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TARJA T. HARTIKAINEN

Biological Purification of Odorous Sulphide Compounds from Effluent Gases

Doctoral dissertation

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ABSTRACT

Anthropogenic emissions of odorous sulphide compounds, which have an unpleasant odour even at low concentrations and a very low odour threshold value, have increased during the last century. Sulphide odours can cause direct health effects, which may be non-specific, multi-systemic symptoms that are not easily traceable to a specific chemical or odour. In addition, an odorous environment can cause decreases in property values.

With the rising concern over environmental conditions, air pollution control (APC) technologies have been developed to handle emissions of harmful and odorous chemicals. Biological air purification research has increased during recent decades, because this approach represents a natural, quite simple and cheap way to purify emissions. Such biological methods include biofiltration, bioscrubbing and biotrickling filtration systems, in which harmful substances are adsorbed onto biofilms, where they are oxidised and degraded to less harmful substances by specific microorganisms. The usual end product of the oxidation of sulphur compounds is a sulphate (SO_4^{2-}). The research reported here is concerned with the biofiltration and biotrickling filtration of odorous sulphide: hydrogen sulphide (H_2S), methyl mercaptan (MeSH) dimethyl sulphide (Me_2S) and carbon disulphide (CS_2) gases.

Peat was found to be suitable material for biofilm formation and the removal of low loads (< 175 g S m^{-3} day^{-1}) but high loadings lead to overloading of the peat material, resulting in a short lifetime for the biofilter. Industrial emissions usually have high concentrations of odorous gases, and biotrickling filters were developed for these cases. The waste air was passed into the trickle bed through a circulation liquid layer, and this liquid phase treatment increased the efficiency of H_2S and CS_2 removal.

Removal efficiencies of CS_2 and H_2S were studied with several packing materials: apatite-biotite ore, ceramic saddles, lava rock and plastic grit. In the laboratory experiments biotrickling filters removed over 92% of sulphide from effluent gases while removals in the pilot-scale experiment varied between 42-77%. Ceramic saddles were found to be the best packing material for an acid environment achieving highest maximum removal capacities in several experiments (2690-5860 g S m^{-3} day^{-1}).

In addition to suitable packing material effective removal requires also an efficient micro-organism. The *Thiobacillus* strain TJ330, which oxidises CS_2, was isolated from biofilters, enriched and then used as an inoculum to improve a biotrickling filter. The strain was deposited in the DSM (Deutsche Sammlung von Microorganismen und Zellculturen) culture collection, where it was given the registration number DSM 8985.

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Medical Subject Headings: air pollution / prevention & control; air pollutants; gases; odors; filtration; biofilms; sulfides; hydrogen sulfide; carbon disulfide; *Thiobacillus*

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Tampere, February 2003

Tarja T. Hartikainen

ABBREVIATIONS

BAPC	Biological air pollution control
BAT	Economically best available technology
COS	Carbonyl sulphide
CS_2	Carbon disulphide
DSM	Deutsche Sammlung von Microorganismen und Zellculturen
EBRT	Empty bed retention time
EC	Elimination capacity (g S m^{-3} h^{-1} or g S m^{-3} day^{-1})
FGD	Flue gas desulphurisation
FPD	Flame photometric detector
H_2S	Hydrogen sulphide
MeSH	Methyl mercaptan
Me_2S	Dimethyl sulphide
Me_2S_2	Dimethyl disulphide
OCS	Carbonyl sulphide
OEL	Occupational exposure limits
RC	Removal capacity (g S m^{-3} h^{-1} or g S m^{-3} day^{-1})
RE	Removal efficiency (%)
SEM	Scanning electron microscope
VOC	Volatile organic compounds
VSC	Volatile sulphur compounds

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on data presented in the following publications:

I Hartikainen T., Martikainen P.J., Olkkonen M. and Ruuskanen J. (2002) Peat biofilters in long-term experiments for removing odorous sulphur compounds. Water Air and Soil Pollution, 133, 335-348.

II Hartikainen T., Ruuskanen J., Räty K., von Wright A. and Martikainen, P. (2000) Physiology and taxonomy of Thiobacillus strain TJ330, which oxidizes carbon disulphide (CS_2). Journal of Applied Microbiology, 89, 580-586.

III Hartikainen T., Ruuskanen J. and Martikainen, P. (2001) Carbon disulfide and hydrogen sulfide removal with a peat biofilter. Journal of the Air and Waste Management Association, 51,387-392.

IV Hartikainen T., Martikainen P.J., Ruokojärvi A., Nummela J., Salmi J. and Ruuskanen J. A biotrickling filter to remove carbon disulphide and hydrogen sulphide from waste gases. Manuscript

V Hartikainen T., Ruokojärvi A., Martikainen P.J. and Ruuskanen J. Removal of carbon disulphide and hydrogen sulphide by a biotrickling filter with various packing materials. Manuscript

CONTENTS

1 INTRODUCTION

Carbon disulphide (CS_2), carbonyl sulphide (COS), dimethyl sulphide (Me_2S) and hydrogen sulphide (H_2S) are the main sulphurous trace gases found in the atmosphere (Watts 2000). These volatile sulphur compounds (VSC's) originate from natural and anthropogenic processes. Volcanoes and seas are among the most important natural sources, while anthropogenic emissions are produced when organic matter is treated in anaerobic biological processes (Smet et al. 1998b) and by certain industrial processes (production of viscose, pulp and paper). Anthropogenic emissions of these compounds have increased significantly over the last century, most notably from waste water treatment, composting and rendering plants, in which organic material containing sulphur is decomposed on purpose or unintentionally anaerobically, especially where the sulphurous amino acids methionine and cysteine are involved (de Zwart et al. 1992, Nakasaki et al. 1998). The reduced sulphur gases are oxidised in the atmosphere, causing sulphate particles or acid deposition (acid rain) in addition to local odour and health effects. Anthropogenic emissions account for about 92% of total sulphur emissions in the northern hemisphere. Sulphurous gases participate in the formation and growth of aerosol particles, which alter the optical properties of clouds and contribute to the atmospheric radiation budget (Lovelock et al. 1972, Turco et al, 1980, Charlson et al. 1987, Bates et al. 1992, Shooter 1999, Watts 2000), and these volatile compounds also have toxic (Milby et al. 1999a, 1999b) and corrosive effects, a very low odour threshold value and a negative hedonic value, in that emissions of very small amounts contribute greatly to odour pollution (Smet et al. 1998a, 1998b). There is a "grey line" between odour nuisance and health effects, because the latter largely entail non-specific symptoms, such as headaches, nausea, gastro- indestinal distress, fatigue, eye and throat irritation, sleep disturbance, inability to concentrate, classical stress response. (McGinley and McGinley 1999).

Public concern about air pollution has been growing, and efficient air pollution control (APC) techniques are needed to remove emissions that cause locally unpleasant odours and harmful health effects. Odour regulations in many countries are expected to alter in the near future as measurement and control techniques improve (Mahin 2001). Both biotechnological and physico-chemical technologies can be efficient in controlling emissions of volatile sulphide compounds, whereas incineration is an expensive cleaning technology when flow rates are high and odorous pollutant concentrations are low. There is no waste gas treatment technology that can effectively and economically be applied to every appli-

cation (Devinny et al. 1999). As physico-chemical technologies are not always possible, the applications of biotechnological methods have increased markedly since around 1980. A demand for more economical and ecological purification method – eco-efficient method has increased (Ruokojärvi A 2002). The term BAT (economically best available technology) is included also in the environmental legislation. However use these biological methods is not new idea. The oldest biological air purification methods were published in the 1950s and 1960s, including some of the first known biofilters, which were soil beds with an air distribution system of perforated pipes at the base of the soil (Pomeroy, 1957). Biofiltration was developed for the reduction of odorous waste gases arising from waste water treatment (Pomeroy, 1957, Carlson et al. 1966), and this marked the beginning of biological air pollution control (BAPC), which is based on the oxidation of harmful compounds by suitable micro-organisms. These biotechnological methods range from simple odour control systems (soil bed biofilters) to technically controlled units (biotrickling filters, bioscrubbers) for removing harmful compounds issuing from industrial sources (Swanson et al. 1997, Devinny et al. 1999). Research into environmental biotechnology will continue, particularly with respect to clean-up processes and the development of sustainable technology (Scragg 1999).

More knowledge is still needed on micro-organisms and technical facilities capable of transforming volatile sulphur compounds, in order to optimise the biological purification process. Since mixtures of sulphur gases in effluent air have caused problems in removal processes, the work reported here examines principally the purification of mixtures of odorous sulphur gases (H_2S, CS_2, Me_2S and MeSH) and further development of biological gas purification. Biofiltration experiments were commenced using Finnish peat as the filter material (results presented in Papers **I** and **III**). Special attention was then paid to the biodegradation of CS_2, as little research had been done into its microbial treatment. Thus Paper **II** reports on a newly isolated microbial strain capable of oxidising CS_2 in an acidic environment. Biotrickling filters and new purification method were studied in Paper **IV**, and filter bed materials investigated more intensively in Paper **V**. Thus Papers **I**, **II** and **III** are concerned with conventional biofiltration for odour control, while Papers **IV** and **V** concentrated on biotrickling filtration for the purification of industrial emissions.

2 REVIEW OF THE LITERATURE

2.1 Odorous sulphide gases

The biogeochemical sulphur cycle involves a large variety of organic and inorganic sulphur compounds with oxidation states ranging from –2 (sulphide: H_2S, HS^-, S^{2-}) to +6 (sulphate: SO_4^{2-}). There are still many uncertainties concerning the chemical species concerned and the magnitude of the natural emission of sulphur gases into the atmosphere, and the full global budget is still unknown (Watts 2000).

