

## Two-stage gas-phase bioreactor for the combined removal of hydrogen sulphide, methanol and $\alpha$ -pinene

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### Abstract:

Biological treatment systems have emerged as cost-effective and eco-friendly techniques for treating waste gases from process industries at moderately high gas flow rates and low pollutant concentrations. In this study, we have assessed the performance of a two-stage bioreactor, namely a biotrickling filter packed with pall rings (BTF, 1<sup>st</sup> stage) and a perlite + pall ring mixed biofilter (BF, 2<sup>nd</sup> stage) operated in series, for handling a complex mixture of hydrogen sulphide (H<sub>2</sub>S), methanol (CH<sub>3</sub>OH) and  $\alpha$ -pinene (C<sub>10</sub>H<sub>16</sub>). It has been reported that the presence of H<sub>2</sub>S can reduce the biofiltration efficiency of volatile organic compounds (VOCs) when both are present in the gas mixture. Hydrogen sulphide and methanol were removed in the first stage BTF, previously inoculated with H<sub>2</sub>S-adapted populations and a culture containing *Candida boidinii*, an acid-tolerant yeast, whereas, in the second stage,  $\alpha$ -pinene was removed predominantly by the fungus *Ophiostoma stenoceras*. Experiments were conducted in five different phases, corresponding to inlet loading rates varying between 2.1 and 93.5 g m<sup>-3</sup> h<sup>-1</sup> for H<sub>2</sub>S, 55.3 and 1260.2 g m<sup>-3</sup> h<sup>-1</sup> for methanol, and 2.8 and 161.1 g m<sup>-3</sup> h<sup>-1</sup> for  $\alpha$ -pinene. Empty bed residence times were varied between 83.4 and 10 s in the first stage and 146.4 and 17.6 s in the second stage. The BTF, working at a pH as low as 2.7 as a result of H<sub>2</sub>S degradation, removed most of the H<sub>2</sub>S and methanol but only very little  $\alpha$ -pinene. On the other hand, the BF, at a pH around 6.0, removed the rest of the H<sub>2</sub>S, the non-degraded methanol and most of the  $\alpha$ -pinene vapours. Attempts were originally made to remove the three pollutants in a single acidophilic bioreactor, but the *Ophiostoma* strain was hardly active at pH <4. The maximum elimination capacities (ECs) reached by the two-stage bioreactor for individual pollutants were 894.4 g m<sup>-3</sup> h<sup>-1</sup> for methanol, 45.1 g m<sup>-3</sup> h<sup>-1</sup> for H<sub>2</sub>S and 138.1 g m<sup>-3</sup> h<sup>-1</sup> for  $\alpha$ -pinene. The results from this study showed the potential effectiveness of a two-stage bioreactor for treating H<sub>2</sub>S together with two hydrophilic and hydrophobic VOCs that are typically emitted from wood industries.

### Keywords

Biotrickling filter, biofilter, inorganic compound, hydrophobic compound, hydrophilic compound, fungi.

## Introduction

Rapid industrial proliferation in the last few decades has contributed to an enormous increase in the generation of waste gas from many industries and production processes. This has led to increasingly stringent regulatory requirements in many countries in recent years. Biological treatment systems such as biotrickling filters (BTF) and biofilters (BF) have shown promising results for handling waste gases at low concentrations and at moderately high flow rates. Bioprocesses depend on the activity of microorganisms to decontaminate polluted air, through a series of complex phenomenological steps comprising absorption, adsorption, diffusion and biodegradation. The pollutants are thereby oxidized to CO<sub>2</sub>, H<sub>2</sub>O and other harmless end products [1]. To date, most lab-scale biofiltration studies address the removal of single pollutants under relatively constant operating conditions. Such conditions are highly unusual at wastewater treatment plants and in other full-scale applications.

