Formation and colloidal behaviour of elemental sulphur produced from the biological oxidation of hydrogensulphide

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Proefschrift
ter verkrijging van de graad van doctor
op gezag van de rector magnificus,
dr. C.M. Karssen
in het openbaar te verdedigen
op woensdag 11 september 1996
des namiddags te half twee in de Aula
van de Landbouwuniversiteit te Wageningen

Omslag: 'Artist impression' van een zwavel uitscheidende bacterie (*Thiobacilli*)

Stellingen:

1 Indien een universiteit bang is voor de publieke reaktie op de stellingen van de promovendus, lijkt het raadzaam de paranimfen te vervangen door juristen.

Intermediair, 28 juni 1996, Een heuse universiteit

2 Steudel veronderstelt ten onrechte dat de kolloidchemische eigenschappen van alle biologisch gevormde zwavel-typen gelijk zijn aan die van het synthetisch gevormde 'LaMer' zwavel.

Dit proefschrift

Steudel R., 1989. On the nature of the "Elemental Sulfur" (S°) produced by sulfur oxidizing bacteria- a model for S° globules. in: Autotrophic Bacteria. H.G. Schlegel and B. Bowien (Ed.), Science Tech Publishers, Madosin, WI

3 De hypothese van Buisman dat 2 verschillende bacterie-typen (Type A en Type B) verantwoordelijk zijn voor respectievelijk sulfaat en zwavelvorming moet op grond van de huidige inzichten worden verworpen.

Buisman C.J.N., et al., 1991. Kinetic parameters of a mixed culture oxidizing sulfide and sulfur with oxygen. Biotechnol. Bioeng. **38:** 813-820

4 De vorming en aanwezigheid van thiosulfaat in een sulfide-oxyderende bioreaktor duidt op een gebrek aan biologische oxydatiecapaciteit.

Dit proefschrift

5 Onderzoeksscholen hebben slechts tot doel om minder geld anders te verdelen.

6 Vruchtbare samenwerking tussen onderzoeksgroepen kan niet bestuurlijk worden opgelegd maar moet op 'de werkvloer' tot stand komen.

7 Wanneer vormfouten worden gemaakt in een rechtszaak moet niet alleen de verdachte maar ook de officier van justitie worden ontslagen.

Hans Helgers, 1995

8 De term 'Fuzzy Logic' voor 'knowledge-based' regelstrategien doet vermoeden dat de betreffende onderzoekers niet weten waar ze mee bezig zijn.

9 In de milieu-biotechnologie is het zinvoller om te zoeken naar reeds aanwezige micro-organismen (extremofielen) voor specifieke conversies dan het genetisch manipuleren van reeds voorhanden zijnde cultures.

10 De hooghartigheid van de Federatie van Katholieke Muziekbonden leidt ertoe dat de top "amateur" blaasmuziek in Limburg enkel nog beoefend wordt door professionele muziekanten, die veelal werkzaam zijn in symfonie-orkesten.

11 De in stelling 10 bedoelde "amateur" muziekkorpsen hebben bij concertwedstrijden veelal meer dan 20 ingehuurde professionele muziekanten doch zijn vaak niet in staat om de reguliere dorpsaktiviteiten op te luisteren.

12 Niemand is perfect

Stellingen behorende bij het proefschrift 'Formation and colloidal behaviour of elemental sulphur produced from the biological oxidation of hydrogensulphide'.
Albert J.H. Janssen, 11 september 1996

CONTENTS

CHAPTER 1

INTRODUCTION

Sulphur compounds in the environment

The increase in global population is inevitably associated with a continued industrialization, urbanization and motorization. Increased pollution effects are recorded in both industrial regions and on a global scale due to an increase in industrial activities.[23] Some of these environmental problems are related to the emission of organic and inorganic sulphur compounds, such as sulphur dioxide (SO_2) and hydrogen sulphide (H_2S). Fossil fuel combustion accounts for approximately 90 per cent of the global man-made emission of SO_2. Other major sources of SO_2 are the processing of sulphide ores, oil refining and sulphuric acid production.[6] In 1989, the global anthropogenic emission of sulphur to the atmosphere was estimated at 93 to over 200 million tons per year[1,6] whereas over 90 per cent of all anthropogenic emissions of SO_2 occur in the northern hemisphere.[22] In tropical regions natural emissions from soils, plants, the burning of biomass and volcanoes are believed to be the predominant sources of SO_2.[78] When released to the atmosphere, sulphur dioxide is a severe acidifying compound causing acid-rain. Fortunately, since the 1970s in many industrialized nations SO_2 levels declined as a result of various emission control strategies such as coal desulphurisation, selection of fuels with a low sulphur content, specialized combustion processes and waste gas treatment. Hydrogen sulphide is emitted into the environment as dissolved sulphide in wastewaters and as H_2S in waste gases. Emission into the atmosphere is mainly a result of volcanic activities and evaporation from oceanic waters.[6] In the atmosphere hydrogen sulphide causes acid rain due to its reaction with ozone to sulphuric acid.

Sulphide containing wastewaters are generated by a number of industries such as petrochemical plants, tanneries, viscose rayon manufactures and as a result of the anaerobic treatment of sulphate containing wastewaters.[6,43,56,58] The latter are mainly produced by paper- and pulp industry. Since this thesis deals with the microbiological oxidation of aqueous sulphide more information is given about the physico-chemical properties of this detrimental compound.

Hydrogen sulphide

Hydrogen sulphide is a weak acid which dissociates into HS^- (pK_1=7.04) and S^{2-} (pK_2=11.96) (pK values at 18°C).[46] As usual, we will apply the term "sulphide" for all three entities. Removal of hydrogen sulphide from gases or wastewaters is required for reasons of health, safety and corrosion. The toxicity of hydrogen sulphide gas is well documented (Table 1). In the USA the worker exposure limits are 10 ppm (14 mg/m^3) TWA (time weighted average) and 15 ppm (21 mg/m^3) SEL (single exposure limit). Hydrogen sulphide becomes progressively more dangerous as the H_2S level incurs above toxic limits (70 ppm), becoming lethal at 600 ppm. In bioreactors, increased sulphide levels may lead to the inhibition of the anaerobic bacteria, e.g. acidifiers and methanogens[40] and aerobic *Thiobacilli*.[7,10] Because of its detrimental characteristics, it is forbidden to drain sulphide containing effluents to sewer pipes or surface waters.

Table 1 Hazard levels associated with releases of H_2S

H_2S concentration	Effects
1 ppm	Rotten egg smell, odour complaints
10 ppm	Occupational exposure limit for 8 hours
20 ppm	Self-contained breathing apparatus required
100 ppm	May cause headaches/nausea, sense of smell lost in 2-15 minutes
200 ppm	Rapid loss of smell, burning eyes and throat
500 ppm	Loss of reasoning and balance, respiratory distress in 200 minutes
700 ppm	Immediate unconsciousness, seizures
	Without immediate resuscitation breathing will stop, leading to death

In order to remove sulphide from waste streams, a number of physico-chemical processes are in common use today, which involve direct air stripping, chemical precipitation and oxidation. The relatively high energy requirements or the high chemical and disposal costs, e.g. for FeS or MnO_2 sludge, constitute important drawbacks of these methods. Oxidation processes used for sulphide removal are aeration (catalyzed and uncatalyzed), chlorination, ozonation, potassium permanganate treatment and hydrogen peroxide treatment.[13,14,16,45,49,53,79] In all these processes, apart from elemental sulphur, also thiosulphate ($S_2O_3^{2-}$) and sulphate (SO_4^{2-}) may be formed as end-products. For the removal of H_2S from sour gases, various well-established physico-chemical techniques are available. Many of the processes that are in use at present may be grouped into the categories listed in Table 2.

Table 2. Some examples of physico-chemical processes available for treating sour gases (After Jensen *et al.*[33])

Category	Example	Reagents	Products
Liquid phase chemical reaction	Amines	Alkanolamine	H_2S and CO_2
	Alkaline salts	Potassium carbonate	H_2S and CO_2
Liquid phase physical absorption	Sulfinol	Sulfolane and diisopropanolamine	H_2S and CO_2
	Selexol	Dimethyl ether of polyethylene glycol	H_2S and CO_2
Dry bed	Iron sponge	Iron oxide	S°
	Molecular sieve	Crystalline alkali-metal aluminosilicates	S°
Direct conversion	Stretford	Sodium carbonate, sodium vanadate, anthraquinone	S°
	Lo-Cat	Iron complexes	S°
	Claus	H_2S and SO_2, alumina	S°
	SCOT	Cobalt/molybdenum catalyst and alkanolamine solvent	H_2S

Since these physico-chemical methods require large investment and operational costs, e.g. high pressures, high temperatures or special chemicals, the continuing search for more economical methods has led to investigations into microbiological solutions for purifying H_2S and SO_2 containing gases, as well as coal and petroleum desulphurization.[69,70] Microbiological processes proceed around ambient temperatures and at atmospheric pressure, thus eliminating the need for heat and pressurization power, cutting the energy costs to a minimum. At this moment, all commercially available processes for treating SO_2 containing waste gases are based on physico-chemical methods. In 1992, a joint-venture between the Dutch companies Paques B.V. and Hoogovens was initiated for the development of a new biotechnological process for treating SO_2-containing flue gases.[11] A major advantage of this new process is that a potentially valuable end-product is produced, in the form of elemental sulphur, whilst in the traditionally applied limestone process, huge amounts of gypsum are formed which are mostly deposited as a chemical waste material.

Microbiological treatment of H_2S by using the biological sulphur cycle

Besides the carbon- and nitrogen cycle, the sulphur cycle is important in nature. It has an oxidative and a reductive side which in a natural ecosystem should be in balance. On the reductive side, sulphate and sulphur function as an electron acceptor in the metabolic pathways, used by a wide range of anaerobic bacteria.[80] On the oxidative side of the cycle, reduced sulphur compounds serve as electron donors for anaerobic phototrophic bacteria or provide growth energy for the colourless sulphur bacteria.[59] The biological sulphur cycle is presented in Figure 1.

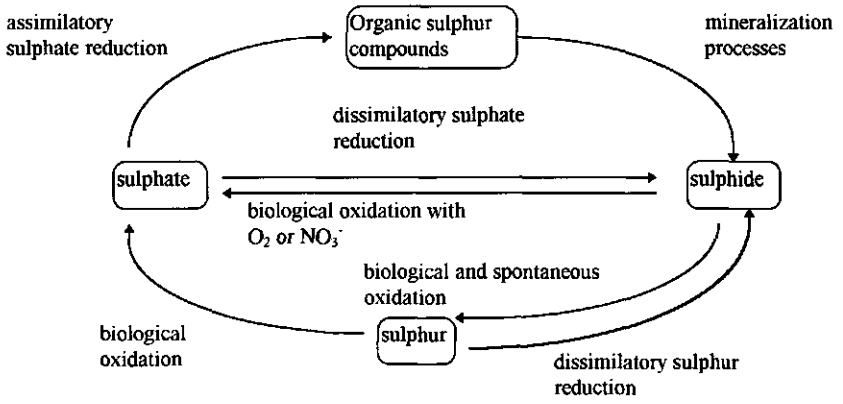

Fig. 1 Biological sulphur cycle

In principle, two different biotechnological processes can be distinguished for the removal of hydrogen sulphide from waste streams, e.g. wastewaters and waste gases. Firstly, genera of the family *Chlorobiaceae* and *Chromatiaceae* catalyze under anaerobic conditions, the photosynthetic Van Niel reaction:[52]

$$2n\ H_2S + n\ CO_2 \xrightarrow{h\nu} 2n\ S^\circ + (CH_2O)_n + n\ H_2O$$

Cork *et al.* proposed a process, using the green sulphur bacteria *Chlorobium limicola* based on this equation.[21] Of the vailable H_2S 67 % was converted to elemental sulphur while the rest was completely oxidized to sulphate. The major disadvantages in using photosynthetic bacteria on a large scale lie in their anaerobic nature and their requirement for radiant energy and hence extremely transparent solutions. Moreover, many of these organisms accumulate elemental sulphur internally, which would make the separation of sulphur and biomass difficult. Therefore, Kim *et al.* immobilized cells of *Chlorobium limicola* in strontium alginate beads in

order to entrap the formed sulphur.[37-39] Several researchers described the oxidation of sulphide to elemental sulphur or sulphate using chemolithoautotrophic bacteria belonging to the genus *Thiobacillus*.[3,8-12, 15,19,26,42,44,55,69-72] Under sulphide limitation in the reactor, *Thiobacilli* can succesfully compete with the chemical oxidation of sulphide because of their high affinity for this compound. Members of the genus *Thiobacillus* are classified as Gram-negative, rod-shaped, colourless sulphur bacteria which utilize reduced inorganic sulphur compounds as their energy source and CO_2 as their main source of carbon. Some species are able to use organic carbon as a supplementary carbon source.[35, 36, 42, 59] Due to their simple nutritional requirements the use of chemolithotrophs for the removal of H_2S is advantageous. Cho *et al.* extensively reported on H_2S removal by the heterotrophic bacterium Xanthomonas sp. strain DY44.[18] The specific H_2S removal rate of this bacterium is however lower than those of purified *Thiobacillus* spp.[17] Furthermore, application of a heterotrophic organism is not favourable if organic compounds are not readily available, e.g. in gas-purification. Table 3 provides examples of the many types found among the colourless sulphur bacteria, together with some of their environmental requirements.

Table 3 Sulphur oxidizing bacteria (After Schlegel[61])

Species	pH of growth	Electron donor	Type[a]
Thiobacillus thiooxidans	2-5	S^{2-}, $S_2O_3^{2-}$, S	o
Thiobacillus ferrooxidans	2-6	Fe^{2+}, $S_2O_3^{2-}$, S	f
Thiobacillus thioparus	6-8	CNS^-, $S_2O_3^{2-}$, S	o
Thiobacillus denitrificans	6-8	CNS^-, $S_2O_3^{2-}$, S	o
Thiobacillus intermedius	2-6	$S_2O_3^{2-}$, S, glutamate	f
Thiobacillus novellus	6-8	$S_2O_3^{2-}$, S, glutamate	f
Thiomicrospira pelophila	6-8	S^{2-}, $S_2O_3^{2-}$, S	o
Sulfolubus acidocaldarius	2-3	S, glutamate, peptone	f

[a] o, Obligately autotrophic; f, facultatively autotrophic

According to Kuenen the following two biological overall reactions may occur in an aerobic sulphide removal system:[41]

$$2\ HS^- + O_2 \rightarrow 2\ S^\circ + 2\ OH^- \qquad \Delta G^\circ = -169.35\ kJ/mol \qquad (1)$$

$$2\ HS^- + 4\ O_2 \rightarrow 2\ SO_4^{2-} + 2\ H^+ \qquad \Delta G^\circ = -732.58\ kJ/mol \qquad (2)$$

Since the formation of sulphate yields most energy, this reaction is preferred by the micro-organisms. The formation of sulphur will only proceed under oxygen-limiting circumstances or high sulphide loading rates whereas sulphate is the main product in the presence of an excess amount of oxygen.[10, 31] High sulphate levels are undesirable as they unbalance the natural

sulphur cycle and can cause taste and alimentary problems in drinking water. For this reason, at the department of Environmental Technology (WAU), a sulphide removing process was developed in which sulphur is the main end-product. Since the non-soluble sulphur can be removed, this process enables a reduction of the total sulphur content from the wastewater.[12] Moreover, the sulphur can be re-used as a valuable raw-material in for instance soil-bioleaching processes[73] or it can be purified by melting at high temperatures. Ironically, several researchers consider the formation of sulphur as unwanted because it clogs tubings and pumps while it also attaches to the reactor wall.[15, 26, 54] Other oxidative reactions of various reduced-sulphur compounds used by the colourless sulphur bacteria to gain energy for growth are:[59]

$$2\ S^{\circ} + 3O_2 + 2\ H_2O \rightarrow 2\ H_2SO_4$$
$$Na_2S_2O_3 + 2\ O_2 + H_2O \rightarrow Na_2SO_4 + H_2SO_4$$
$$4\ Na_2S_2O_3 + O_2 + 2\ H_2O \rightarrow 2\ Na_2S_4O_6 + 4\ NaOH$$
$$2\ Na_2S_4O_6 + 7\ O_2 + 6\ H_2O \rightarrow 2\ Na_2SO_4 + 6\ H_2SO_4$$
$$2\ KSCN + 4\ O_2 + 4\ H_2O \rightarrow (NH_4)_2SO_4 + K_2SO_4 + 2\ CO_2$$
$$5\ H_2S + 8\ KNO_3 \rightarrow 4\ K_2SO_4 + H_2SO_4 + 4\ N_2 + 4\ H_2O$$
$$5\ S^{\circ} + 6\ KNO_3 + 2\ H_2O \rightarrow 3\ K_2SO_4 + 2\ H_2SO_4 + 3\ N_2$$

Contrary to the biological oxidation of thiosulphate and tetrathionate into sulphate, only a little information is available about the biological oxidation of sulphide into elemental sulphur.

Sulphur Chemistry

Elemental sulphur has been known and used for several thousands of years, in Genesis it is referred to as *brimstone*. The use of burning sulphur for disinfection was already mentioned by Homerus. Dioscorides describes its use in medicine.[57] It is the element with the largest number of allotropes, most of which consist of cyclic nonpolar molecules.[63] The stable STP form is orthorhombic α-sulphur, consisting of cyclo-octa-S molecules. At 95.3°C α-sulphur converts into monoclinic β-sulphur, which melts at 119.6°C. Other allotropes of cyclooctasulphur can be obtained from solution. Of these, only monoclinic γ-sulphur, which has a needle-shape, is well characterized. Other well-established solid allotropes containing cyclo-octa-S, cyclododeca-S and other sulphur rings have been prepared by reaction of sulphur compounds. Another class of allotropes, made by the decomposition of sulphur compounds in aqueous solution or by quenching hot liquid or gaseous sulphur, comprises insoluble and other types of sulphurs. All contain long helices of polymeric sulphur but their structures are still incompletely characterized, as they contain helices mixed with other molecular species.[50, 51] Regardless of molecular size, all sulphur allotropes are hydrophobic, are not wetted by water

and barely dissolve in water. The solubility of α-S_8 in water of 25°C is only 5 $\mu g \cdot kg^{-1}$.[5] The densities of all crystalline sulphur allotropes fall within the range of 1.9-2.2 $g \cdot cm^{-3}$.

Biologically produced sulphur

In 1887 Winogradsky described the build-up and disappearance of sulphur inclusions by *Beggiatoa*, depending on whether or not the aqueous medium contained H_2S.[76] Many authors have since described the formation and the properties of this "elemental" sulphur for both phototrophic bacteria[27,28,62,64,67,77] and aerobic *Thiobacilli*.[30,32,34,63,65,66] According to these reports, S° forms transparent droplets (globules) which are deposited inside or outside the cells. The droplets reach diameters of up to 1 μm, are of spherical or ellipsoidal shape and dissolve at least partly in various organic solvents like acetone, chloroform, ethanol and carbon disulfide. Biologically produced sulphur is hydrophilic and has a white or pale-yellow colour. It shows a higher refractive index than water and has been described as liquid-like and amorphous on the basis of X-ray diffraction results.[28] The buoyant density of S° produced by *Chromatium* has been determined at 1.22 $g \cdot cm^{-3}$.[27] When allowed to stand in the liquid state or on drying, the sulphur globules eventually convert to crystalline S_8. However, it has never been demonstrated that the sulphur globules consist of 100 percent sulphur. Surprisingly, many of the properties reported above do not match the properties of any known chemical allotrope of elemental sulphur, indicating that biologically produced sulphur is a not a standard sulphur form.

Colloidal stability of sulphur particles

Colloids are ubiquitous in relatively large concentration (more than 10^9 colloidal particles per liter) in fresh surface waters, in groundwater, in oceans and in interstitial soil and sediment waters.[68,74] They are often defined on the basis of size, e.g. entities having at least in one direction a dimension between 1 nm and 1 μm. Although this classification is somewhat arbitrary, a common property of colloidal particles is that they are so small that they can resist the influence of gravity, e.g. they will remain is suspension due to Brownian motion. Since microbiologically excreted sulphur particles have a size of about 1 μm, they can be defined as a colloid as well.[30,63] Most colloidal dispersed phases are classically referred to as hydrophobic or lyophobic colloids. In addition to these hydrophobic colloids, there are also hydrophilic colloids which are hydrated macromolecules whose colloidal properties arise from their large molecular size. They are stable in solution because the (Van der Waals') cohesive forces between the polymer segments are counteracted by the conformational entropy of the polymer coil and the solvation energy of the polar groups (ionic or non-ionic). The longer the hydrophobic chains, the poorer the solubility in water and the greater the tendency of the molecule to escape from the aqueous solution and to associate at interfaces. Hydrophobic colloids generally owe their stability, e.g. their ability to resist aggregation, to an "electrical

double layer" which results from an electrical surface charge. This double layer causes an electrical repulsion between the particles.[48] Charge can originate either from isomorphic substitution, as in clay minerals, from adsorption of lattice constituing ions, as in AgI, or from the dissociation of surface functional groups. The synthetically produced 'LaMer' sulphur, for instance, owes its surface charge to the dissociation of sulphonic groups.[66] The double layer is usually treated as an inhomogeneous region composed of two parts: (1) a non-diffuse Stern-layer and (2) a diffuse or Gouy layer in which charge and potential obey the Poisson-Boltzmann equation. The thickness of the Stern-layer is in the order of magnitude of 0.5 nm, i.e. the size of a hydrated ion, while that of the diffuse double layer cannot be sharply defined because of the gradual potential decay with distance. For a flat, unperturbed diffuse layer, the thickness is usually given as the reciprocal Debye length, κ^{-1}. This is the distance over which the potential Ψ decays to the e^{-1} part of its value Ψ_d at the Stern-plane. This thickness depends on the concentration and valencies of the ions present in the system ($\kappa^2=5.411\cdot\Sigma_j c_j.z_j^2$; in nm^{-2}, c_j in M).[48] At higher salt concentrations, c_j, or at higher valencies of the counterions, z_j, the charge on the surface is screened more effectively, leading to a compression of the diffuse double layer. Under these circumstances the electrostatical repulsion between the colloidal particles reduces which allows a rapid coagulation as a result of long-range, attractive, Van der Waals forces. These interaction-types are included in the DLVO-theory.[48] In Figure 2 the potential decay in a electrical double layer is schematically represented.

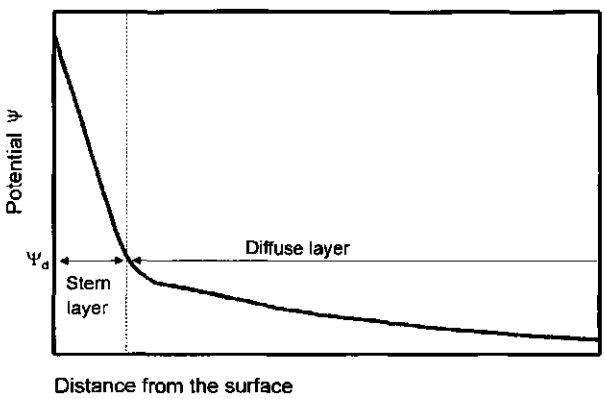

***Fig. 2** Schematic picture of an electrical double layer.*

Two other interactions play a role in colloid chemistry: (1) solvent structure-based short range forces and (2) osmotic pressure interactions related to macromolecular adsorption that may be attractive or repulsive. Solvent structure interactions are a new development of which the understanding is still in its inceptive stage. The existence of interactions of type (2) have already been known for more than a century: Small amounts of added polymer may destabilize colloids and larger amounts can have a stabilizing effect. These compounds tend to adsorb on

surfaces since they gain adsorption-energy due to long-range Van der Waals forces or hydrophobic bonding. In nature, the adsorption of macromolecules such as humic acids on colloidal particles, is well known.[20,25,68,74,75] The adsorption of dissolved macromolecules to microbiologically produced sulphur particles may have a stabilizing effect as well. Results of a sulphide removing pilot-plant have shown that any sedimentation of the formed sulphur colloids only proceeds very slowly.[9] In laboratory-studies it was also found that microbiological sulphur colloids can be stabilized in the presence of a suitable polymer, e.g. polyvinyl-alcohol.[29] Consequently, the removal-efficiency of the sulphur fraction formed in the presence of an adsorbing polymer by means of sedimentation, e.g. in a tilted-plate settler, decreases. Only in the presence of a suitable flocculant, will the sulphur particles rapidly settle. The use of flocculants, however, should be minimized in order to avoid high operational costs and to enable the re-use of the high-purity sulphur. Therefore more knowledge is required concerning the physico-chemical properties of the microbiologically produced sulphur and the stabilization by dissolved macromolecules. The relation between the amount of charged adsorbed macromolecules, the hydrodynamic thickness and the stabilization of the colloids is very complex because in addition to chemical and electrical interactions, conformational entropy effects also contribute to the adsorption behaviour.[4,24] This has for instance been found for the effect of pH on the adsorbed layer thickness of humic acids on iron-oxide (α-Fe_2O_3) particles.[74] There is a continuous change in the conformations of humic substances from uncoiled macromolecules (i.e. expanded conformations) at low ionic strength, i.e. fresh water, to fully coiled macromolecules (i.e. compact conformations) which extend further from the solid surface at high ionic strength. Adsorbed macromolecules mostly unfavourably increase the entropy of approaching colloids which results in a steric stabilization.[48] However, according to Filella *et al.* steric repulsion plays little or no role in the stabilization of hematite particles in the presence of natural organic matter. The stabilization of these colloids is mainly due to the electrostatical repulsion of the adsorbed polyelectrolytes.[25] In low concentrations natural organic matter may also destabilize colloids as a result of bridging flocculation, especially at high calcium concentrations.[2,60,81]

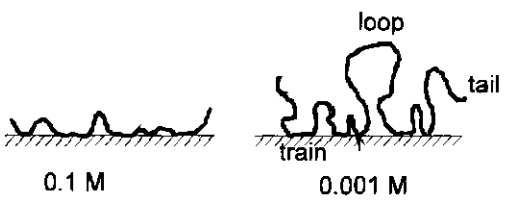

***Fig. 3** Configuration of humic acids at different ionic strength*

With respect to the ionic strength of the colloidal suspension, it is known that electrostatical interactions between colloidal particles are usually already entirely screened at salt

concentrations of about 0.1 *M*. However, a suspension of dissolved polymers can be destabilized at salt concentrations up to 2 *M* as a result of dehydration, i.e. salting-out effect.[47]

Scope of the thesis

In this thesis experimental studies concerning the formation and removal of elemental sulphur from the biological oxidation of sulphide are discussed. The objective of these investigations was to optimize the biological sulphide removing process which means the development of 1) an oxygen control strategy for maximizing the sulphur production and 2) a sulphur removal method. In order to achieve the objectives, it was necessary to understand the colloidal properties of the biologically produced sulphur particles. In Chapter 2 experiments are described that demonstrate the biological oxidation of sulphide into sulphur and sulphate. In Chapter 3 an oxygen suppletion method is developed which is based on the measurement of the redox-potential of the solution. Also a mathematical model of the biological sulphide oxidation process will be presented. Chapter 4 describes investigations in which the colloidal properties of biologically produced sulphur are compared to those of a synthetically formed 'LaMer' sulphur sol. A more detailed description concerning the surface properties of the biologically produced sulphur particles is presented in Chapter 5. Finally, Chapter 6 describes the development of a reactor system in which the aeration of the liquid-phase and the oxidation of sulphide to sulphur are spatially separated in order to form large, well-settleable sulphur sludge.

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CHAPTER 2

Biological sulphide oxidation in a Fed-Batch Reactor[A]

ABSTRACT

This study shows that in a sulphide oxidizing bioreactor with a mixed culture of *Thiobacilli*, the formation of sulphur and sulphate as end-products from the oxidation of sulphide can be controlled instantaneously and reversibly by the amount of oxygen supplied. It was found that at sulphide loading rates of up to 2.33 $mmol \cdot L^{-1} \cdot h^{-1}$, both products can be formed already at oxygen concentrations below 0.1 $mg.L^{-1}$. Because the micro-organisms tend to form sulphate rather than forming sulphur, the oxygen concentration is not appropriate to optimize the sulphur production.[1] Within less than 2 h, the system can be switched reversibly from sulphur to sulphate formation by adjusting the oxygen flow. This is below the minimum doubling time (2.85 h) of e.g. *Thiobacillus neapolitanus* and *Thiobacillus O*,[12] which indicates that one metabolic type of organism can probably perform both reactions. Under highly oxygen-limited circumstances, that is at an molar (O_2/S^{2-}) consumption ratio below 0.7, thiosulphate is abundantly formed. Because the chemical sulphide oxidation results mainly in the formation of thiosulphate, it is concluded that under these circumstances the biological oxidation capacity of the system is lower than the chemical oxidation capacity. The oxidation rate of the chemical sulphide oxidation can be described by a first-order process ($k = -0.87\ h^{-1}$).

[A] The results presented in this chapter have been published in Biotechnol. Bioeng. (1995) **47**:327-333, by A.J.H. Janssen, R. Sleyster, C. van der Kaa, A. Jochemsen, J. Bontsema and G. Lettinga.

INTRODUCTION

Organisms belonging to the group of colourless sulphur bacteria can oxidize sulphide to elemental sulphur or to sulphate, according to the following reactions:[1,9]

$$2\,HS^- + O_2 \rightarrow 2\,S^\circ + 2\,OH^- \qquad \Delta G^\circ = -169.35\ kJ\cdot mol^{-1} \qquad (1)$$

$$2\,HS^- + 4\,O_2 \rightarrow 2\,SO_4^{2-} + 2H^+ \qquad \Delta G^\circ = -732.58\ kJ\cdot mol^{-1} \qquad (2)$$

Here, ΔG° is the Gibbs free energy per mol sulphide. Under oxygen limitating conditions sulphur is the major end-product of the sulphide oxidation (1), while sulphate is formed under circumstances of sulphide limitation (2). In environmental technology, the formation of sulphur is preferred. Firstly, sulphur is nonsoluble and thus can be removed from the water stream, leading to a reduction of the total sulphur content of the wastewater. Secondly, sulphate formation would lead to a four-times-higher oxygen consumption and consequently a higher energy consumption for aeration. However, the Gibbs-Free energy for the sulphate formation is higher than for the formation of sulphur. Consequently, this reaction yields more energy for microbial growth and is therefore preferrently carried out if no biomass-limitation or oxygen-limitation occurs.

Besides the biological sulphide oxidation, the chemical oxidation of sulphide also has to be taken into account. Especially in highly loaded bioreactors, not all sulphide may be converted into sulphur due to a limitation in biological activity. Under these circumstances, chemical auto-oxidation of sulphide becomes relatively more important, resulting in the formation of thiosulphate, according to the following equation:[3]

$$2\,HS^- + 2\,O_2 \rightarrow H_2O + S_2O_3^{2-} \qquad \Delta G^\circ = -387.35\ kJ\cdot mol^{-1} \qquad (3)$$

Aim of this Study

The object of this study was to determine how the relation between oxygen and sulphide consumption affects the type of product formed in a sulphide-oxidizing bioreactor. An additional objective was to determine the chemical stability towards further oxidation into sulphate of the formed sulphur particles.

MATERIALS AND METHODS

Fed-Batch Reactors

Two fed-batch reactors were used to study the oxidation of sulphide. This reactor type was chosen because a dynamic system yields more information about the kinetics than experiments performed under steady-state conditions.