2.1.1 Sources of sulphide gases

The main sources of sulphur gases are biogenic fluxes from the oceans and from soils. The generalised global emissions of sulphur gases (excluding sulphur dioxide) are estimated to consist of 75% Me_2S, 15% H_2S and 10% CS_2 + COS, with minor contributions from other gases such as methanethiol (methyl mercaptan), dimethyl disulphide, dimethyl sulphoxide and higher molecular weight alkyl sulphides (Kelly et al. 1990). The present atmospheric concentrations, lifetimes and global sources and sinks of sulphur compounds are shown in Table 1. The global sources also include anthropogenic emissions. The budgets for COS and Me_2S are relatively well known, but those for H_2S and CS_2 entail greater uncertainties (Watts 2000). Anthropogenic emissions are estimated to be largest relative to total flux in the case of H_2S and CS_2, 42.7% and 51.5%, respectively, but the budgets involve many uncertainties and more information on emissions is needed (Watts 2000).

Table 1. Global mass budgets for the main sulphurous trace gases.

	Atmospheric Concentration ppb	Atmospheric Lifetime days	Global Sources Tg a^{-1}	Anthropogenic Sources Tg a^{-1}	Global Sinks Tg a^{-1}
H_2S	0.2[1]	1[2]	7.72 ±1.25[3]	3.30 ± 0.33[3]	8.50 ±2.80[3]
MeSH	n.d.a.	0.2[2]	1.3-3.4[5]	n.d.a.	n.d.a.
Me_2S	0.001[4]	1[5]	24.49 ±5.30[3]	0.13 ±0.04[3]	n.d.a.
COS	0.5[4]	360[6]- 730[4]	1.31 ±0.25[3]	0.12 ±0.06[3]	1.66 ±0.79[3]
CS_2	0.02[4]	12-40[4]	0.66 ±0.19[3]	0.34 ±0.17[7]	1.01 ±0.45[3]

[1] Lovelock et al. 1972, [2] Miller et al. 1988,[3] Watts 2000, [4] de Zwart et al. 1992, [5] Kelly et al. 1990,[6] Turco et al. 1980, [7] Chin et al. 1993

n.d.a.= no data available

Volatile sulphur compounds are produced by both terrestrial and marine biota (Charlson et al. 1987). Me_2S is the major sulphur gas released into the atmosphere from the oceans (Andreae et al. 1983, Watts 2000), while freshwater (Caron et al. 1994) and terrestrial emissions include a variety of sulphur species (Yang et al. 1996), because the microbial decomposition of organic matter produces a complex mixture of volatile sulphur species, whereas the planktonic algae and associated microbial activities in the oceans produce mainly Me_2S (Charlson et al. 1987). Rates of emission and expiration of volatile sulphur gases from terrestrial ecosystems are high at high temperatures (Yang et al. 1996), and the tropical regions generate 61% of all biogenic sulphur emissions (Bates et al. 1992). Volcanoes are non-biological natural sources of sulphur gases, their emissions being highly being variable in space and time (Charlson et al. 1987). Organic compounds are considered to be the dominant atmospheric component of the biogeochemical sulphur cycle (Lovelock et al. 1972).

Global anthropogenic sulphur emissions have increased with industrialization during the last century, so that while emissions were estimated to be 1.2 M tonnes in 1850, the value for 1990 was 71.5 M tonnes. The world's main sulphur emitters in 1990 were the USA, China and the former Soviet Union. The combustion of coal is estimated to be the predominant anthropogenic source of sulphur entering the atmosphere, mainly as SO_2 (Lefohn et al. 1999). The changeover from high to low-sulphur coals and the implementation of flue gas desulphurisation (FGD) have reduced sulphur emissions from combustion, but only a few countries have succeeded in reducing their emissions significantly (Lefohn et al. 1999). Other anthropogenic sources are waste water treatment (Kangas et al. 1986) and

bio-industries in which organic matter (e.g. proteins, carbohydrates or fats) is produced or converted into products in anaerobic or high-temperature processes (Smet et al. 1998a), mostly releasing hydrogen sulphide and organic sulphur compounds. CS_2 is produced by heating methane with sulphur under high pressure, and it is used in the manufacture of carbon tetrachloride and viscose, for example, and as a solvent for the extraction of oils, fats and waxes. Chemical uses are estimated to be the largest anthropogenic source of CS_2 (Khalil et al. 1984). Anthropogenic sources account for about 92% of total sulphur emissions in the northern hemisphere (between 35° and 50°N), while natural sulphur emissions are more important in the southern hemisphere (Bates et al. 1992).

2.1.2 Properties of sulphide gases

Odour threshold values, i.e. concentrations at which the odours attached to sulphide compounds can be noticed, are at ppb levels (Table 2). At low concentrations, Me_2S is a flavour component of the smell of the sea (de Zwart et al. 1992) and of many foodstuffs, including tea, cocoa and beer, but at higher concentrations it indicates spoilage (Kelly et al. 1990). The unpleasant smell of sulphide compounds constitutes a warning signal emitted from decaying material. Sulphide compounds become toxic well above their odour threshold, at the ppm level, but the sense of smell for the detection of H_2S becomes fatigued by continuous or high (150-250 ppm) exposure (Milby et al. 1999a). The odour of H_2S can create community nuisance problems at air concentrations approaching 0.25 ppm (Marttila et al. 1994, Partti-Pellinen et al. 1996) and the gas has irritating effects on the eye and the respiratory tract at around 10 ppm. Loss of consciousness after a few breaths occurs when inhalation exposure is about 500-1000 ppm, and higher concentrations, of the order of 1000-2000 ppm, are lethal (Milby et al. 1999a, 1999b). Exposure to CS_2 usually has occupational and neurotoxic effects (WHO 1996), and there is a decrease in libido and potency among males (Vanhoorne et al. 1994). Colour vision effects have also been reported (Vanhoorne et al. 1996). The Finnish occupational exposure limits (OEL) for these sulphur compounds are presented in Table 2. No OEL value has been determined for Me_2S in Finland, but De Zwart and Kuenen (1992) give a threshold limit value of 20 ppm.

Table 2. Odour thresholds, occupational exposure limits, Henry's coefficient and boiling points for odorous sulphur compounds (CRC Handbook of Chemistry and Physics 2000, De Bruyn et al. 1995 , http://www.occuphealth.fi/ttl/projekti/htp/english/oel_eng.htm 3.2.2002).

Compound	Odour threshold (ppb)	OEL 8 h /5 min (ppm)	Solubility (water, at 22°C) mg l^{-1}	Henry's coefficient at 25°C	Boiling point (°C)
H_2S	8.5-1000	10 / 15	i	0.087	- 60.71
MeSH	0.9- 8.5	0.5 /1.5	n.d.a	0.203	6.2
Me_2S	0.6-40	n.d.a	6 300	0.478	109.7
COS	n.d.a	n.d.a	1000 cm^3 l^{-1}	0.022	-50
CS_2	100-200	5 / 20	2 300	0.549	46.2

OEL = Finnish Occupational Exposure Limits , i= infinite, nda = no data availble

The most common route for exposure to these compounds is inhalation, as they are usually in gaseous form, hydrogen sulphide, carbonyl sulphide and methyl mercaptan being gases at 25°C, while carbon disulphide is a volatile liquid which can also penetrate through the skin. Henry's law coefficients describe water solubility, which increases under alkaline conditions in the case of H_2S (Millero et al. 1987, De Bruyn et al. 1995) but is unaffected by pH in the case of the other sulphur gases (De Bruyn et al. 1995). These compounds are always poorly soluble in water, however.

2.2 Microbial transformation of sulphur compounds

Biological purification methods are possible if pollutants can be transformed or biodegraded by micro-organisms in a suitable treatment system. The degraded compound may serve as an energy or material source for the micro-organisms. Carbon compounds usually serve as building materials, leading in the long run to an accumulation of biomass and causing clogging of the reactor (Devinny et al. 1999), whereas many micro-organisms use organic sulphur compounds as energy sources.

Sulphur can exist in various oxidation states, and many bacteria are able to obtain all the energy they need for growth from the oxidation of inorganic sulphur compounds (Kelly 1987). Micro-organisms oxidise sulphide, sulphur and sulphite progressively, usually to sulphate, which is a stable form in the presence of oxygen (Charlson et al. 1987, Kelly

1988), although some are able to produce elemental sulphur, usually under conditions of oxygen limitation or high substrate loading (Buisman et al. 1989a, Buisman et al. 1991, Stefess et al. 1996, Visser et al. 1997). Elemental sulphur is a favourable end product in technical processes, because it can be removed from the biological air purification system mechanically (by sedimentation) and used in other processes (Buisman et al. 1989ab, 1991, Basu et al. 1996). Apart from the microbial oxidation of sulphur compounds, it is also claimed that the chemical oxidation of sulphide to elemental sulphur is possible (Gamson et al. 1953, Whitcomb et al. 1989).

Chemolithotrophic bacteria, especially those of the genus *Thiobacilli*, which are found in natural soils and waters (Smith et al. 1991), have the ability to use energy gained from sulphur oxidation to support completely autotrophic growth, without any organic nutrients (Kelly 1988). The general characterization of thiobacilli is that they are gram-negative, small, aerobic rods that are able to oxidise reduced sulphur compounds, leading to the production of sulphuric acid (Harrison 1984, Kelly et al. 1989).

Colourless filamentous sulphur bacteria such as *Beggiatoa* and *Thiothrix* also oxidize sulphides. The cells of *Beggiatoa* are arranged in single filaments that move rapidly by gliding (Smith et al. 1991), and this can cause problems in biological purification systems by interfering with the settling of suspended solids or of sulphur itself (Buisman 1991). *Beggiatoa* is also microaerophilic, achieving its optimum growth at a low oxygen concentration (Smith et al. 1991).

The anaerobic phototrophs *Clorobiaceae* and *Chromatiaceae* use sulphide as electron donors for photosynthesis (Kelly 1988), while *Sulfolobus* can oxidize sulphur at high temperatures, the optimum being above 90°C (Smith et al. 1991). *Thiobacillus* TK-m (Kanagawa et al. 1989) and *T.* DW44 (Cho et al. 1991a) were capable of degrading gas mixtures containing H_2S, MeSH, Me_2S and Me_2S_2.

The biodegradation and transformation rates of compounds in biotechnological systems depend on the types of micro-organisms involved and the environmental conditions (temperature, pH, moisture content, salts, nutrients and oxygen) (Hartmans 1994). An exhaust gas flow containing several compounds can also prove to possess a more attractive alternative substrate, so that the biodegradation of a specific substrate can be disturbed

(Alexander 1994). On the other hand, methylotrophic micro-organisms may need co-substrates such as methane as a source of carbon and energy (co-metabolism) (Van Groenestijn et al. 1993).