Waste gases arising from wood industries consist of a complex mixture of H<sub>2</sub>S and other reduced sulphur compounds (RSCs), such as dimethyl sulphide, dimethyl disulphide and methyl mercaptan, as well as volatile organic compounds, such as methanol, terpenes, alcohols, phenol, ketones, and formaldehyde [1]. Hydrogen sulphide, methanol and α-pinene are representative inorganic, hydrophilic and hydrophobic pollutants present in these emissions, respectively. Emissions of H<sub>2</sub>S give rise to nuisance odours, as they smell like rotten eggs, and have been shown to cause negative effects on the environment. Exposure to H<sub>2</sub>S is harmful to crops and vegetation and is highly corrosive to materials. According to the US Occupational Safety and Health Administration, a maximum safe exposure limit for this compound is 20 ppm at the workplace, but the odour from this gas can be detected down to concentrations below 0.5 ppb [2]. Methanol (CH<sub>3</sub>OH) is a hydrophilic compound and one of the 189 hazardous air pollutants listed in Title III of the 1990 Clean Air Act Amendment proposed by the US Environmental Protection Agency [3]. α-Pinene (C<sub>10</sub>H<sub>16</sub>), on the other hand, is a hydrophobic volatile organic chemical (VOC) that is hardly water soluble, and is emitted by forest product industries, particularly sawmills and composite board mills, as well as the pulp and paper industry (vapour pressure: 10 mm Hg at 25 °C, water solubility: 2.5 ppm at 25 °C) [3–5]. It can cause acute health effects, which include irritation due to direct eye contact, ingestion or inhalation. However, its carcinogenic, mutagenic and tetragenic effects are unknown. The presence of methanol and α-pinene, as well as other VOCs, in air emissions from wood industries has been the subject of recent environmental regulations, and the industries are required to apply an appropriate technology to reduce their emissions.

Most of the reported research focuses either on biofiltration of mixtures of VOCs or mixtures of RSCs [6–8]. Only a few researchers have worked on the co-treatment of H<sub>2</sub>S and VOCs [9–11]. One problem encountered is that the pH of the biofilm will drop when hydrogen sulphide is converted to sulphuric acid. The biological activity in the BF

may temporarily be inhibited with the rapid pH decline, thereby reducing the effectiveness of the treatment of organic compounds and sulphides. Another problem with acid production is that acids degrade organic media, such as compost, and can open channels in some areas and cause compaction in others. In addition, the head loss will rise, thereby increasing the operating costs and decreasing the efficiency. A two-stage BF for the treatment of waste air from wastewater treatment plants has been successfully tested [12]. The first stage, a BF packed with lava rock, was optimized for removal of H<sub>2</sub>S, whereas the second stage was a conventional BF using wood chip. However, it was observed that the pH of the wood chip medium in the second-stage BF had fallen to 2.5 near the inlet by the end of the study. When treating air polluted with a high H<sub>2</sub>S concentration, this may reduce the efficiency of the second-stage BF. Also, the results indicated that there was a considerable flow heterogeneity in both the acidic gas-phase BF and the wood chip BF. The co-treatment of H<sub>2</sub>S and organic vapour investigated in a lava-rock pilot-scale BF, operating at a pH of 4.0, showed high removal efficiencies (REs) for H<sub>2</sub>S (96%) and VOC (53.2%) at an empty bed residence time (EBRT) of 12.2 s [13]. The results showed that microbial species in a low pH environment were also capable of degrading organic compounds. The feasibility for sequential biofiltration of H<sub>2</sub>S and a VOC vapour (methyl *tert*-butylether, MTBE) from waste air from a wastewater treatment plant was evaluated using laboratory-scale and field studies [14]. The results showed complete removal of both H<sub>2</sub>S and MTBE at a loading rate of 8.3 g H<sub>2</sub>S m<sup>-3</sup> h<sup>-1</sup> (EBRT 15 s) and 33 g MTBE m<sup>-3</sup> h<sup>-1</sup> (EBRT 60 s), respectively. In a BTF coupled with a BF, the co-treatment of H<sub>2</sub>S with low concentrations of aliphatic and aromatic VOCs, predominantly toluene vapours, did not exhibit any inhibition in removal profiles [15]. Toluene removal efficiencies were greater than 90% at a concentration of 41 ppb, and average H<sub>2</sub>S removal efficiencies were about 75% at a concentration of 314 ppm.

