR sheets were not tested at a rotation speed of 46 rpm.
Figure 7 shows the performance of the biorotor with Rasschig rings during a few weeks of continuous operation at different HRT's (and consequently different loading rates) and always at a rotation speed of 46 rpm.

**Table 3.** influence of the use of different carrier materials on the sulphide and acetate removal in the biorotor

| carrier material | $S2^-$ infl (mg/l) | $S^{2-}$ effl (mg/l) | % $SO_4$ (%) | removal conversion rates (%) | acetate (mg/l.h) | sulphide (mg/l.h |
|---|---|---|---|---|---|---|
| **ROTATION SPEED 14 rpm, HRT 13 min** | | | | | | |
| rings | 140 | 38 | <1 | 73 | 52 | 491 |
| PUR part. | 134 | 21 | <1 | 85 | 98 | 536 |
| sheets | 134 | 36 | <1 | 73 | 84 | 465 |
| **ROTATION SPEED 46 rpm, HRT 13 min** | | | | | | |
| rings | 138 | 6.9 | 8 | 95 | 144 | 620 |
| PUR part. | 154 | 21 | <1 | 87 | 96 | 710 |
| **ROTATION SPEED 46 rpm, HRT 26 min** | | | | | | |
| rings | 144 | 2.7 | 37 | 98 | 94 | 301 |
| PUR part. | 148 | 11 | <1 | 93 | 96 | 365 |

**effect of air supply in the biorotor**

The air flow through the biorotor reactor during the experiments amounted to 22 liter/minute. Upon lowering the air flow to respectively 12 and 4 liter/minute the sulphide conversion rate decreased with 5% and 13% respectively. We did not follow the oxygen concentration in the effluent gas, but at a sulphide removal rate of 710 mg/l.h and an air flow of 22 liter/minute, it can be calculated that the oxygen concentration in the air decreases only less than 0.5 %. The small size of the reactor does not allow prediction of the air needs in a full scale configuration, the more, because very likely several other factors certainly will be important in that respect.

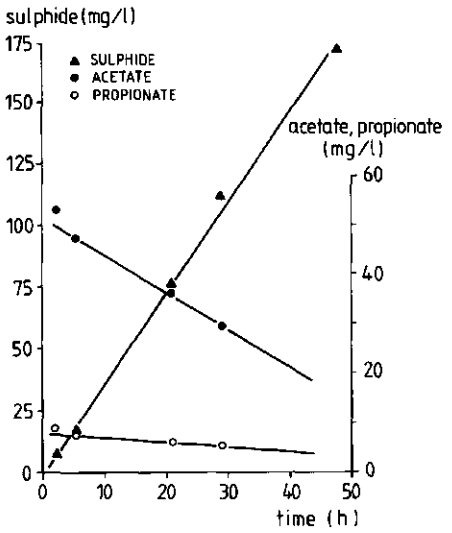


**Figure 6.** The sulphide production rate and acetate and propionate removal rate under anaerobic conditions in a batch experiment in a 5.5 liter vessel at pH 8.0 and 30°C using Rasschig rings removed from the upflow reactor

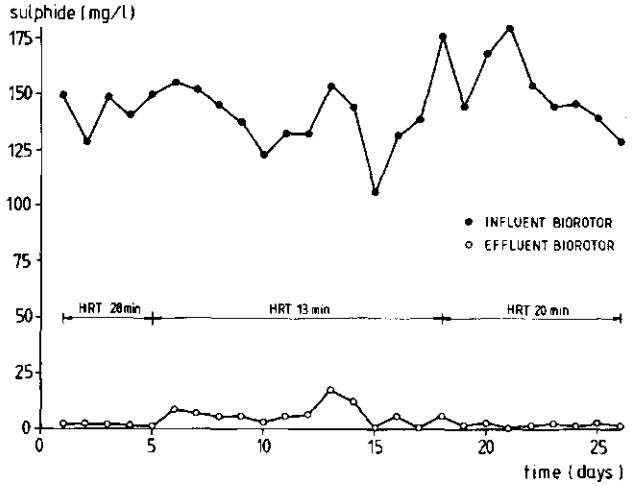


**Figure 7.** The influent and effluent sulphide concentrations at a rotation speed of 46 rpm with Rasschig rings as carrier material at a pH of about 8 and a temperature of 27°C; the weekend are not shown in this figure

## DISCUSSION

The results in Figure 4 and 5 show that the removal efficiency of the reactors deteriorate once the removal of acetate starts, viz. in the second week for the upflow reactor and third week for the biorotor reactor. The removal efficiency during the first week (upflow 96% and biorotor 96%, see Figure 4) is similar as found at laboratory scale with these reactors (98% and 99% respectively) with a composite waste water without organic compounds (Buisman et al., 1989c). The removal efficiencies following the first week (about 85% for both systems) are therefore significantly lower than in these lab studies with composite solutions. It is clear from Table 2 that these differences must be attributed to sulphur and or sulphate reduction. It is known from literature that Desulfuromonas acetoxidans (Pfennig & Biebl, 1976) can convert sulphur to sulphide using acetate according to the following equation:

$$CH_3COOH + 2H_2O + 4S^0 \quad ---> \quad 2CO_2 + 4H_2S$$

No freshwater Desulfuromonas species are known that can use propionate instead of acetate for reducing sulphur to sulphate (Widdel, 1988). On the other hand it is known that Desulfobulbus propionicus can convert propionate into acetate and sulphate into sulphide according to the following equation:

$$4CH_3CH_2COO^- + 3SO_4^{--} \quad ---> 4CH_3COO^- + 3HS^- + H^+ + 4HCO_3^-$$

The results shown in Figure 6 show that acetate and propionate are removed while sulphide is produced. The sulphide production rate in this experiment is 0.111 mmol/l.h and the acetate and propionate removal rates are 0.013 and 0.002 mmol/l.h. The acetate and propionate removal rates are too low to explain the sulphide production and therefore another carbon source must have been available, i.e. equivalent to an amount of 0.012 mmol/l.h acetate, which is missing in the balance. In absence of fatty acids also sulphide production occurs (not shown in the present study). Also the results shown in Tabel 2 clearly indicate that the fatty acid balance does not fit. Probably some usable organic substrates are present in the seed sludge. For a more acurate balance study it is therefore necessary to use very diluted sludge as inoculum. We will investigate this matter in more detail in the near future.

