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Personal Review:

Sources of sulfide in waste streams and current biotechnologies for its removal

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Abstract: Sulfide-containing waste streams are generated by a number of industries. It is emitted into the environment as dissolved sulfide (S^{2-} and HS^-) in wastewaters and as H_2S in waste gases. Due to its corrosive nature, biological hydrogen sulfide removal processes are being investigated to overcome the chemical and disposal costs associated with existing chemically based removal processes. The nitrogen and sulfur metabolism interacts at various levels of the wastewater treatment process. Hence, the sulfur cycle offers possibilities to integrate nitrogen removal in the treatment process, which needs to be further optimized by appropriate design of the reactor configuration, optimization of performance parameters, retention of biomass and optimization of biomass growth. The present paper reviews the biotechnological advances to remove sulfides from various environments.

Key words: Hydrogen sulfide, Sulfide utilizing microbes, Nitrogen and sulfur metabolism, Biotechnologies for sulfide removal
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INTRODUCTION

Hydrogen sulfide (H_2S) is used as a reagent and an intermediate in the preparation of other reduced sulfur compounds (HSDB, 1999). It is also a by-product of desulfurization processes in the oil and gas industries and rayon production, sewage treatment, and leather tanning (Ammann, 1986). The annual statewide industrial emissions from point sources at facilities reporting under the Air Toxics Hot Spots Act in California (USA) based on the most recent inventory were estimated to be 5688172 pounds of hydrogen sulfide (CARB, 1999).

Sulfide-containing waste streams 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 sulfate containing wastewaters (Brimblecombe *et al.*, 1989; Kuenen and Robertson, 1992; Rinzema and Lettinga, 1988).

It is emitted into the environment as dissolved sulfide (S^{2-} and HS^-) in wastewaters and as H_2S in waste gases. Hydrogen sulfide is generated in relatively stagnant wastewater systems as a result of the biological breakdown of sulfates (SO_4^{2-}) in anaerobic wastewater environments as shown in the following biochemical reaction (Elizabeth, 2005).

$$SO_4^{2-} + \text{organic matter} + \text{anaerobic bacteria} \rightarrow S^{2-} + H_2O + CO_2. \quad (1)$$

Sulfate is present in great abundance in municipal wastewater systems and primarily stems from household cleaning detergents. Once the anaerobic bacteria reduce the sulfates to sulfide (S^{2-}), it reacts with hydrogen to produce hydrogen sulfide according

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to the following reaction:

$$S^{2-}+2H^{+}\rightarrow H_2S. \quad (2)$$

Hydrogen sulfide prefers to exist in the gas phase; thus, once produced in the aqueous phase, it will rapidly partition to the gas phase once the headspace in the pipe is available. Once hydrogen sulfide is present in the headspace of the pipe, aerobic bacteria lining the interior surface of the pipeline convert the hydrogen sulfide into sulfuric acid as shown in the following biochemical reaction (Elizabeth, 2005):

$$H_2S+2O_2+\text{aerobic bacteria}\rightarrow H_2SO_4. \quad (3)$$

The hydrogen sulfide produced in various sulfureta (the habitats in which the oxidation and reduction of sulfur compounds take place) may undergo further reactions in the environment, i.e.

(1) Different aerobic microorganisms can oxidize sulfide with oxygen.

(2) Sulfide is one of the few bacterial degradation products that are autooxidizable (i.e. that can react with oxygen at normal temperature). The autooxidation of sulfide is as fast as biological reactions (Cypionka *et al*., 1985; Jørgensen *et al*., 1979; Sorokin, 1972). The mechanism and rate of autooxidation depends upon the pH value, concentration of the reactants, and the presence of catalytic concentrations of heavy metals. At lower pH values (<7), elemental sulfur is the major oxidation product. While at higher pH values (>7), thiosulfate and sulfite are major oxidation products (Almgren and Hagström, 1974; Chen and Morris, 1972a; 1972b; Cline and Richards, 1969; Zehnder and Zinder, 1980). Heavy metals especially manganese(II), nickel(II) and cobalt(II) compounds stimulate autooxidation of sulfide. The sulfur, thiosulfate, or sulfite formed may be oxidized further by colorless sulfur bacteria. Under anaerobic conditions these sulfur compounds can change biologically to sulfide; thiosulfate and sulfite are also microbially misappropriated to sulfide and sulfate.

(3) Under anaerobic conditions phototrophic purple sulfur bacteria and green sulfur bacteria convert S^{2-} to S^0, $S_2O_3^{2-}$, and SO_4^{2-}. Methane and CO_2 are the main products in anaerobic environments where sulfate is absent, but sulfide and CO_2 are the main products if sulfate is present.

(4) Another characteristic reaction of hydrogen sulfide is its binding with heavy metals especially Fe^{2-} found in the cytochromes to form metal sulfides. Depending on the environmental conditions, the heavy metal sulfides may be oxidized after a while or may be preserved, sometimes over geological periods (Zehnder, 1988).

Treatment of sulfide rich waste streams is highly desirable due to its toxic effects upon environment. It causes an irritating, rotten-egg smell above 1×10^{-6} (1.4 mg/m^3), and at concentrations above 10×10^{-6} the toxicological exposure limits are exceeded (WHO, 1981).