2.2.1 Microbial oxidation of gas mixtures containing H_2S

The demand for improving the economics of treating gas mixtures containing H_2S has stimulated research into microbiological alternatives to the physico-chemical techniques available (Jensen et al. 1995). Various micro-organisms are capable of oxidising H_2S, and phototrophic (Kim et al. 1990), chemoautotrophic (Wada et al. 1986, Gadre 1988, Cho et al. 1992a, Chung,Y-C et al. 1997) and heterotrophic micro-organisms (Chung,Y-C et al. 1996a) have been employed in biological desulphuration processes. The use of chemoautorophic *Thiobacillus* species is advantageous due to their simple nutritional requirements, and a great number of biological purification processes employing such strains for the oxidation of H_2S have been published (Sublette et al. 1986, Wada et al. 1986, Das et al. 1993, Halfmeier et al. 1993, Park et al. 1993, Sublette et al. 1994, Chung,Y et al. 1996b, Chung,YC et al. 1996c, Huang et al. 1996, Pagella et al. 1996, Chung,Y-C et al. 1997, Chung,BY-C et al. 1998, Chung,Y et al. 2000). Since the genus *Thiobacillus* includes bacteria that prefer a pH near neutral and acidophilic bacteria growing at low pH, efficient H_2S oxidation can be achieved over a wide pH range (Kasakura et al. 1995). H_2S is easily decomposed by acidophilic bacteria which prefer a pH range of 2–3, and the resulting sulphate has only a minor effect on the activity of these acidophilic bacteria (Kasakura et al. 1995). Sulphide removal at low pH has many advantages. Because sulphide oxidation naturally leads to acidification and acidophilic bacteria can tolerate higher sulphate concentrations. When operations are lead under pH 7 less rinsing water is needed to carry the sulphuric acid away than at neutral pH (Devinny et al. 1999), and it has been shown on several occasions that H_2S removal efficiency is not reduced in the presence of other sulphide compounds such as Me_2S and MeSH in acidic applications (Hirai et al. 1990, Cho et al. 1991a, Zhang et al. 1991a).

2.2.2 Microbial oxidation of CS_2

CS_2 is known to inhibit soil nitrification (Malhi et al. 1982) and bacterial growth in the rhizosphere (Hartel et al. 1992), but there are some microbial strains that have the ability to oxidize it (Rothschild et al. 1969, Smith et al. 1988c, Plas et al. 1993, Jordan et al. 1995). The capacity of micro-organismsfor oxidising CS_2 seems to be more limited than that for oxidising methylated sulphides or inorganic sulphur compounds (Smith et al. 1988c), however, so that out of the nine *Thiobacillus* species tested by Smith and Kelly (1988), only one was able to grow with CS_2. The number of known bacteria using CS_2 as their sole energy substrate is very low, and most of them belong to the genus *Thiobacillus*, although the property is not exclusive to this genus, as *Thiothrix ramosa* is also reported to behave in this way (Odinsova et al. 1993). The intermediate oxidation products are carbonyl sulphide (COS) and H_2S, of which COS is known to inhibit autotrophic CO_2 assimilation, but *Thibacillus thioparus* TK-m is able to grow autotrophically on it. COS undergoes hydrolysis to CO_2 and H_2S, and the end product of oxidation is a sulphate (Kelly 1988, Smith et al. 1988c, Kelly et al. 1990).

2.2.3 Microbial oxidation of methylated sulphides

The oxidation of Me_2S as a energy source was first demonstrated in a *Thiobacillus* strain isolated from a biofilter used in gas desulphurization (Sivelä et al. 1975, Sivelä 1980). This organism was obligately chemolithotrophic, being unable to grow methylotrophically. Later several methylotrophic *Thiobacillus* species using Me_2S, Me_2S_2 and MeSH as their energy sources were isolated (Kanagava et al. 1986, Smith et al. 1988a, Smith et al. 1988b, Kelly et al. 1990). These had the property of being able to obtain energy from oxidation of the methyl groups as well as from the sulphide. Methylotrophic *Hyphomicrobium* species also have the ability to degrade methylated sulphides (De Bont et al. 1981, Zhang et al. 1991a, Cho et al. 1992b, Smet 1996, Smet et al. 1996, Ruokojärvi et al. 2000). Some *Pseudomonas* species also degrade Me_2S under certain circumstances, although a pure culture of *Pseudomonas* AK-2 required co-cultivation with *Thiobacillus* in order to grow on Me_2S (Kanagava et al. 1986) and *P. acidovorans* DMR-11 co-degraded Me_2S in an organic medium but did not use Me_2S as a sole source of either carbon or energy (Zhang et al. 1991b). Methylated sulphides are often metabolized by bacteria which prefer a narrow pH range, from six to seven (Smith et al. 1988a, Smet et al. 1996) and are

inactivated by the acidification of the medium that is associated with sulphate formation (Kasakura et al. 1995, Smet et al. 1996). Gas mixtures containing methylated sulphides can also contain H_2S, and this may cause problems for Me_2S removal because the degradation of H_2S is favoured over that of Me_2S (diauxie). Thus the coexistence of H_2S reduced the efficiency of Me_2S removal in biofilms (Tanji et al. 1989, Cho et al. 1991a, Zhang et al. 1991a, Ruokojärvi et al. 2000) because effective oxidation of the H_2S caused acidification, which reduced the removal of the methylated sulphides (Kasakura et al. 1995). This problem can be solved by using a two-stage process with separate acid and neutral units (Ruokojärvi et al. 2001).

2.3 Biological methods for removing sulphide gases

Biological methods are based on the absorption of volatile compounds in an aqueous phase or on a biofilm, followed by oxidation by means of micro-organisms (Van Groenestijn et al. 1993, Devinny et al.1999), as shown in Figure 1. There is no standard technique that can effectively and economically be applied to every industrial situation, because the optimal solution depends on several factors, e.g. the composition, flow rate and temperature of the waste gas, the space available in the plant, the continuity of gaseous emissions and the purification requirements contained in existing legislation (Lang et al. 1992, Smet et al. 1998a, Devinny et al. 1999). The composition and high flow rates of odorous, low-concentration (< 5g m^{-3}) waste gas streams favour biotechnological techniques and scrubbers (Smet et al. 1998a, Devinny et al. 1999), and biological air pollution control (BAPC) technology has been expanding recently on account of its economic and environmental advantages (Swanson et al. 1997). The incineration is suitable for higher gas concentrations (from 10 to over 100 g m^{-3}) (Devinny et al. 1999).

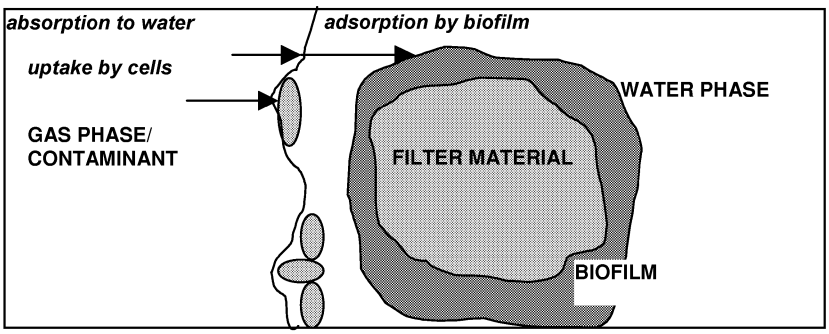

Figure 1. The absorption process in a biofilter

The removal capacity (RC) of a purification unit is affected by physical and microbiological processes taking place on three levels: the reactor, the biofilm and the microorganism (Diks and Ottengraf 1994a). All biotechniques are a combination of mass transfer from the gas to the liquid phase followed by biodegradation (Figure 1), and their effectiveness depends on the biodegrability of the contaminant, the mass transfer rates at the gas-to-liquid interface and the diffusion coefficients of the substrate and oxygen (Van Groenestijn et al. 1993). The amount of biomass that can be readily accessed by the diffusing contaminant is more important than the total biomass (Alonso et al. 1997), and developments in biotechnological H_2S abatement have been focused on the selection of better carrier materials and the use of specific inocula, since the removal of organic sulphide compounds calls for the isolation and breeding of more effective micro-organisms for purifying various gas mixtures under a variety of conditions (Smet et al. 1998b). The biotechnological methods available, chiefly biofilters, biotrickling filters and bioscrubbers, will be described below.

2.3.1 Biofilters

Biofiltration represents the oldest and most economical biotechnological method for the removal of odorous, undesired gaseous components (Williams et al. 1992b, Govind 1999). The very first biofilters used to remove odorous compounds, e.g. H_2S from waste gases in waste water treatment, were open soil beds (Carlson et al. 1966). Filter beds are usually enclosed (Figure 2), and in some cases also insulated, in order to prevent weather effects, and traditionally consist of organic material such as peat (Furusawa et al. 1984, Martin

1991, Park et al. 1993, Rothenbühler et al. 1994, Wu et al. 1996, Hartikainen et al. 1996, Elsgaard 1998, Wu et al. 1999, Hartung et al. 2001), compost (Yang,Y et al. 1994a, Wani et al. 1998b, Lee et al. 1999, Hartung et al. 2001, Nicolai and Janni 2001), soil (Carlson et al. 1966, Prokop et al. 1985) or a wood based material (Zhuang, L. et al. 2001), through which the gas flow to be purified is forced. The organic filter material can also include an additional coarse fraction, e.g. polystyrene, coconut fibre, wood bark or chips, or heather, which serves as a support material preventing compression of the filter (Van Groenestijn et al. 1993, Heslinga 1994, Hartung et al. 2001, Nicolai and Janni 2001). A ratio of 30% compost to 70% wood chips was recommended for treating swine odours with a biofilter (Nicolai and Janni 2001), but the selection of the organic filter material is in practice governed by availability, price and the possession of a suitably large surface area for microbial growth (Nicolai and Janni 2001). The material can also serve as a nutrient source, enabling a quick start-up and high removal efficiencies to be attained. Biofilters should tolerate starvation periods and high loading shocks (Wani et al. 1998b). On the other hand, the oxidation of sulphide compounds produces sulphate and lowers the pH of the medium, which may cause deterioration of the organic packing material and limit its lifetime (Williams et al. 1992a, Yang,Y et al. 1994a, Yang,Y et al. 1994b, Wani et al. 1998a). Synthetic and inorganic filter materials have also been tested in biofilters recently, and lava rock was found to have a good acid resistance, high sulphur elimination capacity and low pressure drop (Yang,Y et al. 2000). Some common filtermaterial properties are presented in Table 3.