Anyhow from the experiments obtained so far we learn that only little sulphate is reduced, this corresponding to the low propionate removal rates.
Moreover the sulphur reduction seems to be uninhibited by sulphide up to at least 170 mg $S^{--}$/l. Pfennig & Biebl even did not observe any sulphide inhibition up to concentrations of 900 mg $S^{--}$/l.
The sulphur reduction rate can be calculated from the dat in Figure 6. We find a sulphur reduction rate of 6.5 mg/h.ring over the first 48 hours, which means that in the upflow reactor (78 rings) the sulphide production rate is about 25 mg/l.h. The sulphur reduction might even be higher than measured in the experiment shown in Figure 6, for reason that the reduction of continuously formed dispersed (colloidal) sulphur will proceed faster than of coarse sulphur particles (higher activation energy). Unfortunately we could not measure the sulphur reduction rate inside the reactor and therefore we can not confirm this idea. Using the results in Table 2 a sulphur reduction rate in the upflow reactor of 106 mg/l.h can be calculated, assuming that all the oxygen removed is used for the oxidation of sulphide to sulphur. It will be only 60 mg/l.h when no oxygen is used for the sulphide oxidation. The values for the sulphide production rate 25 - 106 mg/l.h are 6 - 25 % of the calculated sulphide removal rate (422 mg/l.h) in the upflow reactor. These values can indeed explain the loss of removal efficiency of the upflow reactor in the presence of fatty acids. It is also possible that a part of the acetate removal is due to oxidation with oxygen instead of sulphur.

Clogging problems were not observed in the lab experiments with composite influent solutions. However, when using anaerobically treated waste water, clogging problems manifested clearly in the upflow reactor (in the biomass support particles),

but not in the biorotor reactor; in the latter reactor turbulence suffices preventing clogging. According to visual observations the clogging problems in the upflow reactor with the anaerobically treated waste water were caused by anaerobic sludge present in the waste water, precipitation of calcium salts and the formation of a slimy biofilm.

The differences found among the various carrier materials appear from the results shown in Table 3. The PUR sheets are inadequate support materials due to the occurence of clogging problems. At a rotation speed of 14 rpm the PUR particles apparently are the most appropiate carrier material. At rotation speeds exceeding 46 rpm the rings perform the best. No clear clogging problems manifest when using the PUR particles, but in this case the anaerobic conditions prevailing in the inner part of the particles cause a too high sulphur reduction. Assumming that only the outer 1 to 1.5 mm of the particles are aerobic (Buisman et al., 1989) approximately 30% of the total surface area is available for aerobic bacteria. The rotation speed itself has only a slight influence on the performance of PUR particles. It is also clear that the performance of systems using PUR particles is not optimal when organic compounds are present in the waste water, because significant sulphur reduction can occur in that case in the available large anaerobic inner surface area of the particle.

The Rasschig rings do not perform satisfactory at rotation speeds of 14 rpm, because the biofilm becomes too thick in this case and anaerobic conditions can prevail in the reactor. In applying rotation speeds of 46 rpm the biofilm remains thin and little if any anaerobic conditions prevail, while the aeration efficiency will improve. Of the various carrier materials the best sulphide removal results are found with the Rasschig rings at a rotation speed of 46 rpm.

## CONCLUSIONS

Upon treating anaerobically treated papermill waste water using an upflow and a biorotor reactor, sulphur reduction and clogging problems occur. These problems did not occur in lab experiments using the same reactors. Due to these problems we concluded that the upflow reactor, at least in the configuration investigated, was not suitable for this type of waste water.
In the biorotor reactor sulphur reduction and clogging could be prevented by using Rasschig rings as carrier material at a rotation speed of 46 rpm. In this configuration of the biorotor reactor a sulphide removal rate of 620 mg/l.h was found at a HRT of 13 minutes with a removal efficiency of 95%.

## ACKNOWLEDGEMENTS

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## CHAPTER 10

### Summary

Since the early 1980s, anaerobic wastewater treatment has become more and more accepted as an attractive alternative to conventional aerobic biological purification systems. In treating sulphate containing wastewater anaerobically significant problems may occur. Sulphate reducing bacteria can use sulphate in the anaerobic mineralization process as electron acceptor, which leads to the formation of hydrogen sulphide. The toxicity, corrosive properties, unpleasant odor and high oxygen demand of sulphide dictate stringent control of its release into the environment.

This thesis describes research on a new biotechnological process for sulphide removal, in which it is attempted to convert sulphide into elemental sulphur by colourless sulphur bacteria. The goals of the research were:

A to develop an efficient and reliable high rate treatment system for purification of sulphide containing wastewaters.
B to assess the conditions that will lead to sulphur instead of sulphate formation.

In order to achieve these goals the research was devided into five research themes, i.e.

1. process conditions necessary for optimal sulphide oxidation
2. factors determining the sulphur and sulphate formation
3. prevention of the growth of unwanted bacteria like Thiothrix and sulphide producing bacteria
4. kinetics of chemical and biological sulphide oxidation
5. application of the process

**Process Conditions**

Sofar no literature is available about the oxidation of sulphide to elemental sulphur in mixed cultures in a wastewater purification process. More information is necessary about the optimal process parameters, like pH, oxygen and sulphide concentration. Also an effective start up procedure has to be found. We need to know what kind of sulphur compounds will be produced in a sulphide oxidation reactor. Also the effect of polysulphide formation on the biomass and the sulphur settling characteristics has been investigated.

In order to assess the optimal process conditions for sulphide oxidation, stirred polyurethane carrier reactors were used. No organic compounds were present in the feed solutions. Chapter 2 describes the effect of the pH, temperature, sulphide concentration and oxygen concentration. The results found with adapted biomass show that a satisfactory sulphide removal takes place in the pH range 6.5 to 9.0. Above pH 9.0 sulphide removal detoriates. pH values below 6.5 were not investigated. The optimal pH was found to be situated in the pH-range 8.0 - 8.5. The effect of the temperature on the sulphide removal capacity of the system was investigated using biomass adapted to a temperature of 20°C. The optimal temperature range is found between 25 and 35 °C, which was measured at a sulphide concentration of only 2 mg/l. Increasing the oxygen or the sulphide concentration leads to a higher sulphide removal rate.

In a free cell suspension no sulphide substrate inhibition was found up to a concentration of 100 mg/l. However, we found substrate inhibition when the activity of the biomass was measured using the biological oxygen monitor and when these activity measurements were corrected for the chemical oxidation (Chapter 7). An explanation for the fact that no substrate inhibition is found in Figure 8 (Chapter 2) is that the decrease in biological activity is compensated by the increase in chemical sulphide oxidation.

Chapter 2 describes a fast start-up procedure, which shows that no special steps have to be taken in order to have a successful rapid start-up. After inoculation with 3 PUR particles (1%) the sulphide effluent concentration went down from 80 to 2 mg/l in five days at a HRT of 22 minutes.