The most energy is released when sulfide is oxidized completely to sulfate as seen in Eq.(4). Sulfide oxidation often occurs in steps with elemental sulfur as an intermediate product, as seen with Eqs.(4)~(7) (Yang, 1992), and in oxygen-limited environments, oxidation may proceed only to elemental sulfur, producing less energy. Cells can either deposit sulfur inside or outside their cell membranes. Other reduced sulfur compounds, such as thiosulfate, can also be oxidized for energy as seen in Eq.(7):

$$H_2S+2O_2\rightarrow SO_4^{2-}+2H^{+} \quad (\Delta G^{0'}=-798.2\ \text{kJ/mol}), \quad (4)$$

$$HS^{-}+\frac{1}{2}O_2+H^{+}\rightarrow S^0+H_2O \quad (\Delta G^{0'}=-209.4\ \text{kJ/mol}), \quad (5)$$

$$S^0+H_2O+\frac{3}{2}O_2\rightarrow SO_4^{2-}+2H^{+} \quad (\Delta G^{0'}=-587.1\ \text{kJ/mol}), \quad (6)$$

$$\frac{1}{2}S_2O_3^{2-}+\frac{1}{2}H_2O+O_2\rightarrow SO_4^{2-}+H^{+} \quad (\Delta G^{0'}=-409.1\ \text{kJ/mol}). \quad (7)$$

Sulfide is used as electron donor for cell synthesis in the presence of light by green and purple sulfur bacteria during a process called anoxygenic photolithoautotrophic growth (Zehnder, 1988). During this process sulfate is produced as final product. Significant amounts of sulfur are also produced as intermediate. Green sulfur bacteria and *Ectothiorhodospira* species form extracellular sulfur, whereas *Chromatium* species store intracellular sulfur globules (Zehnder, 1988). Normally oxygenic cyanobacteria were shown to perform anoxygenic

photosynthesis in the presence of sulfide as electron donor that is oxidized to extracellular sulfur (Castenholz, 1977; Jørgensen *et al*., 1979; Oren and Padan, 1978; Oren and Shilo, 1979).

CURRENT BIOTECHNOLOGIES TO REMOVE SULFIDE FROM POLLUTED ENVIRONMENTS

Hydrogen sulfide is emitted into the environment as dissolved sulfide in wastewaters and as H_2S in waste gases. Hydrogen sulfide and sulfur dioxide can be converted into elemental sulfur via the biological sulfur cycle (Buisman *et al*., 1990). The insoluble sulfur can be removed from the treated water, leading to reduction of the total sulfur content of the waste stream. The recovered sulfur is a potentially valuable compound which can be reused, e.g. it can be purified by melting at high temperatures. It is suitable for the production of sulfuric acid or it can be applied in bioleaching processes (Tichý *et al*., 1994).

In order to remove sulfide from wastewater streams, a number of physicochemical 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 sulfide removal are aeration (catalyzed and uncatalyzed), chlorination, ozonation, potassium permanganate treatment and hydrogen peroxide treatment (Butler and Nadan, 1981; Cadena and Peters, 1988; Chen and Morris, 1972a; 1972b; Martin and Rubin, 1978; Watkins, 1977). In all these processes, apart from elemental sulfur, thiosulfate ($S_2O_3^{2-}$) and sulfate (SO_4^{2-}) may also be formed as end products.

Since the conventional physicochemical methods for removing hydrogen sulfide from wastewaters and sour gases 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-containing gases, as well as for the desulfurization of coal and petroleum (Sublette, 1987; Buisman and Prins, 1994). Microbiological processes can proceed around ambient temperatures and at atmospheric pressure, thus eliminating the need for heat and pressure, reducing energy costs to a minimum.

To biologically address the problem of malodorous air, open-bed soil filters began to be used in the 1920's and industrial soil biofilters first appeared in the United States during the 1950's, but operation was not well understood (Carlson and Leiser, 1966). Sulfur compounds are a major component of bad odor in gases and are produced during biochemical reduction of inorganic or organic sulfur compounds. Many soils exhibit a small chemical adsorption capacity for H_2S that is heavily dependent on the iron content of the soil (Bohn *et al.*, 1989). It has since been determined that sustained effectiveness of soil or other biofiltration beds arises primarily from microbial oxidation of organic compounds, leading to biomass formation and nontoxic odorless products, or oxidation of inorganic compounds (such as sulfides), which supply energy to cells and produce odorless compounds like elemental sulfur and sulfate in the process (Ottengraf, 1986).

Biologically active agents have since been used in a variety of process arrangements, such as biofilters, fixed-film bioscrubbers, and suspended-growth bioscrubbers (Dawson, 1993). These processes may also be effective at removing multiple contaminants from a gas stream, increasing their functionality. Fluidized-bed bioreactors have recently been tested for simultaneous removal of H_2S and NH_3 with promising results (Chung *et al*., 2001). It is also possible to achieve co-treatment of volatile organic compounds and H_2S in the same biofilter (Devinny and Chitwood, 1999).

Because of toxic effects, sources of aqueous sulfide such as petroleum refineries face effluent limits. Canadian petroleum refinery wastewaters discharges must not exceed 0.3 kg S^{2-} per 1000 m^3 of oil refined per day (Losier, 1990). The Municipal Industrial Strategy for Abatement Program in Ontario set loading limits to the surface water such that oil refineries must have a monthly average concentration less than 0.2 mg S^{2-}/L with their daily concentration not exceeding 0.3 mg S^{2-}/L (MOE, 1992).

Petroleum refineries typically recover elemental sulfur from sour water by the Holmes-Stretford process or by steam stripping followed by the Claus process. Elemental sulfur, S^0, is a non-corrosive solid that is easy to handle and transport. In addition, it has

a commercial value exceeding that of sulfuric acid, although both are used in chemical processing and fertilizer production. Physicochemical production of elemental sulfur is costly and energy intensive due to the need to replace poisoned catalysts, contaminated reactor liquids and corroded reaction vessels (Cork *et al*., 1986). In contrast, biological methods have several potential advantages including high removal even at low sulfide concentrations, and high elemental sulfur recovery.

BIOREACTORS FOR H_2S REMOVAL INVOLVING CHEMOTROPHIC BACTERIA

Various kinds of bioreactors used for hydrogen sulfide removal are shown in Fig.1. Further applications and specific examples are described below.