In our previous work [11], a low-pH single-stage BTF was successfully designed for the co-treatment of H<sub>2</sub>S and methanol. An *Ophiostoma stenoceras* strain that utilizes  $\alpha$ -pinene as a sole carbon and energy source was also isolated. It has been reported that introducing methanol to an already existing bacterial community (*Thiobacillus* sp. and *Sulfobacillus* sp.) enhanced bacterial diversity, which led to increased biodegradation of both H<sub>2</sub>S and methanol [16]. In a recent study, a hybrid system combining a BF and a BTF section, with different microbes and operating pH levels, for the overall removal of H<sub>2</sub>S,  $\alpha$ -pinene, and methanol from polluted air was tested in our lab. However, the performance of the hybrid system for  $\alpha$ -pinene was not good because of the low fungal growth in the BF section due to the only moderate tolerance of the fungus to acidification. A two-stage system consisting of a low-pH BTF stage and a fungal BF stage may solve this problem. The fungal BF stage can be protected from acidification because most of the H<sub>2</sub>S and methanol can be removed in the first stage. Also, even if a small amount of H<sub>2</sub>S enters the second stage, this will not be a problem because the fungus can resist a moderately acidic pH. Therefore, the purpose of the present study

was to test the two-stage system for the overall removal of H<sub>2</sub>S, methanol and  $\alpha$ -pinene from polluted air. The bioreactors were operated under different conditions in order to maximize the performance of the system.

## **Materials and methods**

### **Microorganisms and media composition**

A mixture of an autotrophic H<sub>2</sub>S-degrading culture and an acid-tolerant methanol-degrading yeast (*Candida boidinii*) was used as inoculum for the BTF stage. The inoculum was obtained from the previous co-treatment of H<sub>2</sub>S and methanol in a BTF operated at a low pH of 2.0. A new fungal isolate, *Ophiostoma stenoceras*, was isolated as dominant strain from a BF originally used for treating  $\alpha$ -pinene. The composition of the mineral salt medium used in the BTF was (in g L<sup>-1</sup> of deionized water): KH<sub>2</sub>PO<sub>4</sub> 2.0, K<sub>2</sub>HPO<sub>4</sub> 2.0, NH<sub>4</sub>Cl 0.4, MgCl<sub>2</sub>·6H<sub>2</sub>O 0.2, FeSO<sub>4</sub>·7H<sub>2</sub>O 0.01. The medium used in the fungal BF had the following composition (g L<sup>-1</sup>): K<sub>2</sub>HPO<sub>4</sub> 0.5, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.1, KH<sub>2</sub>PO<sub>4</sub> 4.5, NH<sub>4</sub>Cl 2.0 and 2 mL of a trace element and vitamin solution [17]. The bed's moisture content was maintained constant by periodic addition (once every three days) of fresh mineral salt medium (pH 5.9) from the top of the BF.

### **Experimental set-up**

The two-stage bioreactor comprising a BTF and a BF is shown in Figure 1. The first stage is a 2.78 L BTF, in which a mixed H<sub>2</sub>S-degrading culture and an acid-tolerant yeast were present. The BTF was constructed using glass, 75 mm in diameter and 700 mm in height. The active height of the packed column, filled with polypropylene pall rings, was 640 mm. The pall ring bed had an initial porosity of 91% and a specific surface area of 350 m<sup>2</sup> m<sup>-3</sup>. The BF stage consisted of a cylindrical glass column with an inner diameter of 100 mm and a total height of 700 mm, provided with four gas sampling ports located along the reactor at 50 (outlet), 250, 450 and 650 (inlet) mm from the bottom of the reactor. The length of the BF bed was 600 mm, leading to a working volume of approximately 4.88 L. In the BF, irregular grains of perlite with a mean diameter of 4.5 mm were mixed with 50% (weight) of the same polypropylene pall rings used in the former BTF. The bioreactors were provided with four equidistant gas sampling ports and two filter-material sampling ports uniformly distributed on the other side of the column. All fittings, connections and tubings were made of either glass or teflon.