We found that the concentrations of other sulphur compounds besides elemental sulphur and sulphate were negligible (Chapter 2).

Sulphide concentrations exceeding 10 mg/l in the sulphide removal reactors cause polysulphide formation, which leads to a decrease of the sulphur settling properties. No specific effect of polysulphide on the biomass could be detected (Chapter 2).

**Sulphur or Sulphate Formation**

It seems obvious that bacteria would prefer to produce sulphate rather than elemental sulphur, because sulphate formation provides 8 electrons, while sulphur formation will provide only 2 electrons. Despite this difference in energy yield there seem to be three factors which force the biomass into sulphur production viz. sulphide-to-oxygen ratio (found for chemical sulphide oxidation), sulphide concentration (found for purple bacteria) sulphide sludge loading (transiently found for Thiobacillus denitrificans).

In order to assess the more specific conditions that promote sulphur instead of sulphate formation, we used stirred reactors with and without polyurethane carrier material. In addition we also investigated the sulphur formation in a biorotor and a upflow reactor. Chapter 3 describes the influence of the carrier material, oxygen concentration and sulphide concentration.

The sulphide concentration appears to be a very important factor with respect to the sulphate production. In free cell suspensions little if any sulphate formation was found at sulphide concentrations exceeding 5 mg/l. Probably sulphide is a more preferred substrate than sulphur or sulphide is toxic for the sulphate producing bacteria. This conclusion is supported by the fact that in a system with polyurethane carrier material sulphate formation was found up to a sulphide concentration of 20 mg/l. It can be estimated (Chapter 3) that the sulphide concentration inside the PUR particles decreases faster than the oxygen concentration and therefore it seems possible that inside the PUR particles sulpate production can occur at sulphide bulk concentrations exceeding 5 mg/l. Chapter 7 describes the presence of two types of bacteria. Type A can produce sulphate but is inhibited by sulphide concentrations exceeding 10 mg/l. Type B produces elemental sulphur but is not sensitive to sulphide inhibition. Therefore we conclude that sulphide is not a more preferred substrate than sulphur but that sulphide is toxic to the sulphate producing bacteria.

Lowering the oxygen concentration leads to a drop in the sulphate production in both a free cell suspension and a reactor system with carrier material (Chapter 3). This effect is of less importance at higher sulphide loading rates. It was found (Chapter 7) that the activity of the sulphate producing bacteria (type A) decreases with decreasing oxygen concentrations. This bacterial behaviour explains the relation between the oxygen and sulphate concentrations.

Chapter 7 describes the relation between the sludge loading rate and the sulphur formation. In free cell suspensions at sludge loading rates below 10 mg $S^{2-}$/mg N.hour no sulphur is formed, while no sulphate is formed when the sludge loading rate exceeds 50 mg $S^{2-}$/mg N.hour. Between 10 and 50 $S^{2-}$/mg N.hour both sulphur and sulphate are formed. Chapter 3 describes an experiment which supports these observations. In a PUR particles containing reactor, it is shown that a decreasing number of PUR particles leads to less sulphate formation.

Chapter 8 deals with the effect of the liquid hydraulic regime on the sulphate formation. It was found that in the upflow reactor and in the biorotor reactor less sulphate was formed than in the CSTR mentioned in Chapter 3, under the same operational conditions. This phenomenon can be explained because the sulphide concentrations in the upflow and biorotor reactor (more plugflow) are higher than

the sulphide concentrations in the CSTR, while the effluent concentrations are the same.

**Unwanted Bacteria**

In a sulphide reactor merely two groups of unwanted bacteria can develop, viz. bacteria that store the sulphur inside the cell like Thiothrix and bacteria that produce sulphide like Desulfuromonas acetoxidans (sulphur reducer) and Desulfobulbus propionicus (sulphate reducer). Both groups of bacteria can only develop, when **organic compounds are present** in the wastewater. We investigated the possibilities to prevent the growth of these bacteria.

Chapter 4 deals with the growth conditions of Thiothrix bacteria in the sulphide removing reactor, because they can represent serious problems for two main reasons: a. Thiothrix accumulates the produced sulphur inside the cell, which makes the reuse of sulphur more difficult and consequently a lot of biomass will be produced and b. Thiothrix can cause serious sludge bulking problems. We found that growth of Thiothrix can be prevented by applying high sulphide volumetric loading rates. The reason for this is not clear, because hardly anything is known about the sulphide affinity and sulphide inhibition of Thiothrix. We presume that at higher sulphide loading rates Thiothrix is unable to compete against the sulphur producing organisms. The shearforce, the type of carrier material used, the sulphide influent concentration and the HRT have hardly any influence on the development of Thiothrix in these reactors. Thiothrix growth is found at pH values ranging from 7 to 8.5.

We found that sulphur and sulphate reducing bacteria play an important role in the sulphide removing reactors used for papermill wastewater (Chapter 9). The sulphide removal efficiency deteriorates due to the activity of these bacteria. Therefore more research on this subject was necessary. Chapter 5 describes growth conditions of the sulphur and sulphate reducing bacteria found in the sulphide removal reactors. It was shown that acetate-degrading sulphur-reducing but no acetate-degrading sulphate-reducing bacteria were present in the sludge from the sulphide-oxidation reactor. With propionate as electron donor no degradation occurred in the presence of sulphur, but sulphate-dependent propionate oxidation did occur.
During a start-up phase sulphide production as a result of the sulphur/sulphate reducing bacteria started after six days, while already 95% of the sulphide was removed after one day. In a reactor system with suspended cells the sulphide production was only 0.6% of the sulphide oxidation, while in reactors using carrier material such as Rasschig rings and polyurethane this was 2% and 4% respectively. The optimal temperatue and pH for sulphide production in the reactors were 30°C and 8.0.

**Kinetics of Sulphide Oxidation**

So far little relevant kinetical data are known on the biological oxidation of sulphide into elemental sulphur. Such information is very desirable in order to describe and understand the oxidation process. Chapter 6 deals with the chemical oxidation of sulphide. We studied the uncatalyzed sulphide oxidation in a phosphate buffered system as a function of the oxygen and sulphide concentration. Based on the results obtained the following emperical equation was found:

$$R_j = k\,[S]^m[O]^{n\log[S]} \qquad (mg/l.h)$$

The values for the constants m, n, k were found to be 0.408, 0.391 and 0.566 respectively for sulphide concentrations in the range 2 to 600 mg/l and for oxygen concentrations in the range 0.1 to 8.5 mg/l. Previously published research data on chemical sulphide oxidation only deal with distinctly lower sulphide concentrations, i.e. ranging from 0.8 - 39 mg/l and signifanctly other equations are found.