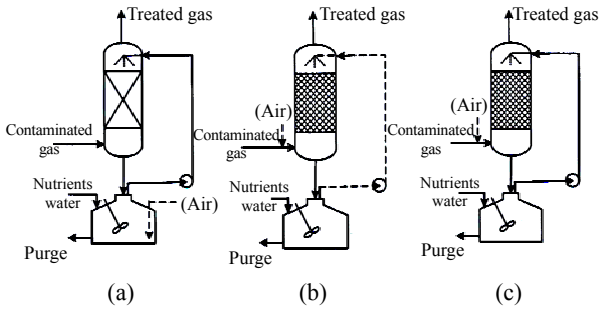

Fig.1 Bioreactors for removal of hydrogen sulfide. (a) Bioscrubber; (b) Biofilter; (c) Biotrickling filter (Syed *et al*., 2006)

Gas-fed batch reactors

Janssen *et al*.(1995) used two batchfed reactors to study the oxidation of sulfide using a mixed culture of *Thiobacilli*. Pure oxygen was supplied to the reactors. The maximum sulfur production (73%±10%) occurred at oxygen to sulfide ratio of 0.6 to 1.0 mol/(L·h). At lower oxygen to sulfide ratios, the lower biological oxidation capacity resulted in the production of more thiosulfate. At higher oxygen to sulfide ratios, more sulfates were produced because more energy was consumed for bacterial growth than for the formation of elemental sulfur.

Continuous-flow reactors

Sublette and Sylvester (1987a; 1987b) reported on a continuous stirred-tank reactor (CSTR) system using *Thiobacillus denitrificans* to remove H_2S from gas streams. Ninety-seven percent of the H_2S bubbled was removed and oxidized to sulfate.

Buisman *et al*.(1990) tested three different continuous-flow reactor configurations: fixed-film CSTR, biorotor (a rotating cage containing reticulated polyurethane biomass support particles, partly immersed in the reactor liquid), and a fixed-film upflow reactor. For the upflow and biorotor reactors, 95% to 100% sulfide removal efficiencies were achieved for loading rates up to 500 mg H_2S/(L·h). The removal efficiency decreased rapidly above this loading rate. At 938 mg/(L·h) (biorotor) and 1040 mg/(L·h) (upflow) loadings, sulfide removal efficiencies were 69% and 73%, respectively. At a 500 mg/(L·h) sulfide loading rate, the stirred-tank reactor's removal efficiency was approximately 62%.

Bioscrubbers

Removal of H_2S using bioscrubbers (Fig.1a) involves a two stage process, firstly absorption of H_2S by a liquid followed by biological oxidation of H_2S in the liquid (Syed *et al*., 2006). Nishimura and Yoda (1997) used a multiple bubble-tray airtight contact tower (bioscrubber) to scrub hydrogen sulfide from the biogas produced by an anaerobic wastewater treatment process. A two-reactor system (a gas-liquid contact tower and an aeration tank) was used to separate the oxidation process from the absorption process to prevent air from mixing with the biogas. Mixed liquor from the activated sludge process was continuously fed to and withdrawn from the contact tower, where H_2S from the biogas was absorbed into the mixed liquor and subsequently oxidized to sulfate by sulfur oxidizing bacteria after returning to the aeration tank. Based on their preliminary results, a full scale plant treating potato processing wastewater was constructed. When treating 2000×10^{-6} of H_2S in 40 m^3/h of biogas, more than 99% removal efficiency was achieved.

Mesa *et al*.(2002) described a bioscrubber system which can be integrated into a system to remove H_2S from biogas by a combination of chemical and biological processes. H_2S removal can be achieved by absorption in a ferric sulfate solution producing ferrous sulfate and elemental sulfur. Ferric sulfate can be regenerated by biological oxidation using *Acidithiobacillus ferrooxidans*. Relevant reactions are shown in Table 1. The study investigated the oxidation of ferrous iron by *A. ferrooxidans* which was immobi-

Table 1 Reactions involving chemotrophic bacteria (Syed *et al*., 2006)

Bacteria	Reactions	Reference
Thiobacillus thioparus	$2HS^-+O_2\rightarrow 2S^0+2OH^-$	Chung *et al*.(1996)
	$2S^0+3O_2+2OH^-\rightarrow 2SO_4^{2-}+2H^+$	
	$H_2S+2O_2\rightarrow SO_4^{2-}+2H^+$	Kim *et al*.(2002)
Thiobacillus denitrificans	$3HS^-+3.9NO_3^-+0.2NH_4^++HCO_3^-+1.7H^+\rightarrow CH_{1.8}O_{0.5}N_{0.2}+1.9N_2+3SO_4^{2-}+2.3H_2O$	Kleerebezem and Mendez (2002)
	$14.5HS^-+5NO_3^-+0.2NH_4^++HCO_3^-+20.5H^+\rightarrow CH_{1.8}O_{0.5}N_{0.2}+2.5N_2+14.5S+17.5H_2O$	Lampe and Zhang (1996)
	$55S+20CO_2+50NO_3^-+38H_2O+4NH_4^+\rightarrow 4C_5H_7O_2N+25N_2+55SO_4^{2-}+64H^+$	McComas and Sublette (2001)
	$5HS^-+8NO_3^-+3H^+\rightarrow 5SO_4^{2-}+4N_2+4H_2O$	
Thiobacillus ferrooxidans	$2FeSO_4+H_2SO_4+0.5O_2\rightarrow Fe_2(SO_4)_3+H_2O$	Mesa *et al*.(2002)
	$2FeS_2+7.5O_2+H_2O\rightarrow Fe_2(SO_4)_3+H_2SO_4$	Takano *et al*.(1997)

lized on a polyurethane foam support with the support particles being placed in an aerated column. Ferric precipitates were accumulated on the support and on the air diffusers which necessitated periodic interruptions of the process for cleaning. Precipitation, air supply, and chemical cost are the potential constraints for this process.