A schematic diagram of the reactor system is shown in figure 1a. An air-lift loop reactor was chosen because of its good mixing properties. The wet volume of the glass reactor was 9.0 L. The temperature and the pH of the culture were maintained at 30°C and 8.0, respectively. The gas flow (300 $L \cdot h^{-1}$) was completely recycled to prevent H_2S escaping from the system. Pure oxygen was supplied by means of a mass-flow controller (Brooks Thermal Mass Flowmeter, type 5850E, 0 to 15 $mL \cdot min^{-1}$). In this manner, a constant oxygen concentration or a constant oxygen gasflow could be maintained. The computer registered the data collected from the pH- and oxygen electrodes. Carbon-dioxide was added to the recirculating gas-flow for pH control. A Na_2S stock solution (5 to 10 $mL \cdot h^{-1}$) was supplied via a peristaltic pump (Gilson, Minipuls 2) to the recirculating stream to obtain a constant addition of sulphide to the reactor. This was done to prevent accumulation of sulphide in the interconnecting point at the base of the reactor. In the calculations of the production of the various sulphur species the increase in volume (about 3%), as a result of the addition of Na_2S solution, was neglected.

Start-up

The fed-batch reactors were filled with 7.75 L tapwater, 250 mL nutrient solution (composition described below) and 80 g $NaHCO_3$ as an initial buffer. After stabilization of the pH, 1.0 L inoculum from a sulphate producing CSTR was added (loading rate CSTR: 1.6 mmol $S^{2-} \cdot L^{-1} \cdot h^{-1}$; dilution rate D = 0.2 h^{-1}; pH = 8; $[O_2]$ = 5 $mg \cdot L^{-1}$). The inoculum was collected on ice (4°C) and consisted of a mixed culture of *Thiobacilli*.[1,2] After temperature stabilization (30 min) the sulphide addition was started. During the experiments, the only sulphur-compounds that could be detected were sulphide, sulphur, thiosulphate and sulphate. Tetrathionate was not found. The presence of polysulphides was deduced from the greenish colour of the reactor suspension at the beginning of some of the experiments. These polysulphides were not determined quantitatively. The green colour always disappeared within 3 h. The suspension then became whitish due to sulphur formation.

Continuous Reactor

One 5.0-L continuous-flow, stirred-tank reactor (CSTR) was used for cultivation of *Thiobacilli* which was described previously (Fig. 1b).[1] The process temperature was maintained at 23°C and the pH at 8.0.

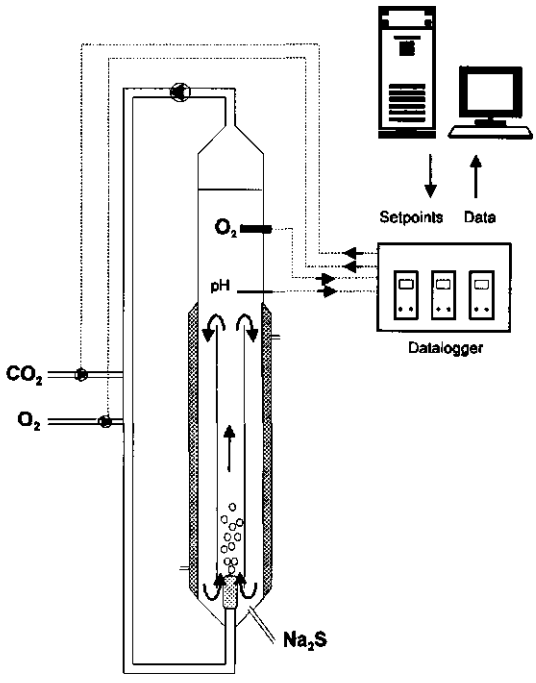

Fig. 1a Laboratory fed-batch reactor for sulphide oxidation.

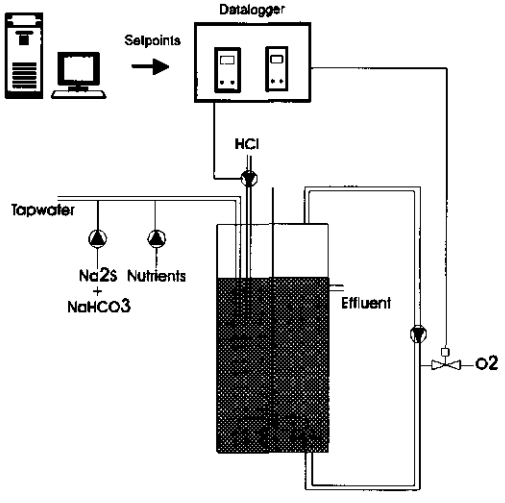

Fig. 1b A 5.0 L CSTR for the cultivation of Thiobacilli.

Chemicals

The nutrient solution contained ($g \cdot L^{-1}$): NH_4Cl, 4; $MgSO_4.7H_2O$, 1; KH_2PO_4, 2; and 10 mL of trace element solution according to Vishniac and Santer.[14] All the chemicals used for the nutrient solution were analytical grade, supplied by Merck (Darmstad, FRG). The sulphide solution was of a technical grade and supplied by BASF (FRG). The saturated sulphide concentration in the stock vessel was determined at 1.875 mol $S^{2-} \cdot L^{-1}$ and did not contain any thiosulphate or sulphate. Pure oxygen, supplied by Hoekloos (Schiedam, The Netherlands), was used as an electron acceptor.

Measurements and Analyses

The pH was measured using a combined glass electrode (Ingold, 425-60-s7 Ing.No. 104253313). The oxygen concentration was measured with an oxygen sensor (WTW; DU 600 201). Sulphide was determined photometrically by the method described by Trüper and Schlegel.[13] The sulphate concentration in the medium was determined by ion-exchange HPLC after a high-speed centrifugation (5 min, 15,000*g*) to remove colloidal and suspended solids. The samples were frozen (-18°C) and stored before analysis. Possibly present sulphide was fixed by adding the sample to a 0.1 M zinc-acetate solution (dilution 1:1). A Chrompack column was used, filled with Vydac 302-IC (250 x 4.6 mm). Potassium hydrogen phthalate (0.018 M) with 2.5% (v/v) acetonitrile was used as an eluent at a flow of 1.2 $mL \cdot min^{-1}$ and the detector was a Waters 431 conductivity detector. The injection volume was 20 mL. With this method thiosulphate can be detected as well. The presence of sulphite was estimated by using a test-strip (Merckoquant 10013). Tetrathionate was measured after cyanolysis followed by the colorimetric assay of the thiocyanate formed.[8]

Samples for the analysis of elemental sulphur were centrifuged (5 min, 15,000*g*) followed by careful decantation and drying overnight at 30°C. The residues obtained were extracted with acetone for 3 days, after which the extracts were analyzed by reversed-phase chromatography using the procedure of Möckel.[10] The samples were diluted to give a maximum sulphur concentration of 200 $mg \cdot L^{-1}$. The HPLC equipment contained a C_{18} column (2*10 cm), 96:4 methanol/water as a mobile phase (flow is 1.00 $mL \cdot min^{-1}$) and UV detector at 254 nm. Standard solutions of S_8 (orthorhombic sulphur) in acetone were used for calibration. To improve the solubility of the standard sulphur flower a few drops of CS_2 were added before the measuring flask was filled with acetone.

RESULTS AND DISCUSSION

So far, little attempts have been made to study the biological oxidation of sulphide in a chemostat bioreactor. For practical reasons, most researchers used dissolved elemental sulphur, thiosulphate or tetrathionate as a reduced sulphur source.[12] An important reason for not using sulphide, is its poor chemical stability and its toxicity toward microorganisms. Biological sulphide oxidation processes so far were mostly investigated in studies on microbial marine ecosystems.[4,5,7,15]

Description of a Fed-Batch Experiment

The results of a typical fed-batch experiment are depicted in Figures 2. Fig. 2a shows that during the first 17 h of the experiment the oxygen concentration in the reactor was controlled at 3.0 mg/L at a constant sulphide loading rate (2.33 mmol $S^{2-}\cdot L^{-1}\cdot h^{-1}$). Initially, the only oxidation products formed were thiosulphate and sulphur (Fig. 2b). However, after 5 h the thiosulphate concentration started to decrease while from this moment onward, all supplied sulphide was oxidized to sulphate. This switch from sulphur and thiosulphate formation into sulphate formation obviously is accompanied by an increase in oxygen demand. The results in Figure 2a indicate that the amount of oxygen supplied becomes almost twice the amount of sulphide added, that is 4.44 mmol $O_2\cdot L^{-1}\cdot h^{-1}$ and 2.33 mmol $S^{2-}\cdot L^{-1}\cdot h^{-1}$, which is in good agreement with Eq. (2). In this regard, it should be taken into account that approximately 9% of the available electrons is required for CO_2 fixation[12]; the theoretically required amount of oxygen therefore becomes 0.91*2.33*2 = 4.24 mmol$\cdot L^{-1}\cdot h^{-1}$. The slightly higher measured oxygen consumption may very likely be caused by oxidation of the formed sulphur and thiosulphate, as will be discussed below.

The oxygen supply rate was decreased at t = 17 h from 4.44 mmol$\cdot L^{-1}\cdot h^{-1}$ to 1.33 mmol$\cdot L^{-1}\cdot h^{-1}$. The system responded immediately, the oxygen concentration dropped steeply from 3 mg/L to below the detection limit of 0.1 mg$\cdot L^{-1}$ (Fig. 2a). As a result of the imposed oxygen limitation, the sulphide oxidation shifted from sulphate to sulphur formation. As can be seen from the results in Fig. 2b, from t = 19 h onward the sulphate concentration did not increase any further while sulphur was formed.

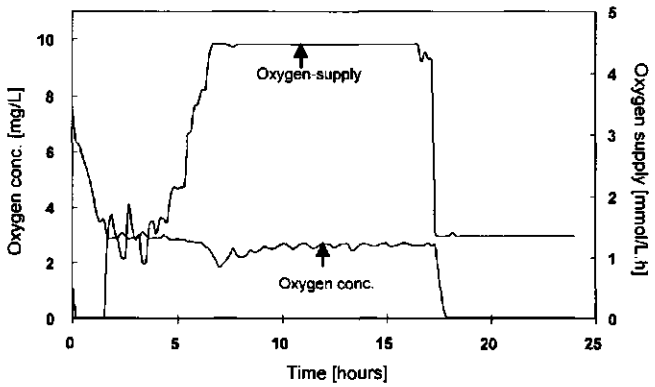

Fig. 2a *Oxygen concentration and oxygen supply during a 24 h fed-batch experiment at a constant sulphide loading rate of 2.33 mmol$\cdot L^{-1}\cdot h^{-1}$. From t = 0 until t = 17 h the oxygen concentration is controlled at 3.0 mg$\cdot L^{-1}$. From t = 17 until t = 24 h the oxygen supply is reduced from 4.33 to 1.33 mmol$\cdot L^{-1}\cdot h^{-1}$.*

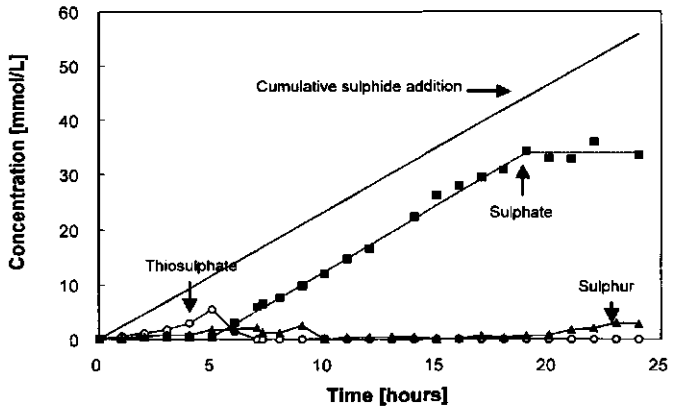

***Fig. 2b** Sulphur, sulphate and thiosulphate concentration belonging to the same experiment as described in Fig. 2a.*

The Oxidation Rate of Sulphur

When the system becomes underloaded, the freshly formed sulphur particles can be oxidized to sulphate. In practice, such a situation is obviously undesirable. To study the rate of sulphur oxidation, the following experiment was carried out. Sulphur was formed at a sulphide loading rate of 2.0 mmol·L^{-1}·h^{-1} during 10 h. After 10 h, sulphide addition was ceased and the reactor became underloaded. This resulted in oxidation of the formed sulphur to sulphate.

The results of the experiment are depicted in Figure 3. During the whole experiment the oxygen concentration was maintained at 3.0 mg·L^{-1}. Directly from the start of the experiment the bacteria form sulphur and sulphate. At t = 10 h the sulphide addition was terminated. The system responded by a rapid drop in the sulphur concentration and a sharp increase of the sulphate concentration (Fig.3). During the first 10 h of the experiment the sulphate production rate, determined from figure 3, amounted to 0.35 mmol.L^{-1}.h^{-1}. Besides sulphur and sulphate no other sulphur compounds could be detected; therefore, the sulphur production rate could be calculated at 1.65 mmol·L^{-1}·h^{-1}. As can been seen from Figure 3, from t = 5 till t = 10 h, the measured sulphur production corresponded very well with the calculated value, i.e. 1.66 mmol·L^{-1}·h^{-1}. From t = 0 till t=5 h, the measured sulphur production was lower than the calculated value, viz. 0.6 mmol·L^{-1}·h^{-1}, which is most likely caused by the fact that a fraction of the formed sulphur attaches to the reactor wall. Therefore, the only the calculated sulphur production was used in the following calculations. During the first 10 h the bacteria oxidized (0.35*8+1.65*2) = 6.1 mEq·L^{-1}·h^{-1}, but after termination of the sulphide supply, this value increased significantly as appears from the increase in sulphate: between t = 10-11 h (6.85 - 3.32) * 6 = 21 mEq·L^{-1}·h^{-1} are oxidized. Apparently, the oxidation capacity of the bacteria after termination of the sulphide addition increased considerably. A possible reason may be that already at very low sulphide concentrations serious inhibition of the bacteria prevails.

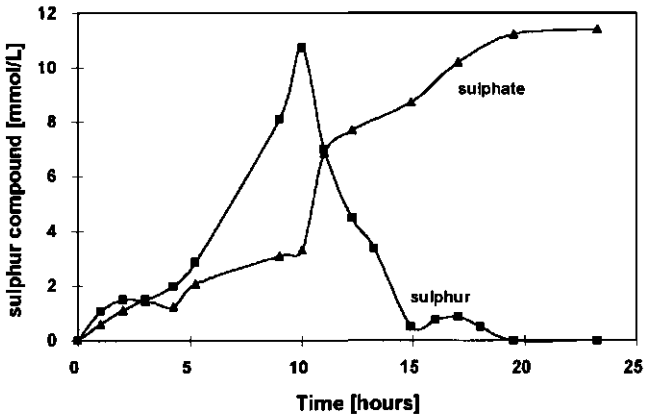

Fig. 3 Sulphur and sulphate concentrations during a 24-h fed-batch experiment. From t = 0 h till t = 10 h the sulphide loading rate was 2.00 mmol·L^{-1}·h^{-1}. At t = 10 h the sulphide supply was terminated.

According to Hirayama *et al.*[6] and Buisman *et al.*[2] sulphide inhibits *Thiobacilli* in the range of 5 to 30 mg·L^{-1}. However, in the present experiment the sulphide concentration in the reactor was well below 5 mg·L^{-1}, indicating that the presence of very low concentrations of sulphide already are quite inhibiting. Because the results of Buisman *et al.*[2] indicate that biological oxidation of sulphide to sulphur proceeds much faster than the oxidation of sulphide to sulphate, the increased electron production cannot be explained by the shift from sulphur to sulphate formation. After cessation of the supply of sulphide, presumably easily oxidizable sulphur present on the cell surface is rapidly converted into sulphate. Once these easily available sulphur particles have been oxidized, the sulphur particles in suspension will serve as substrate. Due to the reduced contact between the surface of the bacteria and the sulphur present in the particles, during the period t = 11 to 19.5 h, the average sulphate production rate drops down to 0.18 mmol SO_4-S·L^{-1}·h^{-1}, corresponding with 0.18*6 = 1.1 mEq·L^{-1}·h^{-1}.

Product formation at different oxygen to sulphide consumption ratios

To investigate the existence of the relationship between the oxygen consumption, sulphide consumption ratio and $(O_2/S^{2-})_{consump.}$ in moles O_2 per mol S^{2-}, and the formed sulphur compounds in our system, different ratios of oxygen to sulphide were applied. In these experiments, the sulphide loading rate varied between 1.2 and 2.33 mmol·L^{-1}·h^{-1}. At higher sulphide loading rates, the start-up of the system failed because of too high initial sulphide levels.

Thiosulphate formation

According to Kuenen[9], the biological formation of thiosulphate will not occur in a sulphide-oxidizing-system but recently van den Ende *et al.*[4] suggested biological thiosulphate production from the oxidation of sulphide under oxygen-limiting conditions. However, it cannot be excluded that the formation of thiosulphate resulted from the sulphide auto-oxidation in van der Ende's experiments. In our reactor, occasionally large amounts of thiosulphate were found, mainly in the beginning of a fed-batch experiment when sulphide accumulated or when the oxygen consumption was interrupted.

The results in Figure 4 reveal that under oxygen limited conditions, i.e. at $(O_2/S^{2-})_{consump.} \approx 0.5$, thiosulphate is abundantly formed. It can be hypothesized that, under highly reducing circumstances, the specific biological activity drops due to a too-low supply rate of oxygen and the sulphide concentration increases. Then, the chemical sulphide oxidation may become more important. As a result, a larger amount of sulphide will be chemically converted into thiosulphate rather than sulphur or sulphate. To elucidate this matter, we measured the reaction rate and the product formation of the chemical oxidation of sulphide. For this purpose, a 60-mg·L^{-1} sulphide solution was added to the reactor keeping the pH and oxygen concentration constant at 8.0 and 3.0 mg·L^{-1}, respectively. To ensure that no biological activity was present, 5 m*M* of sodium-azide (NaN_3) was also added. Because the effect of ionic strength on the chemical sulphide oxidation is substantial,[11] the composition of the solution was kept the same as in the biological oxidation experiments.

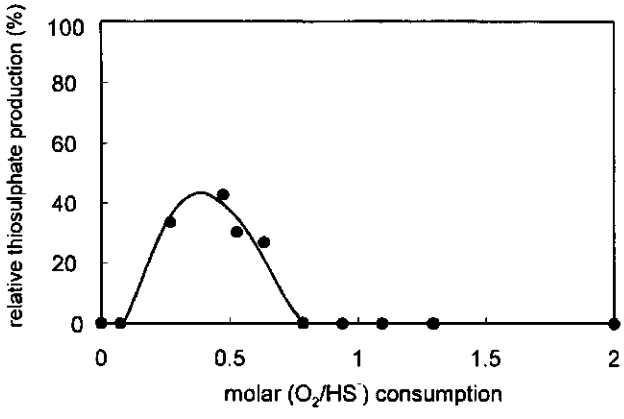

Fig. 4 *Relative thiosulphate production at different molar O_2/S^{2-} consumption ratios.*

As can be seen from the results in Figure 5, thiosulphate indeed appears as the major end-product under these conditions where merely chemical oxidation can proceed. Only during the first hour of the experiment a small amount of sulphite, i.e. less than 10 mg·L^{-1}, appears in the solution, whereas sulphate could not be detected during the first 10 h. Also Chen and Morris[3] found that thiosulphate

is the main product of the chemical sulphide oxidation under slightly alkaline conditions. In our experiment, during the first 15 h about 60% of the total amount of added sulphide is converted to thiosulphate (Fig. 5). From the appearance of a whitish suspension after 10 h of reaction time, it is concluded that the remaining fraction, which is about 40% of the initial sulphide concentration, is oxidized into elemental, colloidal sulphur. It is also likely that a very small amount, maximally 4% of the initial sulphide concentration, of metal sulphide precipitates are formed with the metals present in the nutrient solution. The chemical formation of elemental sulphur at high sulphide to oxygen ratios is in agreement with results reported by Chen and Morris.[3] From Figure 5, it can be calculated that the chemical sulphide oxidation with thiosulphate as the main end-product proceeds as a first order process with a reaction constant (k) of -0.87 h^{-1}.

The formation of thiosulphate was also observed in a transient-state experiment in a sulphide-oxidizing CSTR. This follows from the results in Figure 6a. The sulphide-loading rate was increased from 1.56 mmol·L^{-1}·h^{-1} to 6.25 mmol·L^{-1}·h^{-1} at t = 20 h and at t = 140 h it was returned to 1.56 mmol·L^{-1}·h^{-1}. The oxygen concentration was controlled at 2 mg·L^{-1} and the hydraulic retention time remained constant at 5 h. At t = 20 h, the system is in a sulphate-forming steady state; any sulphur, thiosulphate or sulphide could not be detected. Immediately following the rise in the sulphide loading rate, the formation of sulphate ceased and thiosulphate and sulphur were formed. The presence of sulphur was not determined quantitatively but could be derived from the whitish colour of the effluent. From the results it can be seen that at t = 25 h, the organisms regained their oxidation capacity and started to produce sulphate again. Eventually, from t = 95 h onward the reactor is in a sulphate producing steady state, while the sulphate effluent concentration is even higher than the amount of sulphide in the influent. This can be due to oxidation of accumulated sulphur which was formed from t = 20 h until t = 90 h and was attached to the reactor wall. When at t = 140 h, the sulphide-loading rate returned from 6.25 to 1.56 mg·L^{-1}·h^{-1}, the sulphate production decreased proportionally.

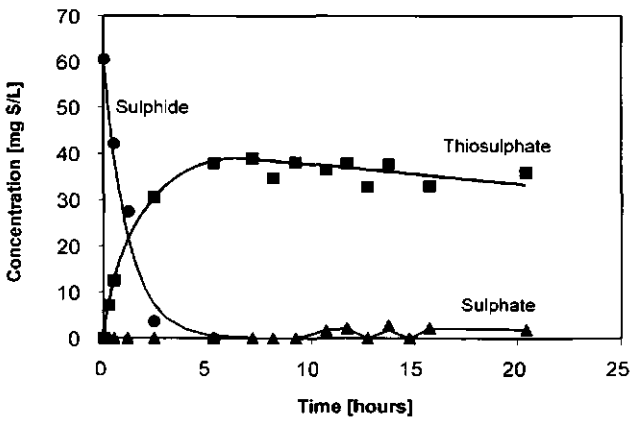

Fig. 5 Sulphide auto-oxidation at pH = 8 and [O_2] = 3.0 mg·L^{-1} in the presence of trace elements.

From the results, it is clear that thiosulphate is merely formed directly after increasing the loading rate. From this moment onward, there is for about 5 hours a limited biological oxidation capacity which leads to an increase of the sulphide concentration (Fig. 6b). Under these conditions of high sulphide concentrations, the chemical sulphide oxidation becomes more important leading to the formation of thiosulphate.

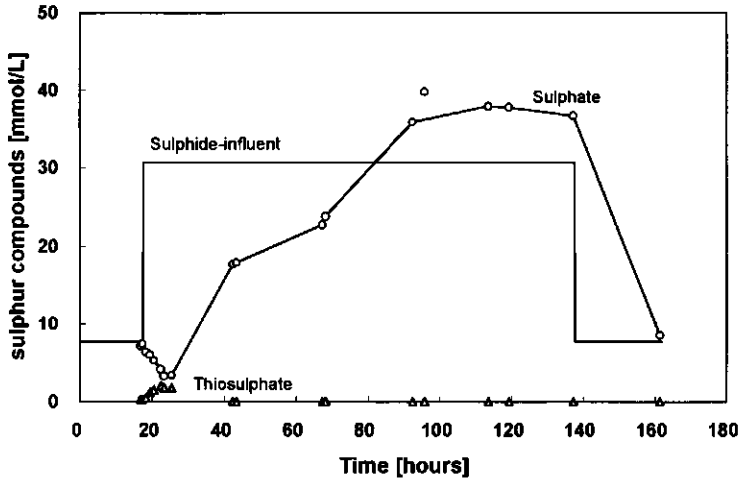

Fig. 6a *Product formation during a transient-state experiment in a CSTR. The sulphide loading rate was 1.56 and 6.25 mmol·L^{-1}·h^{-1}, respectively, at a HRT of 5.0 h. The oxygen concentration was controlled at 2.0 mg·L^{-1}.*

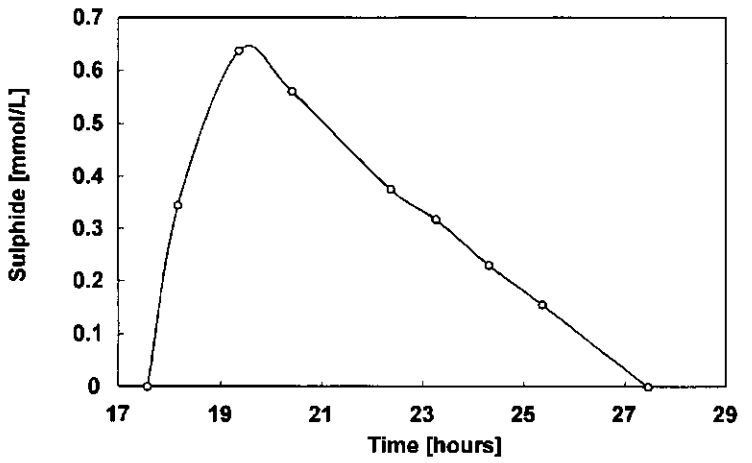

Fig. 6b *Sulphide concentration in a CSTR after increasing the sulphide loading rate, as described in Fig. 6a.*

Sulphur and Sulphate Formation

Under conditions of oxygen limitation, that is at a molar $(O_2/S^{2-})_{consump.}$ between 0.5 and 1.0, the system produces mainly thiosulphate and sulphur whereas at a molar $(O_2/S^{2-})_{consump.} > 1.0$ sulphate is the primary oxidation product (Figs. 4, 7, 8). As can be seen from the results in Fig. 7, a maximal sulphur formation is obtained at a molar $(O_2/S^{2-})_{consump.}$ between 0.6 and 1.0 and not at the stoichiometrical value of a molar $(O_2/S^{2-})_{consump.} = 0.5$, because of the formation of thiosulphate. This means that it is not possible to convert all the sulphide in the influent completely into elemental sulphur but that some sulphate formation will also occur, either directly due to an excess of oxygen or indirectly due to the formation of thiosulphate under oxygen-limiting circumstances. The maximum observed sulphur yield was determined at 73 ± 10%.

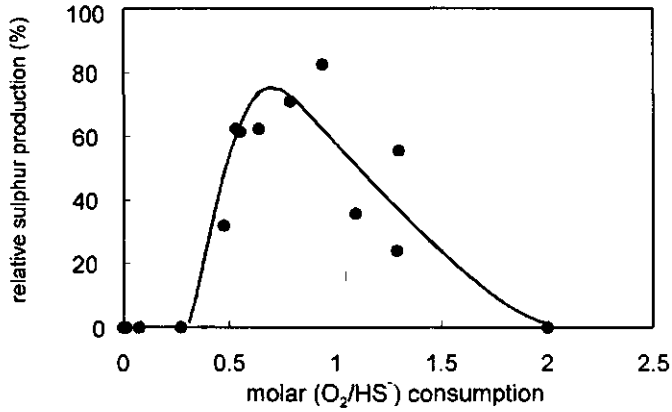

Fig. 7 *Relative sulphur production at different molar (O_2/S^{2-}) consumption ratios.*

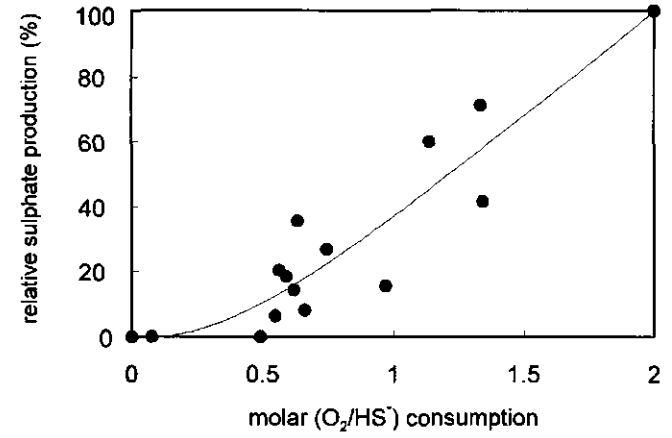

Fig. 8 *Relative sulphate production different molar (O_2/S^{2-}) consumption ratios.*

CONCLUSIONS

In a sulphide-removing bioreactor, only a limited number of oxidation reactions will proceed, that is the biological oxidation of sulphide to sulphur and sulphate respectively, and the chemical formation of thiosulphate as a result of sulphide auto-oxidation. The microorganisms are capable of switching within 2 h from sulphur to sulphate formation and vice versa, whereas the maximal doubling time of i.e. *Thiobacillus neapolitanus* and *Thiobacillus O* is 2.85 h.[12] This means that the biomass may use various electron transport routes, as was already suggested by Stefess.[12] For this reason it is unlikely that, due to changes in specific loading rates, different metabolic types of organisms would become dominant in the mixed population, such as sulphur-forming or sulphate-forming *Thiobacilli,* as was postulated by Buisman *et al.*[2]

The maximal sulphur production (73 ± 10%) occurs at a molar (O_2/S^{2-}) consumption ratio consumption ratio between 0.6 and 1.0. When lower oxygen amounts are supplied, thiosulphate will also be present in the system. Under these conditions, due to the lowered biological oxidation capacity chemical sulphide oxidation becomes relatively more important which generates thiosulphate. At a higher supply of oxygen, sulphate will be produced because then more energy is gained for bacterial growth than from the formation of sulphur. The experiments also revealed that the oxygen concentration is not a suitable parameter to control the system. Both the formation of sulphur as well as the formation of sulphate already proceed at oxygen concentrations below 0.1 $mg \cdot L^{-1}$, which in fact is the lowest detection limit with the available oxygen electrodes.

NOMENCLATURE

CSTR	continuous-flow stirred tank reactor
D	dilution rate (h^{-1})
k	first-order reaction constant (h^{-1})
$(O_2/S^{2-})_{consump.}$	molar ratio between the oxygen and sulphide consumption

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CHAPTER 3

Application of the Redox Potential for real-time control of the oxygen supply to a sulphide oxidizing bioreactor and modelling of the biological sulphide oxidation

ABSTRACT

The investigations described show that the formation of elemental sulphur via the biological oxidation of sulphide can be optimized by controlling the redox state of the solution. It was shown that, by supplying an almost stoichiometrical amount of oxygen to the recirculated gas-phase, the formation of sulphate is minimized. The redox potential is mainly determined by the sulphide concentration because this compound has a high standard exchange current density with the platinum electrode surface. By maintaining a particular redox setpoint value, in fact, the reactor becomes a 'sulphido-stat'. It was shown that in a sulphide-oxidizing bioreactor the measured redox potential, using a polished redox electrode, is kinetically determined rather than thermodynamically. The optimal redox value for sulphur formation is between -147 and -137 mV with a H_2 reference electrode at 30°C. The process of biological sulphide oxidation was mathematically modelled. Kinetic parameters were estimated by comparing results from model calculations with results from laboratory experiments.

INTRODUCTION

In Chapter 2 it was pointed out that the microbiological oxidation of sulphide to elemental sulphur occurs either under oxygen limited circumstances, that is at DO (Dissolved Oxygen) values below, at least, 0.1 $mg \cdot L^{-1}$ or under high sulphide loading rates. In the latter case, the biomass is overloaded and sulphur is formed as intermediate product. Since it is assumed that the formation of sulphur is a faster reaction than sulphate-formation, this mechanism allows the bacteria to remove the harmful sulphide at high rates.[4,9] As follows from the pε-pH diagram for the SO_4-S(s)-H_2S system, elemental sulphur is not a stable sulphur compound at pH=8.[3,11] At pH values below 7, elemental sulphur is formed from the oxidation of H_2S while in the pH range 7-11, HS^- will be completely oxidized to sulphate. However, since a bioreactor is a nonequilibrium system a conceptually meaningful pε cannot be defined.[11] Also the intercellular pH may be different from that of the reactor suspension, resulting in different sulphide species. As a consequence, the redox reactions which occur may differ from the thermodynamically predicted ones.