In measuring the biological activity in the biological oxygen monitor (BOM) we find a combination of the chemical and biological oxidation, because they will occur simutanuously. With the above mentioned equation we can correct these BOM data for the chemical component. Chapter 6 describes the sulphide affinity of two different cell suspensions. It is clear that the biological sulphide oxidation is faster than the uncatalyzed chemical oxidation up to a sulphide concentration of 600 mg/l, but is a factor 75 faster at sulphide concentrations not exceeding 10 mg/l.

Chapter 7 deals with the biological sulphide oxidation. The influence of the sulphide loading rate on the growth yield and on the specific oxidation rate were investigated with free cell suspensions. It was concluded that at least two types of bacteria were present. Type A grows at sulphide loadings rates up to 200 mg/l and produces mainly sulphate. Type B grows at high loading rates and produces mainly sulphur. The characteristics of these two bacterial types are summarized in the table below.

| | A | B |
|---|---|---|
| sulphide affinity | high | low |
| max. oxidation capacity ($mgS^{2-}$/mg-N.min) | 0.8 | 0.5 |
| yield on sulphate (mg-N/mg-S) | 0.011 | - |
| yield on sulphur (mg-N/mg-S) | 0.0039 | 0.0012 |
| substrate inhibition with $S^{2-}$ | yes | no |
| substrate inhibition with $O_2$ | no | yes |

It seems that type A is a kind of autotrophic Thiobacillus which is known from the literature, with a growth yield of 3 g DS/mol S, which is in the same order of magnitude as growth yields of autotrophic sulphide oxidizers found by others (5 - 13 g DS/mol S). Type B has not been described yet and has a significantly lower growth yield (0.3 g DS/mol S).

The difference in growth yield between type A and B explains the phenomenon that at low sulphide loading rates the biomass production is higher than at higher sulphide loading rates. No sulphate is produced anymore at sulphide loading rates exceeding 240 mg/l.h and therefore the growth yield decreases seriously. The maximal biomass production is found at sulphide loading rates ranging from 100 to 150 mg/l.h. At higher sulphide loading rates also the chemical oxidation becomes more important because of the increasing sulphide concentrations in the reactor, which will also lead to a lower biomass production.

In nature type B grows probably in an ecological nische that is characterized by a high sulphide concentration and a low oxygen concentration. At high oxygen concentrations the bacteria probably can not compete with the chemical oxidation. Due to the low oxygen concentration the bacteria of type B have specialized on the formation of sulphur.

**Application of the Process**

Discharge of treated wastewaters in the environment is only allowed when its sulphide concentration is well below 2 mg/l. Therefore a CSTR certainly is not the best choice for a sulphide removal system. Chapter 8 describes and compares the feasibility of three labscale polyurethane carrier reactors, e.g. a biorotor reactor, a CSTR and an upflow reactor, which were fed with composite wastewater. High loading rates must be applied, in order to achieve the required high conversion of sulphide into sulphur andto prevent growth of Thiothrix. However at high loading rates the sulphide effluent concentration of a CSTR generally remains above below 2 mg/l. Therefore a system with a more plug flow character seems more feasible. In the upstream part of the reactor high loading rates can be imposed in order to produce sulphur, while downstream the loading rate can be low enough to achieve a sulphide effluent concentration of 2 mg/l.

The results show that the biorotor and upflow reactor have a much higher sulphide removal capacity and removal efficiency than the CSTR-system. At a sulphide effluent concentration of 2 mg/l less sulphate is formed in the upflow and biorotor reactor than in the CSTR reactor. A comparison is given in the table below.

| reactor type | HRT (h) | $S^{2-}$ load (mg/l.h) | $S^{2-}$ conc. effluent (mg/l) | removal efficiency (%) |
|---|---|---|---|---|
| CSTR | .37 | 375 | 39 | 70 |
| biorotor | .22 | 417 | 1 | 99.5 |
| upflow | .22 | 454 | 2 | 98 |

Therefore we conclude that the upflow and biorotor reactor are more feasible systems for the sulphide removal process than the CSTR.

Besides PUR particles also Rasschig rings have been investigated in the biorotor. The capacity of the system with Rasschig rings was a factor 2 lower than the system with PUR particles.

Chapter 9 deals with the applicability of the biorotor and upflow reactor for removing sulphide from anaerobically treated papermill wastewater. Due to clogging problems in the upflow reactor we concluded that this reactor was less suitable for this type of wastewater. Experiments with the biorotor showed that the removal efficiency was lower than found in the lab study (Chapter 8). This could be attributed to the activity of the sulphur/sulphate reducing bacteria, which can use acetate and propionate to produce sulphide (Chapter 5). The activity of the sulphide producing bacteria can be minimized by using Rasschig rings instead of polyurethane and by applying a higher rotation speed of the rotor. With the biorotor reactor using Rasschig rings a sulphide removal rate of 620 mg/l.h at a removal efficiency of 95% at a HRT of 13 minutes was achieved, at a rotation speed of 46 rpm, while only 8% of the sulphide was converted to sulphate. The influent concentration was 140 mg/l, the pH 8 and the temperature was 27°C. At the same time an acetate removal rate of 144 mg/l.h was achieved. The much higher removal capacity (including the not measured sulphide load of the sulphur reduction) of the papermill wastewater biorotor compared with the lab biorotor probably can be attributed to the higher temperature, viz. 27°C instead of 20°C. The lower sulphate effluent concentrations found with the papermill wasterwater biorotor can be explained by the activity of sulphate reducing bacteria, which are able to convert sulphate to sulphide using propionate.

Presently a pilot plant reactor of 4 $m^3$ is investigated using the same papermill wastewater for assessing the feasibility of the new system on a larger scale.

## CONCLUSIONS

The newly developed biotechnological sulphide removal system accomplishes a removal rate and efficiency which at least is similar to that of other sulphide removal systems, which are based on the oxidation with air. Application of the system is possible in the pH range 6.5 - 9.0 and in a temperature range of approximately 15 - 40 °C. Apart from polysulphides, which can be considered as an association between sulphide and sulphur, only two end-products of the sulphide oxidation could be detected, viz. elemental sulphur and sulphate.

At least two types of sulphide oxidizing bacteria are important in the bioconversion process, viz. a sulphate producing (type A) and a sulphur producing bacteria (type B). Type A is inhibited by sulphide and type B by oxygen. As the conversion of sulphide into sulphur is the main aim of the system, growth of type B should be stimulated. This can be accomplished by imposing high sulphide loading rates (>200 mg/l.h) and maintaining low oxygen concentrations (< 4 mg/l). The

growth yield of type B is a factor 10 lower than that of type A, and therefore little biological sludge will be produced when sulphur is the end-product.