A full scale plant located northeast of Brooks, Alberta, Canada uses Shell-Paques process for natural gas desulfurization (Benschop *et al*., 2002). H_2S is removed from a gaseous stream by absorption into a sodium carbonate/bicarbonate solution. The sulfide containing scrubbing liquid is treated in the bioreactor where it is mostly converted biologically to elemental sulfur. The bioreactor is supplied with a nutrient stream, air, make-up water, and sodium hydroxide. It is reported that normally less than 3.5% of the sulfide is converted to sulfate and a continuous bleed stream is required to avoid accumulation of sulfate. A compost filter is used to treat the trace H_2S present in the spent air from the bioreactor. Less than 4×10^{-6} (v/v) effluent H_2S concentration is achieved when treating natural gas containing 2000×10^{-6} (v/v) H_2S.

Biofilters

A biofilter (Fig.1b) is a three-phase bioreactor (gas, liquid, and solid) made with a filter bed with high porosity, high buffer capacity, high nutrient availability, and high moisture retention capacity to ensure that the target microorganisms can grow on it (Elias *et al*., 2002; Jorio and Heitz, 1999; Dastous *et al*., 2005). The contaminated gas is continuously fed into the biofilter, while a nutrient solution is discontinuously added. Various types of biofilter media have been used by researchers (Syed *et al*., 2006). Table 2 references several studies on biofiltration of H_2S with specific bacterial populations.

Table 2 Specific microorganisms studied for biofiltration of H_2S

Microorganism	Reference
Thiobacillus species	Degorce-Dumas *et al*.(1997); Nishimura and Yoda (1997); Koe and Yang (2000); Oh *et al*.(1998)
Thiobacillus thioxidans	Cho *et al*.(2000); Cadenhead and Sublette (1990)
Thiobacillus denitrificans	Sublette *et al*.(1994); Sublette and Sylvester (1987a; 1987b)
Thiobacillus thioparus	Cho *et al*.(1992); Cadenhead and Sublette (1990)
Thiobacillus ferrooxidans	Jensen and Webb (1995)
Thiobacillus novellus	Chung *et al*.(1998)
Thiobacillus versutus	Cadenhead and Sublette (1990)
Thiobacillus neopolitanus	Cadenhead and Sublette (1990)
Pseudomonas putida	Chung *et al*.(1996; 2001)
Hyphomicrobium	Zhang *et al*.(1991)
Xanthomonas species *DY44*	Cho *et al*.(1992)

Chung *et al*.(1996) immobilized *Thiobacillus thioparus* CH11 with Ca-alginate producing pellet packing material for the biofilter. At a 28 s optimal retention time, the H_2S removal efficiency was more than 98%. Elemental sulfur or sulfate was produced depending on the inlet H_2S concentration.

Gadre (1989) also passed actual biogas from a lab scale anaerobic digester (~55% CH_4, ~42.5% CO_2, and 2.04% H_2S) through a 50-ml glass-bead-packed biotrickling filter washed with inoculum isolated from distillery wastewater. The collection vessel for the wastewater was open to the atmosphere and assumed to contain aerobic *Thiobacillus* species due to

a pronounced drop in pH of 3.0. 69.5% H_2S removal was achieved at a loading rate of 187 mg H_2S/d (Gadre, 1989).

Nishimura and Yoda (1997) performed a similar experiment with a more methane rich biogas (~80% CH_4, ~20% CO_2, and 2000×10^{-6} H_2S), and were able to achieve 99.5 % reduction in H_2S with a gas flow of 40 m^3/h in a bubble column reactor.

Manure composts have been used for biofiltration of other compounds as well. Chou and Cheng (1997) tested compost pig- and cow-manure media mixed with wood chips and activated sludge, for removing methyl ethyl ketone (MEK), and achieved removal rates of around 50 g/(m^3 solids·h).

Chung *et al*.(1997) used *Thiobacillus novellus* in a biofilter for H_2S oxidation under mixotrophic conditions. A removal efficiency of 99.6% was achieved with the products being sulfate (83.6%) and sulfite (12.6%). Little conversion of sulfide to elemental sulfur was achieved. Later, Chung *et al*.(2001) used biofilters packed with co-immobilized cells *Pseudomonas putida* CH11 and *Arthobacter oxydans* CH8 for removal of H_2S and NH_3, respectively, which are often present in off-gases of a livestock farm. In the 5×10^{-6}~65×10^{-6} range, H_2S and NH_3 removal efficiencies were greater than 96%. However, at higher concentrations, H_2S and NH_3 showed inhibitory effects on H_2S removal. They also assessed the environmental risk associated with the release of bacteria when treating large volumes of waste gases. The exhaust gas contained small amounts of bacteria (<19 CFU/m^3 in all cases) and was considered safe.

Degorce-Dumas *et al*.(1997) tested biofilter columns with peat and dry wastewater sludge on actual biogas (characterized as 50%~60% CH_4, 40%~50% CO_2 and 0.5%~2.0% H_2S), mixed 2:1 with air. The H_2S concentration in the gas stream was measured at 3260 mg/m^3 (2375×10^{-6}), and the column maintained 100% removal efficiency for 10 d at a loading rate of 129 g H_2S/(m^3 solids·h). Autoclaved compost, used as a control, showed only 60% H_2S removal under similar conditions. A Henry's law calculation indicated that the abiotic removal efficiency cannot come only from H_2S absorption into water, but must also be from chemisorption.

Cardenas-Gonzalez and Ergas (1999) compared properties of immature and mature yard waste and horse-manure composts for biofiltration of VOC's and found that horse manure compost had higher microbial activity and shorter acclimation time, but was not so stable for long term operation.

Wani *et al*.(1999) described the removal characteristics of H_2S and other reduced sulfur compounds emitted from kraft pulp mills using three different biofilter mediums: compost, hog-fuel (pulverized mixture of raw bark, wood waste, and other materials) and a mixture of compost and hog fuel at 1:1 (w/w) ratio. Dolomitic lime was mixed with each medium to act as a pH buffer. No significant difference was observed in the H_2S elimination capacities of these three media. However, the pH of the media decreased significantly over an operating period of more than six months. At H_2S concentrations up to 250×10^{-6} (v/v), complete removal was observed. The removal efficiency for inlet concentrations higher than 250×10^{-6} (v/v) was above 90%. Compost, hog-fuel, and the mixture media had maximum elimination capacities of 136, 137, and 138 g/(m^3·h), respectively.