At loading rates below 250 mg $HS^- \cdot L^{-1} \cdot h^{-1}$, the organisms tend to produce sulphate rather than sulphur at increasing DO-values because sulphate formation yields more energy for microbial growth.[4] For reasons of environmental protection, the formation of elemental sulphur is preferred because this compound can, in principle, be removed from the wastewater and subsequently be re-used as a raw material, e.g. in bioleaching-processes.[12] Reactors should not be designed to be operated under 'overload conditions' for the sake of process-stability. Therefore, a stoichiometrical oxygen supply is required to oxidize all sulphide into elemental sulphur. Since the detection limit of currently available oxygen sensors is about 0.1 $mg \cdot L^{-1}$, they are not suitable as a measuring device and therefore another parameter should be used. A very promising alternative to control the oxygen supply is the application of the redox (reduction-oxidation) potential of the solution. The successful application of the redox potential as a control parameter for the nitrification/denitrification process in biological wastewater treatment plants and its use for controlling the enhanced biological phosphorous removal has already been demonstrated successfully.[7] The redox potential is a measure of the solution's tendency to accept or donate electrons. The thermodynamic relation of the potential E_H to the composition of the solution is generally known as the Nernst equation:[11]

$$E_H = E_H^o + \frac{2.303 \cdot R \cdot T}{n \cdot F} \log \frac{\prod_i \{ox\}^{n_i}}{\prod_j \{red\}^{n_j}}$$

for the half-reaction: n_i ox_i + n e $\rightarrow$ n_j red_j. One drawback frequently mentioned concerning the application of the redox potential is, that its value is the result of the contribution of a mixture of dissolved components. Several redox couples may prevail and all of them

contribute to the measured, overall, redox value. However, several authors revealed the existence of a linear relationship between the measured redox potential and the logarithm of the hydrogen sulphide concentration in natural environments.[1, 5, 6] The reason for this is that, in comparison to other substances, sulphide has a relatively high standard exchange current density (I_0).[2] In a sulphide oxidizing bioreactor, the measured redox potential therefore will predominantly be determined by the sulphide concentration. Instead of redox potential measurements, one could also consider the use of a commercially available, ion-specific, sulphide electrode which measures the activity of the S^{2-} ion. However, the use of such an electrode is not recommended because the S^{2-} concentration greatly depends on the pH of the solution. In practice, small pH-fluctuations will result in considerable fluctuations in the S^{2-} concentration. The measured S^{2-} concentration should therefore always be correlated to the actual pH-value which complicates its application considerably. The redox potential, however, depends less on the pH of the solutions as will be shown in this chapter. Another reason for not using ion-specific sulphide electrodes (i.s.e.) is that they are not yet available for industrial purposes.

Aim of the study

In this chapter, the controlled oxidation of sulphide to elemental sulphur in a dynamic system will be described. A classical feedback (PI) controller was developed to maintain any desired redox potential value for sulphur formation. The origin of the redox potential was investigated by studying the relation between the redox potential and the oxygen and sulphide loading rates. A mathematical model was developed to describe the relation between the formation of the various sulphur compounds and the sulphide and oxygen loading rates.

MATERIALS AND METHODS

Reactor

A continuous-flow gaslift reactor was used with a liquid volume of 10 L (Fig.1). The influent consisted of tapwater and a nutrient solution (see Chapter 2). The gas flow (300 $L \cdot h^{-1}$) was completely recycled to prevent any release of H_2S-gas into the environment and to reach low oxygen concentrations. Pure (100%) hydrogen sulphide gas was added to this recirculating gas stream via Mass Flow Controllers (Brooks Thermal Mass Flowmeter, type 5850E, 0-75 $mL \cdot min^{-1}$). Under slightly alkaline conditions (pH=8), the H_2S gas was completely absorbed into the liquid phase; in the headspace no H_2S gas could be detected. Pure oxygen was supplied by means of two Mass-Flow Controllers (Brooks Thermal Mass Flowmeter, type

5850E, flow 0-30 mL·min^{-1} and 0-500 mL·min^{-1}). The temperature was controlled at 30°C by a water-jacket.

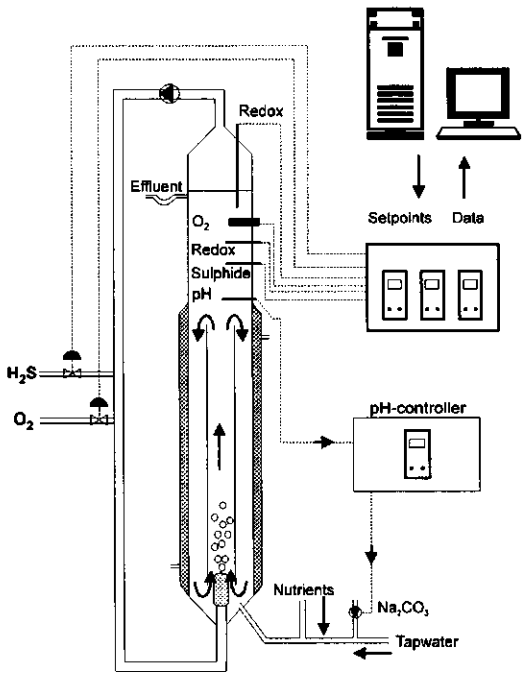

Fig. 1 Laboratory set-up of a continuous-flow gaslift reactor (V=10 L) for sulphide oxidation. The pH and temperature were controlled at 8.0 and 30 °C, respectively.

Measurements and Analyses

In the reactor, the redox potential was measured with two commercially available, polished, platinum electrodes combined with an Ag|AgCl electrode as a reference (WTW, Serolyt Pt). In order to assess the effect of the polished electrode surface, a calibration in a phosphate buffer (KH_2PO_4 = 20 g·L^{-1}, pH = 8.0) was carried out with a platinized electrode (*platinum black*). Platinization of a polished platinum electrode increases the specific surface by a factor 100-1000. Consequently, reactions whereby electrons are transferred to such a platinized electrode surface, i.e. heterogeneous reactions, may proceed faster when the available surface area of the standard polished electrode is the limiting factor.[2] Platinization of the electrode was accomplished according to the following procedure. A polished platinum-electrode was

cleaned for half an hour in a concentrated (65%) nitric-acid solution at 70°C. After thorough rinsing with distilled water, the electrode surface was electrochemically cleaned (10 minutes) in distilled water which was acidified with a few droplets of a concentrated (96%) sulphuric acid solution. The direction of the current (10 $mA \cdot cm^{-2}$) was changed once every minute. Then, the electrode was rinsed with distilled water and electrolysed in a 2% $H_2PtCl_6.6H_2O$ solution. Electrolysis was started for a period of 5 minutes at a current of respectively +10 and -10 $mA \cdot cm^{-2}$. The current was increased in steps of 10 $mA \cdot cm^{-2}$ up to a final value of 50 $mA \cdot cm^{-2}$. Simultaneously, a black deposit was formed on the Pt-surface. All redox values presented in this chapter are expressed relative to the standard hydrogen electrode.

Sulphide was measured on-line with an ion-specific sulphide (i.s.e.) electrode which consisted of a silver-wire which was embedded in solidified resin. The silver tip was first thoroughly cleaned with a detergent solution and polished. Hereafter, the electrode was activated by immersing it for 2 minutes in a 20% $(NH_4)_2S$ solution followed by thorough rinsing with tapwater. In this way, an Ag_2S-coating was created on the silver surface which actually served as electrode surface. Free S^{2-} ions adsorb onto the Ag_2S-crystal and release their electrons. The current was measured with a standard potentiometer. A standard Ag|AgCl electrode was used as a reference. The sulphide-electrode was calibrated in a double wall, air-tight vessel (V=250 mL, T = 30°C) which was filled with 100 mL of an oxygen-free phosphate buffer (KH_2PO_4 = 20 $g \cdot L^{-1}$, pH = 8.0). The headspace was flushed with nitrogen before addition of a 100 mM sulphide stock solution. The sulphide stock solution was added in steps of 0.05 mL, using an automatic burette (Dosimat 665, Metrohm, Hercsau, Switzerland). The following calibration curve for the sulphide electrode was found:

$E = 32.5 \cdot p(HS^-) - 446$ (mV); $p(HS^-) = -\log[HS^-]$ in $mg \cdot L^{-1}$, in a range of 0.5 till 10 HS^- $mg \cdot L^{-1}$. In this way 30 measurements were made and the correlation coefficient found was 0.99. The slope of the line is close to the theoretical slope of 30 mV/p(HS^-). The dissolved oxygen concentration was measured using an oxygen sensor (WTW; DU 600 210). The pH in the reactor was maintained at 8.0 (±0.1) by the addition of a 0.5 *M* Na_2CO_3 solution, using a custom-made pH-controller. The flow of the nutrient solution ranged from 5 $mL \cdot h^{-1}$ till 25 $mL \cdot h^{-1}$ and its composition is described in Chapter 2. The signals from respectively the oxygen, sulphide and redox electrodes and the mass-flow controller were collected via a custom made data-logger (workshop, Dept. of Agricultural Engineering and Physics, WAU). A software PI-controller was developed using the MATLAB software-package (The Mathworks Inc., MA). With this program the measuring data was also collected. The sample interval was set at 1 minute for experiments with constant sulphide and oxygen loading rates whereas the sample interval was set at 30 seconds for the experiments with a computer-controlled oxygen dosage. Sulphate and thiosulphate were measured using a HPLC as described in Chapter 2. The

sulphide concentration was determined using the colorimetrical assay of Trüper and Schlegel.[13]

RESULTS AND DISCUSSION

The relation between the redox potential and the H_2S and O_2 loading rates was assessed from a number of steady-state situations. Four different H_2S-loading rates were applied, viz. 50, 100, 175 and 500 mg $HS^-\cdot L^{-1}\cdot h^{-1}$. At each sulphide loading rate from one up to four different oxygen loading rates were applied. The molar oxygen to sulphide consumption ratio amounted to respectively 0.38, 0.51, 0.77, 1.15 or 1.54. Each steady-state was maintained for at least 24 hours. From the results presented in Fig. 2a, it follows that at a molar $(O_2/HS^-)_{consumption}$ of respectively 0.51, 0.77 and 1.15, the redox potential remained more or less constant. At an oxygen supply of less than the minimal amount required to oxidize all sulphide, i.e. when the value of the molar $(O_2/HS^-)_{consumption}$ is below 0.5, the redox potential decreased from -142 to -161 mV. This is due to the accumulation of sulphide (as will be discussed below). Only at a molar $(O_2/HS^-)_{consumption}$ value of 1.54 was a strong increase of the redox potential observed. It would appear that at this ratio, at a loading rate of 50 mg $HS^-\cdot L^{-1}\cdot h^{-1}$, the sulphide concentration was very low, resulting in small absolute values of the measured redox potential whilst at a loading rate of 500 mg $HS^-\cdot L^{-1}\cdot h^{-1}$ the redox potential fluctuated strongly (± 85 mV). This may be the result of an accumulation of oxygen ($[O_2]$=1.0 mg$\cdot L^{-1}$). Since at this loading rate the biomass becomes overloaded, sulphur is the predominant oxidation product (Fig.2b) and consequently not all oxygen is consumed.

From Fig. 2b it can be seen that at a loading rate up to 175 mg $HS^-\cdot L^{-1}\cdot h^{-1}$ a linear relationship is found between the sulphate formation and the ratio oxygen/sulphide consumption. At a loading rate of 500 mg $HS^-\cdot L^{-1}\cdot h^{-1}$, however, less sulphate is formed than the maximal possible amount due to overloading of the biomass under these conditions, probably resulting in a reduction of the cytochrome chains of the organisms.[9]

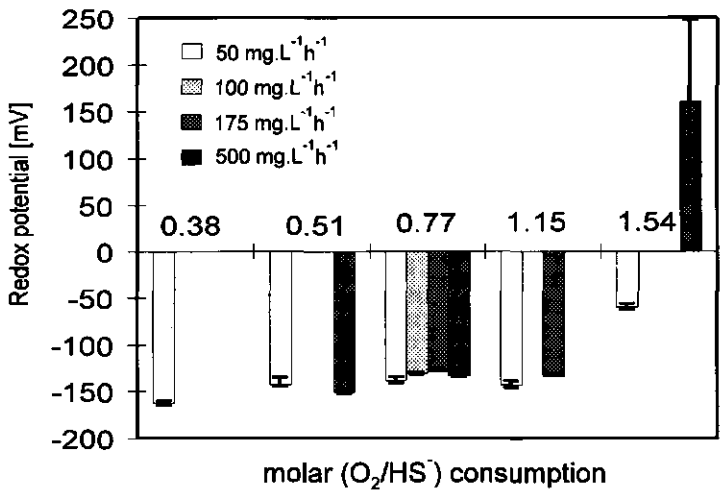

***Fig. 2a** Effect of the sulphide and oxygen loading rate on the measured redox potential in steady-state. The HRT is 6 h and [O_2] was mostly below the detection limit, i.e. 0.1 mg·L^{-1}. The deviation of the redox signal in steady-state from the mean value was mostly below ±3 mV. However, for the experiment at a loading rate of 500 mg HS^-·L^{-1}·h^{-1} and at a molar O_2/HS^-_{cons} of 1.54, [O_2] was 1.0 mg·L^{-1} and the deviation amounted to ±85 mV. In the experiment performed at a loading rate of 50 mg HS^-·L^{-1}·h^{-1} and at a molar O_2/HS^-_{cons} of 0.38, sulphide accumulated ([HS^-]= 1 mg·L^{-1}).*

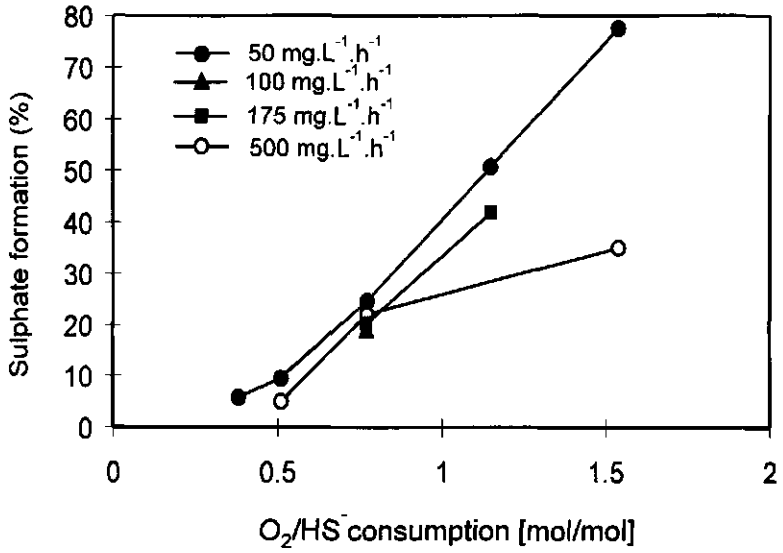

***Fig. 2b** Effect of the sulphide and oxygen loading rate on the relative sulphate production.*

Origin of the redox potential

The redox potential is an 'overall' parameter which means that the oxidation and reduction of a variety of (sulphur) compounds can attribute to its value. The oxidation of sulphide to respectively sulphur and sulphate and the reduction of oxygen in water are the most important redox changes. The measured redox potential therefore will be determined by these reactions. The value of the measured redox potential depends in principle on the standard potentials ($E_h°$) of the half-reactions and the concentration of the reactants. The redox potential can only be calculated by using the Nernst-equation, if a thermodynamic equilibrium exists. In practice however, the measured redox potential is mainly determined by the compound with the highest current exchange density, i.e. the ability to exchange electrons with the platinum surface. This means that the measured redox potential is kinetically determined rather than being dependant on the concentration of all dissolved reactants.[2] Sulphide (S^{2-} or HS^-) is a compound with a relatively high current exchange density at a platinum surface whereas oxygen has a very low value.[5] This means that in a sulphide oxidizing bioreactor the value of the redox potential is determined by the sulphide concentration. In three different experiments the relationship between the redox potential and the sulphide concentration, measured with an i.s.e. electrode, is measured. The results obtained from one of these experiments are depicted in Fig. 3. It can be seen that a linear relationship exists between the sulphide concentration and the redox-potential. The data were collected every 30 seconds from an experiment in which the sulphide and oxygen loading rates were not in a steady-state. Because the redox electrode responds slower to changing sulphide concentrations than the i.s.e. electrode does, a number of redox values were measured at each i.s.e. electrode-potential. The regression line is therefore drawn between the points with lowest redox values. This results in the following relation between the redox-potential and the sulphide concentration: $E = -42*\log(HS^-) - 158$, with $-\log [HS^-]$ in $mg \cdot L^{-1}$. The values measured so far, with a normally polished redox electrode, are not in a thermodynamic equilibrium as becomes clear by comparing the results with those which are obtained with a platinized electrode (*platinum black*). As follows from the titration curve in Fig. 4, the calibration line obtained with a platinized electrode has a slope of 35.0 mV/p(HS^-) which is in closer agreement with the theoretically expected value of 30.2 mV/p(HS^-). Laboratory research has shown that the addition of nutrient solution on the measured redox potential has no detectable effect (data not shown).

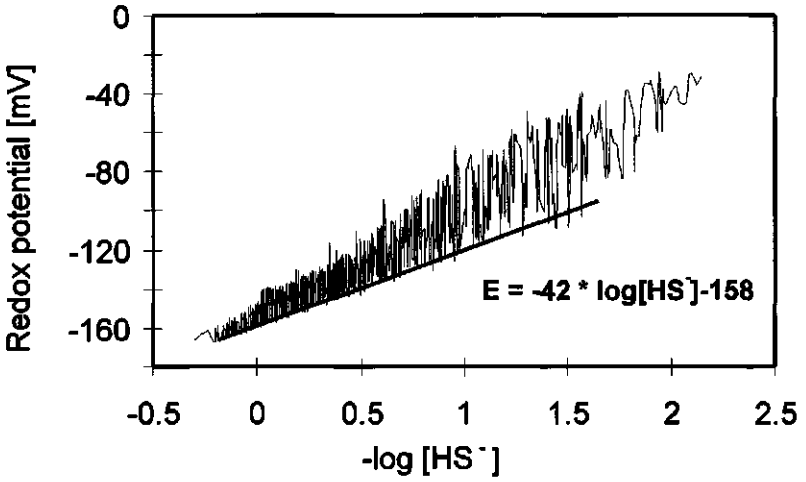

Fig. 3 Response of a polished redox electrode vs. an i.s.e. sulphide electrode in a sulphide oxidizing reactor with [HS⁻] in mg·L⁻¹.

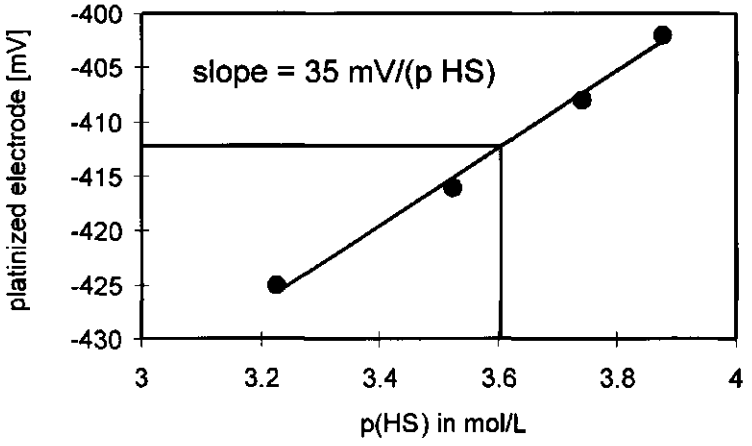

Fig. 4 Redox potential vs. the sulphide concentration (p[HS⁻]) measured with a platinized redox electrode in 20 g·L⁻¹ KH_2PO_4, pH=8.1.

The effect of the pH on the response of both electrodes is substantial, as can be seen in Fig. 5. The response of the i.s.e. electrode depends linearly on the pH, i.e. it drops with 27 mV per pH-unit due to the increase of the concentration S^{2-} ions. Exactly the same value has been found by Visscher *et al.*[14] This value is close to the theoretically expected value, i.e. 30 mV/(pS^{2-}). For the redox electrode, a decrease of 14 mV/pH was found in the pH range 7-10.5.

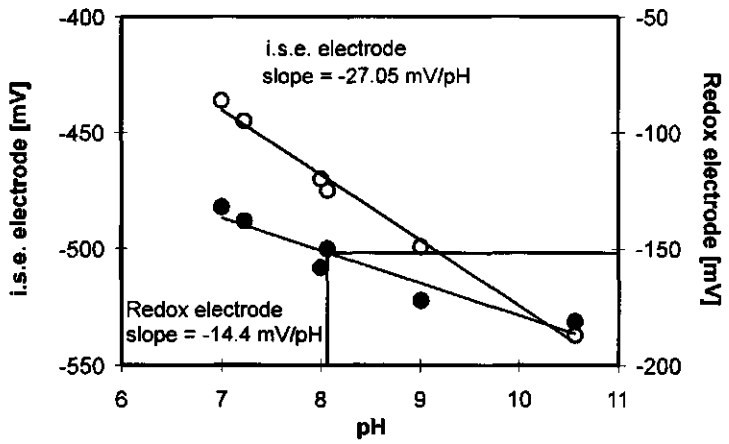

Fig. 5 Effect of pH on the response of a polished redox and i.s.e. electrode at a constant total sulphide concentration of 7.8 mg·L^{-1} (i.e. p(HS$^-$+S^{2-})=3.613). The experiment was carried out in an air-tight vessel (30 °C) in the absence of biomass.

Comparing Figures 4 and 5 shows that the measured values for the blackened electrode are distinctly lower than for the polished electrode, i.e. -412 mV versus -151 mV. This can be attributed to the kinetical limitation of the polished electrode; the blackened electrode accepts more electrons from the sulphide ions.

Real-time control of the oxygen supply at varying sulphide loading rates.

From Fig. 2, it follows that the ratio between the oxygen and sulphide consumption should be as low as possible in order to minimize sulphate formation. However, the redox potential in steady-state should not drop below -150 mV in order to prevent the accumulation of sulphide. In a system operated under a constant sulphide loading rate we investigated which redox levels can be attained. The redox setpoint for the experiment depicted in Fig. 6 amounted to -122 mV. The controler compares the measured values for the redox potential with the desired value, i.e. the setpoint value. From a computer-algorithm an output value for the oxygen-valve was calculated, using the so called P and PI controllers.* It can be seen that the redox potential reached the setpoint value within 4 hours. This time can be reduced by a further optimization of the gain factor (K_c) and time constant (τ) of the used PI-controller.

* See appendix A for more information about the mathematical description of the controllers.

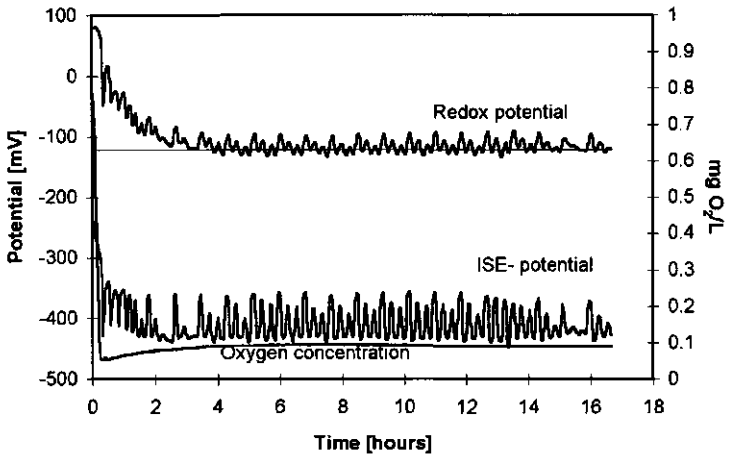

Fig. 6 *PI-controlled oxygen dosage, with K_c=0.4 and τ_i=2900 s. The loading rate and redox setpoint value are 175 mg $HS^-\cdot L^{-1}.h^{-1}$ and -122 mV (s. straight line), HRT=5 h. The sulphate concentration increases from 125 to 200 mg $S\cdot L^{-1}$ and the thiosulphate is between 40 and 75 mg $S\cdot L^{-1}$.*

Tuning of a PI-controller is a precise matter, i.e. chosing the optimal values for the gain factor (K_c) and time constant (τ). Since in our system the amount of biomass varies with changing ratios of oxygen to sulphide consumption and also with changing sulphide loading rates, the time-response of the bioreactor may change as well. This means that the PI-controller also requires different sets of gain factors and time constants. Since the tuning of a P-controller is less troublesome than a PI-controller, some experiments with a P-controller were also performed. Figure 7 shows the results which were obtained with a P-controller with a redox setpoint value of -122 mV.

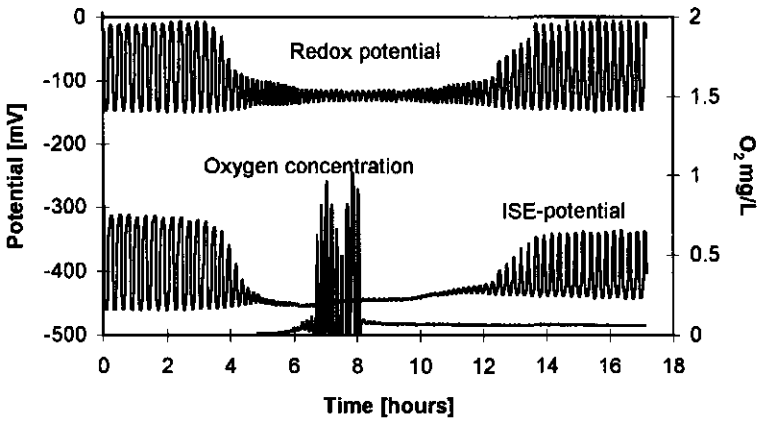

Fig. 7a *P-controlled oxygen dosage (K_c=0.75). The imposed sulphide loading rate was 195 $mg \cdot L^{-1} \cdot h^{-1}$. and redox setpoint value was -122 mV. During the course of the experiment, the sulphate concentration was 200 mg $S \cdot L^{-1}$ and the thiosulphate concentration decreased from 300 to 100 mg $S \cdot L^{-1}$.*

From the results in Fig. 7a, it can be seen that during the first 3.5 h of the experiment, the redox potential oscillated around its setpoint value of -122 mV whereafter it converged to this value. However, after about 11 h of operation, the system apparently became unstable regarding the fact that the redox potential started to oscillate heavily. By repeating the same experiment at a setpoint value of -147 mV it was found that the measured redox-potential converges faster to its desired value (Fig. 7b). Unfortunately, the time-scale between the experiments presented in respectively Fig. 7a and Fig. 7b are not the same which makes a good comparison not possible. However, from the results described in Figs. 7a and 7b it can be concluded that the process can be better controled at lower redox values, which in fact is rather obvious because the sulphide concentration is higher at lower redox levels. Since the measured redox potential depends linearly on the logarithm of the sulphide concentration, fluctuations around the lower setpoint are therefore smaller and the process stability is therefore higher.

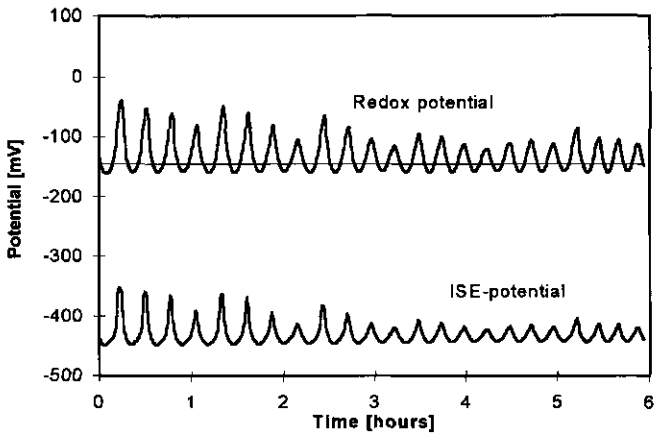

Fig. 7b *P-controlled oxygen dosage (K_c=0.75). The imposed sulphide loading rate was 195 mg·L^{-1}·h^{-1} and the redox setpoint value was -147 mV. During the course of the experiment, the sulphate concentration in the effluent was 150 mg S·L^{-1} and the thiosulphate concentration decreased from 100 to 60 mg S·L^{-1}. The straight line represents the setpoint value.*

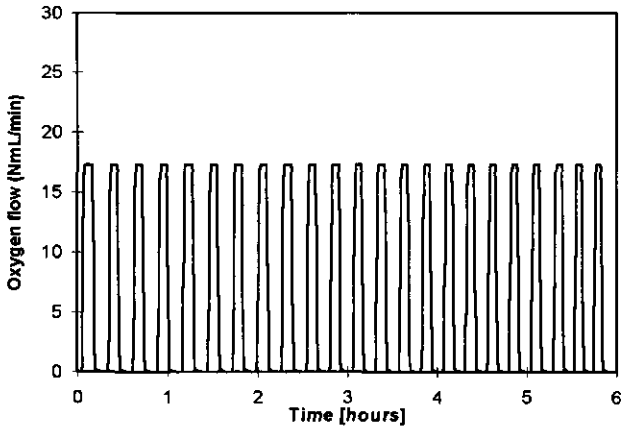

Fig. 7c *Oxygen flow belonging to the experiment described in Fig. 7b.*

As follows from Fig. 7b it is hardly possible to maintain a redox setpoint of -147 mV using a P-controller although this is accompanied with large fluctuations in the oxygen flow (Fig. 7c). Apparently, the controller becomes an on/off switch. The reason for this is, that the value for the gain factor chosen may be too high. Since the maximum oxygen-flow rate was truncated the system did not become unstable. Regarding the fact that a PI-controller functioned well in

experiments with a constant sulphide loading rate (data not shown), it is expected that a combined P and PI controller is the best control-strategy for experiments with abrupt changes in the sulphide loading rate. The P-controller forces the redox potential to a value within a narrow band around the setpoint value, e.g. -25 mV<setpoint<+50 mV, and from then onwards a PI controller provides an almost constant oxygen flow. Such a combined controller was used for controlling the oxygen flow in an experiment in which the supply of sulphide was changed stepwise (Fig. 8).

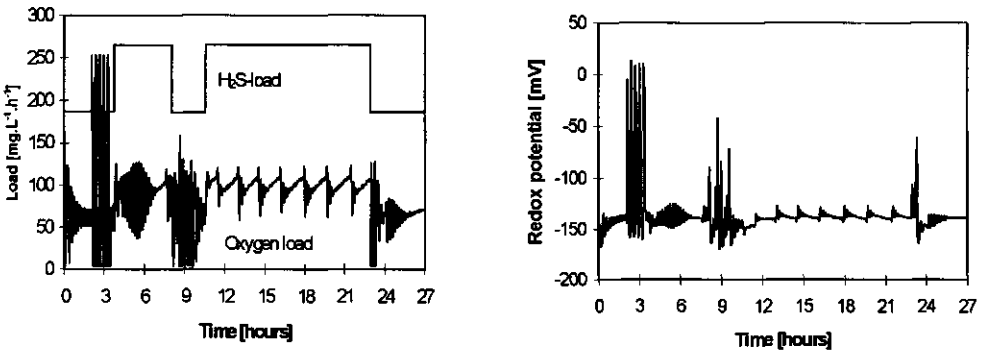

***Fig. 8** The effect of a stepwise change in the sulphide loading rate from 185 mg $HS^-·L^{-1}·h^{-1}$ to 265 mg $HS^-·L^{-1}·h^{-1}$. The oxygen supply is controlled with a combined P/PI-controller (K_c=1.5, τ_i=3280 s). The redox potential setpoint value was –137 mV.*

While the experiment in Fig. 7c shows that the oxygen-flow oscillated vigorously from 0 to 17.5 $mL·min^{-1}$, i.e. 0-150 $mg·L^{-1}·h^{-1}$, a much more constant oxygen-supply rate is obtained in the experiment with the combined P/PI controller (Fig. 8, left). It follows from the results in Figs. 8 that the bioreactor plus P/PI controller responds better to an increase than to a decrease in the sulphide loading rate, i.e. the off-set from the redox setpoint value at t=4 h and t=10 h is considerably smaller than at t=8 h and t=23 h. The explanation for this is that decreasing the loading rate results in a larger net-change of the redox potential than an increase. As a consequence, the calculated deviation between measured value and the setpoint value is bigger in the former case. The results in Fig.8 (right) also show a periodic change in the redox potential during the period 11-23 h, which cannot yet be explained. In practice, the loading rate probably does not change as sharply as in Fig.8, but generally smooth fluctuation will occur, e.g. in a sinusoidal way, as shown in Fig. 9.