The start-up of the system is fast, viz. 5 days in a CSTR (1% inoculum), when applying a sulphide influent concentration of 100 mg/l and a HRT of 22 minutes. The oxygen concentration should not exceed 4 mg/l, because inhibition of type B should be prevented.

The noncatalyzed chemical sulphide oxidation (at oxygen concentration 4 mg/l) is considerably slower (75 times) than the biological sulphide oxidation at sulphide concentrations below 10 mg/l and about a factor 6 at sulphide concentrations up to 600 mg/l. We found a new emperical equation describing uncatalyzed chemical oxidation, as a function of the oxygen and sulphide concentration, in a phosphate buffered system at pH=8.

The problems caused by unwanted bacteria can be minimized by applying high sulphide loading rates (prevention of Thiothrix growth) and a high rotation speed (prevention of the sulphide producing bacteria). The sulphur reducing bacteria, present in the sludge, can use acetate but not propionate, while the sulphate reducing bacteria use propionate but not acetate.

With anaerobically treated papermill wastewater using a biorotor reactor, the system can accomplish a removal rate of 620 mg/l.h at a removal efficiency of 95% and at a HRT of 13 minutes, while only 8% of the sulphide is converted to sulphate.

Sulphate production can be minimized using sufficiently high sulphide loading rates and an adjusted oxygen concentration. When organic compounds (such as propionate) are present in the wastewater the sulphate reduction will also keep the sulphate concentration at a sufficiently low level.

## Samenvatting

Anaërobe biologische zuivering wordt sinds het begin van de jaren '80 in toenemende mate beschouwd als een aantrekkelijk alternatief voor de meer traditionele aërobe biologische zuivering. Bij de anaërobe behandeling van sulfaathoudend afvalwater kunnen zich echter problemen voordoen. Sulfaatreducerende bacteriën kunnen tijdens het anaërobe zuiveringsproces het sulfaat als electronen acceptor gebruiken, wat leidt tot de vorming van waterstofsulfide. De toxiciteit, corrosieve eigenschappen, rotte eieren geur en de hoge zuurstofvraag van sulfide schrijven een strenge controle op de lozing van deze stof in het milieu voor.

Dit proefschrift beschrijft het onderzoek naar een nieuw biotechnologisch proces voor sulfideverwijdering, waarbij getracht wordt sulfide in zwavel om te zetten met behulp van de kleurloze zwavelbacteriën.
De doelstellingen van het onderzoek waren:

A ontwikkeling van een efficiënt en betrouwbaar verwijderings systeem voor de zuivering van sulfide bevattende afvalwaters.
B vaststelling van de condities waaronder sulfide in zwavel wordt omgezet in plaats van in sulfaat.

Het onderzoek is verder onderverdeeld in vijf onderzoeksthema's, namelijk:

1. procesomstandigheden die de optimale sulfide oxydatie bepalen
2. factoren die de zwavel- en sulfaatvorming bepalen
3. de groei van ongewenste bacteriën, zoals Thiothrix en sulfide producerende bacteriën voorkomen
4. kinetische parameters voor de chemische en biologische sulfide oxydatie
5. toepassing van het proces

### Proces Omstandigheden

Vrijwel geen literatuur is bekend over sulfide oxydatie tot elementaire zwavel in gemengde populaties in een waterzuiveringsproces. Meer informatie is daarom nodig over de optimale proces omstandigheden, zoals de pH, zuurstof- en sulfideconcentraties. Bovendien moet een effectieve opstart procedure beschreven worden en moet onderzocht worden welke zwavel verbindingen er tijdens de sulfide oxydatie gevormd worden. Verder moet de invloed van polysulfiden op de bacteriën en op de bezink eigenschappen van zwavel bestudeerd worden.

Gemengde reactoren met polyurethaan als dragermateriaal, werden gebruikt om de proces omstandigheden voor sulfide oxydatie vast te stellen. Er werden geen organische verbindingen aan de reactoren toegevoegd tijdens deze experimenten. Hoofdstuk 2 beschrijft de invloed van de pH, temperatuur, sulfide- en zuurstofconcentratie. Met geadapteerde biomassa werd gevonden dat in het pH gebied 6.5 tot 9.0 voldoende sulfideverwijdering mogelijk is. Boven pH=9 neemt de sulfide oxydatie sterk af. pH waarden onder de 6.5 zijn niet onderzocht. De optimale pH ligt tussen de 8 en 8.5. De invloed van de temperatuur op de sulfide oxydatie was onderzocht met een biomassa die geadapteerd was aan 20°C. Het optimale temperatuur gebied ligt tussen de 25 en 35°C. Dit is bepaald met de activiteitsmeter, bij een sulfide concentratie van 2 mg/l. Verder is gevonden dat de verhoging van de sulfide en zuurstofconcentratie leidt tot een hogere oxydatiesnelheid.

In vrije celsuspensies wordt geen substraat inhibitie gevonden tot aan sulfide concentraties van 100 mg/l. In de activiteitsmeting werd wel substraat inhibitie gevonden, wanneer de waarden gecorrigeerd werden voor de chemische oxydatie (Hoofdstuk 7). Een verklaring voor het ogenschijnlijk afwezig zijn van substraat inhibitie in Figuur 8 (Hoofdstuk 2) is waarschijnlijk dat de afnemende biologische oxydatie wordt gecompenseerd door de toenemende gekatalyseerde chemische oxydatie.

Hoofdstuk 2 beschrijft een snelle opstart procedure. Na het toevoegen van 3 PUR blokjes als ent, daalde de sulfide concentratie van 80 tot 2 mg/l in vijf dagen bij een verblijftijd van 22 minuten.

De concentraties van zwavelverbindingen anders dan sulfide, zwavel en sulfaat zijn verwaarloosbaar in de sulfide oxydatie reactoren (Hoofdstuk 2).

Sulfide concentraties boven de 10 mg/l veroorzaken aanzienlijke polysulfidevorming in de reactoren, wat leidt tot een vermindering van de bezinkeigenschappen van zwavel. Geen specifieke effecten van polysulfiden op de biomassa werden gevonden (Hoofdstuk 2).