A comparison between removal efficiencies of inorganic (H_2S) and organo-sulfur (methyl mercaptan, dimethyl sulfide, and dimethyl disulfide) odor compounds by immobilized *T. novellus* is presented in the study of Cha *et al*.(1999). They observed *T. novellus* can degrade H_2S>methyl mercaptan>dimethyl disulfide>dimethyl sulfide with removal efficiency being 100% for H_2S and methyl mercaptan, 87% for dimethyl disulfide, and 73% for the dimethyl sulfide. The final metabolic product was sulfate.

To investigate inorganic media supports for durability during low-pH H_2S biofiltration, Cho *et al*.(2000) specifically immobilized *Thiobacillus thioxidans* on lava rock. The rock showed favorable moisture retention and resisted excessive pressure drops. Increased removal capacities up to 428 g S/(m^3 solids·h) were reported with space velocities of 300 h^{-1} (Cho *et al*., 2000). In a previous study, Cho *et al*.(1992) reported 89% removal of dimethyl-sulfide, methanethiol, dimethyl-disulfide, and H_2S with a *Thiobacillus thioparus* biofilter treating exhaust gas from a night-soil (septic sludge) treatment plant.

Shareefdeen *et al*.(2002) reported the operation of a commercial biofilter for the treatment of an air stream containing hydrogen sulfide, ammonia, dimethyl sulfide, methanethiol, and ethylamine. This proprietary wood-based (BIOMIX™) biofilter

achieved 96.6% removal of H_2S at an inlet concentration of 1.07 mg/m^3.

Elias *et al*.(2002) used packing material made up of pig manure and sawdust for biofiltration purposes. More than 90% H_2S removal efficiency was attained at a loading rate of 45 g/(m^3·h). No nutrient was added to the system with the porosity of the packing material decreasing from 23.1% to 12.9%. However, this change in porosity did not affect the removal efficiency significantly and it was claimed that the biofilter could be easily cleaned by flushing water through the inlet. The main by-product of the biodegradation process was sulfur (82% of total sulfur accumulation), accompanied by sulfates and thiosulfates (<18%).

Kim *et al*.(2002) investigated the simultaneous removal of H_2S and NH_3 using two biofilters, one packed with wood chips and the other with granular activated carbon (GAC). A mixture of activated sludge (as a source of nitrifying bacteria) and *Thiobacillus thioparus* (for sulfur oxidation) was sprayed on the packing materials and the drain solution of the biofilter was recirculated to increase the inoculation of microorganisms. Initially both of the filters showed high (99.9%) removal efficiency. However, due to the accumulation of elemental sulfur and ammonium sulfate on the packing materials removal efficiency decreased over time to 75% and 30% for H_2S and NH_3, respectively.

Kleerebezem and Mendez (2002) proposed the simultaneous degradation of H_2S and NH_3 using *T. denitrificans* and nitrifying bacteria. *Thiobacillus denitrificans* is able to degrade H_2S in both aerobic and anaerobic conditions using oxygen or nitrate as an electron acceptor. Nitrate (NO_3^-) can be obtained from the nitrification of ammonia by nitrifying bacteria. *Thiobacillus denitrificans* simultaneously oxidizes H_2S to elemental sulfur or sulfate and reduces nitrate to nitrogen gas. Stoichiometric equations for autotrophic denitrification by *T. denitrificans* are presented in Table 1 (Kleerebezem and Mendez, 2002; Lampe and Zhang, 1996).

Malhautier *et al*.(2003) also performed an experiment involving H_2S and NH_3 using two laboratory scale biofilters packed with granulated digested sludge. One unit was fed mainly with H_2S and the other unit with NH_3. Complete H_2S removal (100%) was obtained and no influence on NH_3 or H_2S removal was observed. An 80% NH_3 removal efficiency was obtained, however, the authors concluded that the oxidation of high levels of H_2S might have a negative effect on the growth and activity of nitrifying bacteria.

Morgan-Sagastume *et al*.(2003) investigated changes in the physical properties of a compost biofilter treating hydrogen sulfide. Bench-scale biofilter columns filled with compost media consisting of mature compost derived from food, leaf, yard waste, and horse manure were used. H_2S removal efficiency decreased from 100% to 90% over 206 d of operation. They concluded that the variation in moisture content and specific surface area can explain the decrease in removal efficiency over time. They also mentioned that SO_4^{2-} accumulation which reduced the pH of the compost media from 7.5 to 4.5 could be an important factor. After re-mixing of the compost media, the H_2S removal efficiency returned to near 100%.

Oyarzún *et al*.(2003) used peat for the filter bed of a biofiltration system inoculated with *Thiobacillus thioparus*. Supplemental nutrient was added and the initial moisture content was adjusted to 92%. The pH was also adjusted to 6.0. Full removal was achieved when fed with 355×10^{-6} H_2S at 0.03 m^3/h. The removal efficiency decreased with increasing inlet H_2S concentrations and a maximum removal capacity of 55 g/(m^3·h) was obtained.

Schieder *et al*.(2003) described the "BIO-Sulfex" biofilter to remove H_2S from biogas which uses thiobacteria attached on fixed bed material. The biomass was aerated and the filter was flushed with nutrient containing liquid to remove sulfur from the system. Six BIO-Sulfex modules to treat biogas containing up to 5000×10^{-6} H_2S were operated at flow rates of 10 to 350 m^3/h with 90% or more H_2S removal achieved.