Table 3. Summary of some properties of common biofilter materials (Devinny et al. 1999).

	Compost	Peat	Inert material*
Natural micro-organisms density	high	medium - low	none
Surface area	medium	high	low –high
Air permeability	medium	high	high
Assimilable nutrient content	high	medium	none
Cost	low	low	medium-high
Lifetime	2-4 years	2-4 years	> 5 years

* activated carbon, plastic and others

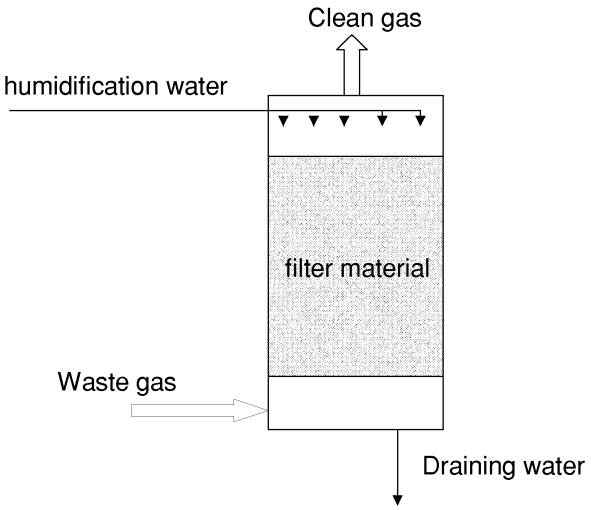

Figure 2. Schematic diagram of a biofilter.

The filter bed packing medium supplies a surface for microbial biomass growth and is of importance for the distribution of air and water. The waste gas can be passed through the filter bed in a downflow or upflow direction. Downflow operation is better for preventing drying in the lower parts, because filter bed is usually moistened by the top sprinkler. The depth of the filter bed is usually 0.5-1.5 m, as shallower beds may cause channelling and deeper beds may increase the pressure drop (Van Groenestijn et al. 1993). The typical volumetric loads are 250-580 m^3 m^{-3} h^{-1} (Van Groenestijn et al. 1993). The micro-organisms are attached to the surfaces of the filter material, forming a biofilm, and the biodegradable compounds are absorbed into the biofilm by virtue of the moist bed material and are oxidised.

Inoculation with effective micro-organisms (Park et al. 1993, Smet et al. 1996) and the selection of better carrier materials (Smet et al. 1996) will improve biofiltration efficiency. Biofilter material will inevitably suffers from acidification, and pH regulation will always be needed in long-term biofiltration systems (Yang et al. 1994a, b, Smet et al. 1996). In addition to toxification of the bed material by the oxidation products, biofilters may suffer from moisture problems, the latter being estimated to account for 75% of all biofiltration problems (Heslinga 1994). Good design of the biofilter may prevent many problems, and some models that have been introduced recently simulate long-term biofilter performance by taken into account moisture, nitrogen availability and the amount of biomass. These models are useful when screening design alternatives and operational schemes (Krailas et al. 2000, Mysliwiec et al. 2000, Chitwood et al. 2002).

2.3.2 Bioscrubbers

In a bioscrubber the biomass is suspended in a water phase, which is recirculated between a scrubber section and a regeneration section (Schippert 1994). The gas flow comes into contact with the water in a spraying tower, which may have an internal structure consisting of perforated plates, for example. In contrast to biofilters, the liquid phase is recirculated, and this allows better control over the system (temperature, pH, ionic strength) (Figure 3). Nutrients and buffers can be added to the liquid phase and the liquid can be refreshed and discharged in order to remove undesired products (Shoda 1991, Van Groenestijn et al. 1993, Schippert 1994). The main drawback relative to biofilters is the lower specific gas/liquid surface area, and the usefulness of the method is also restricted by the poor solubility of the substrates in water (Berzaczy et al. 1988, Shoda 1991), so that it is suitable method largely for substrates with a Henry coefficient lower than 5-10 (Schippert 1989). The water solubility of sulphur compounds can be enhanced by rendering the scrubbing liquid alkaline (H_2S, MeSH), but this restricts the use of micro-organisms with a low pH optimum (Smet et al. 1998b). The use of conventional (non-biological) scrubbers is well known in waste gas treatment, especially for SO_2, but the method is consi-dered expensive (Srivastava et al. 2000, Van Groenestijn et al. 1993, Morton et al. 1997). A scrubber integrated with a bioreactor has nevertheless been found to be a highly cost-effective means of removing H_2S (Buisman et al. 1993).

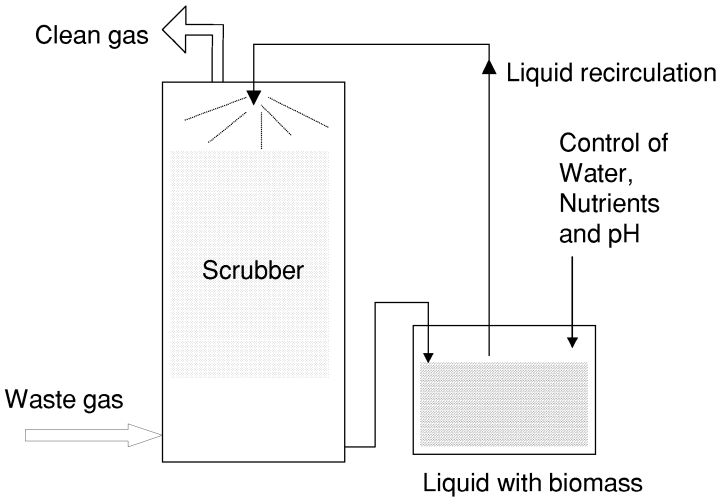

Figure 3. Schematic diagram of a bioscrubber.

2.3.3 Biotrickling filters

Biotrickling filters (Figure 4), which employ an immobilised biomass in the trickle bed (biofilm) to degrade pollutants (Diks et al. 1994a), are regarded as an intermediate between biofilters and bioscrubbers (Van Groenestijn et al. 1993). The waste gas is forced through the bed, which is packed with inert, usually inorganic material that must have appropriate characteristics for the immobilisation of micro-organisms and should ideally also be inert to physical, chemical and biological influences, have a very low pressure loss and offer adequate storage of water and nutrients (Loy et al. 1997). Many packing materials have been tested in recent years, including porous rock media, polyurethane foam, polypropylene, PVC, polyethylene, polyvinyl, activated carbon and ceramics (Loy et al. 1997, Higuchi et al. 2000). The search for a packing material is continuing, as the surfaces of most media tend to change with time through growth of the biofilm or the accumulation of sulphur particles (Higuchi et al. 2000).

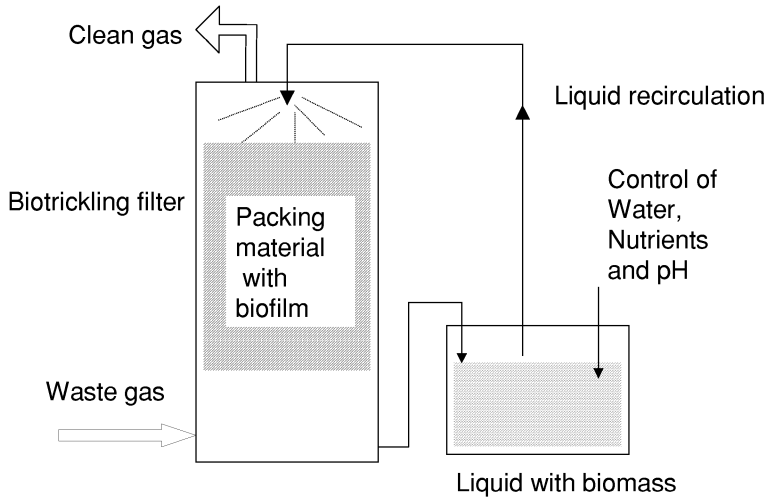

Figure 4. Schematic diagram of a biotrickling filter.

The liquid circulates in a biotrickling filter in a similar to that in a bioscrubber, and the recirculating liquid is initially inoculated with suitable micro-organisms, which in the course of time form the biofilm layer on the surfaces of the packing material (Figure 4). The pollutant is absorbed into the liquid and transferred to the biofilm, where it is transformed or degraded (Diks et al. 1994a). Both absorption and biodegradation of harmful pollutants can occur in one column comprising the biofilm and circulating liquid (Van Groenestijn et al. 1993). The rate-limiting step at low substrate concentrations is the mass transfer at the gas-liquid interface, while when the substrate concentration in the liquid is close to its equilibrium state, the rate limiting step is the oxidation taking place in the biofilm (Lobo et al. 1999). Recirculation can cause the stripping of solvent substrates in countercurrent (upflow air stream) operation, as the air flow may pick up pollutants from the concentrated liquid just before leaving the trickle bed and this can reduce the removal efficiency even when the trickle bed unit is working properly (Cox et al. 1998, Lobo et al. 1999).

Continuous control and adjustment of liquid phase parameters such as pH, salinity and nutrient content is possible in biotrickling filters, and this enables higher performance to

be achieved than with biofilters, but the industrial deployment of biotrickling filters is still limited (Cox et al. 2000), because they suffer from clogging of the bed by the growing biomass, especially when used for VOC control (Diks et al. 1994b, Schönduve et al. 1996, Smith et al. 1998). The biomass consists of an active and an inactive part, and its excessive accumulation in the bioreactor will reduce removal efficiency (Alonso et al. 1997). Growth of the biofilm can be regulated by limiting nutrient availability, but this may lead to a decrease in biodegradation in the trickle bed (Schönduve et al. 1996). Mineralization of the biomass, if possible, is a better way of preventing clogging (Hartmans 1994), and predatory protozoa have been also used to control biomass growth (Cox et al. 1997).