**Zwavel en Sulfaat Vorming**

Het lijkt duidelijk dat de bacteriën liever sulfaat dan zwavel vormen, omdat sulfaatvorming 8 electronen levert, terwijl zwavelvorming slechts 2 electronen levert. Ondanks dit verschil in energie opbrengst, zijn er blijkbaar drie factoren die de biomassa dwingen zwavel te produceren in plaats van sulfaat, namelijk sulfide-zuurstof ratio (gevonden bij chemische oxydatie), sulfide concentratie (gekleurde zwavelbacteriën) en de sulfide slib belasting (tijdelijk gevonden bij Thiobacillus denitrificans).

Om proces omstandigheden vast te stellen die de zwavelvorming bevorderen in plaats van sulfaatvorming, zijn gemengde reactoren gebruikt met en zonder dragermateriaal. Bovendien is de zwavelvorming in een opstroom en biorotor reactor bekeken. Hoofdstuk 3 beschrijft de invloed van dragermateriaal, zuurstof en sulfide concentratie.

De sulfaatvorming blijkt zeer gevoelig te zijn voor de sulfide concentratie in de reactor. In vrije celsuspensies wordt vrijwel geen sulfaat meer gevormd als de sulfide concentratie boven de 5 mg/l komt. Waarschijnlijk is sulfide toxisch voor de sulfaatvormende bacteriën. Deze conclusie wordt ondersteund door het feit dat in een reactor met dragermateriaal sulfaatvorming werd gevonden tot sulfide concentraties van 20 mg/l. Verwacht kan worden (Hoofdstuk 3) dat de sulfide concentratie in de PUR blokjes sneller afneemt dan de zuurstofconcentratie. Het lijkt daarom mogelijk dat in de PUR blokjes sulfaatvorming optreedt bij sulfide concentraties boven 5 mg/l. Hoofdstuk 7 beschrijft de aanwezigheid van twee soorten bacteriën. Sulfaatvormers (type A) die geremd worden door sulfide bij concentraties boven de 10 mg/l en zwavelvormers (type B) die niet geremd worden door sulfide. Uit deze waarnemingen blijkt dat sulfide inderdaad toxisch is voor de sulfaatvormende bacteriën.

Verlaging van de zuurstofconcentratie heeft een verlaging van de sulfaatproduktie tot gevolg, zowel in een reactor met als zonder dragermateriaal (Hoofdstuk 3). Dit effect is minder belangrijk bij hoge sulfide belastingen. In Hoofdstuk 7 wordt beschreven dat de activiteit van de sulfaatproducerende bacteriën (type A) afneemt bij lagere zuurstofconcentraties. Dit bacterie gedrag verklaart de relatie tussen de zuurstof- en sulfaatconcentratie.

Hoofdstuk 7 beschrijft de relatie tussen de slibbelasting en de zwavelvorming. In celsuspensies wordt geen zwavel gevormd bij slibbelastingen lager dan 10 mg$S^{2-}$/mg-N.uur, terwijl geen sulfaat gevormd wordt bij slibbelastingen hoger dan 50 mg$S^{2-}$/mg-N.uur. In het gebied tussen deze waarden worden zowel zwavel als sulfaat gevormd. In Hoofdstuk 3 wordt een experiment beschreven dat deze waarneming ondersteund. In een reactor met PUR deeltjes blijkt de sulfaatproduktie sterk te verminderen wanneer een gedeelte van de PUR deeltjes verwijderd worden uit de reactor.

Hoofdstuk 8 wordt het effect van het propstroomachtige karakter van de vloeistof op de sulfaatvorming beschreven. Het blijkt dat in de opstroom en biorotor reactor beduidend minder sulfaat gevormd wordt dan in de gemengde reactor, bij dezelfde omstandigheden. Dit verschijnsel kan verklaard worden doordat bij dezelfde effluentconcentraties de sulfide concentraties in de opstroom en biorotor reactor hoger zijn dan in de gemengde reactor.

**Ongewenste Bacteriën**

In een sulfide reactor kunnen voornamelijk twee soorten ongewenste bacteriën groeien, namelijk bacteriën die het gevormde zwavel intracellulair opslaan, zoals Thiothrix en sulfide producerende bacteriën, zoals Desulfuromonas acetoxidans (zwavel reduceerders) en Desulfobulbus propionicus (sulfaat reduceerders). Beide groepen bacteriën kunnen alleen groeien, wanneer **organische stoffen aanwezig zijn** in het afvalwater. Wij hebben de mogelijkheden onderzocht om de groei van deze bacteriën te voorkomen.

Hoofdstuk 4 behandelt de groeiomstandigheden van Thiothrix bacteriën in de sulfide verwijderingsreactoren. Deze bacteriën zijn ongewenst omdat zij het gevormde zwavel intracellulair opslaan, waardoor het hergebruik van zwavel bemoeilijkt wordt en omdat Thiothrix een bekende licht slib veroorzaker is. De groei van Thiothrix kan voorkomen worden door hoge sulfide volumebelastingen toe te passen. Een verklaring hiervoor is nog niet gevonden, omdat vrijwel niets bekend is over de sulfide affiniteit en inhibitie van Thiothrix. Voorlopig veronderstellen wij dat bij een hoge sulfidebelasting Thiothrix niet kan concurreren met de zwavelvormende organismen. De turbulentie, het soort dragermateriaal, de influentconcentratie en de verblijftijd hebben nauwelijks invloed op de ontwikkeling van Thiothrix in deze reactoren. Thiothrix groei werd gevonden in het pH gebied 7 tot 8.5.

Sulfaat- en zwavelreducerende bacteriën zijn van grote betekenis in een sulfide oxydatie reactor (Hoofdstuk 9). Als gevolg van de sulfideproductie van deze bacteriën verslechtert het sulfide verwijderingsrendement. In Hoofdstuk 5 worden de groeiomstandigheden van de zwavel- en sulfaatreducerende bacteriën, gevonden in de sulfide verwijderingsreactor, beschreven. Het blijkt dat de zwavelreduceerders wel acetaat, maar geen propionaat kunnen gebruiken, terwijl de sulfaatreduceerders juist wel propionaat, maar geen acetaat kunnen gebruiken. Tijdens de opstartfase bleek dat de sulfideproductie als gevolg van de zwavel/sulfaat reductie op gang kwam na 6 dagen, terwijl reeds 95% van de sulfide verwijderd werd na 1 dag. In een reactor met celsuspensie was de sulfideproductie slechts 0.6% van de sulfide oxydatie, terwijl in reactoren met dragermateriaal, zoals Rasschig ringen en PUR blokjes, dit 2% en 4% was resp. De optimale temperatuur en pH voor sulfide produktie in de reactoren is 30°C en 8.0.