Clark *et al*.(2004) operated a pilot-scale agricultural biofilter to reduce odors from a swine manure treatment plant's exhaust air. Biofilters were packed with polystyrene particles and peat moss (3:1 ratio by volume). The packing volume was 1.89 m^3. The gas flow rate was 100 L/s. The inlet load contained 2×10^{-6}~60×10^{-6} hydrogen sulfide and 2×10^{-6}~30×10^{-6} ammonia. The addition of nutrients did not play an important role in the overall system performance. Average odor reduction was approximately

38% without addition of nutrients and 45% when nutrients were added. Increasing the temperature had a favourable effect during the acclimatisation phase only.

Biotrickling filter

The working principle of a biotrickling filter (Fig.1c) is the same as for a biofilter except that the packed bed is continuously trickled over by an aqueous phase nutritive solution (Cox and Deshusses, 2001). Cox and Deshusses (2001) used two laboratory scale biotrickling filters made of polypropylene, inoculated with biomass from a toluene biodegrading filter operating at pH of 7.0 and 4.5 to treat H_2S and toluene in a gas stream. There was no significant difference between the performances of the two reactors in terms of H_2S removal. At an inlet concentration of approximately 50×10^{-6} (v/v), complete consumption of H_2S was observed. However, the removal efficiency decreased to 70%~80% when the inlet concentrations were raised to 170×10^{-6} (v/v).

High removal efficiency for H_2S, in comparison to other reduced sulfur compounds was obtained by Gabriel and Deshusses (2003) using *Thiobacillus* sp. in a biotrickling filter. For inlet H_2S concentrations as high as 30×10^{-6}, typical removal efficiency was 98%. Methyl mercaptan, arbonyl sulfide, and carbon disulfide removal efficiencies were 67%, 44%, and 35% at inlet concentrations of 67, 193, and 70×10^{-9}, respectively.

Sercu *et al*.(2005) studied the aerobic removal of hydrogen sulfide using a biotrickling filter packed with 1 L-polyethylene rings (73% volume free) inoculated with *Acidithiobacillus thiooxidans* ATCC-19377. The inlet H_2S concentration was varied between 400×10^{-6} and 2000×10^{-6} and the airflow rate was varied between 0.03 and 0.12 m^3/h. However, the system performance was not affected by changing the operational conditions and a maximal removal efficiency of 100% was obtained. During the experiment, the pH of the nutritive solution decreased to 2~3, but this did not affect the process performance.

Soreanu *et al*.(2005) developed a laboratory-scale biotrickling system to remove H_2S from digester biogas under anaerobic conditions. In these experiments, polypropylene balls inoculated with anaerobically digested sludge were used as packing material in the bioreactor (packing volume of 0.0062 m^3, 90% volume free). Sodium sulfite was added in the nutritive solution as an oxygen scavenging agent. Nitrate was used as electron acceptor in the absence of oxygen. Removal efficiency greater than 85% was achieved for an H_2S inlet concentration of 500×10^{-6} and a gas flow rate of 0.05 m^3/h. Of particular interest, inhibition of the biological process by trace amounts of O_2 was noticed when a nitrate solution was used as the sole nitrogen/nutrient source.

Chemotrophic bacterial sulfide removal

A number of studies were conducted using chemotrophic (Nishimura and Yoda, 1997; Chung *et al*., 1997; Oh *et al*., 1998) and photoautotrophic (Basu *et al*., 1996; Kim and Kim, 1996) bacteria to convert H_2S to S^0 since Henshaw *et al*.(1998) summarized the work done in this area prior to 1995. The photoautotroph of choice is the naturally occurring green sulfur bacterium (GSB) *Chlorobium thiosulfatophilum*. A sulfide loading of 104 mg/(h·L) was achieved by Basu *et al*.(1996) in a continuous stirred-tank reactor equipped with a sulfur separator. A 20 min gas retention time was required to remove the hydrogen sulfide from a gas stream containing 2.5% H_2S at 1.01×10^5 Pa and converted up to 99.2% to elemental sulfur. Kim and Kim (1996) used a plate-type gas-lift photoreactor with LEDs emitting at a wavelength tuned to one of the GSB infrared absorption peaks for enhanced light efficiency. In each of these latter two investigations, sulfide was fed into the reactor as a gas as opposed to removing sulfide directly from wastewater.

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. Also, phototrophic bacteria generally store the produced sulfur internally, making a separation of cells and sulfur impossible. Secondly, the oxidation of sulfide to elemental sulfur or sulfate by using chemolithoautotrophic bacteria belonging to the genus *Thiobacillus* is frequently described (Buisman *et al*., 1990; Jensen and Webb, 1995).

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 colorless sulfur bacteria looks to be the

most reliable biotechnological approach for H_2S removal (Buisman *et al*., 1990; Jensen and Webb, 1995), because these organisms have very simple nutritional requirements; i.e., they utilize reduced inorganic sulfur compounds as their electron donors and carbon dioxide as carbon source. The two most important bioconversions in an aerobic sulfide-oxidizing bioreactor are (Kuenen, 1975):

$$2HS^-+O_2\rightarrow 2S^0+2OH^- \quad (\Delta G^{0'}=-129.50\ \text{kJ/(mol HS}^-)), \tag{8}$$

$$2HS^-+4O_2\rightarrow 2SO_4^{2-}+2H^+ \quad (\Delta G^{0'}=-772.43\ \text{kJ/(mol HS}^-)). \tag{9}$$

The presented $\Delta G^{0'}$ values (STP) are given for physiological conditions, i.e., at pH 7. Under oxygen-limiting circumstances, i.e., at oxygen concentrations below 0.1 mg/L, sulfur is the major end-product of the sulfide oxidation (Eq.(8)), whereas sulfate is formed under sulfide-limiting circumstances (Eq.(9)) (Janssen *et al*., 1995). In biological treatment plants, the formation of sulfur is preferred for several reasons. First, elemental sulfur is nonsoluble and can therefore, in principle, be removed from the water stream. Moreover, the recovered sulfur can be purified and reused as a valuable raw material; e.g., in bioleaching processes (Tichý *et al*., 1994). Second, sulfate formation requires four fold more oxygen and, consequently, higher energy consumption is needed for aeration.