The organic filter material may support high microbial diversity. Compost material, for example, may itself contain micro-organisms that have the ability to oxidize sulphur compounds from gas mixtures (Shoda 1991). Biotrickling filters always need inoculation with suitable micro-organisms.

The costs of constructing a biotrickling filter are higher than for a chemical scrubber, but the former is cheaper to operate (Morton et al. 1997). Operation costs have been reduced further by minimising the amount of technical equipment (pumps, gas flow meters and controllers) by periodically rotating between several biotrickling filter beds using the same technical equipment (Cox et al. 2000). A recent economic comparison of biofiltration and chemical scrubbing in Ohio showed that the unit costs of a biofilter relative to the design flow rate were much lower than for a wet scrubber, although surprisingly, the actual unit cost of the wet scrubber after operation for over one year (131 US$/m^3 H_2S removed) was lower than for the biofilter (160 US$/m^3 H_2S removed) (Gao et al. 2001).

2.3.4 Comparison of different methods

There is no waste gas treatment technique that is suitable for every application. Some general advantages and disadvantages of different biological methods are described in table 4. The cost-effectiveness is site spesific for all techniques and the optimal solution depends on the particular application, qualitative and quantitative composition of the waste gas, the flow rate and temperature of the waste gas, materials required for constructions, monitoring and adjusting systems, available space etc (Smet et al 1998b, Devinny et al 1999). Biofiltration technology has low operating costs and secondary pollutant waste streams are minimal. However, it is not suitable technology for high concentrations. The control and

adjustment of pH and nutrient concentration is possible in trickling filter and scrubber. It is highly recommended to perform pilot plant experiments in order to select the optimal solution for specific problem.

Table 4. Comparison of biological waste gas treatment techniques (Devinny et al 1999)

	Advantages	**Disadvantages**
Biofiltration	Low operating and capital costs Moderate pressure drop No further waste streams produced	Deterioration of medium Less suitable for high concentrations Moisture and pH difficult to control
Biotrickling filter and Bioscrubber	Medium operating and capital costs Low pressure drop Suitable for higher concentrations Suitable for acid producing contaminants Nutrient and pH control possible	More complex to construct and operate Clogging by biomass Further waste streams produced

3 AIMS OF THE RESEARCH

Research into biological purification methods has increased in many countries during recent decades, as this old-established, natural methodology has many advantages and there is demand for odour removal technology in general. The main object here was to develop a biological purification method for odorous, occupationally and locally deleterious sulphur emissions from waste water treatment, composting and industry. The more specific goals were:

- to examine the possibility and capacity of peat biofilters to remove the most common odorous sulphur gases (H_2S, MeSH, Me_2S) in long-term experiments (**I**),
- to isolate and characterise a CS_2-oxidizing micro-organism in order to improve the biological removal of CS_2 (**II**),
- to develop the biofiltration of industrial H_2S and CS_2 emissions by means of peat biofilters (**III**),
- to further develop the biological purification of H_2S and CS_2 emissions by means of a biotrickling filter with a liquid aeration unit (**IV**), and
- to study the suitability of various filter materials for a biotrickling filter (**V**).

4 MATERIALS AND METHODS

4.1 Experiments with peat biofilters

The biofiltration experiments with peat were performed on two scales (**I**): first H_2S filtration experiments *in situ* in a waste water pumping station, and then removal of a mixture of H_2S, MeSH and Me_2S in laboratory experiments. The packed filter bed of fibrous peat consisted of 50% very coarse horticultural peat and 50% peat fibres. The material was selected on the basis of its physico-chemical properties, to ensure that the pressure drop over the filter bed was as low as possible. Naturally acid peat (pH 4) was neutralised in some experiments to pH 6.2 with 170 g $Ca(OH)_2$ kg^{-1} peat (dry wt). The peat used had its natural nutrient content (0.32 mg g^{-1} P, 0.53 mg g^{-1} Mg, 0.25 mg g^{-1} K, 8.8 mg g^{-1} N and 1.1 mg g^{-1} S including 0.03 mg g^{-1} water soluble SO_4-S).

4.1.1 *In Situ* biofiltration for H_2S removal

The ventilation air of the waste water pumping station contained a mixture of several gases, but as the concentration of hydrogen sulphide was low (0.002-0.1 mg m^{-3}), supplementary hydrogen sulphide was added in order to study the removal of higher concentrations (**I**). The filtration experiments were started at low hydrogen sulphide concentrations, 3 mg m^{-3}, and these were then gradually increased up to about ten times after two months, the maximum being 35 mg m^{-3}. The first experiment with both untreated and limed peat lasted three months altogether, after which the peat filters were exposed to the ventilation air of the pumping station for a month. Thereafter experiments were restarted with the same filter material, increasing the H_2S concentration rapidly to 50 mg m^{-3}. These experiments were continued for 85 days. The temperature in the pumping station varied during the 232 days of the experiment depending on the season, being at its maximum (31°C) in summer and at its minimum (10°C) in winter. Only H_2S removal was studied in the *in situ* experiments.

The biofilters were washed once a week by spraying 1000-2000 ml of deionized water onto the top of them. The soluble substances were removed by the water flowing through the filter. Sulphate concentrations in the leached water were analysed with an ion cromatograph (see section 4.5.1), and air samples were collected in laminated plastic bags (volume 10 litres) and analysed with a gas chromatograph in the laboratory (see section 4.5.2).

4.1.2 Laboratory biofiltration experiments for H_2S, Me_2S and MeSH removal

Biofiltration of Me_2S was started in the laboratory with column of diameter 100 mm and a bed of depth 250 mm (volume 2 litres) (**I**). The filter material was similar to that used in the H_2S experiment at the pumping station. Again both untreated and limed materials were tested, the limed material being inoculated with sludge that had originated from a Me_2S-contaminated environment (a paper mill). The Me_2S was evaporated into the humidified air flow that was driven into the biofilter (downflow). The biofiltration experiments were performed with a mixture of Me_2S and MeSH (10%, Teknohaus), the latter being added from a gas cylinder through a mass flow controller (Bronkhorst F200C-FA-11-E). Gas samples were taken with a gas-tight syringe and analysed immediately with a gas chromatograph.

4.1.3 Isolation of micro-organisms able to oxidize CS_2

The biofiltration technique involves the removal of pollutants by immobilized mixed microbial populations located in biofilms, which are formed at the beginning of the biofiltration process (acclimation). These biofilms probably contain several microbial species, as the system is open. A CS_2-degrading microbe was enriched (**II**) from a pilot-scale peat biofilter which had been used for H_2S removal in a waste water pumping station for eight months and then for the removal of CS_2 and H_2S from waste gases in the viscose industry for two months (**I**). The pH of biofilter dropped from pH 6.2 to 1.4 in the later filtration experiments. A sample of the peat from the biofilter was transferred to a liquid-enrichment medium aerated with an air flow containing 500 ppm CS_2 at room temperature (**II**). Finally, a pure culture was obtained by serial dilutions and its purity was checked by electron mi-

croscopy and any heterotrophic contaminants tested on R2A agar plates and in a nutrient solution, as described in detail in Paper **II**. The nutrient solution was also used to study whether the strain was able to grow heterotrophically. A characterisation of the isolated microorganism is reported in Paper **II**.

4.1.4 CS_2 and H_2S removal with a peat biofilter

Simultaneous removal of CS_2 and H_2S from a gas mixture was studied using a peat biofilter inoculated with the previously isolated *Thiobacillus* strain TJ330 (**III**). The most important parameters tested were the empty bed retention time (EBRT) for the gas and the total inlet sulphide concentration, in order to determine the possibilities for using such a peat biofilter for full-scale CS_2 removal. The EBRT was studied in a short-term experiment (lasting nine days) with an increasing total gas flow rate. Gas inlet concentrations of less than 500 mg m^{-3} CS_2 and 250 mg m^{-3} H_2S were used to study removal capacity (RC) in a 41-day experiment with the same peat material. About 30% of the material was changed before a subsequent high-load experiment designed to test the impact of extremely high concentrations (1500-6000 mg m^{-3}).

4.2 Experiments with a biotrickling filter

4.2.1 Laboratory experiments for H_2S and CS_2 removal (**IV**)

The filter bed of the laboratory-scale biotrickling filter consisted of glass beads. The inlet gas was first bubbled through a porous nozzle into the liquid aeration unit located in the bottom of the reactor (**IV**). This unit contained a nutrient solution with *Thiobacillus* TJ330 (DSM 8985), which was able to oxidize both CS_2 and H_2S. The incoming gas was effectively moistened in the liquid unit before it contacted the filter bed. The liquid was recycled to the top of the filter with a peristaltic pump, thus moisturizing the upper part of the bed. The pH of the liquid dropped to 1 during operation, and either $Ca(OH)_2$ or NaOH was used to keep it in the range 1-2.

4.2.2 Comparison of filter materials (**IV, V**)

The trickle bed bioreactor experiments in the laboratory commenced with glass beads, and later other materials were used (**V**) in order to acquire further information on biofilm formation and the technical and chemical suitability of various materials for use in an acid trickle bed bioreactor. The experiments were carried out with four parallel filters packed with various filling materials: ceramic saddles, porous lava rock, a grit composed of irregularly shaped particles of recycled plastic, and apatite-biotite ore (**V**). The apatite-biotite ore and lava rock represented natural materials, while the ceramic saddles were a commercial product. Apatite-biotite ore was also selected because it contains the necessary nutrients: phosphate, magnesium and calcium. The technical properties of the packing materials are presented in Table 5 (**V**).

Table 5. Technical properties of the filter materials (**V**).