**Kinetische Parameters van de Sulfide Oxydatie**

Er zijn praktisch geen kinetische gegevens bekend over de biologische oxydatie van sulfide in elementaire zwavel. Deze informatie is echter wel gewenst om het proces te kunnen beschrijven en te begrijpen. Hoofdstuk 6 behandelt vooral de kinetiek van de chemische oxydatie van sulfide. We hebben de niet gekatalyseerde sulfide oxydatie bestudeerd in een fosfaat gebufferd systeem, als functie van de sulfide en zuurstofconcentratie. Gebaseerd op de verkregen resultaten is een empirische vergelijking opgesteld:

$$R_i = k\,[S]^m[O]^{n\log[S]} \qquad (mg/l.h)$$

De waarden voor de constanten m, n en k zijn bepaald op 0.408, 0.391 en 0.566 resp. voor sulfide concentraties van 2 tot 600 mg/l en voor zuurstofconcentraties van 0.1 tot 8.5 mg/l. In eerder gepubliceerde onderzoeken over chemische sulfide oxydatie, werden de metingen bij veel lagere sulfide concentraties uitgevoerd (0.8 tot 39 mg/l) en werden andere empirische vergelijkingen gevonden.

Wanneer de biologische sulfide oxydatie gemeten wordt in de activiteitsmeter, vinden we een combinatie van biologische en chemische activiteit, omdat beiden tegelijk plaatsvinden. Met de hierboven genoemde vergelijking kunnen we deze meetgegevens corrigeren voor het chemische aandeel. Hoofdstuk 6 beschrijft de sulfide affiniteit van twee verschillende celsuspensies. Duidelijk wordt dat de biologische sulfide oxydatie sneller is dan de niet gekatalyseerde chemische

oxydatie tot aan sulfide concentraties van 600 mg/l, maar bij sulfideconcentraties beneden 10 mg/l is de biologische oxydatie een factor 75 sneller.

Hoofdstuk 7 behandelt de biologische sulfide oxydatie. Het effect van de sulfidebelasting op de celopbrengst en specifieke oxydatiesnelheid werd onderzocht met celsuspensies. Geconcludeerd werd dat er tenminste twee soorten bacteriën aanwezig moesten zijn in de reactoren. Een sulfaat produceerder (type A), die groeit tot sulfidebelastingen van 200 mg/l.h en een zwavelvormer (type B) die vooral groeit bij hoge sulfidebelastingen. De karakteristieken van deze twee bacterie typen zijn samengevat in de volgende tabel.

| | A | B |
|---|---|---|
| sulfide affiniteit | hoog | laag |
| max. oxydatie capaciteit ($mgS^{2-}$/mg-N.min) | 0.8 | 0.5 |
| celopbrengst op sulfaat (mg-N/mg-S) | 0.011 | - |
| celopbrengst op zwavel (mg-N/mg-S) | 0.0039 | 0.0012 |
| substraat inhibitie met sulfide | wel | niet |
| substraat inhibitie met zuurstof | niet | wel |

Type A lijkt een soort autotrofe Thiobacillus bekend uit de literatuur, met een celopbrengst van 3 g DS/mol S, wat in dezelfde orde van grootte is als de celopbrengst gevonden voor autotrofe sulfide oxideerders (5 -13 g DS/mol S). Type B is nog niet beschreven en heeft een veel lagere celopbrengst (0.3 g DS/mol S).

Het verschil in celopbrengst tussen type A en type B, verklaart dat bij lage sulfidebelastingen de biomassa produktie hoger is dan bij hoge sulfidebelastingen. Boven een sulfidebelasting van 240 mg/l.h wordt geen sulfaat meer gevormd en daardoor neemt ook de celopbrengst af. De maximale biomassa produktie wordt gevonden bij sulfidebelastingen tussen de 100 en 150 mg/l.h. Bij hogere sulfidebelastingen wordt ook de chemische oxydatie belangrijker, omdat de sulfide concentratie in de reactor toeneemt. Hierdoor wordt ook de biomassa produktie lager.

Onder natuurlijke omstandigheden groeit type B waarschijnlijk in een ecologische nis, die gekarakteriseerd wordt door een hoge sulfide concentratie en een lage zuurstofconcentratie. Bij hoge sulfide en zuurstofconcentraties kunnen de bacteriën waarschijnlijk niet concurreren met de chemische oxydatie. Als gevolg van de lage zuurstofconcentratie zijn de bacteriën van type B zich waarschijnlijk gaan specialiseren op zwavelvorming.

**Toepassing van het Proces**

Lozing van sulfidehoudend afvalwater is alleen toegestaan wanneer de sulfideconcentratie beneden de 2 mg/l is. Daarom is een gemengde reactor niet de beste keuze voor een sulfide verwijderingssysteem. Hoofdstuk 8 beschrijft en vergelijkt de toepasbaarheid van drie reactortypen, namelijk een gemengde reactor, een opstroom reactor en een biorotor reactor, allen met polyurethaan als dragermateriaal. Er is gewerkt met kunstmatig afvalwater zonder organische stoffen. Hoge belastingen moeten toegepast worden om procentueel veel sulfide in zwavel om te zetten en om Thiothrix groei te voorkomen. Bij hoge sulfidebelastingen voldoet een gemengde reactor niet meer aan de effluenteis van 2 mg/l. Daarom lijken systemen met een meer propstroom karakter meer geschikt, omdat aan het begin van de reactor een hoge belasting wordt toegepast, waardoor sulfaatvorming wordt voorkomen. Aan het einde van de reactor is de belasting laag genoeg om een effluentconcentratie van 2 mg/l te bereiken.

Gevonden is dat de opstroom en biorotor reactoren inderdaad een veel hoger verwijderingscapaciteit en zuiveringsrendement hebben dan de gemengde reactor. Dit wordt geïllustreerd in de volgende tabel.

| reactor type | verblijftijd (h) | $S^{2-}$ load (mg/l.h) | $S^{2-}$ conc. effluent (mg/l) | rendement (%) |
|---|---|---|---|---|
| CSTR | .37 | 375 | 39 | 70 |
| biorotor | .22 | 417 | 1 | 99.5 |
| opstroom | .22 | 454 | 2 | 98 |

Verder is de sulfaatproduktie in de opstroom en biorotor reactor bij een sulfide effluentconcentratie van 2 mg/l veel lager dan die in de gemengde reactor.

Behalve PUR blokjes zijn ook Rasschig ringen getest in de biorotor reactor. De capaciteit van het systeem met Rasschig ringen is een factor twee lager dan met PUR blokjes.