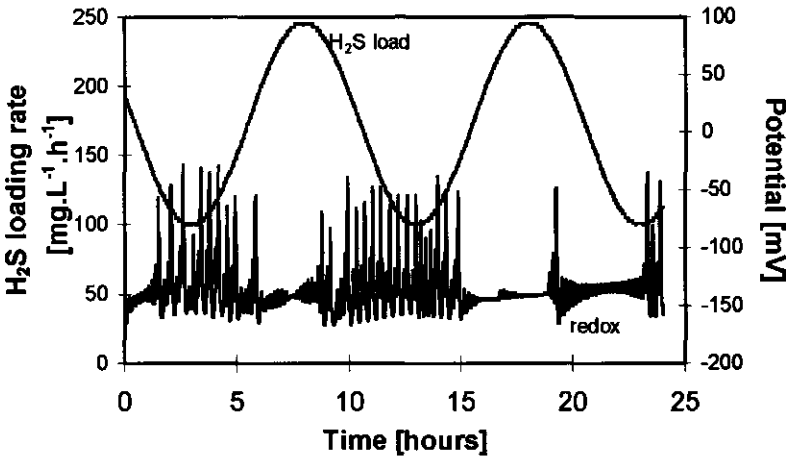

***Fig. 9a** Effect of an imposed sinusoidal change in the H_2S loading rate at a HRT=5 h; The redox setpoint was −137 mV and a combined P/PI controller (K_p=0.75, K_c=1.5, τ_i=3280 s) was used.*

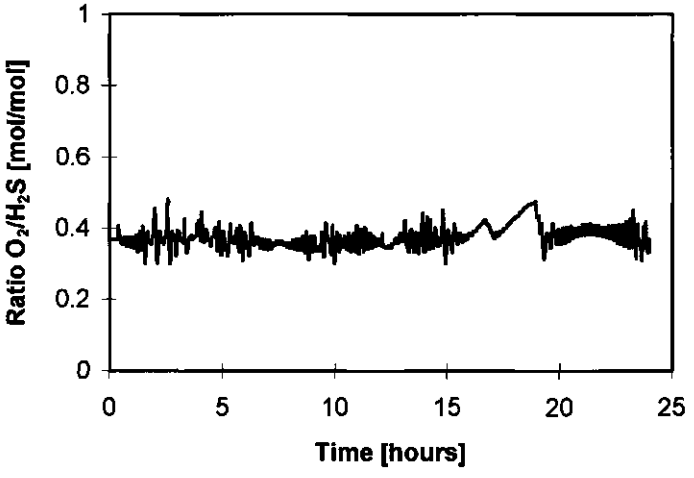

***Fig. 9b** Ratio between oxygen and sulphide consumption belonging to the experiment depicted in Fig. 9a. This ratio has been calculated using a moving average value for the oxygen consumption (n=15).*

From Fig. 9b it is clear that it is possible to control the oxygen over sulphide consumption under dynamic circumstances, although a decrease in the loading rate results in large fluctuations of the redox potential. The ratio of the molar oxygen over sulphide consumption

was found to be 0.38 which is below the stoichiometrical minimum value of 0.5 (Fig. 9b). As reported in Chapter 2, the reduction of carbon dioxide to biomass is presumably responsible for this effect. Although a very limited amount of oxygen is consumed, still, to some extent sulphate formation occurs (Fig. 9c). Apparently, the system is incapable of completely preventing the formation of sulphate. This might be caused by small fluctuations in the dissolved oxygen concentration because at slightly higher DO-values the organisms immediately may switch to sulphate formation.

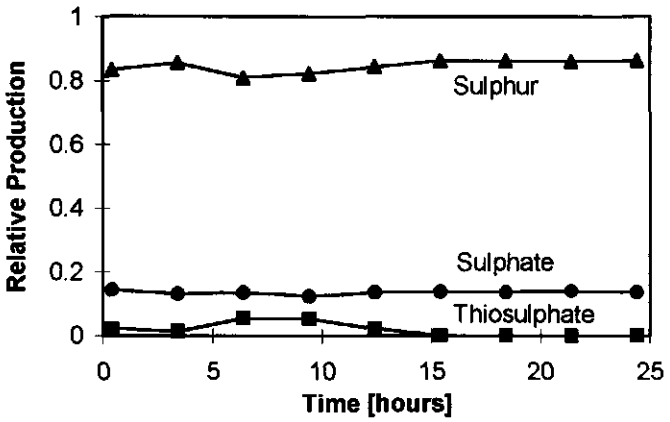

Fig. 9c Relative production of sulphate, thiosulphate (measured) and sulphur (calculated) during the experiment shown in Fig. 9a.

Modelling of the biological sulphide oxidizing process.

In this section we want to present a mathematical model describing the process of biological sulphide oxidation. It is assumed that both sulphur and sulphate are formed by the same metabolic type of organisms (s. Chapter 2). This does not necessarily mean that the biological oxidation of sulphide is carried out by a pure-culture. The mass-balances for sulphide, sulphur, thiosulphate, sulphate, biomass and oxygen are given below. In the model, the formation of sulphite (SO_3^{2-}) is neglected. It is assumed that both the gas and liquid phase are ideally mixed and that the volume of the reactor and liquid flow are constant. The gas-phase is recirculated to prevent the escape of H_2S gas. The energy which is released from the oxidation of sulphide is only used for growth of the micro-organisms, i.e. no maintenance-energy is taken into account. The decay of biomass is neglected and the formed sulphur is considered to be inert, i.e. no sulphur is oxidized to sulphate.

Sulphide balance:

$$\frac{d[HS]}{dt} = D \cdot ([HS]_{in} - [HS]) - (R_s + R_{SO_4}) \cdot [X] - 0.29 \cdot [HS] \cdot [O_{2,liq}]$$

Sulphate balance:

$$\frac{d[SO_4]}{dt} = -D \cdot [SO_4] + R_{SO_4} \cdot [X]$$

Sulphur balance:

$$\frac{d[S]}{dt} = -D \cdot [S] + R_S \cdot [X]$$

Thiosulphate balance:

$$\frac{d[S_2O_3]}{dt} = -D \cdot [S_2O_3] + 0.29 \cdot [HS] \cdot [O_{2,liq}]$$

Oxygen balance (gas phase):

$$\frac{d([O_{2,gas}])}{dt} = \frac{\Phi_{O_{2,in}}}{V_{gas}} - K_L A \cdot (\frac{[O_{2,gas}]}{m} - [O_{2,liq}])$$

Oxygen balance (liquid phase):

$$\frac{d[(O_{2,liq})]}{dt} = D \cdot ([O_{2,liq,in}] - [O_{2,liq}]) + K_L A \cdot (\frac{[O_{2,gas}]}{m} - [O_{2,liq}]) - (R_s \cdot 0.45 + R_{so_4} \cdot 1.82) \cdot [X] - R_{S_2O_3} \cdot 2$$

The oxygen consumption can be calculated according to the reaction stoichiometry of the reactions for sulphide oxidation:

$$HS^- + \frac{1}{2} O_2 \rightarrow S^o + OH^-$$

$$HS^- + 2O_2 \rightarrow SO_4^{2-} + H^+$$

$$2HS^- + 2O_2 \rightarrow S_2O_3^{2-} + H_2O$$

For the formation of 1 mol of sulphur or sulphate, 0.5 and 2.0 mol of oxygen are stoichiometrically necessary. However, according to Stefess the required molar amounts of oxygen are 0.45 and 1.82 due to the fact that 9% of the available electrons are required for CO_2 fixation.[9]

Biomass balance:

$$\frac{d[X]}{dt} = (R_S \cdot Y_S + R_{SO_4} \cdot Y_{SO_4}) \cdot [X] - D \cdot [X]$$

It was found that the organisms in the reactor can switch from sulphur to sulphate formation. Since the formation of sulphate yields more energy than that of sulphur, the former reaction is preferentially carried out. However, at high sulphide concentrations the system produces

elemental sulphur, probably because the formation of sulphate is inhibited by high sulphide concentrations.[4] The oxidation of sulphide to sulphur is therefore described by a standard Monod equation while the formation of sulphate is described by a Haldane equation which contains an inhibition term for sulphide. Since in a sulphide oxidizing bioreactor the prevailing oxygen and sulphide concentrations are very low, it was impossible to measure the K_m and K_i values. The values used for these parameters in the model are therefore 'best-guesses'.

Sulphur formation according to Monod:[8]

$$R_S = R_{max,S} \cdot \left(\frac{[HS]}{K_1 + [HS]}\right) \cdot \left(\frac{[O_{2,liq}]}{K_2 + [O_{2,liq}]}\right)$$

Sulphate formation according to Haldane:[8]

$$R_{SO_4} = R_{max,SO_4} \cdot \left(\frac{[HS]}{K_3 + [HS] + \frac{[HS]^2}{K_4}}\right) \cdot \left(\frac{[O_{2,liq}]}{K_5 + [O_{2,liq}]}\right)$$

Table 1: Parameters used in model for biological sulphide oxidation.

D=0.2 h^{-1}	$R_{max,S}$=436 mg(HS)/mgN.h [b]
Vgas=1 L	K_1=1.0 mg HS^-/L
m=32	K_2=0.05 mg O_2/L
K_LA=180 h^{-1} [a]	
Y_S=0.0015 mg N/mg (HS^-) [b]	$R_{max,SO4}$=149 mg(HS)/mgN.h [b]
Y_{SO4}=0.021 mg N/mg (HS^-) [b]	K_3=0.03 mg HS^-/L
	K_4=5 mg HS^-/L
	K_5=0.05 mg O_2/L

[a] reference from van 't Riet and Tramper. Basic Bioreactor Design. 1991, Marcel Dekker, New York.
[b] reference from Buisman *et al.* (1991). Biotechnol.Bioeng. **38**:813-820

Parameter sensitivity

The sensitivity of the parameters involved was investigated to determine the accuracy of these constants. The calculations presented in Fig. 10 show that K_1 = 1.0 mg $HS^-\cdot L^{-1}$ is a reasonable estimate for the K_m value for sulphur formation. At K_m-values below 0.1 mg $HS^-\cdot L^{-1}$ the sulphur production increases. Consequently, less sulphate is produced so the calculated oxygen concentration increases drastically. At K_m values above 1.0 mg $HS^-\cdot L^{-1}$, the affinity of the organisms for sulphide is too low which results in calculated sulphide concentrations that are higher than the measured sulphide concentration, which is about 0.3 mg $HS^-\cdot L^{-1}$.

Table 2: Sulphate-effluent concentrations [mg·L^{-1}] measured at various imposed sulphide loading rates and oxygen over sulphide consumption ratios. The hydraulic retention was 5.0 h and the pH was 8.0. The oxygen concentration was always below the detection limit, i.e. below 0.1 mg·L^{-1}, except for a loading rate 500 mg HS^-·L^{-1}·h^{-1} and a ratio of 1.54. The oxygen concentration then was 1.0 mg·L^{-1}.

molar O_2/HS^- - consumption	50 [mg·L^{-1}·h^{-1}]	100 [mg·L^{-1}·h^{-1}]	175 [mg·L^{-1}·h^{-1}]	500 [mg·L^{-1}·h^{-1}]
0.38	18	-	-	-
0.51	29	-	-	150
0.77	75	117	217	660
1.15	155	-	448	-
1.54	237	-	-	1050

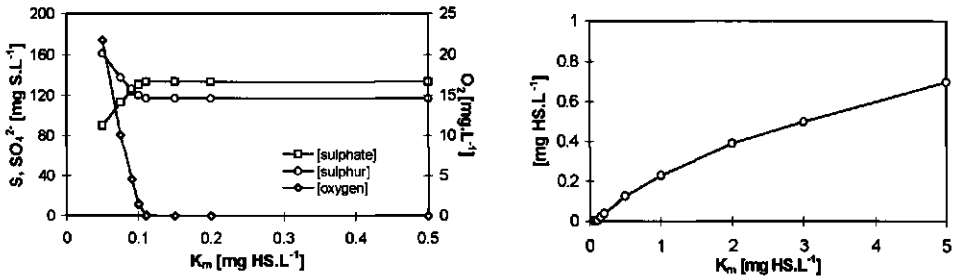

Fig. 10 The calculated sulphur, sulphate, oxygen and sulphide under steady-state concentrations at increasing K_m values (K_1) for sulphur formation at a loading rate and hydraulic retention time of 50 mg HS^-·L^{-1}·h^{-1} and 5 h, respectively. The molar oxygen to sulphide consumption was 1.15.

The Monod-constant for the oxygen concentration (K_2) in the equation which describes the formation of sulphur was determined using the results depicted in Fig. 11.

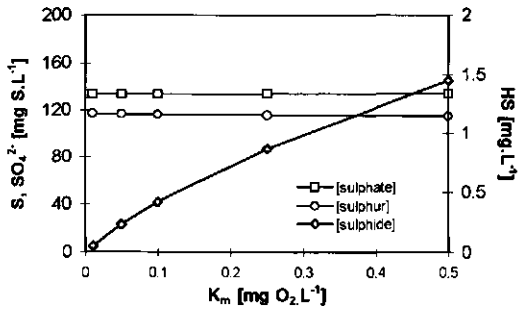

Fig. 11 The calculated sulphur, sulphate and sulphide concentrations under steady-state conditions at increasing K_m-values (K_2) for sulphur formation at a loading rate and hydraulic retention time of 50 mg HS^-·L^{-1}·h^{-1} and 5 h respectively. The molar oxygen to sulphide consumption was 1.15.

Since the measured sulphide concentration in steady-state is about 0.3 mg·L^{-1}, the K_m value for sulphur formation with respect to the oxygen concentration is estimated at 0.05 mg O_2·L^{-1}.

The kinetic parameters in the Haldane-equation for sulphate formation were estimated in the same manner. Figure 12 shows the effect of K_m value for the sulphide concentration on the sulphur, sulphate, oxygen and sulphide concentrations.

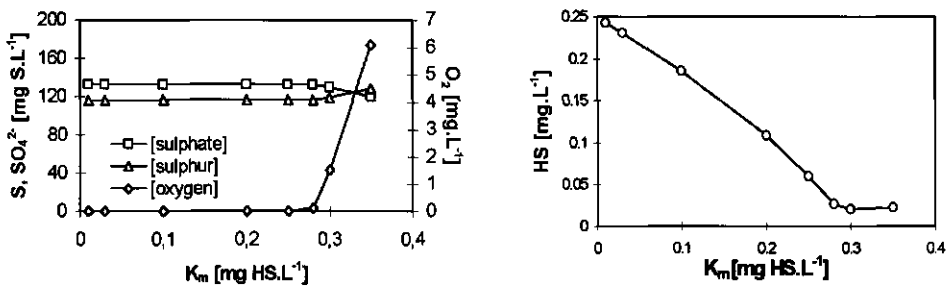

Fig. 12 The calculated sulphur, sulphate, oxygen and sulphide concentrations under steady-state conditions at increasing K_m-values (K_3) for sulphate formation at a loading rate and hydraulic retention time of 50 mg HS$^-$·L^{-1}·h^{-1} and 5 h, respectively. The molar oxygen to sulphide consumption was 1.15.

From figure 12 it follows that at a K_m value for sulphate formation of 0.03 mg HS$^-$·L^{-1} the calculated sulphide effluent concentration is 0.23 mg·L^{-1} which lies within the range of the measured values. At K_m values exceeding 0.30 mg·HS$^-$·L^{-1}, the affinity of the system for sulphate formation becomes too low which results in the presence of considerable, calculated, amounts of dissolved oxygen.

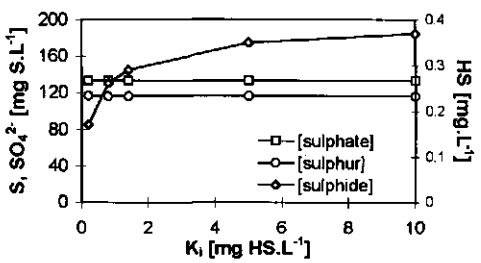

Fig. 13 The calculated sulphur, sulphate and sulphide concentrations under steady-state conditions at increasing K_i values (K_4) for sulphate formation with respect to the sulphide concentration at a loading rate and hydraulic retention time of 50 mg HS$^-$·L^{-1}·h^{-1} and 5 h, respectively. The molar oxygen to sulphide consumption was 1.15.

The K_i-value (K_4) for sulphate formation with respect to the sulphide concentration is estimated at 5 mg $HS^-\cdot L^{-1}$ since lower estimated values for K_i would also lead to lower sulphide effluent concentrations which were not measured during the experimental period.

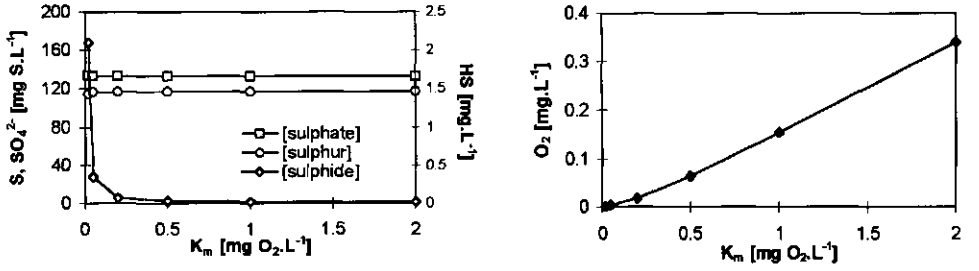

Fig. 14 The calculated sulphur, sulphate, sulphide and oxygen concentrations under steady-state conditions vs. the estimated K_m values (K_5) for sulphate formation with respect to the oxygen concentration. The loading rate and hydraulic retention time were 50 mg $HS^-\cdot L^{-1}\cdot h^{-1}$ and 5 h, respectively and the molar oxygen to sulphide consumption was 1.15.

For sulphate formation, the K_m value (K_5) with respect to oxygen concentration was estimated at 0.05 mg $O_2\cdot L^{-1}$. At lower values substantial amounts of sulphide are present in the effluent while at values exceeding 1 $mg\cdot L^{-1}$ the calculated dissolved oxygen concentration becomes 0.15 $mg\cdot L^{-1}$ which is higher than the measured value. The latter value was always below 0.1 mg $O_2\cdot L^{-1}$

Table 3: Calculated sulphate-effluent concentrations [$mg.L^{-1}$] at various loading rates and oxygen over sulphide consumption ratios.

molar (O_2/HS^-) consumption	50 [$mg\cdot L^{-1}\cdot h^{-1}$]	100 [$mg\cdot L^{-1}\cdot h^{-1}$]	175 [$mg\cdot L^{-1}\cdot h^{-1}$]	500 [$mg\cdot L^{-1}\cdot h^{-1}$]
0.38	1	-	-	-
0.51	20	-	-	116
0.77	64	122	209	584
1.15	133	-	451	-
1.54	205	-	-	1993

Most of the values calculated with the model for the sulphate concentration (Table 3) are in good agreement with the measured data, as presented in Table 2. Only the calculated sulphate concentration at a sulphide loading rate of 500 $mg\cdot L^{-1}\cdot h^{-1}$ is much higher than the measured concentration, viz. 1993 mg SO_4-$S\cdot L^{-1}$ calculated versus a measured concentration of 1050 mg SO_4-$S\cdot L^{-1}$. This may be attributed to the use of a value too high for the specific sulphate production rate in the model.

CONCLUSIONS

The results described in this chapter, demonstrate that it is very well possible to oxidize sulphide into elemental sulphur by creating an oxygen-limited environment. This can be achieved by controlling the redox state of the solution using a classical PI-controller so that nearly stoichiometrical amounts of oxygen are supplied to the system. In this way, the supplied oxygen suffices for oxidizing sulphide to sulphur, although still about ten per cent of sulphide is oxidized to sulphate. Since sulphide has a high 'current exchange density', i.e. the ability to exchange electrons with the platinum electrode surface, the redox potential is determined by the sulphide concentration. Other compounds, e.g. dissolved oxygen, are less important. The measured redox potential therefore is kinetically determined rather than thermodynamically. It was also shown that the effect of the pH on the redox state is substantial. Higher pH-values lead to lower redox potentials, probably due to a shift in the $H_2S/HS^-/S^{2-}$ equilibrium.

A mathematical model was developed that describes the biological sulphide oxidation. The model relies on the presence of only one metabolic type of organism, as explained in Chapter 2. In the model, the sulphur formation was described with a double-Monod equation (for respectively oxygen and sulphide). Sulphate formation is inhibited by increasing sulphide concentrations.[4] Therefore, the formation of sulphate was modelled by multiplying a Haldane equation for sulphide with a Monod term for oxygen. A sensitivity-analysis was carried out to determine the kinetic constants, e.g. Monod-terms for the sulphide and oxygen concentrations. The following kinetic parameters were estimated for sulphur formation: K_m=1.0 mg $HS \cdot L^{-1}$ and K_m=0.05 mg $O_2 \cdot L^{-1}$. The estimated kinetic parameters for sulphate formation were: K_m=0.03 mg $HS \cdot L^{-1}$, K_i= 5 $HS \cdot L^{-1}$ and K_m=0.05 mg $O_2 \cdot L^{-1}$. The model calculations for sulphate formation are in good agreement with the measured values. In future, this model can be used to develop and tune controllers for oxygen suppletion prior to the performance of biological experiments.

Appendix A: Mathematical Description of a P and PI-controller

The controller is placed between the measuring device, e.g. the redox electrode, and the final control element, e.g. a mass-flow controller. Its function is to receive the measured output signal ($y_m(t)$) and after comparing it with the setpoint (y_{sp}), it produces the actuating signal ($c(t)$) in such a way as to return the output to the desired value (y_{sp}). Therefore, the input to the controller is the error $\varepsilon(t)=y_{sp}-y_m(t)$, while its output is $c(t)$. In our research the error was calculated by subtracting the measured redox potential from its desired value, i.e. the setpoint value. However, it would have been better to calculate the oxygen supply according to the following reasoning. The relation between the measured redox potential and the sulphide concentration is:

$E_t = -42 \cdot \log[HS^-] - 158$, which gives:

$$[HS^-] = 10^{-(\frac{E_t+158}{42})}$$

Since the output of the oxygen dosage should be proportional to deviations in the sulphide concentration, it follows that:

$$\varepsilon_t = 10^{-(\frac{y_{sp}+158}{42})} - 10^{-(\frac{E_t+158}{42})}$$

The output signal of a feedback controller depends on its construction. In our research, two basic types of feedback controllers were used: (1) a Proportional (P) controller and (2) a Proportional-Integral (PI) controller. For a P-controller, its actuating output is proportional to the error:

$$c(t) = K_p \cdot \varepsilon(t) + c_s$$

where K_p = proportional gain of the controller and c_s = the bias of the controller's signal (i.e., its actuating signal when ε=0). A proportional controller is described by the value of its proportional gain K_p. It is clear that the larger the gain K_p, the higher the sensitivity of controller's actuating signal to deviations $\varepsilon(t)$ will be.

For a PI-controller, its actuating signal is related to the error by the equation:

$$c(t) = K_c \cdot \varepsilon(t) + \frac{K_c}{\tau_i} \int_0^t \varepsilon(t) \cdot dt + c_s$$

where τ_i is the integral time constant. The integral action causes the controller output c(t) to change as long as an error exists in the process output. Therefore, such a controller can eliminate even small errors.

The controllers were tuned, using the empirical method developed by Cohen and Coon.[10]

NOMENCLATURE

τ_i	integral time constant of a PI controller
$\Phi_{O2,in}$	oxygen flow to the gas-phase, ($mg \cdot h^{-1}$)
ε_t	input error to the contoller
{ox}	activity of oxidizable compounds ($mol \cdot L^{-1}$)
{red}	activity of reducible compounds ($mol \cdot L^{-1}$)
c(t)	output controller
D	dilution rate (h^{-1})
DO	dissolved oxygen ($mg \cdot L^{-1}$)
E_H	half-potential (V)

E_H^o	standard half-potential (V)
E_t	measured redox potential at time t
F	Faraday constant ($9.6485 \cdot 10^5$ C·mol^{-1})
HRT	hydraulic retention time (h)
i.s.e.	ion selective sulphide electrode
$K_{1\text{-}5}$	kinetic constants
K_p	proportional gain of the P-controller
K_c	proportional gain of the PI-controller
K_LA	oxygen transfer rate (h^{-1})
m	oxygen partition coefficient
n	number of electrons transferred
n_i	moles of oxidizable compounds
n_j	moles of reducible compounds
pε	= $(E_H \cdot F)/(2.3 \cdot R \cdot T)$
R	gasconstant (8.31 J·mol^{-1} K^{-1})
Rs	specific sulphur formation rate (mg S°·mg N^{-1}·h^{-1})
R_{SO_4}	specific sulphate formation rate (mg SO_4-S·mg N^{-1}·h^{-1})
T	Absolute temperature (K)
V_{gas}	volume of the gas-phase (L)
V_{liq}	volume of the liquid phase (L)
X	biomass concentration (mg N·L^{-1})
$y_m(t)$	measured output signal, i.e. measured redox potential
Ys	biomass yield on sulphur formation (mg N·mg S^{-1})
Y_{SO_4}	biomass yield on sulphate formation (mg N·mg S^{-1})
y_{sp}	redox setpoint value

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CHAPTER 4

Colloidal properties of a microbiologically produced sulphur suspension in comparison to a LaMer sulphur sol. [A]

ABSTRACT

The colloidal characteristics of a biotechnologically produced hydrophilic sulphur suspension have been compared to those of a synthetically formed LaMer sulphur sol. Biologically produced sulphur is the end-product of the microbiological sulphide oxidation, a process carried out by mixed cultures of *Thiobacillus*-like bacteria.

Electrophoretic mobility measurements and flocculation experiments revealed differences in the colloidal properties of the two sols. This observation was explained by the assumption that negatively charged (bio)polymers are attached to the microbiologically produced sulphur particles, having an orthorhombic sulphur nucleus, while a LaMer sol has a vesicle structure which is built up of long-chain polythionates.

As shown by dynamic light scattering measurements, the diameter of the biological sulphur particles decreases with increasing salt concentration, indicative for an inward shift of the adsorbed polymers at high electrolyte concentrations.

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INTRODUCTION

Sulphide has to be removed from wastewater because of its toxicity, corrosive properties, high oxygen demand and unpleasant odour. Several chemical oxidizing agents can be used to do this but the high chemical and disposal costs are the major drawbacks of the methods based on this principle.[5, 6] As described in chapter 2, bacteria belonging to the group of the colourless sulphur bacteria, in presence of an oversupply of substrate, oxidize sulphide into sulphur under aerobic conditions. To make a biotechnological sulphide removal process applicable in practice, an economically and technically satisfactory sulphur separation method should be developed. Results of pilot-plant research studies, have shown that in the absence of polyelectrolytes the sedimentation of the sulphur fraction proceeds very slowly, but in the presence of a suitable polyelectrolyte the sulphur particles settle rapidly.[4] The use of polyelectrolytes, however, should be minimized in order to avoid high operational costs and to enable the reuse of the high purity biological sulphur obtained. Therefore more knowledge is required about the physical-chemical properties of the microbiologically produced sulphur.

Cultures of the sulphur bacteria tested, excrete small hydrophilic sulphur particles with a diameter up to 1 μm.[12] In a bioreactor, these sulphur particles form complex aggregates consisting of elemental sulphur, biomass and biopolymers.[1, 11] In order to assess any matrix effects on the physical-chemical properties of a biological sulphur suspension, a comparison was made with a synthetically made LaMer sulphur sol, produced in the absence of bioproducts. Like biological sulphur, LaMer sol particles are hydrophilic in nature. According to Steudel, in an acidic environment biological sulphur globules dispersed in water look similar to a LaMer sol.[14] The latter sol consists of polythionate vesicles, which contain a certain amount of elemental sulphur dissolved in the hydrophobic membrane. Condensation of polythionic acids during the preparation of a LaMer sol produces particles which are essential composed of elemental sulphur.[17, 19]

To characterize the biological sulphur particle and to validate the vesicle model postulated by Steudel [14], the effect of the nature and the concentration of the counterions on the colloidal stability of the biological sulphur sol was studied.

MATERIALS AND METHODS

Reactor

The microbiological sulphur particles studied have been produced in a 8.3 L volume tank reactor under complete mixing at room temperature (22°C).[3]

LaMer sol

The hydrophilic LaMer sol was prepared following the procedure described by Weitz *et al.*.[18] According to this procedure, an amount of 10 mL sulphuric acid (96%) is slowly added within a period of 15 minutes to a solution of 30 mL of a 6 *N* sodium thiosulphate under constant cooling in an ice bath, the temperature being kept below 25°C. The sulphur formed was coagulated with 50 mL of 4 *M* sodium chloride solution after which the yellow coloured solution was centrifuged for 10 min. at 4,500 r.p.m. and decanted to remove all soluble substances. Subsequently the precipitate was repeptized in 200 mL distilled water. This washing procedure was repeated at least five times. The stock solution was then diluted with distilled water to a final volume of three litres. The specific conductivity was 0.15 $mS \cdot cm^{-1}$, corresponding to 1.2 m*M* NaCl. For each series of experiments a freshly prepared suspension was used.

Microelectrophoresis

The electrophoretic mobility of the particles was measured by Laser Doppler velocimetry using a ZetaSizer (Malvern Instruments, Malvern England). To prevent the biological oxidation of sulphur into sulphate the solution was sedimented for three days under continuous UV radiation in order to kill the micro-organisms present. The suspension was then decanted to remove the biomass plus the suspended salts, and was reconstituted by an aliquot of distilled water under the assumption that the biomass is not sedimented. Dialysation followed for a period of 10 days in order to remove the rest of the salt fraction, leading to a final specific conductivity of 40 $\mu S \cdot cm^{-1}$, which corresponds to approximately 0.33 m*M* NaCl. 10 mL amount of stock solution was mixed with the same amount of electrolyte solution, leading to a final concentration as presented in Figs. 2-4. Since no buffer was used, the pH value after mixing was about 6.

After addition of the sulphur suspension to the electrolyte solution the samples were left to stand overnight so that only the colloidal fraction was sucked into the capillary of the instrument and precipitation in the measuring cell could be avoided. Electrophoretic mobility data were obtained from at least three separate experiments.

Flocculation experiments

Because of the negative charge of both sulphur sols, aliquots of three salt solutions, viz sodium- and calcium chloride and lanthanum nitrate, were added to the sols. The cation concentration in the test tubes ranged from 5.10^{-5} up to 2 *M*. The sulphur concentration in the test tubes amounted to 10 m*M*. After waiting for three hours at room temperature, flocs were clearly visible. The experiments described were all performed in duplicate.

Particle size determination

A sample of 250 mL taken from the reactor suspension was allowed to form sediment overnight, then successively decanted and passed over a filter (Whatman Glass Microfibre Filters, GF/C, pore size 1.2 μm) to remove aggregates in order to obtain a monodisperse solution. Dynamic light scattering measurements (ALV apparatus, angle: 60°, wavelength: 632.8 nm) were used to determine the particle size of the biological sulphur particles in different salt solutions. Each result has been calculated as the average of 10 measurements.

Total specific area

After freeze-drying for 12 hours the specific area of the particles was determined by nitrogen adsorption (Quanta Chrome, NOVA-1000 BET Surface Area Analyser). The result presented is an average of three independent measurements.