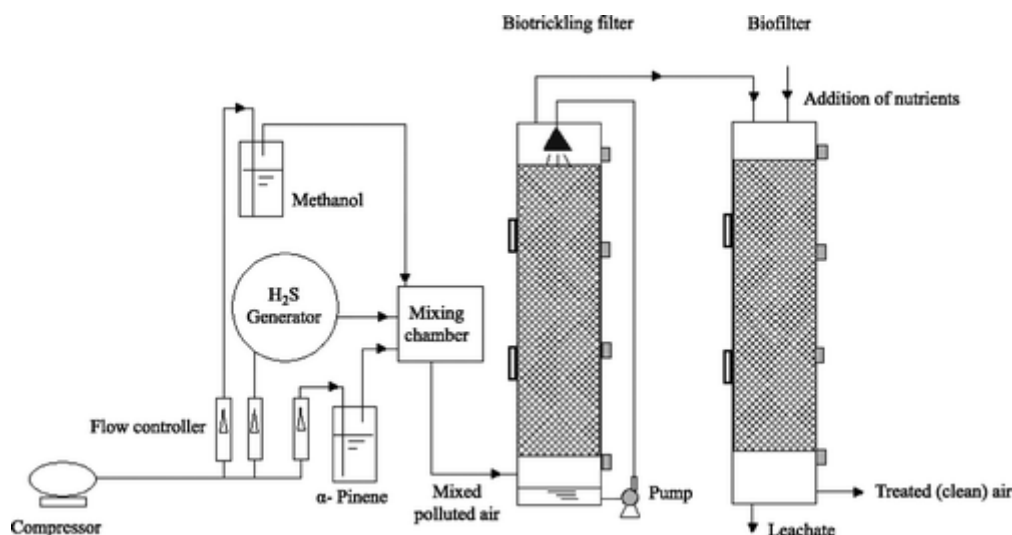


Figure 1 Schematic of the two-stage bioreactor (BTF followed by BF).

A compressed air stream was split into three flows. Hydrogen sulphide was generated by passing the major portion of the air stream over an  $\text{H}_2\text{SO}_4$  solution into which a solution of  $\text{Na}_2\text{S}$  was dripped. Different gas-phase  $\text{H}_2\text{S}$  concentrations were obtained by changing the  $\text{Na}_2\text{S}$  concentration and/or dripping rate. The other two minor air streams were bubbled through liquid methanol and  $\alpha$ -pinene, respectively, in flasks. The three streams were combined in a mixing chamber and fed to the bottom of the BTF column in a countercurrent flow mode. The aqueous mineral medium described above was continuously recirculated over the packed bed using a peristaltic pump (323E/D, Watson-Marlow Limited, Falmouth, England) at a constant volumetric flow rate of  $2.77 \text{ L h}^{-1}$ . The exit air from the top of the BTF was later fed through the fungal BF in a downflow mode. The two-stage bioreactor's performance was estimated by calculating the elimination capacity of the filter bed and removal efficiency of the corresponding pollutant at different inlet loading rates, according to equations defined elsewhere in the literature [1].