Reaction products from many of these microorganisms include sulfates and H^+, which form sulfuric acid in the leachate and reduce the pH. Some *Thiobacillus* species are acidophilic and therefore function adequately at low pH, but organic media tend to degrade under these conditions causing plugging and increased pressure.

A comprehensive study of operational parameters, design, and basic kinetic modeling for removing H_2S from air with composted sewage sludge and yard waste was conducted by Yang and Allen (1994a). Variables studied included temperature, residence time, concentration, loading rate, compost sulfate level, acidity, and water content. H_2S removal efficiencies greater than 99.9% were noted using yard waste composts and inlet concentrations ranging from 5×10^{-6} to 2650×10^{-6}. Maximum elimination capacities for the composts ranged from 11.5 to 130 g S/(m^3 solids·h) (Yang and Allen, 1994a; 1994b).

H_2S levels up to 10000×10^{-6} were oxidized by Sublette *et al*.(1994) with pure cultures of *Thiobacillus denitrificans* in less than 2 s under anoxic conditions. Here, added nitrate, rather than oxygen, served as the terminal electron acceptor. These reaction times indicated that limitations in H_2S removal were due to mass transfer rather than biological limitations. Up to 97% reduction in inlet H_2S levels was achieved (Sublette *et al*., 1994).

Koe and Yang (2000) also tested *Thiobacillus thioxidans* with plastic packing and found that for gas-retention times greater than 5 s and a loading rate below 90 g S/(m^3 solids·h), 99% H_2S removal was obtained.

Vaiopoulou *et al*.(2005) treated hydrogen sulfide from oil-refining wastewater through autotrophic denitrification. Anoxic sulfide to sulfate oxidation, with nitrate as a terminal electron acceptor, proved very successful, as incoming concentrations of 110 mg S^{2-}/L were totally converted to sulfate. At complete denitrification, the concentration of S^{2-} in the reactor effluent was less than 0.1 mg/L. Fluctuating S^{2-} concentration in the feed could be tolerated without any problems, as the accumulated sulfide in the effluent of the denitrification stage is oxidized aerobically in a subsequent activated-sludge treatment stage. This alternative new treatment scheme was further introduced at the refinery's wastewater processing plant. Thus, complete H_2S removal is now accomplished by the combination of the proposed biological method and the existing stripping with CO_2. As a result, stripping, and thus its cost, is reduced by 70%.

Mathioudakis *et al*.(2005) tested a biological method for controlling odor problems caused by H_2S originating from sewer networks under anaerobic conditions. The proposed method is based on the continuous addition of nitrate which oxidizes dissolved sulfide according to an autotrophic biological procedure and inhibits further sulfide production by sulfate reducing bacteria, until complete denitrification. The proposed method was first laboratory tested in an anaerobic batch reactor simulating municipal wastewater. Addition of nitrate in non-septic (not sulfide containing) wastewater inhibits the production of sulfide until complete denitrification. Heterotrophic denitrification rate was found to be 4.5 and 3.9 mg NO_3-N/(L·h) at 25 °C and 30 °C respectively.

Higher C/N ratio is, probably, responsible for the increased denitrification rate of the lower temperature. The addition of nitrate into septic wastewater led to preferential autotrophic denitrification with sulfide as electron donor at a rate of 0.8 and 1.5 mg NO_3-N/(L·h) at 25 °C and 30 °C, respectively. After complete sulfide oxidation, heterotrophic denitrification takes place inhibiting any further sulfate reducing activity. Based on these experimental results, a continuous addition of 10 kg NH_4NO_3/h is proposed for practical implementation as the optimal dosing, considering sufficient odor control and tolerable increase of the ammonia load. The proposed method is not only effective but also financially interesting taking into account the facility cost and the monthly operational cost, during the summer months of the year (Mathioudakis *et al.*, 2005).

FUTURE PROSPECTS OF SULFIDE REMOVAL

Biotreatment of heavy metals

Sulfate-rich wastewaters from various sources, e.g. spoil leachate, acid mine drainage and landfill percolates, are often also contaminated with heavy metals (Tichý *et al*., 1998). The extremely low solubility of metal sulfides produced in sulfidogenic bioreactors, allows the removal of heavy metals from the water stream by precipitation (Gadd and White, 1993). Hence, maximization of sulfide production in sulfate-reducing reactor units is an attractive way to remove both sulfate and heavy metals from wastewater (Hao *et al*., 1996). As these types of wastewater usually contain little or no organic matter, an alternative electron donor should be added (Hulshoff Pol *et al*., 1998). Full scale processes are currently applied for heavy metal removal from Zn and Cd (effluent concentrations below the $\times 10^{-9}$ range) contaminated groundwater at a long-standing smelter site (Scheeren *et al*., 1991).

Nitrogen removal

Nitrogen and sulfur metabolism interact at various levels of the wastewater treatment process (Hulshoff Pol *et al*., 1998). Sulfide is toxic as it nitrifies (Hooper and Terry, 1973) and thus can upset nitrification in the aerobic post treatment. Besides, Sulfate Reducing Bacteria (SRB) can readily utilize most of the carbon sources added to denitrify wastewater (e.g. methanol and lower volatile fatty acids), thus leading to loss of carbon source for the denitrification step. However, methanol utilization by SRB can be avoided by proper selection of the hydraulic residence time (van der Hoek *et al*., 1988). On the other hand, sulfide can also contribute to nitrogen removal. As electron donor it can be reoxidized to S^0 or sulfate by *Thiobacillus denitrificans* using nitrate as electron acceptor. This type of denitrification reduces the overall requirements for carbon at a nutrient removal plant (Garuti *et al*., 1992). Also SRB can be involved in alternative denitrification routes, as some SRB can use nitrate, instead of sulfate, as terminal electron acceptor (Widdel, 1988). However, SRB convert nitrate to ammonium, and thus other treatment steps are needed if complete nitrogen removal is required. Hence, the sulfur cycle offers possibilities to integrate nitrogen removal in the treatment process, which need to be further optimized by appropriate design of the reactor configuration (Hulshoff Pol *et al*., 1998).