RESULTS AND DISCUSSION

General description of biological sulphur

X-Ray measurements confirmed the data of Hageage[7], indicating that a biological sulphur suspension contains orthorhombic sulphur crystals. It is well known that these crystals (S_8) are strongly hydrophobic. However, biological sulphur has a hydrophilic character.[14] This can be clearly seen in the photographs in Figure 1.

Fig. 1 Hexadecane-water partition-test. Biologically produced sulphur remains in the, lower, water phase (left) whereas standard hydrophobic yellow 'sulphur flower' remains in the upper hexadecane-phase (right).

Under acidic circumstances possibly long-chain polythionates are adsorbed to the orthorhombic sulphur nucleus; this could be responsible for its hydrophilic character.[15] Polythionates have been

reported to be stable only at pH values below 6,[13, 17] therefore their presence is very unlikely in our reactor which was operated at slightly alkaline conditions. It seems that another compound is responsible for the hydrophilic character of the sulphur produced in our reactor.

Effect of different cations on the electrophoretic mobility

To compare a biological sulphur suspension with a LaMer sol by assessing the relationship between salt concentration and electrophoretic mobility, three different cations (Na^+, Ca^{2+}, La^{3+}) were used. The results of these experiments are shown in Figs. 2-4.

The possible influence on the electrophoretic mobility of components which might be produced in the bioreactor was studied in a control experiment. For this purpose the supernatant from the bioreactor mass was added to an aliquot of a LaMer sol and the mobility was compared to that of a genuine LaMer sol (Fig. 2).

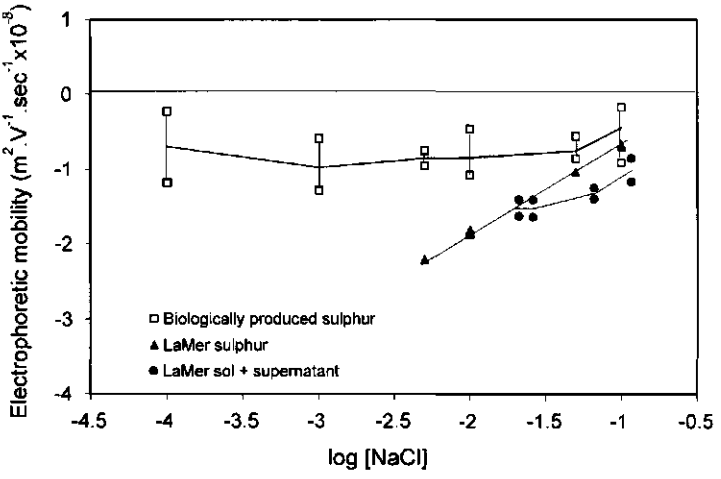

Fig. 2 Electrophoretic mobility of a biological sulphur sol, a LaMer sol and a LaMer sol with bioreactor mass, at different NaCl concentrations

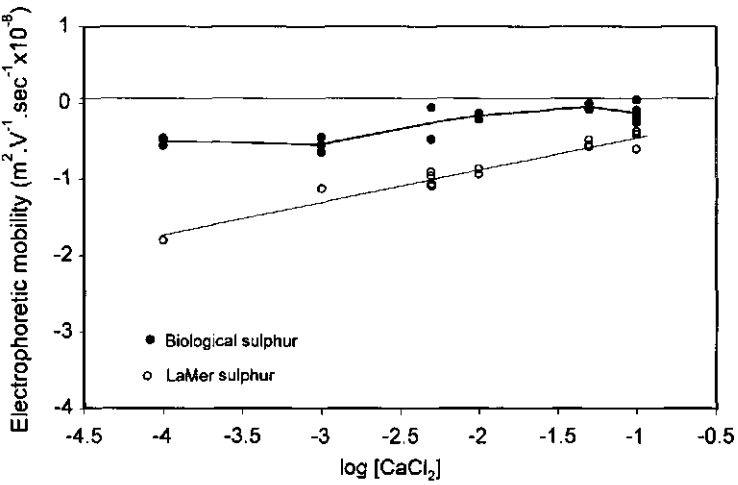

Fig. 3 Electrophoretic mobility of a biological sulphur sol and a LaMer sol at different $CaCl_2$ concentrations

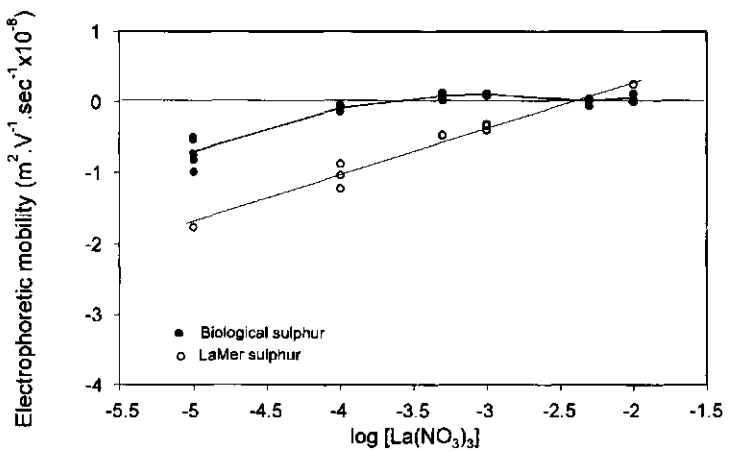

Fig. 4 Electrophoretic mobility of a biological sulphur sol and a LaMer sol at different $La(NO_3)_3$ concentrations

The results in Fig. 2 reveal that the absolute value of the electrophoretic mobility of a LaMer sol decreases almost linearly with the log of the Na^+ ion concentrations. The same trend is observed when Ca^{2+} and La^{3+} ions are used as counterion (Figs. 3 and 4). At increasing electrolyte concentrations the double layer of the LaMer sol is apparently compressed, resulting in a lower ζ potential and consequently in a lower electrophoretic mobility. For the same reason, at higher valencies a decrease in the electrophoretic mobility is also observed, i.e. at 10^{-2} *M* Na^+, Ca^{2+} and

La^{3+} ion concentrations, the mobilities are $-2x10^{-8}$, $-1x10^{-8}$ and $+0.3x10^{-8}$ $m^2 \cdot V^{-1} \cdot s^{-1}$ respectively. For high concentrations of La^{3+} ions, charge reversal is even observed which can be explained by assuming specific adsorption of this ion.

The behaviour of biological sulphur differs considerably from that of the LaMer sol; for example, increasing the Na^+ ion concentration hardly affects its electrophoretic mobility (Fig. 2). When Ca^{2+} ions are used, the electrophoretic mobility decreases slightly only at high concentrations, with a tendency for charge reversal (Fig. 3). The electrophoretic mobility in presence of Ca^{2+} ions is slightly lower than that in presence of Na^+ ions. If La^{3+} acts as a counterion, even at concentrations as low as 10^{-4} *M* could hardly any mobility be measured and a barely noticeable charge reversal occurs at a concentration of $5x10^{-3}$ *M* La^{3+} ions (Fig. 4).

A comparison of the results for Na^+, Ca^{2+} and La^{3+} ions indicates that a large difference in mobility exists between both sulphur sols at low electrolyte concentrations. Probably the LaMer sol has a higher negative electrical charge within its stagnant layer than the biological sulphur particles.

From the results presented in Figure 2, it may be concluded that in the presence of Na^+ ions, the differences between a biological sulphur sol and a LaMer sol are not caused by the adsorption of soluble biological excretion products, since the addition of the supernatant of the bioreactor mass to a LaMer sol does not change significantly the behaviour of the sol.

Flocculation experiments

The relation between the flocculation value and the electrolyte concentration was established for the same three cations (Table 1).

Table 1: Flocculation values ($mol \cdot L^{-1}$) for a LaMer sol and a biological sulphur sol

Cation	LaMer sol	Biological sulphur sol
Na^+	0.1	-
Ca^{2+}	5.10^{-4}	$2.5\ 10^{-1}$
La^{3+}	5.10^{-4}	$2.5\ 10^{-5}$

For both sols it can be seen that at higher valencies, z, the coagulation shifts towards a low concentration. The measured cation concentration ratio, C, at which a LaMer sol flocculates corresponds reasonably well with the theoretically predicted ratio of $C \approx z^{-6}$.[10] This is not the case for the biological sulphur sol. Even at a 2 *M* concentration of sodium, biological sulphur does not coagulate within a period of 3 h, although for La^{3+} ions there is hardly any difference between a LaMer sol and a biological sulphur suspension.

Dynamic light scattering measurements

Table 2 provides the results of the particle size measurements of the biological sulphur sol in different salt solutions in a concentration range from 10^{-5} *M* to 10^{-2} *M*. At higher Na^+ ion concentrations, the light reflection was disturbed by the formation of aggregates, leading to heterodispersity. A slow flocculation also occurred at concentrations above 10^{-2} *M* Ca^{2+} ions and 10^{-4} *M* La^{3+} ions. The sizes obtained are of the same order of magnitude as earlier published data.[8, 12, 16] It should however be noticed that the values obtained by Nicolson and Schmidt[8] and Strohl *et al.*[16] were obtained for intracellularly stored sulphur particles whereas we measured extracellularly excreted sulphur particles. The calculated particle radii have an accuracy of 17 nm. The specific area (SA) of a dried biological sulphur sol was 2.5 $m^2 \cdot g^{-1}$, as determined by nitrogen adsorption. This corresponds very well with the calculated SA which is in the range of 2.5 - 4.2 $m^2 \cdot g^{-1}$ (average radius r= 600 nm and particle density d=1.2 - 2 $kg \cdot L^{-1}$). Since the measured SA does not exceed the calculated value, the biological sulphur particles are not expected to be porous.

Table 2: Average particle radius r (nm) of biological sulphur suspensions at different electrolyte concentrations.

Concentration	NaCl	$CaCl_2$	$La(NO_3)_3$
($mol \cdot L^{-1}$)			
10^{-5}	-	-	715
10^{-4}	642	609	-[a]
10^{-3}	629	615	-[a]
10^{-2}	608	-[a]	-[a]
10^{-1}	-[a]	-[a]	-[a]

[a] measurements are disturbed by particle aggregation.

It can be inferred from Table 2 that an increase in the Na^+ ion concentration leads to a reduction in the particle size. From the $CaCl_2$ results, it seems that an increase in valency also leads to a smaller particle radius. However, for increasing Ca^{2+} ion concentrations, no further size reduction is observed; this might be explained by the inaccuracy of the measurements.

An explanation for the size reduction may be found in an inward shift of an adsorbed polymer layer. Such a polymer layer could very well be a biological excretion product.[1, 11] At increasing salt concentrations or at increasing valencies a decrease in the electrostatical repulsion between the charged groups of the polymer chains occurs. Therefore the polymer chains become less extended, resulting in an inward shift. The presence of a polymer layer also may explain the difference in flocculation values of the two sulphur sols for Na^+ and Ca^{2+} ions (Table 1). Whereas for a LaMer sol the flocculation values are in agreement with DLVO theory[10], this is not the case for biological

sulphur. The following explanation can be given for this phenomenon. From the electrophoretic mobility measurements it follows that biological sulphur is weakly negatively charged (Figs. 2-4), but rapid flocculation does not occur even in the presence of 2 *M* Na^+. Moreover, to flocculate biological sulphur considerably more Ca^{2+} ions are needed than for a LaMer sol. Apparently, apart from electrostatical repulsion forces, another stabilizing mechanism is involved in the colloidal behaviour of biologically produced sulphur. This could be steric hindrance caused by adsorbed polymers. Steric repulsion causes an increase in entropy when particles approach each other and this will result in an extra stabilization. A third phenomenon, which can only be explained by assuming the adsorption of polymers onto the sol particle surface, is contained in the results of the electrophoretical mobility measurements, as shown below.

From the values presented in Table 1, it has already been mentioned that the coagulation of biologically produced sulphur in the presence of lanthanum ions occurs at a much lower concentration than is expected from the results obtained for Na^+ and Ca^{2+} ions. It is known from literature, that La^{3+} ions easily form complexes with humic acids.[2] Although very high concentrations of Na^+ and Ca^{2+} ions are required to eliminate steric hindrance, this is not the case for La^{3+} because of its potential to bridge more than two particles at the same time, with the possibility of network formation. Hence, the relatively high particle size at which biological sulphur flocculates may be attributed to bridging as well (Table 2). The electrophoretic mobility measurements point in the same direction, i.e. charge reversal is only observed for La^{3+} ions, even at very low concentrations. For this reason, the numerically similar flocculation value of La^{3+} for both sols may be purely coincidental, assuming that only electrostatic repulsion determines the stability of a LaMer sol.[17,19]

The electrophoretic mobility measurements indicate that biological sulphur particles are slightly negatively charged. Since no polythionates or polysulphides are present in the system the particle charge has to be induced by other ions, e.g. negatively charged polymers adsorbed on to the particle surface.

Upon salt addition the repulsion between these polymers becomes smaller and the particle size decreases. At the same time the plane of shear moves towards the bare sulphur particle surface, resulting in an increase in the negative charge. In the case of sodium addition, the added cations are unable to compensate for the increase in negative charge, and the electrophoretic mobility is hardly affected, which is not the case for a LaMer sol. Upon increasing the Ca^{2+} ion concentration, the electrophoretic mobility gradually approaches zero, indicating that the negative charge is screened. From the bridge formation observed in the process of flocculation it follows that La^{3+} ions may be bound specifically to the particle surface, which explains the effect of charge reversal when the concentration exceeds 10^{-4} *M*.

In contrast to a biological sulphur sol, a LaMer sol consists of vesicles with a homogeneous surface so that the results in Figure 2-4 are in agreement with theoretically expected curves.[14] Verstraete[17] found that for this sol, polythionic acids act as the stabilizing agent and consequently these acids are responsible for the negative surface charge of the particle.

Adsorbed polymers may also be responsible for the hydrophilic nature of biological, as will be further discussed in chapter 5.

CONCLUSIONS

The colloidal properties of a biological sulphur sol differ considerably from those of a LaMer sulphur sol, which at least partly can be attributed to the attachment of negatively charged polymers. It is most likely that these attached polymers are also responsible for the hydrophilic character of the particle. Apart from electrostatical repulsion, steric hindrance also has to be taken into account to explain the typical characteristics of biological sulphur. The vesicle structure proposed by Steudel[14] for both a LaMer sol and a biological sulphur sol does not explain the observed differences in electrophoretic mobility and in flocculation values.

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CHAPTER 5

Surface characteristics and aggregation of microbiologically produced sulphur particles[A]

ABSTRACT

The effect of surface properties and effects of several process conditions, e.g. loading rate, ionic strength and the presence of polymers, on the degree of aggregation of sulphur particles were studied. Sulphur is formed under oxygen limiting circumstances during the partial oxidation of sulphide by a mixed culture of *Thiobacillus*-like bacteria. Since the freshly excreted particles are in a colloidal state, with a diameter of approximately 100 nm, their aggregation is a prerequisite in order to obtain a satisfactory sedimentation. Titration experiments revealed that the negative sulphur surface charge is determined by the presence of multiple functional groups. Attention was also paid to the effect of the chain-length, hydrophilicity and charge of a number of dissolved polymers on the degree of sulphur aggregation. The degree of polymer adsorption on the sulphur surface mainly depends on the hydrophobicity and charge of the polymer. Since the charge of biologically produced sulphur is negative at pH 8.0, a highly charged cationic polymer like Q_N-HEC inhibits the sulphur aggregation. For Perfectamyl, a cationic potato starch, and carboxy-methylcellulose no clear effect was measured. Particularly for long-chain polymers a distinct negative effect on the aggregation was found. Steric hindrance, apparently, is an important factor in the aggregation process.

Upon increasing the sulphide loading rate, larger sulphur aggregates were formed while the opposite trend was observed for increasing salt concentrations. In practice, therefore, a sulphide oxidizing bioreactor should be operated at high loading rates to enhance the settleability of the sulphur sludge.

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INTRODUCTION

During the last decade, the application of the biological sulphur cycle in environmental technology has become increasingly popular.[3,4,5,11,16,21,26,34] The processes involved concern the microbiological reduction of sulphate or sulphite, and the oxidation of sulphide. As described in Chapter 2 and by Kuenen[15], under oxygen limiting circumstances bacteria of the genus *Thiobacilli*, oxidize sulphide to insoluble, elemental sulphur which can be separated from the liquid phase. In this way, a reduction of the total amount of sulphur compounds can be achieved. The sulphur is a potentially valuable product which can be re-used, e.g. in soil-bioleaching processes.[34]

Recently, a new biotechnological process based on the biological sulphur-cycle was developed for removing SO_2 from (flue) gases produced by coal combusting power plants.[4] The process consists of three integrated reactors: the first reactor serves to scrub the SO_2 present in flue gas with an alkaline solution to form HSO_3^-/SO_3^{2-}, in the second reactor HSO_3^-/SO_3^{2-} is reduced to HS^-, and in a third reactor sulphide is oxidized under oxygen-limiting circumstances to elemental sulphur. These sulphur particles are separated in a tilted plate settler and the clean water can then be recirculated to the first reactor. In case the sulphur particles are not completely removed, a part of the sulphur fraction will be recirculated and subsequently reduced in the anaerobic reactor. An incomplete separation of sulphur particles therefore leads to (1) a double consumption of the required electron donor, e.g. methanol, ethanol or hydrogen gas and (2) increased sulphide levels in the anaerobic reactor which may cause inhibition of the metabolic processes taking place there.[2, 21] For these reasons, a highly effective sulphur removal step is essential for the successful application of the process.

In comparison with separation techniques such as flotation, filtration, extraction and membrane processes, plain sedimentation of sulphur particles undoubtedly represents the cheapest and technically most attractive method. However, in order to be able to apply this method, the formation of easily settleable sulphur aggregates from the freshly formed -but poor settling- sulphur particles is a prerequisite. It is of importance therefore, to improve our knowledge of the physico-chemical properties of biologically produced sulphur particles. In this respect, the composition of the wastewater should be taken into account. Since macromolecules tend to accumulate at interfaces due to Van der Waals forces and hydrophobic bonding, they may affect the aggregation of sulphur particles.[7]

Biologically produced sulphur has a hydrophilic nature[30,34] whereas orthorhombic sulphur (S_8) is strongly hydrophobic.[9] Steudel and co-workers suggested that sulphur particles produced by acidophilic *Thiobacillus ferrooxidans* and neutrophilic *Chromatiaceae vinosum* have a vesicle structure, like 'La-Mer' sulphur.[32,33] This is a synthetically produced sulphur sol, formed by the acidification of a concentrated sodium thiosulphate solution. It consists of polythionate micelles or vesicles, which contain a certain amount (17%) of elemental sulphur in their inner part whereas sulphonic groups ($-SO_3^-$) cover the outside.[37] These polythionates are reported to be stable only under acidic circumstances.[28] However, as the sulphur particles in our reactor are produced under slightly alkaline conditions, they are expected not to belong to the 'polythionate model'. In Chapter

3 it is explained that the hydrophilic character of the biologically produced sulphur particles is the result of the presence of (bio)polymers on the hydrophobic sulphur nucleus. This corresponds to the observation of Steudel who describes the positive effect of surfactants on the solubility of elemental sulphur.[31] Surfactants cover the orthorhombic sulphur nucleus and give the particle a hydrophilic character.

Aim of the study

The objective of this study is to assess the effects of process conditions, such as loading rate, ionic strength and presence of polymers, on the degree of aggregation of sulphur particles in relation to their surface properties.

MATERIALS AND METHODS

Fed-batch Reactor

The oxidation of sulphide to elemental sulphur was investigated using a fed-batch reactor which is extensively described in Chapter 2. In such a reactor all formed sulphur particles are retained in the system which enhances their aggregation. The system was kept at 30°C and pH=8.0.

Continuous Flow Stirred Tank Reactor

To assess the effect of the sulphide loading rate on the sulphur-aggregation, a completely mixed 5.0-L continuous-flow, stirred-tank reactor (CSTR) reactor was used.[3] The process temperature was approximately 23°C, (room temperature) and the pH was controlled at 8.0. The hydraulic retention time was 5 h. In contrast to the fed-batch system, the ionic strength in a CSTR remains constant when it is operated under steady-state conditions. Since the ionic strength may influence the degree of aggregation[22], in this respect a continuous system is better suited for assessing the effect of the sulphide loading rate on the aggregation of the sulphur particles. At decreasing sulphide loading rates, the specific conductivity in the continuous reactor was kept at 14 $mS \cdot cm^{-1}$ (≈0.14 *M* NaCl) by adding a 2.5 *M* NaCl solution to the reactor.

Start up of fed-batch reactor

The start-up of the fed-batch reactor is described in chapter 2. Prior to sulphide addition, 1.0 L of a particular polymer solution (as described below) was supplied to the reactor

Chemicals

The nutrient solution contained ($g \cdot L^{-1}$): NH_4Cl, 4; $MgSO_4.7H_2O$, 1; KH_2PO_4, 2; and 10 mL trace element solution according to Vishniac and Santer.[35] All chemicals used for the nutrient solution were analytical grade supplied by Merck (Darmstad, FRG). Na_2S was of technical grade (BASF,

FRG). The concentration of sulphide in the stock vessel was determined as 60 g $S^{2-}\cdot L^{-1}$. Pure oxygen, supplied by Hoekloos (Schiedam, The Netherlands) was used as an electron acceptor.

Potentiometric titrations

As described by the DLVO-theory[22], the coagulation of colloids depends strongly on the surface charge. Potentiometric titrations were performed to assess the relationship between the negative surface charge, determined from the electrophoretic mobility (s. Chapter 3), and the presence of functional groups on the sulphur surface.

Sample preparation

Potentiometric titrations were performed with an automatic titration apparatus.[8] Biologically-produced sulphur was collected from a high-loaded, 500 mg $S^{2-}\cdot L^{-1}\cdot h^{-1}$, sulphide-oxidizing CSTR.[2,5] Sodium azide (NaN_3, 0.0015 *M*) was added to the effluent to prevent oxidation of the formed sulphur particles due to biological activity, whereafter the suspension was allowed to settle for 2 days. After careful decantation, the sulphur containing residue was dialysed under constant nitrogen bubbling in order to prevent chemical sulphur oxidation. The dialysis was finished when the specific conductivity dropped below 40 $\mu\cdot cm^{-1}$, which corresponds to a NaCl concentration of $3\cdot10^{-4}$ *M*.

Titration experiment

Each titration cycle was started by adding HNO_3 down to a pH of 3.0, followed by N_2-scrubbing for half an hour in order to remove dissolved CO_2. Salt solutions without sulphur (blanks) were titrated in the same way. An amount of 0.5 g of sulphur, present in a volume of 50 mL was titrated with 0.1 *M* HCl and 0.1 *M* NaOH. After each addition of acid or base, an equilibration time of 15 min was allowed. The pH values investigated ranged from 4.0 to 10.0. A complete titration needed about 7.5 h. After one acid and base titration the salt concentration was increased, resulting in three different NaCl concentrations, i.e. 10^{-3}, 10^{-2} and 10^{-1} *M*. The change, $\Delta\sigma$, in surface charge density per unit weight (Coulomb$\cdot g^{-1}$) at a particular pH was calculated using the equation:

$$\Delta\sigma = \frac{\Delta mol\ H^{+}\cdot F}{m} = \frac{\Delta V\cdot c\cdot F}{m} \qquad [C/g]$$

where Δmol H^+ is the amount of protons released from sulphur, i.e. the difference in the amount of base between the blank and the sulphur suspension required to accomplish an equal pH increase, F is the Faraday constant, ΔV is the difference in volume (in litres) of acid added to obtain a particular pH between the blank and the sulphur dispersion, c is the concentration of the titrant in moles$\cdot L^{-1}$ and m is the amount of sulphur in grams. The volume difference between blank and suspension was accounted for. Since the nature of ionic groups present on the sulphur surface is unknown, the absolute point of zero charge is not known a priori. It is assumed, however, that the point of zero

charge is located at the common intersection point of the four titration curves, i.e. the point where no salt effect can be found, which means that no net charge is present.

The position of the titration curves relative to each other was determined in a separate experiment. For this purpose, the pH of a dispersion of sulphur was set at a value of 7.5 or 6.0, and then an amount of salt was added. From the resulting drop in pH (concentration a, before and concentration b after addition) the amount of released protons can be calculated, using:

$$\Delta\sigma = \frac{\Delta mol\ H^{+}_{(a\ -\ b)} \cdot F}{m} = \frac{(10^{-pH_a} - 10^{-pH_b}) \cdot F}{m} \qquad [C/g]$$

Microelectrophoresis at different pH values

The electrophoretic mobility of the sulphur particles was measured by laser Doppler velocimetry using a Zetasizer III (Malvern Instruments, Malvern, UK). After addition of sodium azide, the suspension was allowed to sediment overnight in order to remove relatively large sulphur aggregates. Thereafter, the suspension was decanted and subsequently dialysed, leading to a final specific conductivity of 40 $\mu S \cdot cm^{-1}$. Addition of acid or base resulted in three different final pH-values, namely 3.8, 7.3 and 10.9. The ionic strength of the solution was adjusted to 0.01 *M* by addition of NaCl.

Elemental Analysis

Analysis of the carbon, hydrogen and nitrogen content of a freeze-dried sulphur powder was performed with a Carlo-Erba C, H, N analyzer (model 1106).

Single Particle Optical Sizing (SPOS)

The aggregation and disintegration of sulphur particles smaller than 1 μm was measured by Single Particle Optical Sizing, using the instrument described earlier by Pelssers *et al.*[23] A perpendicular laser beam illuminates a continuous stream of sulphur particles dispersed in water. As the particles pass one-by-one through the detection volume, the light pulses are detected under a scattering angle (Θ) of 5°. The intensity of the reflected light $I_s(h)$ at wavefactor h (i.e. at angle Θ) depends on the refractive index of the solids (n_d) and on their radius (r_d in nm) in the following way:

$$I_s(h) = \frac{8\pi^4}{r^2} \cdot \left[\frac{n_d^2 - n_s^2}{n_d^2 + 2n_s^2}\right]^2 \cdot \frac{r_d^6}{\lambda^4} \cdot (1 + \cos^2\Theta) \cdot p(h)$$

In this formula r represents the distance between the detector and the particle, n_s is the refractive index of the solvent, namely water, λ is the wavelength in the medium and p(h) a form factor. The equation can be simplified to:

$$I_s(h) \approx r_d^n \cdot \left[\frac{n_d^2 - n_s^2}{n_d^2 + 2n_s^2}\right]^2$$

From calibration with a standard material the empirical constant n is obtained in which the form factor is also included. The value of n is determined by measuring the scattered light intensity of five polystyrene latices with known diameters. From the slope of the log-log plot of the intensity ($I_s(h)$) against the diameter (r_d), it followed that the value of n is 4.16 (data not shown). The simplified formula was validated for a monodispers LaMer sulphur sol which was formed according to the procedure of Weitz *et al.*[37] The refractive indices for sulphur and water are respectively $n_{s,water}$ = 1.33 and $n_{d,sulphur}$ = 2.0.[18]

Electron Microscopy

The morphology of the bacteria (*Thiobacilli*) has been examined with high resolution scanning electron microscopy (JEOL 6300 F). To prevent preparation and observation artifacts the material was examined in a hydrated state. Bacteria were placed on Millipore filter paper (GS 0.22 μm). To liberate the bacteria from the surrounding water, the surplus of water on this filter and around the bacteria was sucked away with filter paper. The filter was subsequently frozen in liquid nitrogen and mounted on a clamp holder. The sample was brought into a cryotransfer unit (CT 1500 HF, Oxford Instruments, Oxford, UK). This cryotransfer unit consists of a cryo transfer chamber at high vacuum (1.10^{-6} Pa) and a cryo-holder inside the scanning electron microscope. The specimen was placed inside the cryo-chamber, etched at -85°C for two min and subsequently sputter-coated (Denton) with 3 nm platinum. The coated specimen was placed inside the SEM and observed at 5 kV. The temperature of the specimen inside the SEM was kept at -180°C.

Sedimentation experiments

Sedimentation experiments were carried out to assess the effect of polymer adsorption and sulphide loading rate on the colloidal stability of the sulphur-containing solutions. In a draught free box a homogeneously mixed suspension was added to a one liter measuring cylinder. The sulphur particles settle on a scale which is hanging from a balance while the measuring data are recorded with a computer.

Determination of dry-weight in suspension

Dry-weight was measured by passing an aliquot of 30 mL suspension over a membrane filter (nitro-cellulose, pore size 0.45 μm) and then drying the filter overnight at a temperature of 40°C. The weight increase was subsequently measured.

Adsorption experiments

Since the composition and concentrations of dissolved components in wastewaters vary strongly, sulphide was oxidized to elemental sulphur in the presence of a number of well-defined polymers. The effects of charge, chain length and hydrophobicity of the macromolecules on the sulphur aggregation was studied.

The effect of chain length and hydrophobicity of polymers on the sulphur aggregation was studied for four uncharged polyvinyl alcohols (PVAs). The concentration of these water-soluble polymers, is relatively easy to measure.[40] Its chemical structure is relatively simple, its basic unit being -(CH_2-CHOH)-. Koopal and Lyklema characterized the physico-chemical properties of the PVAs used.[14] Table 1 gives an outline of their results.

Table 1: Properties of the PVA samples

Sample Code	Trade name Polyviol	Hydroxyl content (%)	Acetate content (%)	Degree of polymerization
3-98	V 03/20	98.5	0.27	300
48-98	V 48/20	98.7	0.15	2000
3-88	V 03/140	87.9	-	300
40-88	W 40/140	88.4	0.09	2000

The hydrophilic character of the molecule increases at a higher hydroxyl content, e.g. from 88% to 99%. In the paper industry PVA is frequently used as a pulp stabilizer and therefore it represents a potential component in these wastewaters.[1] Since at the end of all experiments a considerable amount of PVA could be measured in the solution, the adsorption is apparently at its plateau value.

The effect of the charge on the dissolved organic matter on the degree of aggregation of the sulphur particles was investigated by studying the formation of sulphur particles in the presence of carboxymethylcellulose (CMC), Perfectamyl and quaternary-hydroxy ethylcellulose (Q_N-HEC). Some relevant characteristics of the polyelectrolytes used are summarized in Table 2. Perfectamyl, a commercially available cationic starch, is used in the paper industry to improve dry-strength.[10,19] The cationic charges in this compound originate from quaternary ammonium groups (2-hydroxy,3-trimethylammonium-propyloxy starch). Perfectamyl is prepared by allowing starch granules to react with compounds containing amino or ammonium groups at pH 11-12. The degree of substitution (D.S.) is mostly below 0.05, a maximum of 5 out of 100 glucose units have a cationic group. Like Perfectamyl, Q_N-HEC is a cationic polymer but it contains more charged groups per molecule. HECs (*O*-(2-hydroxyethyl)cellulose) are commercially produced cellulose derivatives, i.e. polysaccharides, which are widely used as adhesive and thickening agents.

As a representative for the large group of anionic polymers, carboxymethylcellulose (CMC) was used. This compound is frequently used as a stabiliser, for instance in the food and paper industries, the viscose-industry and the mining-industry.

Table 2: Some properties of polyelectrolytes used.

Name	Abbreviation	Charge	Degree of substitution	Molecular substitution
Carboxy methyl cellulose	CMC	Neg.	0.7	
Quaternair-hydroxy ethylcellulose	Q_N-HEC	Pos.	0.4	1.9
Perfectamyl (PW)		Pos.	0.035	

RESULTS AND DISCUSSION

Characterisation of the surface charge density of biologically produced sulphur

The surface charge density as a function of the pH for biologically produced sulphur is depicted in Figure 1. With increasing pH, the negative surface charge gradually increases. The surface charge also increases with increasing salt concentration. This effect is a consequence of a stronger screening of the surface charge at higher electrolyte concentrations, enabling a higher desorption of protons at a given surface potential (or pH). At pH 5.8 a clear salt effect is absent, indicating the absence of a diffuse electrical double layer. Apparently, this pH value corresponds to the point of zero charge (*pzc*), provided that any specific adsorption does not prevail. The effect of ionic strength is distinctly smaller than can be expected from the salt effect on a pure diffuse double layer which can be observed for an oxide-surface.[8] This is analogous to results obtained for humic acids[39] and bacteria, indicating similar surface properties for these compounds and biologically produced sulphur (Dept. Physical & Colloid Chemistry, WAU, unpublished results).