### Analytical methods

The pH was measured with a Crison 507 pH meter (Crison Instruments S.A., Barcelona, Spain), using a combined glass electrode. A glass U-tube water manometer was used to measure the pressure drop across the filter bed height. The moisture content (%) in the filter bed (stage II) was measured according to the procedure described elsewhere [18]. Hydrogen sulphide concentration was determined using a hand-held sensor (Dräger Sensor XSEC  $\text{H}_2\text{S}$  HC6809180, Dräger Safety Hispania S.A., Madrid, Spain). Gas phase concentrations of methanol and  $\alpha$ -pinene were measured via gas chromatographic analysis using a Hewlett-Packard 5890 series II GC. The GC was equipped with a flame ionization detector. The following flow rates were used:  $\text{H}_2$  –  $30 \text{ mL min}^{-1}$ , air –  $300 \text{ mL min}^{-1}$ . The inlet and outlet streams were sampled. The GC was equipped with a 50 m tracer column (TR-WAX, ID: 0.32 mm, film thickness:  $1.2 \mu\text{m}$ ,

Thermo Scientific, USA) and helium was used as the carrier gas (flow rate: 2.0 mL min<sup>-1</sup>). The temperatures at the GC injection, oven and detection ports were 150, 150 and 150 °C, respectively. Similarly, CO<sub>2</sub> concentrations were measured using another Hewlett-Packard 5890 GC fitted with a thermal conductivity detector (TCD). The CO<sub>2</sub> concentrations were determined at an injection temperature of 90 °C, an oven temperature of 25 °C and using a TCD at 100 °C [11]. Pall ring and perlite samples, immobilized with biomass and exposed to methanol, H<sub>2</sub>S and α-pinene vapours, were prepared for observations under the electron microscope according to the procedure described elsewhere [17]. Examinations were performed with a JEOL JSM-6400 scanning electron microscope (JEOL Ltd., Japan) working at a voltage of 20 kV and a working distance of 15 mm, and with Oxford Instruments (Abingdon, England) energy-dispersive x-ray equipment.

## **Results and discussion**

### **Performance of the biotrickling filter and biofilter during start-up**

During the first 14 d, the concentrations of methanol were kept relatively high (1.5–5 g m<sup>-3</sup>) because of the good removal of that pollutant, while H<sub>2</sub>S concentrations were less than 0.05 g m<sup>-3</sup>. In the BTF, though nearly 82% methanol was removed, giving rise to ECs as high as 205 g m<sup>-3</sup> h<sup>-1</sup>, the removal of H<sub>2</sub>S was just about 80% at low loading rates. In that reactor, an uneven biomass distribution was noticed along the bed height, with a more clustered biomass in the top section and a fairly meagre biomass in the bottom section of the filter bed. On the 15th day, the BTF was stopped for six hours and repacked manually to distribute the biomass evenly along the bed height. The concentration of methanol was lowered to 0.6 g m<sup>-3</sup>.

After this new start-up and from the 16th day, the concentration of H<sub>2</sub>S and methanol were increased slowly. The H<sub>2</sub>S and methanol removal efficiencies in the BTF improved slowly, reaching an EC of 197 g m<sup>-3</sup> h<sup>-1</sup> for methanol and 1.9 g m<sup>-3</sup> h<sup>-1</sup> for H<sub>2</sub>S. The much higher EC reached for methanol was presumably because of the much faster growth and activity of the heterotrophic VOC-degrader. The increase in RE was also well correlated with the decline in pH of the recycled mineral salt medium, presumably because of the production of H<sup>+</sup> and sulphate from the oxidation of H<sub>2</sub>S [19]. The medium was replaced once every four days or when its pH dropped below 2.7. On the other hand, the BF (2nd stage), though fed with low concentrations of α-pinene (<0.2 g m<sup>-3</sup>), showed steady and increasing removal performance, where nearly 100% of the incoming α-pinene was removed at the end of the 28th day, corresponding to an EC of 3.9 g m<sup>-3</sup> h<sup>-1</sup>. The moisture content in the BF was monitored periodically by collecting known amounts of samples, taken two days after medium addition, from the sampling ports. The moisture levels across the BF height varied depending on the gas flow rate, but remained within the optimal range (52–63%) recommended for BFs [1]. After this

start-up period, both the BTF and BF were ready to be tested for their performance under different operating conditions. Media samples were collected after 44 days of continuous operation and subjected to SEM (scanning electron microscopy) analysis. From the SEM photographs shown in Figure 2, it can be seen that a mixed culture composed of bacteria, yeasts (presumably *Candida boidinii*) and filamentous fungi (Figure 2a) was present in the BTF, whereas *Ophiostoma* sp. was clearly predominant in the BF (Figure 2b). These images were confirmed based on previous observations by our research group [11,20].