	Empty space %	Bulk density g dm^{-3}	Particle size mm
Ceramic saddles	60.9	1013	6
Lava rock	41.8	714	5.6–10
Apatite-biotite ore	45.5	1755	5.6–10
Plastic grit	67.3	305	4–10

All the biotrickling filters (**V**) had bed heights of 0.5 m and diameter 0.05 m, and they had the same acidic circulating liquid, containing nutrients and cells of the *Thiobacillus* strain TJ330 (10^7-10^8 cells ml^{-1}) which is capable of oxidising H_2S and CS_2 simultaneously under acidic conditions (pH 2). The filters were operated in a counter-current direction, i.e. the liquid flow was opposite to the air flow. The flow rate of the circulating liquid varied from 1.6 to 5.2 $m^3_{liquid}\,h^{-1}m^{-3}_{filterbed}$ and the gas flow rate from 157 to 367 $m^3h^{-1}m^{-3}_{filterbed}$ (retention times 10-23 seconds). These variations were made systematically in order to study their effects of the running parameters on removal efficiency. CS_2 was evaporated from a diffusion tube into the main gas stream and hydrogen sulphide was added via a mass flow controller. The gas flow rates were checked with a rotameter.

4.2.3 Pilot-scale experiments (**IV**)

A pilot biotrickling filter with liquid aeration unit was designed to treat a gas stream of 200 $m^3 h^{-1}$ (retention time 29 seconds) containing 500 mg CS_2 m^{-3} and 150 mg H_2S m^{-3} (**IV**). The liquid was of a similar composition to that used in the laboratory experiments. The gas passed through the perforated bottom plate into a liquid layer of variable thickness, and from there into the biotrickle bed. Three packing materials were tested: hydrophilised polypropylene Ralu rings, diameter 25 mm, specific surface area 190 $m^2 m^{-3}$ (Raschig, Ludwigshafen, Germany), ceramic saddles, nominal size 25 mm, specific surface area 255 m^2m^{-3} (Raschig, Ludwigshafen, Germany) and a plastic grit (irregularly shaped particles of variable size composed of recycled plastic material). The gas and liquid phases flowed in opposite directions. The liquid was circulated through the biotrickle bed and neutralized with 19% NaOH and partly exchanged to keep the sulphate concentration below 15 g l^{-1}.

4.3 Enumeration of microbial cells and examination of the biofilms

The numbers of microbial cell in the solution were determined microscopically with a Petroff-Hausser counting chamber (**II**, **IV** and **V**).

The biofilm on the packing materials was studied with a scanning electron microscope (SEM model JEOL JSM-35). A piece of packing material was dehydrated with ethanol, dried in 1,1,1,3,3,3- hexamethyldisilazane, attached to an SEM sample stud and coated with gold (**III**, **IV** and **V**).

4.4 Chemical analyses

4.4.1 Sulphate

Sulphate was analysed with a Dionex Series 2010i ion chromatograph equipped with an AG4A P/N 37042 precolumn, an AS4A P/N 37041 analytical column and a Dionex conductivity detector. The eluent consisted of 1.7 mM $NaHCO_3$ and 1.8 mM Na_2CO_3, and the regenerant was 25 mN H_2SO_4. The flow rate was 1.5 ml min^{-1} (Papers **I-V**).

4.4.2 Volatile sulphur gas compounds

H_2S, MeSH and Me_2S concentrations were measured with a gas chromatograph (Analytical Instruments Development, Model 511-19) equipped with a flame photometric detector (FPD) incorporating a sulphur filter that permitted light transmission at 349 nm. Nitrogen with flow rate of 25 ml $min^{-1}$was used as the carrier gas. The air and hydrogen flow rates were 100 and 50 ml min^{-1}, respectively. A teflon column packed with Chromosorb T 40/60 mesh, 12% polyphenyl ether and 0.5% H_3PO_4 was used in the biofiltration experiments for the removal of H_2S and methylated sulphide compounds (**I**). The oven temperature was 65°C and the detection limits were 0.14 mg m^{-3} (0.1 ppm) for H_2S, 0.6 mg m^{-3} (0.3 ppm) for MeSH and 0.78 mg m^{-3} (0.3 ppm) for Me_2S. This column could not separate H_2S and COS, and it was later exchanged for a teflon column packed with Carbopack B 60-80 mesh, 3% CW and 0.5% H_3PO_4 in order to study the biodegradation of CS_2 (II-V). This new column separated H_2S from COS at an oven temperature of 40°C. The detection limits were 0.14 mg m^{-3} (0.1 ppm) for H_2S, 1.7 mg m^{-3} (0.7ppm) for COS and 3.17 mg m^{-3} (1 ppm) for CS_2.

5 RESULTS

5.1 Biofiltration of sulphur compounds with peat

The removal efficiencies of biofiltters depended on the treatment of peat material before experiment. Also the type of sulphur compound affected to removal.

5.1.1 Removal efficiency with given sulphur compounds (**I**, **III**)

Removal efficiencies and acclimation periods varied between the sulphur compounds. The H_2S removal efficiency of the peat biofilters was improved by neutralizing the naturally acid peat material, whereupon 95% of the H_2S (inflow concentration 5 mg m^{-3}) was removed after one day of operation, while the untreated peat reached the same efficiency after one month of operation with the same H_2S load (**I**).

No removal of Me_2S was observed with natural peat during one month of operation, but after receiving an inoculum from a paper mill environment the limed filter achieved over 20% removal immediately and its efficiency increased to 95% after two weeks of operation (**I**). When MeSH was added to the Me_2S, the peat biofilter removed over 99% of the former immediately, but there was a decrease in the Me_2S removal efficiency from 80% to 35% within one day. The maximum removal of Me_2S was 175 g S m^{-3} day^{-1}.

The inoculated peat biofilter was capable of removing a mixture of H_2S, MeSH and Me_2S in a short-term experiment with an average load of 80 g S m^{-3} day^{-1} (range 40 to 150 g S m^{-3} day^{-1}) (**I**). The mean efficiency of H_2S removal during this gas mixture experiment was high (99%), but the gas mixture disturbed the biodegradation of Me_2S at retention times below 30 seconds, the average removal efficiency being 85%. The removal of MeSH was also reduced by 10% when the gas mixture was fed in.

CS_2 removal was poor when a peat biofilter without inoculation was used, but CS_2 and H_2S were oxidized efficiently without any adaptation time when the biofilter was inoculated with the *Thiobacillus* strain TJ330 (**III**). The total sulphur load in the experiments varied from 380 to 1530 g S day^{-1} m^{-3} when inflowing CS_2 and H_2S concentrations were

93-560 mg m^{-3} and 56-200 mg m^{-3}, respectively. Concentrations below 200 mg m^{-3} for H_2S and below 560 mg m^{-3} for CS_2 were effectively oxidised to sulphate by the *Thiobacillus* strain TJ330 in the fibrous peat material, and the filter material showed no signs of overloading. The high sulphur load experiment had concentrations of CS_2 up to 5920 mg m^{-3} and of H_2S up to 2160 mg m^{-3}, with an EBRT of 120 seconds. Removal capacity was not dependent on the total sulphur load up to 4800 g S m^{-3} day^{-1}, but overloading of the peat material was noted at the end of experiment, at which point the removal efficiency declined.

The removal capacities measured in the experiments are presented in Table 6. The lowest removal capacities were recorded in the peat biofiltration experiments, except for the mixture of H_2S and CS_2, for which a high short-term removal efficiency was obtained, but the filter became overloaded in three weeks, losing its efficiency.

Table 6. Removal capacities and efficiencies of given bed materials in laboratory and pilot-scale experiments with odorous sulphide compounds.

Sulphide gas	Filter bed material (article)	Removal capacity (RC)	RE
	PEAT BIOFILTERS	g S m^{-3} day^{-1}	%
H_2S	Peat (limed) (I)	136	99.2
Me_2S	Peat (limed and inoculated) (I)	175	88.0
Me_2S + MeSH	Peat (limed and inoculated) (I)	55	72.7
H_2S + Me_2S + MeSH	Peat (limed and inoculated) (I)	150	95.0
H_2S + CS_2	Peat (limed and inoculated) (III)	4450	93.4
	BIOTRICKLING FILTERS	g S m^{-3} day^{-1}	%
CS_2	Glass beads* (IV)	4030	97.9
H_2S + CS_2	Ceramic saddles* (Pilot) (IV)	2620	77.0
H_2S + CS_2	Ralu rings (Pilot)* (IV)	2140	42.0
H_2S + CS_2	Plastic grit (Pilot)* (IV)	2520	64.0
CS_2 (H_2S+CS_2)	Apatite-biotite ore (V)	2220 (4150)	97.9 (92.9)
CS_2 (H_2S+CS_2)	Lava rock (V)	2590 (3740)	98.2 (99.7)
CS_2 (H_2S+CS_2)	Plastic grit (V)	2550 (4300)	99.5 (98.1)
CS_2 (H_2S+CS_2)	Ceramic saddles (V)	2690 (5860)	93.4 (99.3)

* treatment with liquid aeration unit

5.1.2 Lifetime of a conventional peat biofilter (I)

The lifetime of a peat biofilter depends on the filter material and the sulphur load. Measurement of the pH of the draining water is the most practical way of obtaining information on the sulphate concentration of a filter, but it does not show the amount of elemental sulphur accumulating in it. This pH generally varied in the range 1-2, while pH values below this indicated sulphate concentrations over 20 g l^{-1}. The results suggest that the critical sulphur level for the peat material is 40 mg S g^{-1}, which was the average sulphate sulphur concentration in a properly operating biofilter. The highest sulphur content measured in the peat material was 136 mg SO_4-S g^{-1}, in material overloaded with a high sulphide input. Peat filters have been operated commercially for several years with sulphide loads of about 2 mg m^{-3}.

5.2 Microbial oxidation of CS_2 by Thiobacillus strain TJ330 (DSM 8985) (II)

The *Thiobacillus* strain TJ330 used CS_2 or elemental sulphur as a substrate and was unable to grow in liquid and solid media containing solely organic substrates. The cells were rod-shaped (1-3 x 0.5-0.8 µm) and motile, mostly occurring in pairs. A polar flagellum was observed in transmission electron microscopy. The strain bore many physiological and morphological similarities to *T. thiooxidans*, but the latter is unable to oxidize CS_2. On the other hand, it showed only 44.2 ±11.8% DNA homology with the type strain *T. thiooxidans* ATCC19377. The *Thiobacillus* strain TJ330 was deposited in the DSM culture collection (DSM 8985).