The observed increase of the charge density with increasing pH can be explained by the presence of multi-functional groups with different dissociation constants, on the surface of the sulphur particles. Since the sulphur particles are excreted by the micro-organisms the groups, most likely, originate from lipids or proteins. Results from elemental analysis from a freeze-dried sulphur powder show that carbon, hydrogen and nitrogen are present in the following relative amounts (% of dry-weight): 1.26, 0.16 and 0.25. From these values the calculated molar ratios of C/N, C/H and H/N amount to 5.88, 0.65 and 8.96 which corresponds reasonably well to the ratios found for biomass. According to Pirt[24], the theoretical molar ratios of C/N, C/H and H/N for biomass are 6.25, 0.56, and 11.25, since the chemical formula for a bacterium is $CH_{1.8}O_{0.5}N_{0.16}$. Therefore, presumably the carbon, hydrogen and nitrogen in the sulphur powder at least partly originate from biomass. In the titration experiment, it is most likely that the organic matter present on the surface of the sulphur is titrated. This can be made clear as follows. Assuming all the oxygen in the sample originates from -COOH groups, it follows that, based on the elemental bacterial composition, a quaerter of all measured carbon is located in -COOH groups. Consequently, per gram of sulphur

¼·12.6/12 ≈ 0.26 mmol -COOH is present. This is equivalent to 25 Coulomb·g^{-1} sulphur. From Figure 1, it follows that between pH=4 and pH=8 about 2 Coulomb·g^{-1} sulphur is titrated, which means that the capacity of the organic matter would be about 12.5 times higher than the amount which is actually titrated. The reason for this is that the release of protons becomes increasingly difficult with higher pH values due to an increasing negative surface charge density.

From the measured amount of carbon, it can be calculated that the total amount of organic matter (o.m.) is approximately 2.5% (assuming that the chemical composition for the organic matter is equivalent to that of bacteria). So, the titrated amount of 2 Coulomb·g^{-1} sulphur results from 2/0.0251 g o.m.≈ 80 Coulomb·g^{-1} organic matter in the pH range 4-8. This value is in the range found for cell walls of bacteria and humic acids, namely 35-250 Coulomb·g^{-1} (Dept. Physical & Colloid Chemistry, unpublished results). This means that the titrated protons may originate completely from organic matter attached to the sulphur particles and 'free' organic matter, i.e. bacteria. Consequently, any contribution of the sulphur core to the proton adsorption may be neglected. The presence of organic compounds, such as lipids, on sulphur particles was reported previously by Jones and Benson.[13] They found that *T. thiooxidans* excretes phosphatidyl glycerol, an essential surfactant for metabolic attack of hydrophobic sulphur surfaces.

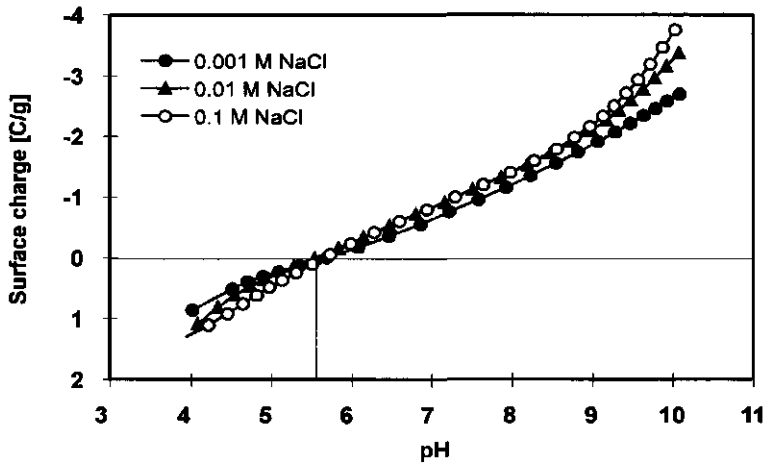

Fig. 1 Surface charge density vs. pH for biologically produced sulphur.

Electrophoretic mobility

The results presented in Figure 2 reveal that the point of zero charge (*pzc*) does not correspond with the iso-electrical point (*iep*). As explained above, the *pzc* is located at a pH value near 5.8 while the *iep* is situated at a pH below 3.8. An increase in pH from 3.8 to 7.5 is accompanied with an increase of the electrophoretic mobility, which is not the case at pH values exceeding 7.5. The electrophoretic mobility most likely results from the dissociation of -COOH groups. At a pH of 4, a considerable amount of carboxyl groups is already dissociated since protein associated

COOH/COO$^-$ groups have a pK_a value between 2.1 and 2.4, the pK_a value for peptidoglycan-associated COOH/COO$^-$ is 2.1, (the value for alanine) and for polysaccharide-associated COOH/COO$^-$ the pK_a value is 2.8.[12] Upon increasing the pH from 3.8 to 7.5, more of the carboxyl groups dissociate and the absolute value of the electrophoretic mobility increases. A further pH increase to 11 does not affect the electrophoretic mobility any further. Probably, the adsorbed (bio)polymers shift outwards as a result of an increased electrostatic repulsion of the dissociated groups present on the (bio)polymers. Hence, also the plane of shear moves outwards and counter-ions may enter the Stern layer. In this way, a possible increase in the charge density on the plane of shear due to the formation of anionic groups is compensated and any clear increase of the electrophoretic mobility is absent. Moreover, an increasing surface conduction with higher pH values will also compensate for the increased negative surface charge, so that the electrophoretic mobility is hardly affected. Since at the *pzc* (pH 5.8) the electrophoretic mobility is not zero, it can be concluded that the Stern layer is not free of charge. An explanation for this could be that the charge within the polymer layer is not homogeneously distributed. Since elemental sulphur has a high electron density it is likely that more cationic groups, e.g. $-NH_2^+$, are present in the neighbourhood of the bare sulphur surface than at the outside of the polymer layer, whereas for anionic groups the opposite distribution is assumed. A small heterogeneity in the charge distribution may cause the observed results. In a pH range 3.5-7, the absolute value of the electrophoretic mobility increases from $-1\cdot10^{-8}$ to $-2\cdot10^{-8}$ $m^2\cdot V^{-1}.s^{-1}$, which corresponds to a decrease in the ζ-potential from -14 mV to -28 mV. These are low potentials which, according to the limiting equation (5), correspond to a charge increase (σ_d) at the outside of the polymer layer of 3.35 $mC.m^{-2}$ which is about 7% of the total charge at pH=10 (Fig. 2), under the assumption that the specific sulphur area is 61 $m^2\cdot g^{-1}$, as will be shown below.

$$\sigma_d = \varepsilon_0 \cdot \varepsilon \cdot (\sqrt{10 \cdot c}) \cdot 10^9 \cdot \Psi_d$$

where σ_d=charge per unit area [$C\cdot m^{-2}$]; ε=80, relative dielectric permittivity of water; ε_o=$8.854.10^{-12}$ [$C\cdot V^{-1}\cdot m^{-1}$], dielectric permittivity of vacuum; c=0.01 *M* NaCl; Ψ_d= 0.014 V, Stern potential.

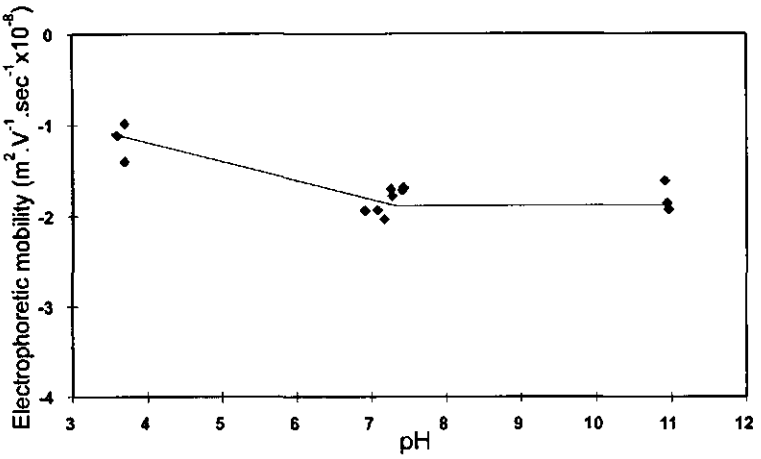

Fig. 2 Electrophoretic mobility of biologically produced sulphur particles at different pH, in 0.01 M NaCl.

Environmental effects on the sulphur aggregation

Loading rate and salt concentration

In the CSTR sulphur is formed under oxygen-limiting circumstances at four loading rates, namely 150, 200, 300 and 400 mg $HS^{-}\cdot L^{-1}\cdot h^{-1}$. From the increase of the initial slope of the curves depicted in Fig. 3, it can be concluded that the sulphur flocks grow bigger at increasing loading rates. This effect is shown in Fig. 4. It is also clear that the density of the aggregates increases with increasing loading rates.

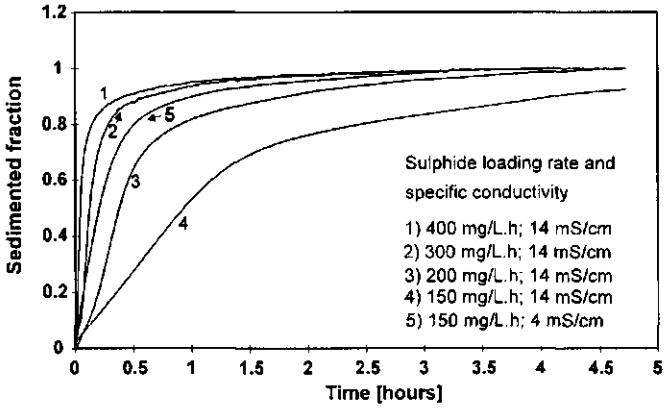

Fig. 3 Analysis of the sedimentation capacity of biological sulphur, produced at four different sulphide loading rates and at two different ionic strengths.

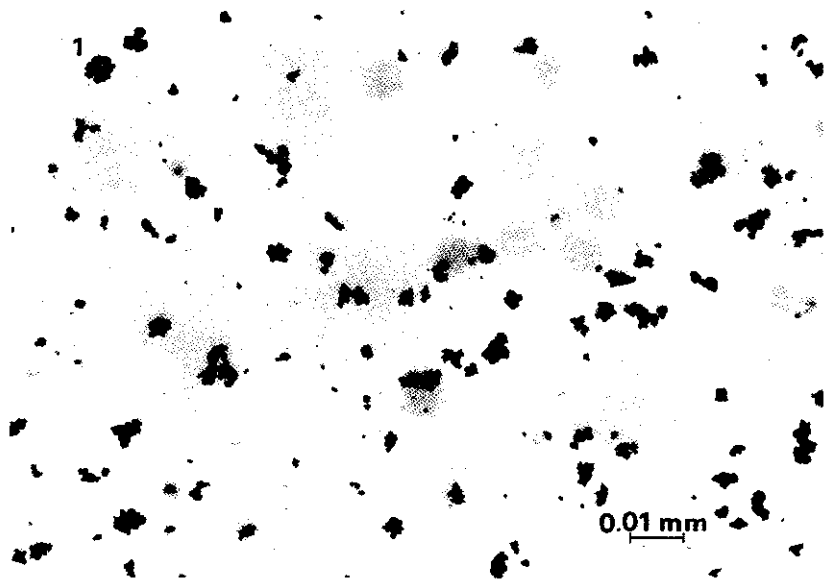

Fig. 4a Photograph of sulphur flocks produced in a CSTR at a loading rate of 150 mg $HS^{-}\cdot L^{-1}\cdot h^{-1}$. The specific conductivity was 14 $mS\cdot cm^{-1}$ at a pH of 8.0 and a hydraulic retention time of 5.0 h.

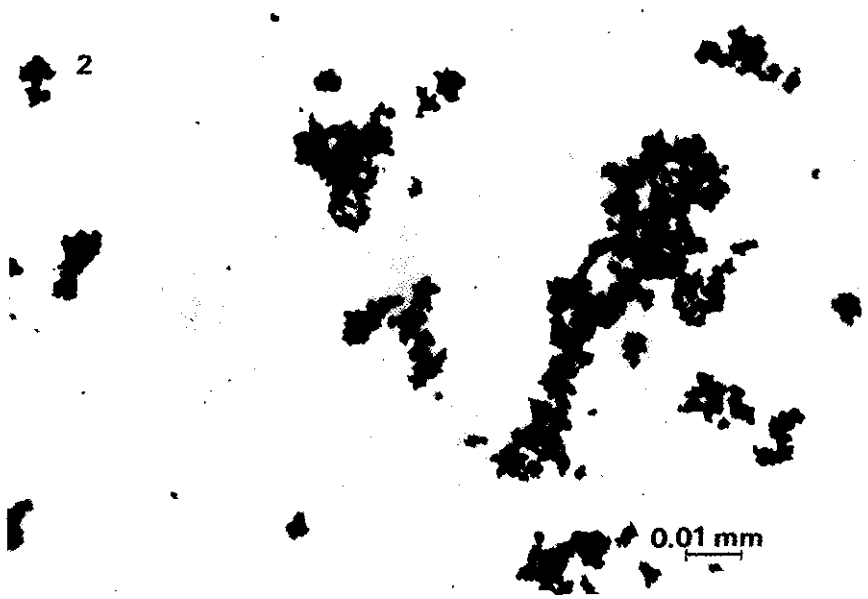

Fig. 4b *Photograph of sulphur flocks produced in a CSTR at a loading rate of 400 mg $HS^{-}\cdot L^{-1}\cdot h^{-1}$. The specific conductivity was 14 $mS\cdot cm^{-1}$ at a pH of 8.0 and a hydraulic retention time of 5.0 h.*

As follows from the settleability of the aggregates, an increase of the specific conductivity from 4 to 14 $mS \cdot cm^{-1}$ (I≈0.04 *M* NaCl and ≈0.14 *M* NaCl respectively) at a loading rate of 150 $mg.L^{-1}.h^{-1}$, leads to the formation of smaller sulphur particles (Fig.3). In case merely DLVO interactions determine the degree of sulphur aggregation, an increase of the salt concentrations would reduce the electrostatic energy barrier, allowing the particles to coagulate as a result of Van der Waals attraction. However, this is not the case for the sulphur particles, indicating that another mechanism is involved. This probably is polymer-bridging between the sulphur particles and/or between sulphur particles and bacteria. It is generally accepted that adhesion of biomass on surfaces is the result of (i) interactions based on the DLVO theory of colloidal stability, and (ii) steric interactions between the outer cell surface macromolecules which reach into the liquid medium and the substratum.[6,12,17,19,25,27] Therefore, very likely that a combination of these factors will determine the formation of sulphur aggregates. In addition to these interactions also the crystallisation of sulphur nuclei will obviously contribute to the formation of aggregates. It is known that at increasing salt concentrations crystal growth can be suppressed e.g. by altering the characteristics of the adsorption layer at the crystal-solution interface and influence the integration of growth units.[20] Scanning electron micrographs show that orthorhombic sulphur crystals of several sizes are present within one aggregate (as will shown below).

Since a number of different physico-chemical mechanisms are involved in forming sulphur-aggregates, an unambiguous explanation can not be provided yet for the reduced aggregation at increasing ionic strength. However, from the results obtained, it is clear DLVO interactions only play a minor role in the aggregation process.

Influence of polymeric compounds on the formation of sulphur aggregates

PVA-adsorption. The results of the experiments with the uncharged PVAs reveal that the short chain polymers PVA3-88 and PVA3-98 adsorb to a higher extent on the sulphur particles than the long chain polymers PVA40-88 and PVA48-98 (Table 3). This is contrary to findings of Koopal and Lyklema[14] concerning the adsorption of PVA to an AgI sol. Due to the presence of longer loops and tails the adsorbed amount of an uncharged polymer generally increases with increasing molecular weight. Decreasing adsorption with increasing tail length is a strong indication that the adsorbent, in our case the biologically produced sulphur including the organic matter, contains pores not accessible for longer chain polymers.

It can be seen that the more hydrophobic PVAs adsorb stronger than the hydrophilic PVAs, i.e. more PVA3-88 than PVA3-98 and more PVA40-88 than PVA48-98 are adsorbed. This increased adsorption of hydrophobic compounds results from a relatively stronger dissipation from the water phase. Theoretical calculations of Fleer *et al.* [7] show that in case that the adsorbed amount of a

polymer remains constant, the hydrodynamic layer thickness (δ_h) will decrease when the solubility of the dissolved compound decreases. This means that the higher PVA adsorption resulting from an increasing hydrophobicity is not necessarily accompanied by an increasing thickness of the polymer layer. Less steric repulsion may occur for the hydrophobic PVAs than for the hydrophilic ones. Consequently this may be a reason for the formation of smaller sulphur aggregates in the latter case, which indeed is in accordance with the results in Table 3.

Table 3: Results of PVA-adsorption to biologically produced sulphur, formed in a fed-batch reactor. The dry-weight of the sulphur suspension before sedimentation is about 500 $mg \cdot L^{-1}$.

PVA Type	Initial conc. PVA($mg \cdot L^{-1}$)	Final conc. PVA($mg \cdot L^{-1}$)	PVA adsorbed ($mg\ PVA \cdot g^{-1}\ S°$)	Dispersed sulphur ($mg \cdot L^{-1}$) after 24 h of sedimentation
3-88	156	84	144	30
3-98	199	150	98	90
40-88	192	133	118	202
48-98	189	157	64	230

It can be seen from the results in Table 3 that the polymer chain length plays a major role in the stability of the formed sulphur colloids. Upon increasing the chain-length from 200 up to 3000 monomer-units, a bigger fraction of the formed sulphur does not sediment within a period of 24 h. This can be attributed to the fact that for sulphur nuclei covered with PVA polymers, an entropically unfavourable compression in overlapping adsorbed layers occurs as a result of an increase in osmotic pressure and therefore an extra colloidal stabilisation.[7]

The specific surface of a freeze dried sulphur-powder was previously determined at 2.5 $m^2 \cdot g^{-1}$, using BET-nitrogen adsorption.[34] When we calculate the specific adsorption of the two hydrophobic PVA's, we find a value of 52 mg $PVA \cdot m^{-2}$ which is about 25 times higher than the value found by Koopal and Lyklema for AgI, namely 1.7-2.5 $mg \cdot m^{-2}$. Apparently, the effective surface area for polymer adsorption is distinctly higher than 2.5 $m^2 \cdot g^{-1}$. Most likely the freeze drying affects the surface structure or state of aggregation, resulting in a lower measured specific surface (by nitrogen adsorption) than the surface which is actually available for polymer adsorption in the bioreactor. Assuming that about 2.1 mg PVA adsorbs per m^2 (the measured average value of Koopal and Lyklema for AgI), the specific surface of biologically produced sulphur is calculated as 61 $m^2 \cdot g^{-1}$.

Adsorption of polyelectrolytes. From the results of the sedimentation experiments conducted in presence of Q_N-HEC, Perfectamyl and CMC, it follows that in all cases, including the blanks, a part of the formed sulphur remains in suspension (Table 4). For Q_N-HEC a clear relation is found between the added amount and the degree of aggregation, i.e. at higher concentrations more sulphur remains in suspension. Apparently, its high charge density leads to stabilisation of colloidal

sulphur particles. For the less charged Perfectamyl this effect is not found. It appears that in the presence of Perfectamyl similar amounts of sulphur are left in suspension. Also in the presence of CMC, no stabilisation of sulphur particles was found. This is in accordance with our expectations since both sulphur and CMC are negatively charged at pH 8.

Since the specific conductivity of the sulphur suspension at the end of each experiment is 20 $mS \cdot cm^{-1}$, i.e. corresponding to 0.16 *M* NaCl, the double layer is essentially non-diffuse and hence no significant electrical repulsion between the sulphur particles will prevail. In comparison with the diffuse double layer, the adsorbed layer is rather thick so that the colloidal stabilisation merely results from steric hindrance by segments of adsorbed polymers extending in the solution.

Table 4 Effect of the presence of polyelectrolytes on the colloidal stability of formed sulphur particles

Polyelectrolyte name	Amount [$mg \cdot L^{-1}$]	Dry-weight before sedimentation [$g \cdot L^{-1}$]	Dry-weight after 24 h sedimentation [$g \cdot L^{-1}$]	Ratio [in %] dispersed S° before and after sedimentation
Q_N-HEC	0.05	0.453	0.040	9
	0.50	0.506	0.080	16
	5.00	0.335	0.166	49
	5.00	0.451	0.252	56
Perfectamyl	0.05	0.219	0.047	21
	0.05	0.521	0.062	12
	0.50	0.307	0.072	23
	0.50	0.351	0.040	11
	25.0	0.515	0.050	10
	25.0	0.652	0.058	9
	25.0	0.770	0.061	8
	25.0	0.901	0.065	7
	100.0	0.493	0.102	21
	100	0.777	0.095	12
CMC	5.00	0.595	0.096	16
Blank		0.304	0.063	21
		0.513	0.033	6

Mechanism of sulphur aggregation

So far, it is not sufficiently clear to what extent the sulphur aggregates will disrupt as a result of liquid shear forces and whether it is possible to stimulate aggregation by adding sulphur nucleii. However, if a significant quantity of active biomass is attached to excreted sulphur particles rather than being freely dispersed, the sulphur aggregates will grow predominantly by the entrapment of freshly formed sulphur into the sulphur/biomass aggregates. In that case, the addition of extra colloidal sulphur would be ineffective in enhancing the formation of large sulphur aggregates. In order to clear this matter, an experiment in the fed-batch reactor was carried out. For a period of 7 days sulphide was oxidized to elemental sulphur. During this period the fraction of small sulphur aggregates was monitored with a laser Single Particle Optical Sizing (SPOS) method. From day 7

onwards, sulphide addition was discontinued. The size of the sulphur aggregates was monitored after 5 days. From the results in Fig. 5, it can be seen that the primary sulphur particles present on day 1, apparently aggregate to bigger particles. To prevent clogging of the fine tubes of the SPOS apparatus with the larger sulphur aggregates, the reactor suspension was allowed to sediment for 3 h before a sample of the supernatant liquid was injected into the apparatus. In this way, only the very fine sulphur fraction from the reactor is measured. It is clear that after cessation of the sulphide addition, no further increase in particle size occurred. On the contrary, the number of particles with a diameter between 185 and 275 nm increased substantially, which means that large aggregates, which were not measured, are falling apart into smaller particles. The latter can be attributed to erosion due to fluid shear forces in the bioreactor. Apparently, in such a mixed reactor system as used in the experiments the formation of aggregates is a dynamic process which consists of both aggregation and erosion.

The formation of sulphur aggregates was also studied by scanning electron microscopy. Fig.6.1 shows an orthorhombic sulphur crystal, present in commercially available, yellow, 'Flowers of sulphur'. Amorphous sulphur particles are also present. Fig.6.2 shows aggregates produced in a sulphur forming bioreactor. Both bacteria with sulphur on their surface (stars) and separate sulphur crystals can be seen (arrows). The sulphur particles on the surface of the bacteria are seen in more detail in Fig.6.3 (arrows). It appears that their minimum diameter is approximately 100 nm but also bigger sulphur needles can also be found (Fig.6.4). From these results, it can be concluded that the sulphur aggregates are created by the excretion of 'sulphur-spheres' which are attached to the surface of the micro-organisms. In this way, large conglomerates consisting of sulphur and biomass with a high biological activity are formed. The images fail to show a flagellum which is expected for *Thiobacilli*-like bacteria. The reason for this might be that such a flagellum, if present, would stick to the bacteria-surface as a result of absorption of the surplus water in the filter paper. The same observation has been made by Wirsen and Jannasch who observed a flagellum for *Thiovulum sp.* using transmission electron microscopy but not with scanning electron microscopy.[38]

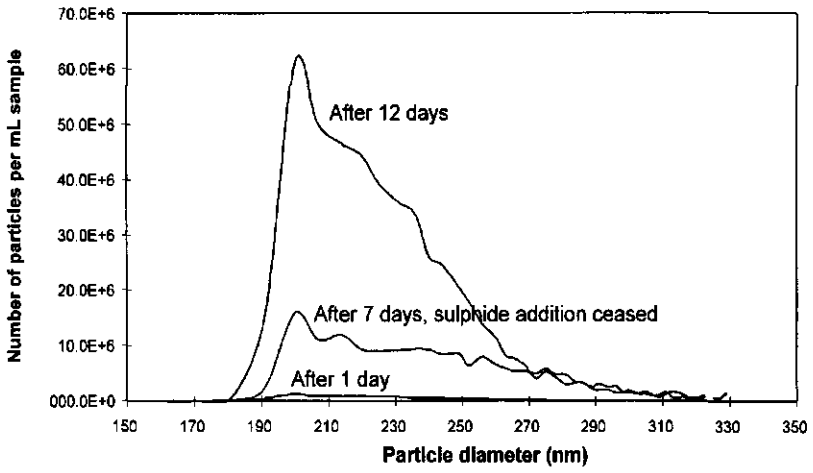

***Fig. 5** Particle-size distribution of biologically produced sulphur at three days in a fed-batch reactor. Loading rate of the reactor was 75 mg $HS^{-}L^{-1}h^{-1}$. The molar (O_2/S^{2-}) consumption was 0.57. At day 7 the sulphide addition was ceased.*

CONCLUSIONS

From the results of the presented experiments, it can be concluded that the colloidal stability of biologically produced sulphur greatly depends on the process and environmental conditions prevailing in the bioreactor. The presence of long-chain polymers disturbs the aggregation of the sulphur particles, resulting in a highly dispersed suspension. The degree of polymer adsorption to the sulphur surface mainly depends on their affinity for the water-phase. More hydrophobic PVAs adsorb stronger than the hydrophilic ones. Higher salt concentrations lead to an inhibition of the sulphur aggregation because, firstly, the bridging between the sulphur particles and the sulphur and biomass reduces and, secondly, the growth of the sulphur crystals deteriorates. To improve the sedimentation capacity of the sulphur, it is necessary to operate the reactor under high loading conditions with a minimum of shear forces so that large, easily settleable sulphur flocks are formed.

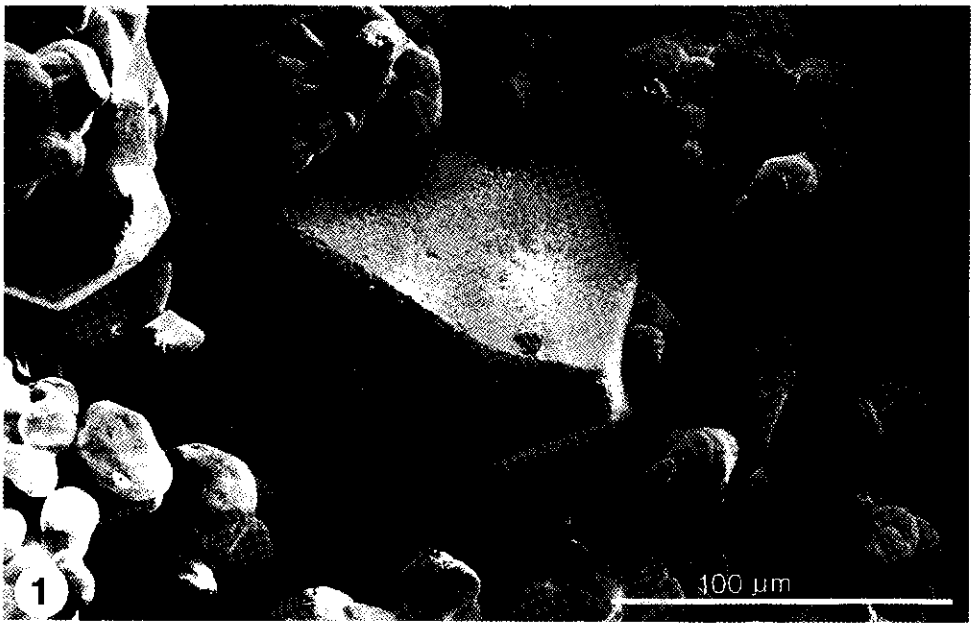

Fig. 6.1 Scanning electron micrograph at room temperature of commercially available orthorhombic sulphur crystals.

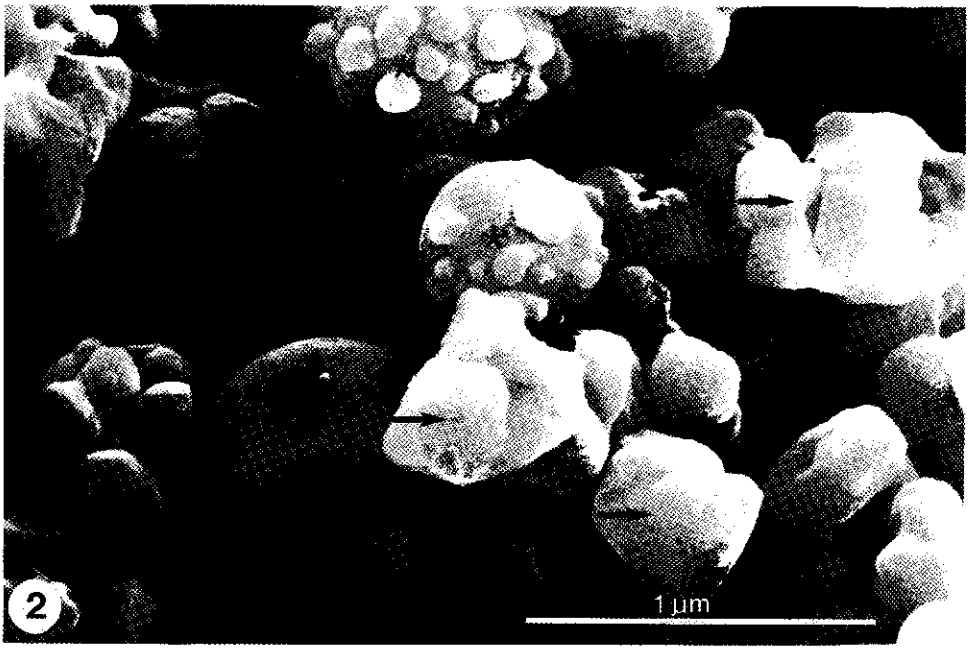

Fig. 6.2 Scanning electron micrograph of sulphur sludge at -180°C in hydrated state. Bacteria (stars) are present between small sulphur crystals (arrows).