There has been a considerable amount of interest in the past 10 to 15 years in the design of nitrification-denitrification systems employing nitrite as the primary intermediate—thereby eliminating the formation of nitrate—in both the nitrification and denitrification steps. Numerous authors (Chen *et al*., 1991; Balmelle *et al*., 1992; Fdz-Polanco *et al*., 1996; Garrido *et al*., 1997; Yoo *et al*., 1999) reported the capital and operational benefits of the process, referred to here as the nitrate shunt, to include a 25% reduction in aeration requirements, a 40% reduction in external carbon addition for denitrification, a potential reduction in anoxic zone volume, and a significant reduction in sludge production.

Partial nitrification techniques, such as the continuously aerated SHARON (single reactor high activity ammonia removal over nitrite) or ANAMOX (anaerobic ammonia oxidation) process, have been denoted for quite a while as very promising for improved sustainability of wastewater treatment (Abeling and Seyfried, 1992). In the SHARON process, partial nitrification of ammonium nitrogen to nitrite nitrogen is established by working at high temperature (above 25 °C) and maintaining an appropriate sludge retention time (SRT) of 1 to 1.5 d, so that ammonium oxidizers are maintained in the reactor,

while nitrite oxidizers are washed out and further nitrification of nitrite to nitrate is prevented. In this way, significant aeration cost savings is realized in comparison with conventional nitrification to nitrate. Partial nitrification techniques are very suitable for reducing the load of streams with high ammonium concentration rather than to meet strict effluent standards.

The principles of partial nitrification and sulfide oxidation can be utilized to investigate simultaneous biological sulfide and nitrite removal from wastewaters. Effluents from partial nitrification processes can be used in anoxic sulfide oxidizing reactor (ASOR) to achieve simultaneous nitrite and sulfide removal. Nitrite can be used as electron acceptor instead of nitrate to test the potential of anoxic sulfide oxidizing reactor (ASOR) because nitrite should be more reactive in aqueous medium as compared with nitrate.

Control of odor from sewers

Because of the severe environmental and economic problems that arise from microbial hydrogen sulfide production, its removal from wastewater has become mandatory (Mathioudakis *et al*., 2005). Hydrogen sulfide is associated with corrosion of metal and cement pipes (USEPA, 1998), operational problems in wastewater treatment plants (WWTP) (Æsøy *et al.*, 1997), as well as with hygiene and odor problems (Hvitved-Jacobsen *et al*., 2002).

Among others chemicals such as oxygen, hydrogen peroxide and ferric salts inhibiting H_2S formation or removing sulfide from wastewater, addition of nitrate seems a promising technique when applied to fields like oil industries—to avoid oil souring or precipitation of metal sulfide in reservoirs (Davidova *et al.*, 2001; Melidis *et al.*, 2004) and sewer systems for odor control (Mathioudakis *et al*., 2005). In regard to sewer networks, microbial utilization of dissolved oxygen and nitrate leads to the prevalence of anaerobic conditions (septicity). A large number of substances, produced under anaerobic conditions, lead to odor problems, e.g. fermentation products, volatile organic compounds and hydrogen sulfide. From an engineering point of view, hydrogen sulfide can be considered the appropriate indicator for odor control in wastewater treatment plants and sewers (Hvitved-Jacobsen *et al.*, 2002; Gostelow and Parsons, 2000).

Although sulfide formation is typically recognized as a problem in pressure mains, it is also observed in gravity sewers with slow flow rates and insufficient reaeration potential at relatively high temperature. Better understanding of chemical and microbial processes taking place in sewers has led to a greater use of nitrate for control of odor due to hydrogen sulfide (Nielsen *et al*., 1992; Hvitved-Jacobsen *et al*., 2002). The effectiveness of the use of nitrate in odor control has been proved recently in sewer networks. Addition of nitrate in septic wastewater biologically oxidizes dissolved sulfide via autotrophic denitrification by sulfur-oxidizing denitrifying bacteria such as *Thiobacillus denitrificans* and *Thiomicrospira denitrificans.* When dissolved sulfide is completely oxidized, excess concentration of nitrate is reduced via heterotrophic denitrification inhibiting any further sulfide production (Mathioudakis *et al*., 2005). Nitrite can also be used as electron acceptor to biooxidize sulfide for odor control in sewer networks.

CONCLUSION

(1) Sulfide in waste streams is generated from various sources and is emitted into the environment as dissolved sulfide (S^{2-} and HS^-) in wastewaters and as H_2S in waste gases. Hydrogen sulfide prefers to exist in the gas phase; thus, once produced in the aqueous phase, it will rapidly partition to the gas phase.

(2) To remove sulfides from wastewater streams, a number of physicochemical processes are in common use today involving 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. Microbiological processes can proceed around ambient temperatures and at atmospheric pressure, thus eliminating the need for heat and pressure, reducing energy costs to a minimum.

(3) Biologically active agents have been used in a variety of process arrangements, such as biofilters, fixed-film bioscrubbers, and suspended-growth bioscrubbers. These processes may also be effective at removing multiple contaminants from a gas stream, increasing their functionality. Fluidized-bed biore-

actors have recently been tested for simultaneous removal of H_2S and NH_3 with promising results.

(4) The extremely low solubility of metal sulfides produced in sulfidogenic bioreactors, allows the removal of heavy metals from the water stream by precipitation, thus maximization of sulfide production in sulfate-reducing reactor units is an attractive way to remove both sulfate and heavy metals from wastewater.

(5) Nitrogen and sulfur metabolism interact at various levels of the wastewater treatment process. The principles of partial nitrification and sulfide oxidation can be utilized to investigate simultaneous biological sulfide and nitrite removal from wastewaters. This type of denitrification reduces the overall requirements for carbon at a nutrient removal plant.

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