The highest rate of CS_2 oxidation to sulphate was achieved at pH 2. This oxidation reaction had a narrow temperature range, with its maximum at 28°C but only very slow growth below 26°C or above 30°C. μ_{max}, measured in terms of the production of bacterial cells in a medium with an initial pH of 4 in the presence of CS_2, was 3.9×10^{-2} h^{-1}, corresponding to a generation time of 17 hours. The half-maximum oxidation rate (Monod constant K_s) was obtained with 0.97 µmol l^{-1} (0.07 mg CS_2 l^{-1}), and the K_s for the formation of sulphate was 2.6 µmol l^{-1} (0.20 mg CS_2 l^{-1}).

5.3 Biotrickling filter

5.3.1 Biotrickling with a liquid aeration unit (**IV**)

The biotrickling filter with liquid treatment removed CS_2 very effectively in the laboratory, and this purification process was patented (Hartikainen et al. 1995). The highest CS_2 removal capacity was 170 g S m^{-3} h^{-1} (4030 g S m^{-3} day^{-1}), giving a removal efficiency of 71-99.8% (Table 6). Pilot-scale experiments performed with three bed materials showed polypropylene Ralu rings to have the lowest removal capacity, 89 g S m^{-3} h^{-1} (2140 g S m^{-3} day^{-1}), while the maximum removal capacities for the mixture of H_2S and CS_2 were achieved with ceramic saddles and plastic grit, 109 (2620 g S m^{-3} day^{-1}) and 105 g S m^{-3} h^{-1} (2520 g S m^{-3} day^{-1}), respectively. The removal efficiencies were higher for H_2S than for CS_2. All the experiments with ceramic saddles as packing material were run with liquid treatment, and the highest removal capacity was 109 g S m^{-3} h^{-1} (2620 g S m^{-3} day^{-1}) with a removal efficiency of 77%.

The importance of the liquid treatment was tested systematically with the pilot reactor. Bubbling the inlet air flow through the liquid increased the removal efficiency of the reactor with plastic polypropylene rings as the bed material, especially at slow air flow rates. Thus liquid treatment increased the removal efficiency of H_2S and CS_2 by 20% at a flow rate of 100 m^3 h^{-1} per filter m^3, but no longer enhanced it when the flow rate was over 350 m^3 h^{-1} m^{-3}.

5.3.2 Biotrickling experiments with various bed materials (**V**)

When ceramic saddles, plastic grit, lava rock and apatite-biotite ore were tested as trickle bed materials in laboratory experiments without liquid treatment the adaptation time needed for biofilm growth varied from 2 days (apatite - biotite ore and lava rock) to two weeks (ceramic saddles and plastic grit). After adaptation, the plastic filter material and ceramic saddles had the highest removal capacities (2500 - 2690 g S m^{-3} day^{-1}).

The retention time of the gas in the filter bed at the highest air flow rates tested was only 10 seconds, and the maximum liquid flow rate was 5.2 m^3_{liquid} m^{-3}_{filter} h^{-1}. The CS_2 concen-

tration in the experiments was 116 ± 16 ppm, the sulphate concentration 12-24 g l^{-1} and the cell density in the circulation liquid 3×10^{8}-1×10^{9} cells ml^{-1}.

The ceramic saddles achieved the highest CS_2 removal capacity in the laboratory tests (maximum 133 g CS_2 m^{-3} h^{-1}) and the apatite-biotite ore the lowest (maximum 109 g CS_2 m^{-3} h^{-1}). The differences were small at low air flow rates, but greater at higher rates. An increase in the liquid flow rate favoured the plastic and ceramic filter materials in terms of RC, but the removal efficiency decreased. Both RE and RC were reduced when the liquid flow rate was increased in the case of apatite-biotite ore, indicating that a high liquid flow rate increases disintegration and disturbes biofilm formation. The pressure drop over the trickle bed after experiments at an air flow rate of 262 m^3 m^{-3} h^{-1} (retention time 14 s) was lowest with the plastic grids, being only 39 Pa, while the ceramic saddles and lava rock had pressure drops of 210 and 250 Pa, respectively. The pressure drop of 500 Pa recorded with the apatite-biotite ore was the highest among the materials studied here.

The removal efficiency of the biotrickling filter was highest in the first ten centimetres of the trickle bed, where the maximum removal rates achieved were 100-280 g CS_2-S m^{-3} h^{-1} (ore and ceramic saddles, respectively). The plastic grit had the highest empty space (67.3%), and filters containing this material also had the most uniform removal rates in the first 30 cm of the trickle bed. The upper 20 cm was of minor importance for removal, because most of the CS_2 had already been oxidised in the lower part of the filter.

Both H_2S and CS_2 removal rates were high in all the trickle beds up to a certain threshold level which was observable with increasing loads. Low concentrations of CS_2 (about 1 ppm) were released from the filters, but H_2S concentrations in the effluent mostly remained below the detection limit. The first 2/5 of the trickle bed had high load rates relative to filter volume, and as the main removal occurred there, the removal rate was highest and removal capacities could be calculated for this area separately. The highest capacity for removing sulphur from a gas mixture was achieved with ceramic saddles, with 76% of the sulphur load removed in the first 20 cm of the trickle bed, giving an RC of 450 g S m^{-3} h^{-1} (sum of H_2S-S and CS_2-S removed).

6 DISCUSSION

6.1 Biofiltration

Our results confirmed that domestic peat serving as a porous support base for the biofilm is a suitable material for the biofiltration of odorous sulphide compounds. The removal efficiency of the neutralised peat was better than that of the untreated peat throughout the eight-month period of operation, although over ten times higher removal rates have been achieved in short-term experiments with an inoculated peat biofilter (Cho et al. 1991b). Our experiments nevertheless emphasised the long-term removal of H_2S and the testing of removal capacity with low loads (Table 6). When higher sulphide loads were used the peat became covered with fine, pale yellow particles observable to the naked eye, which were assumed to consist of biofilm and elemental sulphur formed during the filtration of sulphide compounds. It is also possible that micro-organisms in the biofilm adapt slowly to oxidise elemental sulphur rather than sulphide compounds. Bonnin et al. (1994) noted that a high sulphate load caused some precipitation of ammonium sulphates when their solubility was exceeded, resulting in clogging of the filter. Continuous rinsing with water will prevent such precipitation, but once it has occurred, regeneration of the peat by rinsing is difficult. The optimal use of water is extremely important, because poor control over the moisture of the filter material has been estimated to cause up to 75% of all problems in biofiltration (Reyes et al. 2000). Insufficiently humidification will lead to evaporative drying and high sulphate concentrations, while too much water will increase the pressure drop. Attention should therefore be paid to improving moisture control (Reyes et al. 2000).

A temporary high concentration of H_2S evidently does not interfere with the operation of a biofilter, but continuously high sulphide concentrations (over 100 mg m^{-3}) imply a high loading and will destroy a conventional peat biofilter. Wani et al. (1998b) reported that a return to the original removal rates was achieved within 2-8 hours after a shock loading. On the other hand, if a biofilter is left for a long time without loading, the adaptation time will be longer than required after an extremely high load, and an idle phase without any air flow through the filter will cause the longest re-acclimation periods of all (Wani et al. 1998b). Our in situ experimental peat biofilters exposed to the ventilation air from a waste

water pumping plant reached a high removal efficiency soon after the end of the non-loading phase (**I**), but removal efficiency decreased after the inlet H_2S concentration had been increased gradually from zero to 30 mg m^{-3} over a period of two weeks, this effect being quicker with a natural peat biofilter than with neutralized peat.

Removal of H_2S is efficient in acid circumstances, but Me_2S removal requires an almost neutral environment (**I**) and its removal is more sensitive to pH changes. The maximum removal of Me_2S-S achieved here, 175 g m^{-3} day^{-1}, is more than twice as high as that reported earlier for Me_2S-S with peat biofilters, 74 g Me_2S-S m^{-3} day^{-1} (Zhang et al. 1991a). The inoculated compost biofilter had a removal capacity of 258 g Me_2S-S m^{-3} day^{-1} at pH above 6, which decreased to 52 g Me_2S-S m^{-3} day^{-1} at pH 4.7 (Smet et al. 1996). In some cases (Budwill and Coleman 2000) it has been possible to restore Me_2S removal after a decrease caused by acidification by neutralizing the filter. The highest Me_2S removal efficiencies, 879 g Me_2S-S m^{-3} day^{-1}, have been achieved with two-stage biotrickling filters (Ruokojärvi et al. 2001).

Although earlier studies with a compost biofilter had suggested that a sulphate content of 25 mg S g^{-1} in the filter material is the critical level for microbial activity (Yang,Y et al. 1994a), our results indicate that the critical sulphate content for peat material inoculated with acidophilic *Thiobacillus* is higher than this, possibly 40 mg S g^{-1}. A sulphate concentration of over 100 mg S g^{-1}, however, gave signs of overloading of the organic filter material, requiring occasional replacement. High sulphide concentrations are common in industry (e.g. in viscose production and the pulp and paper industry), and purification of these emissions is not possible with peat biofilters.

About 45 operating peat biofilters have been built in Finland since this research begun. Full-scale peat biofilters have mainly been implemented to date in Europe (Bonnin et al. 1994), where over 500 such installations are now reported to exist (Castro et al. 1997), and a great deal of research is going on in the USA and Japan as well.

6.2 Microbial characterisation

The *Thiobacillus* strain TJ 330 represents a high-affinity bacterium which can effectively remove low CS_2 concentrations in an acidic environment. These properties can be utilised

in biotechnological purification applications where sulphuric acid is produced as a result of CS_2 oxidation. The microbial strains described earlier (Smith et al. 1988c, Plas et al. 1993, Jordan et al. 1995) require a neutral environment for CS_2 oxidation.