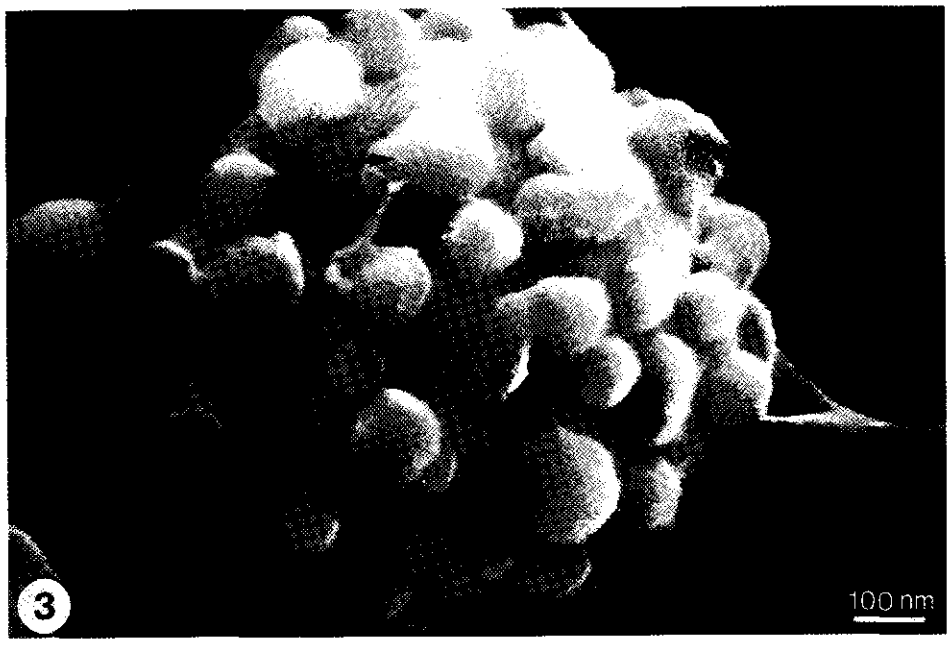

Fig. 6.3 *Scanning electron micrograph of the surface of Thiobacilli at -180 °C in hydrated state. Sulphur excretion on the cell surface is visible (arrows).*

Fig. 6.4 *Scanning electron micrograph of the surface of Thiobacilli at -180 °C in hydrated state. Bacteria are present with relatively large sulphur particles (arrows) on the cell surface.*

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CHAPTER 6

Performance of a sulphide oxidizing expanded bed reactor supplied with dissolved oxygen[A]

ABSTRACT

The performance of a new sulphide-oxidizing, expanded bed bioreactor is described. To stimulate the formation of well-settleable sulphur sludge which comprises active sulphide-oxidizing biomass and elemental sulphur, the aeration of the liquid-phase and the oxidation of sulphide to elemental sulphur are spatially separated. In this manner, turbulences due to aeration of the liquid phase is avoided. The aerated suspension is subsequently recirculated to the sulphide oxidizing bioreactor. It appeared that under autotrophic conditions almost all biomass present in the reactor is immobilized within the sulphur sludge which mainly consists of elemental sulphur (92%) and biomass (2.5%). The particles formed have a diameter of up to 3 mm and can easily be ground down. Within time, the sulphur-sludge obtained excellent settling properties, e.g. after 50 days of operation, 90% of the sludge settles down at a velocity greater than $m \cdot h^{-1}$ whilst 10% of the sludge had a sedimentation velocity above 108 $m \cdot h^{-1}$. Since the biomass is retained in the reactor, higher sulphide loading rates may be applied than those of a conventional free-cell suspension. The maximum loading rate reached was 14 g $HS^- \cdot L^{-1} \cdot d^{-1}$ whereas for a free-cell suspension a maximum loading rate of 6 g $HS^- \cdot L^{-1} \cdot d^{-1}$ was found.[3] At higher values, the upward velocities of the aerated suspension became excessive and this resulted in the accumulation of sulphur sludge in the settling zone above the reactor. When the influent was supplemented with volatile fatty acids, heterotrophic sulphur and sulphate reducing bacteria accumulated within the sludge. This led to a serious deterioration of the system, i.e. the sulphur formed was increasingly reduced to sulphide whilst the aggregation of the freshly excreted sulphur particles also declined.

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INTRODUCTION

Hydrogen sulphide has to be removed from wastewaters because of its toxicity, oxygen demand, corrosivety and unpleasant odour. It is is emitted to the environment as dissolved sulphide (S^{2-} and HS^-) in wastewaters and as H_2S in exhaust gases. Sulphide containing wastestreams are generated by a number of industries such as petrochemical plants, tanneries, viscose rayon manufactures, the gasification of coal for electricity production or by the anaerobic treatment of sulphate containing wastewaters.[1,15,19] The removal of sulphur-compounds from waste streams can be mediated by application of bacteria involved in the natural biological sulphur cycle. Microbial processes operate around ambient temperatures and at atmospheric pressure, thus eliminating high costs for heat and pressure generation as required in a variety of chemical processes. The use of colourless sulphur bacteria appears to be the most reliable biotechnological approach for H_2S removal[2,12] since these organisms have very simple nutritional requirements, i.e. they utilize reduced inorganic sulphur compounds as their electron donors and carbon dioxide as carbon source. The two most important bioconversions in an aerobic sulphide oxidizing bioreactor are:[14]

$$2\ HS^- + O_2 \rightarrow 2\ S^\circ + 2\ OH^- \qquad \Delta G^\circ = -169.35\ kJ/mol\ HS^- \qquad (1)$$

$$2\ HS^- + 4\ O_2 \rightarrow 2\ SO_4^{2-} + 2\ H^+ \qquad \Delta G^\circ = -732.58\ kJ/mol\ HS^- \qquad (2)$$

In Chapter 2, it is demonstrated that under oxygen limiting circumstances, i.e. at oxygen concentrations below 0.1 $mg \cdot L^{-1}$, sulphur is the major end-product of the sulphide oxidation (reaction 1), whilst sulphate is formed under sulphide limiting circumstances (reaction 2). In biological treatment plants, the formation of elemental sulphur is preferred for several reasons. Firstly, elemental sulphur is non-soluble and can therefore in principle be removed from the water stream. Moreover, the recovered sulphur can be purified and re-used as a valuable raw-material, e.g. in bioleaching processes.[21] Secondly, sulphate formation requires a four-times higher oxygen consumption and consequently it has a higher energy demand for aeration.

The removal of SO_2 from flue-gases can be mediated in a biotechnological way as well, using a process which consists of three integrated process steps: the first step serves for scrubbing the SO_2 present in flue-gas, the second step serves for reducing the formed HSO_3^- /SO_3^{2-} to sulphide, which in the third step is oxidized to elemental sulphur under oxygen limiting circumstances.[3] The sulphur is removed by gravity-sedimentation, e.g. in a tilted plate settler, whereafter the sulphur-free water can be recirculated to the scrubber. In case of an inadequate removal of the sulphur particles, they will enter the anaerobic reactor with the recirculation water. Anaerobic conversion of the sulphur particles is obviously detrimental to the efficiency of the system because it leads to: 1) an increased consumption of the required electron donor and 2) increased sulphide levels in the anaerobic reactor which may cause inhibition of the biomass.[13] For these reasons, a highly effective sulphur removal step is essential for a successful application of this process.

The size of freshly excreted sulphur particles is within the submicron range.[9,20] Fortunately, the sulphur particles tend to form aggregates in reactors with a long solid retention time.[10] In our gas-lift loop reactor these aggregates are continuously disrupted due to the turbulence caused by the aeration of the suspension. In the process configuration described in this paper, the aeration of the liquid phase and the oxidation of sulphide to elemental sulphur are therefore spatially separated.

Aim of this study

The objective of this study is to develop a sulphide oxidizing bioreactor in which the turbulencies are minimized in order to enhance the formation of a well-settleable sulphur-sludge. With respect to the biological and physical-chemical properties of the formed sludge, the performance of this system was studied under both autotrophic and heterotrophic conditions.

MATERIALS AND METHODS

Reactor

In order to minimize turbulences, a reactor-system was developed in which oxidation of sulphide to elemental sulphur and the aeration of the liquid phase are spatially separated. The configuration consisted of an expanded bed (E.B.) reactor (V=30 L) for sulphide oxidation combined with a continuously stirred vessel (V=8 L) for aeration of the suspension from the E.B.-reactor (Fig.1). In the lower part (d_i=10 cm, V=12 L) of the expanded bed reactor, sulphide is oxidized to elemental sulphur while the upper part (d_i=30 cm, V=18 L) acts as a settling-zone from which the sulphur sludge can fall back into the lower part. In the aeration tank, the oxygen concentration of the liquid phase amounted to 6.0-7.0 mg·L^{-1}. The liquid solution was recirculated through the system with a centrifugal pump, at a flow-rate controlled between 50 and 500 L·h^{-1} by means of a manually adjustable flow-controller (Brooks GT 1306). The oxygen concentration in the E.B.reactor remained always below 0.1 mg·L^{-1} and the pH was controlled between 7.2 and 7.6 by the addition of hydrochloric acid (1.5%). The system was operated at room temperature, i.e. 22 (±2)°C. The influent of the experimental assembly consisted of a sulphide stock solution (200 g·L^{-1} Na_2S and 50 g·L^{-1} $NaHCO_3$ as a carbon-source) diluted with tap water in order to obtain a final sulphide influent concentration of 240 mg S^{2-}·L^{-1}. This yielded a convenient flow rate of the influent from 0.12 till 2.5 L·h^{-1}. The composition of the nutrient solution is described in Chapter 2. The flow rate of the nutrient solution was equal to the flow-rate of the sulphide stock solution. In most of the experiments, the molar oxygen to sulphide consumption ratio was set at 0.7 by adjusting the recirculation flow. This ratio was chosen because in a sulphide oxidizing fed-batch reactor, at ratios below 0.7, thiosulphate is abundantly formed while at higher ratios sulphate becomes the

main end-product (s. chapter 2). Additionally, in some of the experiments volatile fatty acids (acetate 300 $mg \cdot L^{-1}$ and propionate 300 $mg \cdot L^{-1}$) were supplied to the influent of the E.B. reactor.

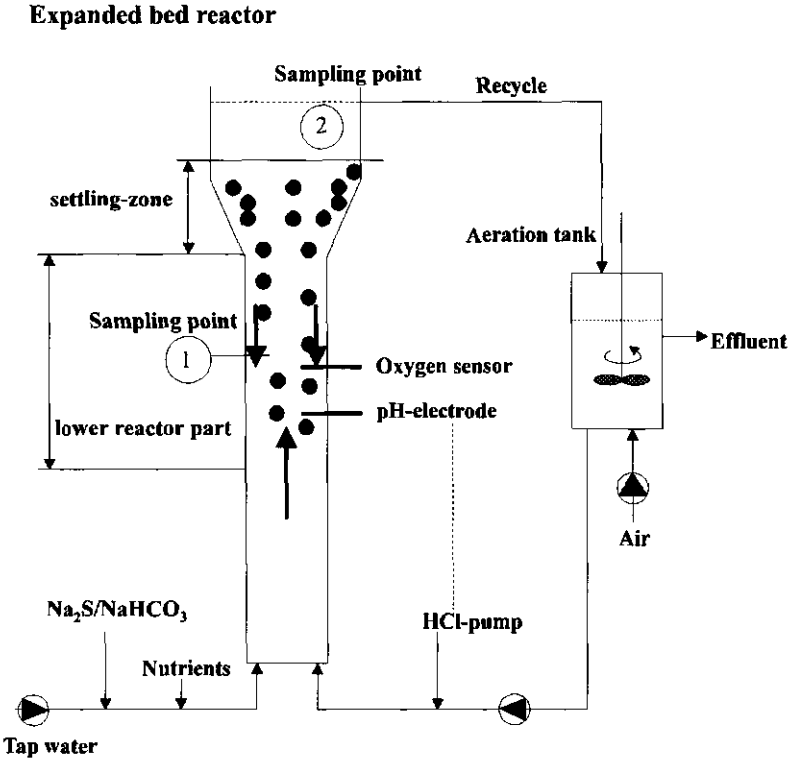

Fig. 1 Expanded bed reactor (V=30 L) for sulphide oxidation with a external aeration of the reactor suspension; (1) and (2) are sampling points.

Start-up

The expanded-bed reactor was inoculated with 2.0 L effluent from a sulphide-oxidizing bioreactor. The inoculum consisted of *Thiobacillus*-like bacteria (s. Chapter 2).

Measurements and Analysis

Sulphide, sulphate and thiosulphate analysis were made according to the methods described in chapter 2. Since the sulphur sludge is not homogeneously distributed over the reactor, the S° production is calculated by subtracting the produced amounts of $S_2O_3^{2-}$-S and SO_4^{2-}-S from

the sulphide loading rate. This seems to be justified since the results presented in chapter 2 show that no tetrathionate or higher polythionates are formed at pH 8. Acetate and propionate were measured gas-chromatographically. The chromatograph (HP 5890A, Palo Alto, USA) was equipped with a 2 m x 4 mm glass column, packed with Supelcoport (100-120 mesh) coated with 10% Fluorad FC 431. Operating conditions are: column, 130°C, injection port, 200°C, flame ionization detector, 280°C. N_2-gas saturated with formic acid at 20°C is used as carrier gas (30 mL·min^{-1}).

Suspended solids (SS) were measured by passing an aliquot of 30 mL reactor suspension over a membrane-filter (nitro-cellulose, pore size 0.45 μm) whereafter the filter was dried overnight at a temperature of 40°C and weighed using a high precision balance. Measurement of the SS was considered as an appropriate alternative for the laborious analysis of elemental sulphur, using the acetone extraction method (s. Chapter 2).

The total sulphur content of the sludge was measured following the procedure of Hordijk *et al.*[7] Sulphur sludge was dried overnight at 40°C and then carefully crushed. An amount of 15 mg of the dried and crushed sulphur-rich powder was thoroughly mixed with 0.5 g KNO_3 and Na_2CO_3 (1:10, w/w) and subsequently transferred into a 20 mL glass bottle. Since SO_2-gas may be formed during the incineration, an additional amount of 0.5g of the KNO_3/Na_2CO_3 mixture covered the sulphur-containing powder to absorb this SO_2-gas. The samples were oxidized to sulphate in a furnace for 4 h at 620°C. After oxidation, the thus formed pellet was dissolved in 10 mL distilled water whereafter the sulphate concentration was measured using inductively-coupled plasma atomic emission spectrometry.

Biomass measurements were carried out by determining the amount of organically bound nitrogen.[21] This method was chosen because the presence of sulphur particles interferes with most standard methods for biomass determination. From these measurements, the total amount of biomass was calculated under the assumption that 12% of the dry-weight consists of nitrogen.

The settleability of the sludge was measured using two sedimentation columns of 0.3 m and 2.9 m, respectively. The sludge settles down onto a weigh-scale which is connected to an electronic balance. The weight-increase is registered with a computer. The set-up for the sedimentation experiments is illustrated further in Tichý *et al.*[21]

Activity measurements

Aerobic and anaerobic batch-tests were conducted to study if bacterial biomass was immobilized within the sulphur aggregates. The aerobic sulphide oxidizing capacity of the aggregates was measured by performing BOM-tests (Biological Oxygen Monitoring). An aliquot of 100 mL reactor suspension was centrifuged and decanted whereafter the remaining pellet was resuspended in 0.5 L phosphate buffer (10 $g \cdot L^{-1}$ KH_2PO_4 and 2.5 $g \cdot L^{-1}$ NaOH, pH=8.0). The buffer solution was oxygenated with air to near saturation and subsequently sealed in an air-tight vessel. The decrease in the oxygen concentration after a sulphide injection was measured with a laboratory electrode and registered with a recorder. The initial sulphide concentration was 50 $mg \cdot L^{-1}$.

The anaerobic activity of sulphur and sulphate reducing bacteria in the presence of acetate and propionate was studied. In sealed bottles (V=0.5 L) 30 mL reactor suspension (suspended solids content 4-5 $g \cdot L^{-1}$) was added to phosphate buffer (as described above), supplemented with 1 $mL \cdot L^{-1}$ trace element solution and substrate (acetate and/or propionate). The trace element solution consisted of a tenfold concentrated trace element solution described by Pfennig and Lippert.[17] To study the role of sulphate reducing bacteria (SRB), all batch experiments were performed in the presence of sulphate (22 m*M*) or in absence of sulphate; blanks (no VFA) were measured as well. The bottles were incubated at 30°C in the dark in a rotary shaker (60 rpm) and sampled daily for sulphide, sulphate and VFA analysis. All batch experiments were performed in duplicate.

Detection of sulphate and sulphur reducing bacteria

The presence of sulphate and sulphur reducing bacteria was detected by a blackening test which relies on FeS formation by sulphide generated during dissimilatory sulphate or sulphur reduction.[18] Sludge samples (1 g wet weight) were transferred anaerobically into screw-capped test tubes (20 mL) containing 9 mL sterile medium. This medium consisted of ($mg \cdot L^{-1}$ demineralized water): KH_2PO_4, 270; K_2HPO_4, 350; NH_4Cl, 530; $MgCl_2.6H_2O$, 100; $CaCl_2.2H_2O$, 75; $NaHCO_3$, 1200; $FeCl_2.4H_2O$: 20. Test tubes to which molybdate (20 m*M* MoO_4^{2-}), a specific SRB inhibitor[16] was added to modified medium were inoculated simultaneously in order to verify if the blackening of the medium was due to microbial activity of SRB. For each substrate tested, detection tubes and molybdate amended tubes were inoculated in duplicate. After inoculation, test tubes were sparged with N_2 gas for 1 minute using anaerobic techniques,[8] sealed and incubated at 37°C. At regular time intervals, the blackening of the medium was visually observed.

RESULTS

Two different experiments were carried out to assess the feasibility of the new sulphide oxidizing reactor system. In the first experiment, the formation of sulphur aggregates was studied under autotrophic conditions at various sulphide loading rates. The second experiment was conducted to study if heterotrophic conditions would deteriorate of the system.

1. Formation of sulphur sludge under autotrophic conditions

The effect of the volumetric sulphide loading rate (VSLR) on the production rate of various sulphur compounds, i.e. sulphur, thiosulphate and sulphate, is depicted in Figure 2. Initially, the VSLR was maintained at a low level of 0.7 HS^- $g{\cdot}L^{-1}{\cdot}d^{-1}$, to prevent inhibition of the biomass due to high sulphide concentrations. Due to the low loading rate the system therefore merely produced sulphate during the first 8 days.[11] Sulphur formation can be enhanced by reducing the availability of oxygen. However, as the recirculation flow could not be reduced below 50 $L{\cdot}h^{-1}$ at this low sulphide loading rate it was not possible to reduce the molar oxygen over sulphide consumption ratio 1.2. For the maximization of the sulphur production previous research revealed that the optimal oxygen over sulphide consumption ratio is about 0.7.[11] At day 9, the VSLR was increased from 0.7 to 1.8 HS^- $g{\cdot}L^{-1}{\cdot}d^{-1}$ and the recirculation flow was adjusted in order to obtain the optimal oxygen to sulphide consumption ratio of 0.7. Figure 2 shows that the increase in the VSLR immediately resulted in the formation of a considerable fraction of elemental sulphur. However, for a period of 3 days thiosulphate could also be detected. The latter is in agreement with our earlier observations that directly after an increase of the VSLR the biological oxidation capacity does not suffice, resulting in a temporary overloading of the biomass.[11] As a consequence, a small fraction of the supplied sulphide is oxidized chemically, which merely results in the formation of thiosulphate. However, within four days of the increase of the sulphide loading rate, the sulphur production gradually increased to 0.5 HS^- $g{\cdot}L^{-1}{\cdot}d^{-1}$ and small-sized, colloidal aggregates became apparent. At day 15, the VSLR was increased to 3.4 HS^- $g{\cdot}L^{-1}{\cdot}d^{-1}$. The results in Figure 2 reveal that the sulphate production only increased slightly, i.e. to approximately 1.9 S $g{\cdot}L^{-1}{\cdot}d^{-1}$ at day 18, meaning that the system produced 1.5 $g{\cdot}L^{-1}.d^{-1}$ elemental sulphur. Beyond day 19, the amount of sulphate produced even slightly decreased. Moreover, at the higher VSLR applied, the sulphate production reached a maximum of 1.9 g $S{\cdot}L^{-1}.d^{-1}$. This is contrary to the expectation that the sulphate production should increase proportionally to the imposed sulphide loading rate when the supplied oxygen over sulphide ratio is maintained at a constant value. A comprehensive explanation for this observation is that entrapped biomass within the sulphur aggregates (as will be discussed below) becomes isolated from the supply of a sufficient amount of oxygen and due to this oxygen depletion the bacteria are forced to form elemental sulphur. Biomass on the outside of the biofilm however, which is not oxygen limited, will preferentially produce sulphate.[11] As the size of the sulphur flocs increases with increasing

loading rates, relatively more biomass becomes oxygen depleted and consequently a higher fraction of the sulphide is oxidized to sulphur whilst the sulphate production will remain more or less constant. The highest VSLR loading at which no sulphide in the effluent could be detected was 14 HS^- $g \cdot L^{-1} \cdot d^{-1}$(data not shown).

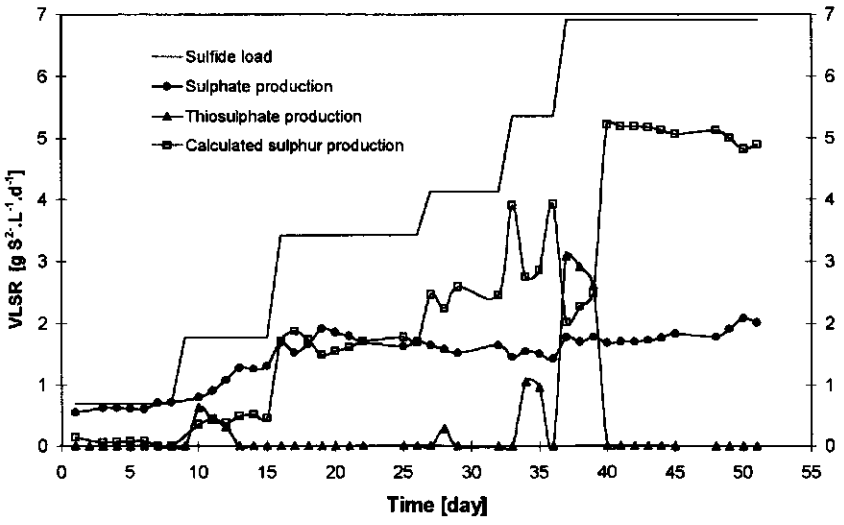

Fig. 2 Sulphate, thiosulphate and calculated elemental sulphur formation in the autotrophic E.B. reactor at increasing sulphide loading rates. During the first 9 days of the experiment the molar oxygen to sulphide consumption ratio was 1.2. From day 10 to day 52 this was set at 0.7.

During the first 13 days of the experiment, the S.S. content in the effluent of the E.B.reactor increased continuously (Fig.3). During this period, sulphur sludge could not sufficiently develop due to the restricted formation of elemental sulphur. As a consequence, almost all sulphur particles formed were washed-out. From day 13 onwards, greater amounts of sulphur were formed as a result of the higher VSLR (Fig.2). The aggregation of the sulphur particles was enhanced and accordingly the amount of SS in the effluent reduced substantially. At sampling point (1) (s. Fig.1) the sludge content in the reactor was maintained at 5 (±2) $g \cdot L^{-1}$ by daily removal of the excess sulphur sludge. Under these conditions, the SS content in the effluent remained well below 45 $mg \cdot L^{-1}$ for the duration of the rest of the experiment (Fig.3).

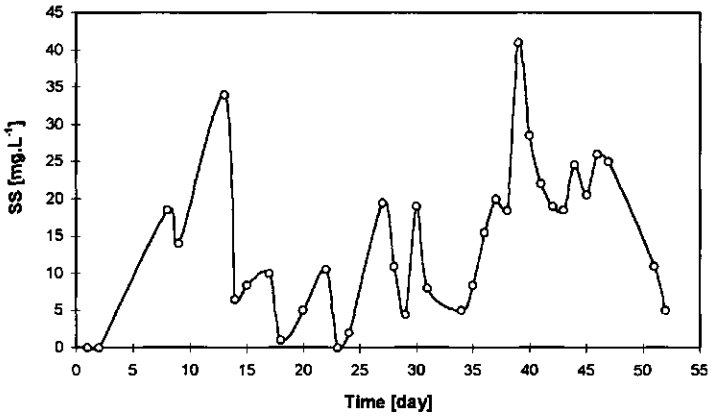

Fig. 3 SS content [mg·L^{-1}] in the effluent of the autotrophic expanded-bed reactor, taken at sampling point (2).

In the E.B. reactor, the sludge is transported from the middle of the lower reactor pipe to the settler from where it settles back due to gravity. The pictures in Fig. 4 show the presence of the sulphur sludge in the settler.

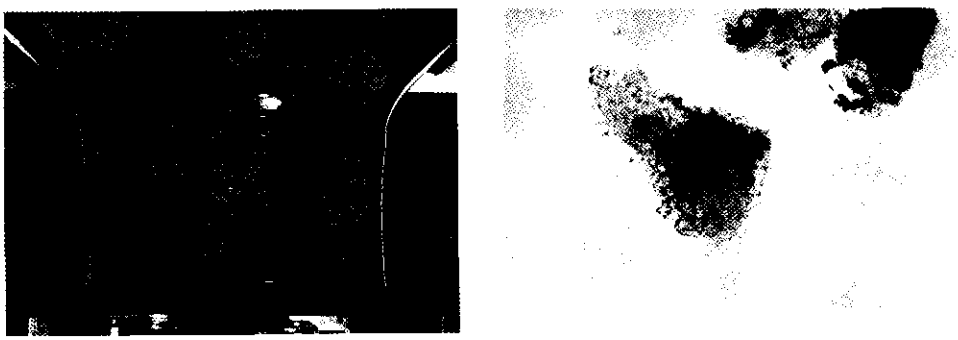

Fig. 4 Photographs from sulphur sludge in the settler (left) and light-microscopy from sulphur sludge (right), taken after 40 days of operation under autotrophic conditions.

It can be seen that a biofilm covers the dark centre of the sulphur aggregates. The presence of biomass in the sulphur aggregates also follows from aerobic activity measurements. The oxygen consumption of 100 mL mixed liquor taken at sampling point (1) was significantly higher than the oxygen consumption of the same amount of effluent. This shows that sulphide

oxidizing biomass was immobilized within the sulphur aggregates rather than free in suspension (Table 1).

Table 1: Oxygen consumption [mg $O_2 \cdot L^{-1} \cdot min^{-1}$] of three different suspensions (100 mL) in a BOM-vessel, initial [S^{2-}]=50 mg$\cdot L^{-1}$ and [O_2]=8.0 mg$\cdot L^{-1}$ at pH=8.0

Suspension	Oxygen consumption [mg $O_2 \cdot L^{-1} \cdot min^{-1}$]
Tapwater (Blank, chemical oxidation)	0.07
Suspension from settler (SS= 4.9 g$\cdot L^{-1}$)	1.48
Endogenous respiration	< 0.01
Effluent (SS= 20 mg$\cdot L^{-1}$)	0.08

Elemental analysis of the sulphur sludge showed that the sulphur content amounted to 92% of the total mass after 40 days of operation. At that time, the biomass content was about 1-5% of the total dry-weight. The unidentified rest-fraction most probably comprises precipitates such as metal sulphides.

The results from sedimentation experiments are summarized in Table 2. From Table 2, it follows that within time the sulphur aggregates obtain better settling properties. The reason for this is that the flocks are becomming increasingly bigger while also the density of the particles may increase. After 50 days of operation 90 per cent of the total sulphur amount has a settling velocity above 25 m$\cdot h^{-1}$ while 10 per cent has a settling velocity higher than 108 m/h. At increasing sulphide loading rates, the recirculation flow had to be increased proportionally in order to supply the required amount of oxygen. As a result, the lighter sludge fraction is washed-out whilst the size of the remaining sludge particles increases. The maximum upward velocity in the lower reactor pipe reached was 66 m$\cdot h^{-1}$. At higher upward velocities most sulphur aggregates remain in the settling-zone because here the upward velocity is lower. This results in a reduced contact-time between the sulphur-sludge and the sulphide containing solution. As a consequence, the sulphide-oxidation-capacity of the system deteriorates.

Table 2: Cumulative minimum sedimentation velocity [m$\cdot h^{-1}$] of sulphur-sludge fractions (%) taken on certain days from the autotrophic expanded-bed reactor at sampling point (1).

Day	Fraction			
	10%	50%	75%	90%
6	-	>10	>4	>2
40	>54	>21	>13	>7
50	>108	>53	>36	>25

2. Performance of sulphur sludge under heterotrophic conditions

According to Hooijmans *et al.*[6] and Wijffels *et al.*[23], oxygen can only penetrate from 150 up to 200 μm into a biofilm. Therefore, very likely, within the sulphur-sludge anaerobic circumstances prevail. This means that in the presence of organic matter, sulphur and sulphate reducing bacteria are expected to grow within the sulphur sludge particles. Under anaerobic conditions, members of the sulphate-reducing genus *Desulfobulbus* oxidize propionate to acetate according to:[22]

(3) $4CH_3CH_2COO^- + 3SO_4^{2-} \rightarrow 4CH_3COO^- + 4HCO_3^- + 3HS^- + H^+$ $\Delta G° = -150.6$ kJ

while *Desulfobacter* species are able to oxidize acetate completely to bicarbonate:

(4) $CH_3COO^- + SO_4^{2-} \rightarrow 2HCO_3^- + HS^-$ $\Delta G° = -47.6$ kJ

So far, very little information is available about the reduction of elemental sulphur to hydrogen sulphide. Members of the genus *Desulfuromonas* use acetate as electron donor for mesophilic sulphur reduction:[22]

(5) $CH_3COO^- + 4S + 4H_2O \rightarrow 2HCO_3^- + 4H_2S + H^+$ $\Delta G° = -6.9$ kJ

Buisman *et al.*[4] reported the occurrence of reactions (3) and (5) in a sulphide-oxidizing reactor which was additionally fed with acetate and propionate.

The presence of anaerobic, sulphide producing, organisms in the autotrophically cultivated reactor was assessed from both blackening tests and anaerobic batch experiments. In the detection test, blackening occurred within 48 h when sludge was incubated with substrates such as lactate, H_2/CO_2, ethanol, acetate, propionate and butyrate. In the presence of isobutyrate and valerate no blackening was found within an incubation period of 3 weeks. Furthermore, in test tubes containing molybdate, no blackening was encountered, indicating that sulphide production is mediated by SRB.

The results from the anaerobic batch experiments with the sludge from the autotrophically operated reactor are summarized in Table 3. From these results it can be seen that in comparison to propionate, acetate was preferentially oxidized. Additionally added sulphate was not consumed and did not significantly affect the conversion rate. It can therefore be concluded that acetate degrading, sulphur reducing, *Desulfuromonas* species were, in fact, the dominant anaerobic organisms present in the autotrophically cultivated sulphur-sludge.

Table 3: Degradation rate [m*M*·g SS^{-1}·d^{-1}] of acetate and propionate in batch experiments (pH=8.0, 37°C, SS=150 mg·L^{-1}) with sulphur-sludge cultivated under autotrophic conditions. Initial concentration of acetate and propionate is 20 m*M* in the experiments with merely one of these substrates. In the experiment with the mixed substrate acetate and propionate the initial concentrations are 10 m*M*, respectively.

Substrate	acetate	propionate	acetate + propionate	
Electron acceptor				
	acetate	propionate	acetate	propionate
sulphur	2.00	0.67	1.33	0.67
sulphur and sulphate [22 m*M*]	2.00	0.67	1.33	0.93

In the experiment with acetate as the sole substrate, the sulphide production rate was 6 m*M* per g SS^{-1}·d^{-1} whilst the acetate degradation rate amounted to 2 m*M*·g SS^{-1}·d^{-1}, yielding a sulphide production to acetate consumption ratio of 3.0. This is below the stoichiometrically expected ratio of 4.0 (s. equation 5). This difference could be attributed to the oxidation of sulphide due to trace levels of oxygen and the formation of acetate due to the hydrolysis of biomass.

Under practical conditions, when organic compounds are also present in the influent, both aerobic heterotrophic and mixotrophic *Thiobacilli* species and anaerobic heterotrophic organisms can catabolize this organic matter. Anaerobic microbial activity could be detrimental to the performance of the system because under oxygen limiting circumstances, sulphur may be reduced into sulphide. Such conditions may prevail in the settling zone of the reactor or within anaerobic zones of the sulphur-sludge. To investigate this matter, experiments were conducted with acetate [290 mg COD·L^{-1}] and propionate [275 mg COD·L^{-1}] at a VSLR set at 3 g·L^{-1}·d^{-1}. The assessed effluent sulphide, sulphate and VFA concentrations taken at sampling point (2) are presented in Figure 5. During the first 15 days of the experiment the molar oxygen to sulphide consumption ratio was 1.0. Under steady-state conditions, about 15 mg·L^{-1} sulphide was present in the effluent (Fig. 5a). This is most likely the result of 1) an increased anaerobic activity leading to the formation of sulphide and 2) the competition for oxygen between autotrophic, mixotrophic and heterotrophic *Thiobacilli*. To reduce the sulphide concentration in the effluent, from day 16 onwards the molar oxygen to sulphide consumption ratio was increased up to a value of 1.6. This resulted in an elevated sulphate production although sulphide could still be detected in the effluent.