Hoofdstuk 9 behandelt de toepassing van de opstroom en biorotor reactoren op anaëroob behandeld papierfabriek afvalwater. Vanwege dichtgroeien van het dragermateriaal in de opstroom reactor, is geconcludeerd dat dit reactor type niet geschikt is voor dit soort afvalwater. Met de biorotor reactor werd gevonden dat de zuiveringsrendement op papierafvalwater lager was dan op kunstmatig afvalwater tijdens de labstudies (Hoofdstuk 8). Dit verschil blijkt het gevolg te zijn van de activiteit van zwavel en sulfaatreducerende bacteriën, die in staat zijn om met acetaat en propionaat sulfide te produceren uit zwavel en sulfaat (Hoofdstuk 5). De activiteit van de sulfide producerende bacteriën kan verminderd worden door Rasschig ringen te gebruiken in plaats van PUR blokjes en door een hogere rotor draaisnelheid toe te passen. De biorotor met Rasschig ringen bereikte een verwijderingscapaciteit van 620 mg/l.h en een zuiveringsrendement van 95% bij een verblijftijd van 13 minuten en een draaisnelheid van 46 rpm (rotordiameter ca. 14 cm). Slechts 8% van het sulfide werd omgezet in sulfaat. De influent concentratie was 140 mg/l, de pH was 8 en de temperatuur was 27°C. Ook werd er 144 mg/l.h acetaat verwijderd. De veel hoger verwijderingscapaciteit (inclusief de niet gemeten sulfidebelasting van de zwavelreductie) van de biorotor op papierafvalwater, vergeleken met het labonderzoek, wordt waarschijnlijk veroorzaakt door de temperatuur, deze is namelijk 27°C in plaats van 20°C. De lage sulfaatconcentraties gemeten in het effluent van de papierafvalwater biorotor zijn waarschijnlijk het gevolg van de sulfaat reducerende bacteriën.

Momenteel wordt een proefinstallatie van 4 $m^3$ gebruikt met hetzelfde papierafvalwater om de geschiktheid van het systeem op een grotere schaal te onderzoeken.

## CONCLUSIES

Het nieuw ontwikkelde sulfide verwijderingssysteem, kan een sulfide verwijderingssnelheid en zuiveringsrendement bereiken, die op zijn minst hetzelfde is als van andere sulfide verwijderingssystemen die gebaseerd zijn op oxydatie met lucht. Toepassing van het systeem is mogelijk in het pH gebied 6.5 tot 9 en in het temperatuur gebied van 15 -tot 40°C. Behalve polysulfides, die wij beschouwen als een associatie van zwavel en sulfide, zijn er slechts twee oxydatieproducten gevonden, namelijk zwavel en sulfaat.

Tenminste twee soorten bacteriën zijn belangrijk voor de biologische oxydatie van sulfide, namelijk een sulfaatvormer (type A) en een zwavelvormer (type B). Type A wordt geremd door sulfide en type B door zuurstof. De omzetting van sulfide in elementaire zwavel is het hoofddoel van het proces en daarom moet de groei van type B bevorderd worden. Die kan bereikt worden door hoge sulfidebelastingen (> 200 mg/l.h) en lage zuurstofconcentraties (< 4 mg/l) toe te passen. De celopbrengst van type B is een factor 10 lager dan die van type A, waardoor er maar weinig biologisch slib wordt geproduceerd wanneer zwavel het eindprodukt is.

De opstart van het systeem is snel, namelijk binnen 5 dagen (1% ent) in een gemengde reactor, bij een sulfide influentconcentratie van 100 mg/l en een verblijftijd van 22 minuten. De zuurstofconcentratie moet beneden de 4 mg/l blijven vanwege inhibitie van type B.

De ongekatalyseerde chemische sulfide oxydatie (bij zuurstofconcentratie 7.5 mg/l) is 75 keer langzamer dan de biologische sulfide oxydatie, bij sulfide concentraties beneden de 10 mg/l en 6 keer langzamer bij sulfide concentraties tot 600 mg/l. Een nieuwe empirische vergelijking die de ongekatalyseerde chemische oxydatie beschrijft als functie van de zuurstof en sulfide concentratie, in een pH gebufferd systeem bij pH=8, is gevonden.

De problemen veroorzaakt door de ongewenste bacterie soorten kunnen verkleind worden door een hoge sulfidebelasting toe te passen (voorkomen van Thiothrix groei) en door een hoge rotor draaisnelheid toe te passen, waardoor de sulfide produktie geminimaliseerd wordt. De zwavel reducerende bacteriën aanwezig in het slib kunnen acetaat wel en propionaat niet gebruiken, terwijl de sulfaat reducerende bacteriën juist propionaat wel en acetaat niet kunnen gebruiken.

Wanneer de biorotor reactor toegepast werd op anaëroob behandeld papierfabriek afvalwater werd een verwijderingscapaciteit van 620 mg/l.h en een zuiveringsrendement van 95% bereikt, bij een verblijftijd van 13 minuten, terwijl slechts 8% van het sulfide werd omgezet in sulfaat.

Sulfaat produktie kan voorkomen worden door hoge sulfidebelastingen en een aangepaste zuurstofconcentratie toe te passen. Wanneer organische verbindingen, zoals propionaat, aanwezig zijn in het afvalwater, wordt de sulfaatconcentratie verlaagd door de activiteit van sulfaat reducerende bacteriën.

## Publications

**Chapter 2** is published in Acta Biotechnologica, (1989) 9(3), 271-283, with a different title, viz "Biotechnological process for sulphide removal with sulphur reclamation"

**Chapter 3** is accepted for publication in Biotechnology & Bioenchineering

**Chapter 4** is submitted for publication in Water Research

**Chapter 5** is submitted for publication in Applied Microbiology and Biotechnology

**Chapter 6** is submitted for publication in Water Research

**Chapter 7** is submitted for publication in Biotechnology & Bioenchineering

**Chapter 8** is accepted for publication in Water Research

**Chapter 9** is submitted for publication in Water Research

## Curriculum vitae

De auteur van dit proefschrift is op 22 juli 1961 geboren in Badhoevedorp. In 1979 behaalde hij het diploma atheneum B aan het Pius X Lyceum te Amsterdam. Daarna begon hij met een studie aan de Landbouwhogeschool te Wageningen. In 1985 legde hij het doctoraalexamen af in de studierichting Milieuhygiëne, met als bijvakken Industriële Bedrijfskunde en Communicatieleer. Vanaf april 1986 werkt hij bij de vakgroep Waterzuivering van de Landbouwuniversiteit als toegevoegd onderzoeker aan het onderzoek naar sulfideverwijdering, dat in dit proefschrift beschreven wordt.