The strain TJ330 has a 3-layer cell wall structure, as reported for *Thiobacillus* species in general (Shively et al. 1970). *T. thiooxidans* possesses a glycocalyx, which may be involved in the adhesion of cells to surfaces and may also protect the cells against harmful environments and compounds (Kelly et al. 1989). The carboxysomes detected here by electron microscopy are also found in many obligate aerobic autotrophic bacteria, including *T. thiooxidans* (Shively et al. 1970). TJ330 and *T. thiooxidans* have similar pH and temperature requirements, both are able to grow down to pH 0.5, and where *T. thiooxidans* has an optimum growth temperature between 28 and 30°C (Kelly et al. 1989), the strain TJ330 has a narrow optimum temperature range close to 28°C. We measured the effect of pH and temperature on growth in relation to sulphate formation, and observed that some gaseous intermediate products, such as carbonyl sulphide (OCS) and H_2S, were generated during oxidation. Some sulphur and polysulphide formation has been reported by Kelly (1982).

Many isolates of extremely acidophilic chemolithotropic sulphur-oxidising bacteria have been proposed as new species of *Thiobacillus,* but most of them lack sufficient documentation to justify a new species designation and are regarded as *T. thiooxidans* in Bergey's Manual of Systematic Bacteriology (Kelly et al. 1989). The strain TJ 330 showed only 44% DNA homology with the type strain for *T. thiooxidans* (ATCC 19377) and was able to oxidise CS_2, in contrast to *T. thiooxidans,* which lack this capacity.

6.3 Biotrickling filtration

When the *Thiobacillus* strain TJ330 was employed in CS_2 biotrickling filtration experiments the rate of biofilm formation and type of biofilm varied between the trickle bed materials tested, although the CS_2 removal capacities were quite similar with all the materials (2220-2690 g S m^{-3} day^{-1})(Table 6). More variable removal capacities were achieved in the experiment with a CS_2 + H_2S gas mixture in short-time experiment, the highest removal being recorded with ceramic saddles (5860 g S m^{-3} day^{-1}) and the poorest

with lava rock (3740 g S m^{-3} day^{-1}). Yang et al. (2000) found lava rock to have a higher H_2S removal capacity (2400 g S m^{-3} day^{-1}) and a lower pressure drop in an acid biotrickling filter than did organic materials (wood products) or synthetic reclaimed waste media (foams, fibres and pieces of plastic) in long-term experiments (100-300 days). They also found that the lower parts of the upflow filter bed removed most of the sulphide at the beginning of the experiment but that the upper part became more important with time. In our short-term experiment (one month) the removal efficiency of a ceramic saddle filter bed was highest in the lower filter bed (the first 10 cm).

Since apatite-biotite ore contains phosphate and mineral nutrients, it was expected to serve as a good growth medium for biofilm bacteria, and the fact that the adaptation period was shortest for the lava rock and the apatite-biotite ore supported this assumption. On the other hand, the ore disintegrated so rapidly in the acid environment that the biofilm was partly damaged at high flow rates. Also, the high density of the material required heavy biofilter constructions. Apatite-biotite can therefore not be recommended as the sole material for use in large biotrickling units. The phosphate and mineral nutrients magnesium and calcium that may have been released from the material into the common liquid suspension served the needs of all the materials equally well, which implies that small amounts of apatite-biotite ore may be used as an additive in other filter bed materials, so that disintegration of the ore will release nutrients from the trickle bed slowly in long-term operation.

Cells of the rod-shaped *Thiobacillus* strain TJ330 which had been inoculated into the liquid phase reached their greatest abundance on the surfaces of the saddles and lava rock (**V**). The biofilm that formed on the surface of the plastic grit had more filamentous growth than the other bed materials, but this growth was almost black in colour, while the rod-shaped *Thiobacillu*s growth was light-coloured. This same difference was observed in the pilot-scale experiments (**IV**).

The packing materials used in the pilot-scale biotrickle bed differed somewhat from those used in the laboratory experiment (**IV**). It is possible that the cylindrical Ralu rings used in the pilot plant had been designed especially for a bioscrubber, as they allowed only minor biofilm formation, whereas the ceramic saddles, having a porous surface, supported good growth. It should be pointed out that the conditions for biofilm growth were extreme in our experiments because of the high acidity in the reactor (down to pH 2). Although bio-

logically excellent, the ceramic material, being both fragile and heavy, required a robust reactor construction in the pilot experiment. The grit composed of irregular-shaped particles of recycled plastic had almost the same removal efficiency as the ceramic saddles and was light and cheap. This material has quite a rough surface structure, but lacks micropores.

The lava rock and ceramic saddles, both porous materials, had good biofilm formation properties in the laboratory. Biofilm formation correlated with removal capacity, the highest removal being achieved with the ceramic saddles and the lowest with the apatite-biotite ore. As a commercial bed material, ceramic saddles gave the highest removal efficiency (5860 g S m^{-3} day^{-1}) for the CS_2 and H_2S gas mixture in the comparative laboratory experiment. Ceramic saddles constituted the most expensive of the tested materials, but they gave a good removal efficiency with a thinner filter bed. Our experiments confirmed that the bed materials greatly affect the removal rates and function of biotrickling systems. Long-term experiments are now needed, because the development of secondary populations and possibly the accumulation of intermediate products (e.g. sulphur) are likely to alter the functioning of the system. The clogging caused by biomass overgrowth, which is common in biotrickling units operating close to neutral pH, e.g. in VOC treatment, is rarer in acid sulphide biotrickling units, and the growth of secondary populations is often high in neutral units.

The removal capacity of the biotrickling filter was almost equal to that of the peat biofilter, but its stability will be better in long-term operation, because the accumulated end products can be removed easily (**IV**). We obtained a higher CS_2 and H_2S removal efficiency with the laboratory reactor than with the pilot reactor, suggesting that any scaling up of the liquid phase biotrickling filter may prove a demanding task. In particular, the size of the air bubbles during liquid treatment increases when the system is scaled up, so that greater amounts of air containing sulphide but having no contact with the liquid will be carried through the liquid layer and gas exchange will be ineffective because of the higher volumes of gases trapped inside the bubbles. This would be one reason for the lower efficiency achieved with a large reactor. Furthermore, liquid flow rates relative to packing volume were lower in the laboratory experiment.

6.4 Selection of appropriate biological purification methods

Bioscrubbers and biotrickling filters allow better control and removal of accumulated compounds, but these methods are more expensive and need more supervision than conventional biofilters, which are suitable for low-concentration sites where they can be run without continuous supervision. On the other hand, this lack of operational control is also a drawback. The accumulated product may disturb the functioning of conventional biofilters, or even destroy the filter bed. Biofiltration is still the most commonly used biotechnological method for full-scale applications (Smet et al. 1998b), and it is widely used for treating emissions from the bioindustries (Smet et al. 1998a), where concentrations of odorous sulphide compounds are low. Bonnin et al. (1994) estimated that the removal capacity of a peat biofilter is approximately 10 g m^{-3} h^{-1} (in the case of sulphur compounds). In our experiments the figures varied from 2 to 185 g S m^{-3} h^{-1}, although the highest rates entailed overloading of the peat (**I**).

Although the biotrickling method allows better control over the operating parameters, its operational costs are higher than for biofilters due to the more complex structure, supervision and chemicals needed to stabilise conditions (nutrients and neutralisation). The choice of method depends mainly on the concentrations and amounts of waste gases involved. The biotrickling method should be preferred when sulphide concentrations are high, as this gave the highest removal capacities in our experiments, provided that the optimum bed material was used. A two-stage biotrickling filter has been developed to enhance the removal efficiency with respect to methylated sulphur compounds and high H_2S concentrations contained in gas mixtures, since without a two-stage strategy the H_2S detracted from removal of the methylated sulphur compounds (Ruokojärvi et al. 2001).

7 CONCLUSIONS

Biological methods for purifying waste gases containing odorous sulphide compounds both separately and in gas mixtures were investigated here, the emphasis being on purification of CS_2 and H_2S, although methylated sulphide compounds were also successfully removed. A *Thiobacillus* strain TJ330 that oxidising CS_2 and H_2S was isolated and characterized in order to optimize the pH, temperature and substrate concentration, all important operational parameters for the biological purification of these compounds.

Simultaneous removal of H_2S and CS_2 from effluent gas is possible in an acid environment, and fibrous peat inoculated with *Thiobacillus* is a suitable biofilter material when concentrations are quite low. Concentrations below 100 mg CS_2-S m^3 (still toxic and odorous) can be removed with a peat biofilter, but industrial emissions with hundreds of milligrams of S m^{-3} need more complicated technology. These high concentrations can cause serious health effects. The peat in a biofilter will become overloaded in time at concentrations of 1300 - 5000 mg S m^{-3}, indicating that organic material is unsuitable for the biofiltration of high concentrations of sulphur gases. This is mainly because elemental sulphur accumulates in the material and the bed is destroyed as a result of high sulphuric acid production. Regeneration of peat filter material is difficult and the overloaded material has to be replaced.

Several filter materials suitable for the formation of a biofilm were studied in order to improve the biological removal capacity at high concentrations of CS_2 and H_2S. The use of recycled plastic as a bed material may be preferred because of its low operation costs and serviceable technical properties, but the biofilms that formed on the plastic grit and ceramic saddles were quite different. *Thiobacillus* cells inoculated into the liquid phase favoured the ceramic material, whereas the plastic material supported the growth of filamentous micro-organisms harboured in the reactor as contaminants. These micro-organisms could belong to the genera *Beggiatoa* and *Thiotrix,* which are likewise able to oxidise sulphides, but they also produce slime, which sometimes disturbs the operation of the biotrickling filter. This filamentous growth may cause clogging and raise the pressure drop over the filter bed with time. The plastic grit made of recycled material required a longer acclimation period but was subsequently comparable with the other materials. The

recycled plastic material is light and entails a low pressure drop, and it is also cheaper than commercial ceramic saddles.

A liquid phase bubbling treatment combined with a conventional biotrickling filter increased the efficiency of H_2S and CS_2 removal. The removal efficiency of this technique is affected by the microbial oxidation capacity and by mass transfer between the bubbles and the microbial cells and between the dissolved gas and the micro-organisms in the biofilms or the liquid. The liquid phase treatment apparently increased the mass transfer of pollutants and oxygen from the gas to the liquid phase, which in turn increased the oxidation rate.

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