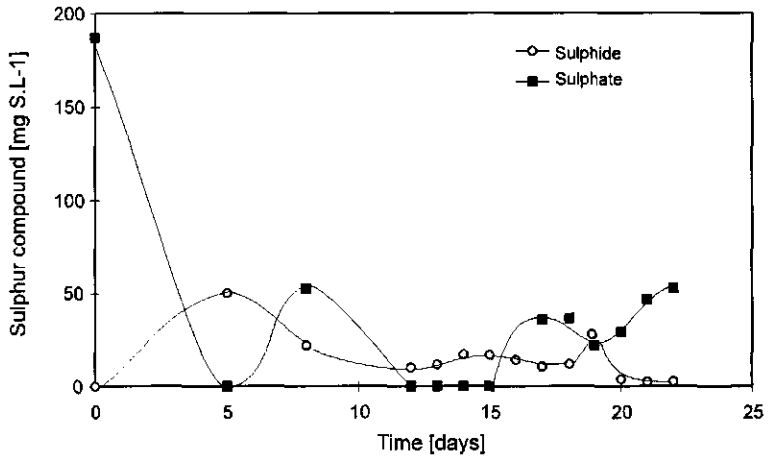

Fig. 5a *Sulphide and sulphate concentration [mg $S{\cdot}L^{-1}$] of the effluent from the expanded-bed reactor operating under heterotrophic conditions. From day 1 till day 15 the molar oxygen to sulphide consumption ratio was 1.0, from day 15 till day 22 this ratio was 1.6.*

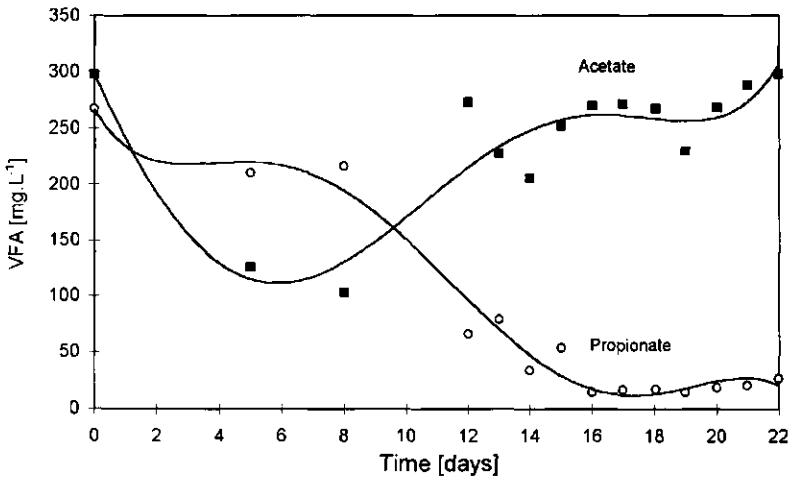

Fig. 5b *Acetate and propionate concentration [mg$\cdot L^{-1}$] with reference to the experiment described in Fig. 5a.*

As follows from Figure 5b, during the first 8 days acetate was degraded better than propionate, but from day 12 onwards, the acetate concentration in the effluent increased to levels greater than the propionate concentration. Apparently from then onwards propionate was incompletely oxidized, most probably by SRB of the genus *Desulfobulbus*. As a result, acetate accumulates because either the availability of sulphur as electron acceptor is becoming a limiting factor or the sulphate reducing *Desulfobulbus* species outcompete the sulphur reducing *Desulphuromonas* species. The relatively good degradation of acetate during the first

8 days of the experiment can probably be attributed to the activity of *Desulfuromonas* species which were then still present and also due to the activity of heterotrophic and mixotrophic *Thiobacilli*. Due to the increased acetate production during the rest of the experiment, the acetate conversion by (facultatively) heterotrophic *Thiobacilli* or by *Desulfuromonas* species then becomes insufficient. Elemental analysis of the sludge revealed that after 20 days of cultivation under heterotrophic growth conditions, the sulphur content dropped from 92% to 12% whilst the amount of biomass increased from 2.5% to 65%. The unidentified rest-fraction (23%) most likely consisted of metal sulphides in the sulphur sludge, as can be deduced from the blackening of sludge particles. Before addition of VFA to the system, the sludge particles had a whitish-grayish colour whilst under heterotrophic conditions the color changed into black, indicating the formation of iron sulphide as a result of anaerobic microbial activity. Microscopic observations of effluent samples confirmed the proliferation of rod-shaped, motile bacteria. These, most probably, are *Desulfobulbus* species.

Activity tests confirmed changes in the biomass-population of the sulphur-sludge as a result of the VFA-addition. Table 4 shows that the sulphur reducing capacity present in the sludge before cultivation with VFA (Table 3), is substituted by a sulphate reducing capacity. Whilst the autotrophically cultured sludge merely showed a sulphur-dependent acetate oxidation (Table 3), the heterotrophic sludge is only capable of degrading both acetate and propionate with sulphate as the electron acceptor. Detection test tubes blackened much quicker for propionate (10 h) than for lactate, H_2/CO_2, ethanol, acetate and butyrate (20 h). As observed for the autotrophically cultivated sludge, no blackening occured in tubes incubated with the substrates iso-butyrate and valerate and the selective SRB inhibitor molybdate.

Table 4: Formation and degradation [m*M*·g SS^{-1}·d^{-1}] of acetate and propionate in batch experiments (pH=8.0, 37°C) with sulphur-sludge grown under heterotrophic conditions. Initial concentration of acetate and propionate is 20 m*M* in the experiments with merely one of these substrates. In the experiment with the mixed substrate acetate and propionate the initial concentrations are 10 m*M*, respectively.

Substrate	acetate	propionate		acetate + propionate	
Electron acceptor					
	acetate	acetate	propionate	acetate	propionate
sulphur	0	0	0	0	0
sulphur and sulphate [22 m*M*]	-5.09	+4.60	-4.43	+8.34	-9.69

Whilst feeding the sludge with VFA, the daily decanting of the excess sulphur from the reactor could be omitted due to the fact that a part of the formed sulphur left the system as suspended matter as became apparent from the SS content, which increased from levels below 45 to approximately 65 mg·L^{-1}. Moreover, the sulphide and sulphate concentrations of the effluent are higher for the heterotrophic system than for the autotrophic system and thus less sulphur is being formed.

DISCUSSION

A sulphide oxidizing E.B. reactor combined with a separate aeration of the liquid phase was developed in order to promote the formation of a well-settleable sulphur sludge. In this manner, the demand of polyelectrolytes to flocculate formerly dispersed sulphur particles can be significantly reduced as compared to a conventional fixed-film system. Results show that at sulphide loading rates exceeding 3.4 $g \cdot L^{-1} \cdot d^{-1}$ and operating under autotrophic conditions, indeed an exceptionally well settleable sulphur sludge is formed which mainly consists of elemental sulphur (92%). After 50 days of operation 90% of the sludge settled out at a velocity greater than 25 $m \cdot h^{-1}$. Since most of the sulphide oxidizing biomass is immobilized within the sulphur aggregates, distinctly higher sulphide loading rates may be applied than in a conventional free-cell suspension.[3] The highest applicable loading rate reached in our experimental assembly amounted to 14 $g\text{-}S \cdot L^{-1} \cdot d^{-1}$. At higher sulphide loading rates, wash-out of the sulphur sludge becomes restrictive due to the very high upward velocities needed to supply the required oxygen. In order to be able to apply higher loads, the design of the reactor system has to be further adapted.

The results obtained reveal that both aerobic sulphide oxidizing biomass and anaerobic sulphur reducing biomass can become immobilized within the sludge. Anaerobic activity tests show that the autotrophically cultivated sludge also contains heterotrophic sulphur reducing bacteria. These heterotrophic organisms may use microbial excretion products as a carbon source. Within the sludge anaerobic circumstances most probably prevail, thus providing the sulphur and sulphate reducing bacteria with the required growth conditions. When besides sulphide also VFA are present in the wastewater, heterotrophic and mixotrophic sulphide oxidizing *Thiobacilli* species and anaerobic bacteria will proliferate. This follows clearly from the increase of the biomass content of the sludge under these conditions. This growth of anaerobic organisms is accompanied with a deterioration of the sulphide removal efficiency. Due to the anaerobic activity of sulphate and sulphur reducing bacteria in the upper part of the reactor, the formed sulphur will be partially reduced to sulphide which leads to increased sulphide levels in the effluent. For this reason, it is clear that the main field of application of this reactor-design lies in the treatment of sulphide containing wastewaters that contain no organic material, e.g. minewaters and scrub-waters.

NOMENCLATURE

d_i	Internal diameter (cm)
E.B. reactor	Expanded bed reactor
SS	Suspended solids ($g \cdot L^{-1}$)
SRB	Sulphate reducing bacteria
V	Volume (L)
VFA	Volatile fatty acids
VSLR	Volumetric sulphide loading rate ($g\ HS^- \cdot L^{-1} \cdot d^{-1}$)

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SUMMARY

The formation and aggregation of elemental sulphur from the microbiological oxidation of hydrogensulphide (H_2S) by a mixed population of aerobic *Thiobacillus*-like bacteria has been investigated. Sulphide is formed during the anaerobic treatment of wastewaters which contain oxidized sulphur compounds such as thiosulphate, sulphite and sulphate.[11] This sulphide has to be removed from the effluent solution of anaerobic reactors because of its detrimental characteristics e.g. toxicity, corrosiveness, oxygen demand and bad odour. Also the biogas produced in the anaerobic treatment plants generally will contain substantial amounts (up to 3% v/v) of hydrogensulphide. For removing the sulphide, conventional physico-chemical sulphide-removing processes can be applied. The processes are based on the oxidation of sulphide with peroxide, hypochlorite or permanganate or the precipitation of sulphide with iron(III)chloride.[2,3,4] Major drawbacks of these methods are the high costs for chemicals and the production of excessive amounts of chemical sludge. An alternative method for the chemical sulphide removal comprises the oxidation of sulphide with bacteria. At the Department of Environmental Technology (WAU) a process was developed in the mid eighties in which sulphide is oxidized into elemental sulphur.[1] Since sulphur is an insoluble compound it can be removed from the water-phase which leads to a reduction of the total S-content. The formed sulphur can be re-used in, for instance, bioleaching processes[10] or it can be used as a raw material for sulphuric acid production after undergoing a purification step. The objective of this PhD-research was to optimize the biological sulphide removing process which concerned the development of 1) an oxygen control strategy for maximizing the sulphur production and 2) a sulphur removal method. In order to achieve the objectives, it was necessary to understand the colloidal properties of the biologically produced sulphur particles.

Chapter 1 presents a general introduction on physico-chemical and biological methods for sulphide removal and a general overview on the sulphur chemistry. In Chapters 2 and 3 experimental results concerning the oxidation of sulphide into sulphur and sulphate are described. The oxidation of sulphide to sulphate yields more energy than the formation of elementary sulphur and consequently the micro-organisms tend to form sulphate rather than sulphur. In environmental technology however, the formation of the non-soluble sulphur is preferred. It was shown that at sulphide loading rates of up to 175 mg $S^{2-} \cdot L^{-1} \cdot h^{-1}$ complete conversion of sulphide into sulphur only proceeds if a stoichiometrical amount of oxygen is supplied, that is 0.5 mol of oxygen per mol of sulphide. At higher oxygen to sulphide consumption ratios increasing amounts of sulphate are formed, even when the oxygen concentration remains below 0.1 mg$\cdot L^{-1}$. This value is in the range of the lower

detection-limit of the currently available oxygen sensors which means that these probes are not suited to the accurate control of the oxygen dosage. An appropriate alternative for the oxygen measurements is the application of the redox-state of the solution. Although the redox-potential is a so called 'mixed-parameter', which means that its value is determined by several dissolved compounds, e.g. sulphide, thiosulphate, oxygen and maybe also certain 'unknown' compounds, it has been shown that a linear relationship exists between the sulphide concentration and the redox-potential. According to Eckert sulphide-ions have a much higher current exchange density than oxygen-ions which means that the electron exchange with the platinum electrode surface is much higher for sulphide than for oxygen.[5] The measured redox-potential is therefore kinetically determined rather than thermodynamically. The optimal redox range for sulphur formation is -147 $\pm$ 5 mV|H_2 at a temperature of 30°C and pH 8.

Dynamic experiments conducted in a fed-batch reactor revealed that the organisms are capable of switching between sulphur and sulphate formation within 0.5 h. This is far below the maximum doubling time of e.g. *Thiobacillus O* and *Thiobacillus denitrificans*, indicating that one metabolic type of organism can perform both reactions. Sulphide auto-oxidation primarily leads to the formation of thiosulphate. Its presence was recognized immediately after an increase of the sulphide loading rate during experiments conducted in a continuous flow reactor. In such a situation the biological oxidation capacity obviously becomes a limiting factor.

In order to develop an appropriate sulphur removal step, the physico-chemical properties of biologically produced sulphur particles had to be known. Steudel *et al.* encountered the presence of long-chain polythionates ($^-SO_3$-S_n-SO_3^-) in a sulphur dispersion formed by acidophilic *Thiobacillus ferrooxidans* species.[8] They formulated a 'vesicle-model' to describe the appearance of these sulphur particles. In such a vesicle the orthorhombic sulphur crystals are included within a network of long-chain polythionates. Synthetically formed 'LaMer' sulphur, which is formed by the acidification of a sodium thiosulphate solution, belongs also to this vesicle model.[9] In more recent papers, Steudel applies the vesicle-model to all types of biologically produced sulphur, formed by e.g. neutrophilic *thiobacilli* and phototrophic *chromatiaceae* species.[6,7] However, electrophoretic mobility measurements and flocculation experiments, as described in Chapter 4, show a clear difference between sulphur originating from our reactor system and 'LaMer' sulphur. Since the sulphur particles in our system are formed under slightly alkaline conditions, i.e. pH 8, they don't belong to the 'vesicle' model. Polythionates were reported to be stable only under acidic conditions.[12] Steudel attributes the hydrophilic character of the biologically produced sulphur to the presence of negatively charged sulphonic-groups, whereas we have evidence that (bio)polymers are attached to the sulphur core. From dynamic light-scattering measurements it can be seen that the particle diameter reduces at increasing salt concentrations which is indicative of an inward migration of an adsorbed layer. These (bio)polymers very likely contain charged groups, such as carboxylic, phosphate and ammonium groups which give the sulphur its overall hydrophilic

carboxylic, phosphate and ammonium groups which give the sulphur its overall hydrophilic character. X-ray measurements of freshly formed sulphur particles indicate the presence of orthorhombic sulphur crystals (S_8) which are known to be hydrophobic. These crystals are therefore present in the inner-part of the sulphur particles. The negative surface charge of the particles can be measured by potentiometric titrations. Results of such titration experiments are described in Chapter 5. The point of zero charge (*pzc*) was determined at pH 5.8. At higher pH-values the surface becomes more negatively charged whilst at pH-values below 5.8 a positive charge was measured. The *pzc* does not however correspond with the iso-electrical point (*iep*), i.e. the pH at which the electrophoretic mobility is zero. The *iep* is located at a pH below 3. A possible explanation is that within the adsorbed polymer layer charge distribution occurs. Although at pH 5.8 the overall surface charge is zero, the charge of the outside of the polymer layer may be slightly negative, as follows from the electrophoretic mobility measurements, while the charge of the inner polymer side is more positive. This positive charge is attracted to the S_8 nucleus because of its high electron density.

In this study it was shown that biologically produced sulphur particles have the ability to aggregate into larger clusters, particularly at high sulphide loading rates. However, increasing salt concentrations lead to a deterioration of the aggregation process, indicating that not only DLVO-interactions are involved but also factors such as entrapment of sulphur particles within the biomass/sulphur film and crystallisation of the elemental sulphur particles attribute to the sulphur aggregation. The effect of certain well defined polymers was investigated in order to improve the understanding of the effect of certain complex dissolved polymers on the sulphur-aggregation such as tannins, humic acids or additives used in the paper industry (Chapter 5). It was found that long-chain polymers especially affect the sulphur-aggregation detrimentally. These compounds are dissipated from the water-phase and adsorb onto the sulphur particles. In the case of approaching sulphur particles covered with these long-chain polymers approach, an increased entropy results, leading to lateral repulsion. This then hampers the aggregation of the sulphur particles. Similar observations have been made for cationic polymers but not for anionic polymers. Besides chemical factors, physical factors also play an important role in the formation of a well-settleable sulphur sludge. Fluid shear forces disintegrate the sludge. For this reason we developed a new reactor-type for sulphide oxidation, i.e. an expanded sludge bed reactor. In this reactor, shear-forces due to aeration of the reactor suspension are avoided (Chapter 6). In this new reactor type, sludge particles are formed which have an average size of about 3 mm and a mean sedimentation velocity exceeding 25 $m \cdot h^{-1}$. The sulphur content of the sludge amounted to 92% whilst the rest fraction presumably consists of active biomass, as follows from aerobic activity measurements. Because biomass is immobilized within the sludge high loading rates are achieved, viz. 14 g $HS \cdot L^{-1} \cdot d^{-1}$ whilst only 6 g $HS \cdot L^{-1} \cdot d^{-1}$ could be obtained for a free cell suspension. The maximal applicable sulphide loading could indeed be higher but in the experimental assembly the recirculating flow, necessary for oxygen suppletion, reached an excessive level resulting in extreme liquid upward velocities. As a consequence, wash-out of biomass occured. Under the condition that fatty acids are present in the

influent, such as acetate and propionate, anaerobic conditions within the sludge prevail, leading to the reduction of sulphur into sulphide.

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SAMENVATTING

Dit proefschrift beschrijft de vorming van elementair zwavel tijdens de microbiologische oxydatie van waterstofsulfide (H_2S) door zuurstofminnende bacteriën, de zg. *Thiobacilli*. Sulfidehoudende afvalstromen worden onder andere gevormd tijdens de anaërobe (zuurstofloze) behandeling van sulfaathoudende (SO_4^{2-}) afvalwaters.[6] Ook andere geoxydeerde zwavelverbindingen zoals thiosulfaat ($S_2O_3^{2-}$) en sulfiet (SO_3^{2-}) kunnen in anaërobe bioreaktoren in sulfide worden omgezet. Het gevormde sulfide is giftig, corrosief, het stinkt naar rotte eieren en het reageert met zuurstof. De verwijdering van deze ongewenste verbinding is hierom noodzakelijk. Conventionele methoden om sulfide te verwijderen zijn gebaseerd op de chemische oxydatie van sulfide met bijvoorbeeld waterstofperoxyde, hypochloriet of permanganaat. Ook is het mogelijk om sulfide neer te slaan met ijzerchloride.[2,3,4] De belangrijkste nadelen van deze methoden zijn de hoge kosten voor chemicaliënverbruik en de vorming van chemisch afval. Een alternatieve methode voor deze chemische omzettingen is de oxydatie van sulfide met behulp van bacteriën. Op de vakgroep Milieutechnologie van de Landbouw Universiteit Wageningen is een proces ontwikkeld waarin het sulfide microbiologisch wordt omgezet in het onschadelijke, elementaire zwavel.[1] Elementair zwavel is een onoplosbare verbinding en kan hierom d.m.v. bezinking in principe uit het afvalwater worden verwijderd. Het teruggewonnen zwavel kan vervolgens worden hergebruikt als grondstof voor bijvoorbeeld de produktie van zwavelzuur. Ook kan het worden gebruikt in zg. *bioleaching* processen.[5] Dit zijn processen waarin met behulp van bacteriën zwavelzuur wordt gevormd om zware metalen uit vervuilde bodems op te lossen.

De microbiologische vorming van elementair zwavel uit sulfide is een proces dat alleen goed verloopt onder zuurstofgelimiteerde omstandigheden. Indien de zuurstofconcentratie in de bioreaktor te hoog is zal sulfaat i.p.v. zwavel worden gevormd. Een belangrijke doelstelling van het uitgevoerde onderzoek betrof dan ook de optimalisatie van de zuurstofdosering om de vorming van sulfaat zoveel mogelijk te voorkomen. Een tweede voorwaarde voor een succesvolle implementatie van deze nieuwe technologie dat het gevormde zwavel gemakkelijk uit de waterfase kan worden verwijderd. De meest eenvoudige afscheidingsmethode is bezinking van de zwaveldeeltjes. Omdat de zwaveldeeltjes die door de bacteriën worden uitgescheiden zeer klein zijn, ze hebben een grootte van ca. 1 µm (=0.001 mm), is het noodzakelijk dat deze allereerst aangroeien tot grotere aggregaten of kristallen voordat ze door middel van bezinking uit het water kunnen worden verwijderd.

Hoofdstuk 1 is een algemene inleiding over de beschikbare fysisch-chemische en biologische methoden voor sulfideverwijdering. Tevens is een globaal overzicht gegeven over het gebied van de biologische zwavelchemie. In de hoofdstukken 2 en 3 staat beschreven onder welke omstandigheden sulfide in respectievelijk zwavel en sulfaat wordt geoxydeerd. De oxydatie van sulfide tot sulfaat levert meer energie op dan de vorming van zwavel. De bacteriën geven hierom de voorkeur aan sulfaatvorming. Er wordt aangetoond dat bij sulfidebelastingen tot 175 $mg \cdot L^{-1} \cdot h^{-1}$ zwavelvorming alleen mogelijk is wanneer stoichiometrische hoeveelheden zuurstof worden gedoseerd. Indien meer zuurstof wordt toegevoegd zal evenredig meer sulfaat worden gevormd, echter de zuurstofconcentratie blijft bijna onmeetbaar laag, d.w.z. beneden de 0.1 $mg \cdot L^{-1}$. Dit betekent dat de zuurstofdosering niet gestuurd kan worden op basis van de gemeten zuurstofconcentratie. Een bruikbaar alternatief is de redox-potentiaal van de oplossing. Er is aangetoond dat zwavelvorming bij een lagere redox-potentiaal optreedt dan sulfaatvorming. Er is gevonden dat de optimale redoxwaarde voor zwavelvorming -147±5 mV bedraagt bij een temperatuur van 30°C en pH 8.

Om een geschikte zwavelafscheidingsmethode te ontwikkelen was het nodig om de fysisch-chemische eigenschappen van de biologisch gevormde zwaveldeeltjes te bestuderen. Het is gebleken dat het biologische gevormde zwavel uit ons systeem bedekt is met een laagje (bio)polymeren (hoofdstuk 4). Deeltjesgroottemetingen tonen aan dat de zwaveldeeltjes kleiner worden bij toenemende zoutconcentraties. Dit duidt mogelijk op het inklappen van een geadsorbeerde (bio)polymeerlaag. De geadsorbeerde (bio)polymeren bevatten verschillende functionele groepen, zoals fosfaatgroepen, carboxylgroepen en ammoniumgroepen. Hierdoor zijn de zwaveldeeltjes negatief geladen onder pH-neutrale omstandigheden. Deze negatieve oppervlaktelading kan worden gemeten m.b.v. potentiometrische titraties (hoofdstuk 5). Het is gebleken dat het ladingsnulpunt (*pzc*) van de zwaveldeeltjes bij pH 5.8 ligt. Dit betekent dat bij hogere pH-waarden het zwaveloppervlak negatief is geladen terwijl bij pH-waarden beneden het *pzc* het oppervlak positief is geladen. Het *pzc* komt echter niet overeen met de pH waarbij geen elektroforetische mobiliteit wordt gemeten, het zg. iso-elektrische punt (*iep*). Dit is de pH waarbij de zwaveldeeltjes niet meer bewegen in een elektrisch veld. In het *iep* geldt dat de buitenkant van de zwaveldeeltjes ongeladen is. Uit metingen is gebleken dat het *iep* van het biologisch gevormde zwavel beneden pH 3 ligt. Een mogelijk verklaring voor het verschil in *pzc* en *iep* is dat er sprake is van ladingsscheiding. Terwijl bij pH 5.8 de totale lading in de (bio)polymeerlaag gelijk is aan nul, is het mogelijk dat de buitenkant van de (bio)polymeerlaag een beetje negatief geladen is terwijl de binnenkant wat meer positief is geladen. Deze positieve lading wordt aangetrokken door het zwavelkristal dat een hoge elektronendichtheid heeft.

Verder is gebleken blijkt dat de biologische gevormde zwaveldeeltjes het vermogen hebben om spontaan te aggregeren tot grotere, goed bezinkbare vlokken. Met name hoge sulfideconcentraties en lage zoutconcentraties bevorderen deze aggregatie. Opgeloste polymeren die als afvalstof uit de procesindustrie in het afvalwater kunnen voorkomen, kunnen deze aggregatie verstoren omdat ze

adsorberen aan het zwaveloppervlak. Vooral lange, hydrofobe polymeren die sterk uit de waterfase worden verdrongen hebben een negatieve invloed op het aggregatieproces.

Naast chemische aspecten spelen ook fysische aspecten een rol in de vorming van een goed bezinkbaar zwavelslib. De zwavelaggregaten worden namelijk voortdurend kapot geslagen indien de vloeistof in de reaktor te hard wordt gemengd. Hierom is een nieuw reaktortype voor sulfide-oxydatie ontwikkeld waarin de reaktorvloeistof buiten de reaktor wordt belucht waarna het zuurstofrijke water wordt gerecirculeerd naar de bioreaktor (hoofdstuk 6). Op deze wijze kunnen de zwaveldeeltjes aangroeien tot vlokken met een diameter van ongeveer 3 mm die uitstekend bezinken. Meer dan 90% van al het zwavel bezinkt met een snelheid groter dan 25 $m \cdot h^{-1}$. Het biomassa-gehalte in dit nieuwe reaktortype voor sulfideoxydatie is hoger dan in de eerder toegepaste gemengde tank reaktoren omdat het zwavelslib voor een gedeelte uit bacteriën bestaat. Dit leidt ertoe dat met dit systeem hogere sulfideomzettingen mogelijk zijn, te weten 14 in plaats van 6 kg $HS \cdot m^{-3} \cdot d^{-1}$. In het geval dat ook vetzuren in het influent aanwezig zijn, zal dit leiden tot anaërobe omstandigheden binnen in de zwavelkorrel waardoor het gevormde zwavel weer tot sulfide wordt gereduceerd. Dit leidt tot een verslechtering van het omzettingsrendement.

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Nawoord

Het werk zit erop en eindelijk is de tijd aangebroken om enigszins relativerend terug te kijken op een bijzonder drukke maar zeer plezierige periode. De totstandkoming van dit proefschrift was zeker niet mogelijk geweest zonder de bijdragen van een aantal personen. Op de eerste plaats zijn dit de doktoraal studenten, stagiaires, post-docs en analisten die een deel van het experimentele werk voor hun rekening hebben genomen. Johan de Kok en Marie-Ellen van Schendel hebben de kolloïdchemische aspekten van het biologisch gevormde zwavel bestudeerd. John Bohnen ontdekte dat zwavelvorming onder zuurstoflimiterende omstandigheden optreedt. Cheryl van der Kaa heeft onder deze omstandigheden het verloop van de redoxpotentiaal bestudeerd. Arco Jochemsen heeft in samenwerking met de sectie Meet- Regel- en Systeemtechniek (MRS) 'optimal control' toegepast. Tenslotte is het Bas Meijer gelukt om, eveneens in samenwerking met MRS, op basis van de redoxpotentiaal de zuurstofdosering te sturen. I am deeply indebted to Dr. He (China) and Mr. S. Ma (Taiwan) for their stimulating contribution to the research. Your presence was both professionally and personally highly appreciated. Ron Sleyster heeft als analist gedurende 1.5 jaar een belangrijke bijdrage geleverd aan de begeleiding van de doktoraal studenten en bij het ondersteunen van het praktische werk. Ilse Bennehey en Johannes van der Laan waren altijd bereid om de HPLC tijdig om te bouwen voor het meten van (thio)sulfaat en elementair zwavel.

Samen met Richard Tichý (Tsjechië) en Remco van Abswoude heb ik met veel plezier gewerkt aan het bestuderen van de hergebruiksmogelijkheden van het biologische gevormde zwavel. Dat een en ander heeft geleid tot een auto-aanrijding was uiteraard niet de bedoeling.

Bij het bouwen van de laboratorium opstellingen heb ik veel ondersteuning gehad van de mechanische werkplaats waar Jan Theunissen en Evert Janssen altijd bereid waren om een helpende hand toe te steken. Ditzelfde geldt voor Gerrit Nieuwboer van de centrale glasinstrumentmakerij.

Het uitvoeren van een multi-disciplinair onderzoek als het deze, is vrijwel onmogelijk zonder het hebben van goede discussiepartners. Wat dit betreft heb ik veel steun gehad aan mijn co-promotor Dr. Arie de Keizer van de vakgroep Fysisch- en Kolloïdchemie (F&K) die altijd bereid was zich in de zwavelmaterie te verdiepen. Arie, ik heb veel geleerd van jouw kritische wijze van onderzoek bedrijven. In een omgeving waar voornamelijk fundamenteel onderzoek

wordt verricht was gelukkig ook plaats voor het toegepastere milieu-onderzoek. Dr. Jan Bontsema van de sectie Meet- Regel- en Systeemtechniek vervulde eenzelfde rol. Jullie enthousiasme voor het onderzoek heb ik altijd als bijzonder stimulerend ervaren. Adriaan van Aelst (Vakgroep Plantencytologie en -Morfologie) heeft geholpen bij het maken van de elektronenmicroscoop foto's en het perfectioneren van de verkregen opnamen. Behalve wetenschappelijke ondersteuning heb ik ook van diverse vakgroepen technische ondersteuning gehad. Remco Fokkink en Ab van der Linden (F&K) waren altijd bereid om hun dure laserapparatuur in te schakelen voor het 'zwavelonderzoek'. Rachel van Ooteghem en Kees van Asselt (MRS) hebben hun expertise regelmatig ingeschakeld bij het bouwen van computeropstellingen voor de diverse zuurstofregelingen.

De behaalde laboratoriumresultaten zijn ook onder praktijkomstandigheden onderzocht. Hiertoe is een pilot-plant onderzoek uitgevoerd bij Industriewater Eerbeek B.V. (IWE). De pilot-plant is ter beschikking gesteld door Paques B.V. en werd mede begeleid door Arie Sopjes en Jurie Wenderich (IWE).

De medewerkers van het Bordes zorgden ervoor dat het hier plezierig toeven was. De jaarlijkse 'Bordesborrel' moeten dan ook zeker worden voortgezet. De 'koffiekamer' van Jules van Lier zorgde in aanwezigheid mijn kamergenote Miriam van Eekert, André Visser, Salih Rebac, Piet Lens Jan Weijma en de steeds wisselende doctoraal studenten en buitenlandse gastmedewerkers voor een gezellige start van de dag.

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Last but not least, ben ik mijn ouders veel verschuldigd. Zij zijn de pijlers waarop ik heb gebouwd en die mij altijd stimuleerden 'mijn best' te doen.

Hiervoor allen hartelijk dank,

J. Albert

CURRICULUM VITAE

Albert Josef Hendrik Janssen was born in Nieuwenhagen on October 17 1966. In 1985 he received his Atheneum-B degree at the Eijkhagen College in Landgraaf. In the same year he began to study 'Environmental Sciences' at the Wageningen Agricultural University. During his studies he specialized in Environmental Technology and Control Engineering. His practical period was completed at BASF A.G. (Ludwigshafen, Germany). In January 1991 he obtained his MSc.-degree whereafter he started a research period of 4.5 years as a researcher at the Department of Environmental Technology (WAU). The results of these investigations are presented in this thesis. Since June 1994 he has been working simultaneously at Paques B.V. Environmental Technology (Balk, The Netherlands).

Tuin van de wereld vlak bij de nieuwe
stad,
In de weg van de holle bergen,
Zal worden gegrepen en in de put lopen,
Gedwongen water te drinken, vergiftigd
met zwavel.

(Nostradamus, 1503)