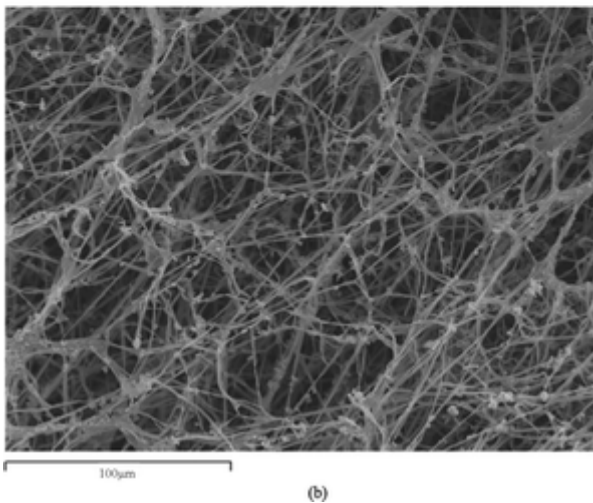
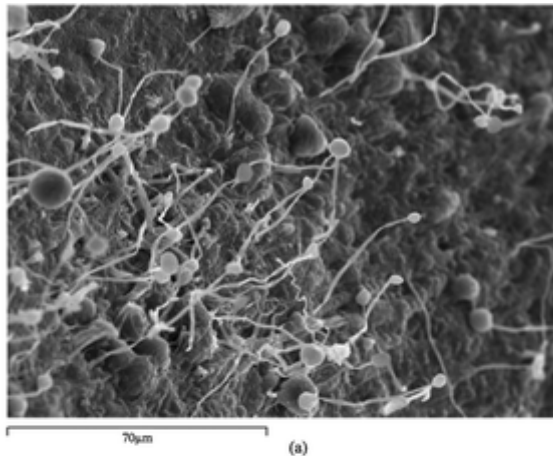


Figure 2 SEM images taken from (a) the first-stage BTF and (b) the second-stage BF.

### **Long-term removal performance of the biotrickling filter and biofilter**

After start-up, experiments were carried out in five phases, at flow rates of 0.12, 0.24, 0.36, 0.5 and 1 m<sup>3</sup> h<sup>-1</sup> corresponding to different EBRTs and inlet loading rates of H<sub>2</sub>S, methanol and α-pinene vapours (Table 1).

		EBRT, s		Inlet loading rate, g m <sup>-3</sup> h <sup>-1</sup>		
Phase of operation	Operation time, d	Stage I	Stage II	H <sub>2</sub> S	Methanol	α-Pinene
Start-up	1–28	83.4	146.4	1.5–2.8	28.9–205.1	0.7–3.9
Phase I	29–44	83.4	146.4	2.1–5.7	76.4–244.1	2.8–4.8
Phase II	45–89	41.7	73.2	6.0–18.4	55.3–1145.2	3.9–33.7
Phase III	90–104	27.8	48.8	4.6–15.1	109.1–553.9	6.7–82.7
Phase IV	105–115	20	35.2	19.0–72.1	140.8–1260.2	64.4–138.7
Phase V	116–132	10	17.6	33.5–93.5	186.1–1139.5	17.2–161.1

## Phase I

During the first experimental phase (days 29–43), at EBRTs of 83.4 s (BTF) and 146.4 s (BF), the concentration of all three compounds was gradually increased: 0.04–0.1 g m<sup>-3</sup> for H<sub>2</sub>S, 4.7–6.8 g m<sup>-3</sup> for methanol and 0.2–0.3 g m<sup>-3</sup> for α-pinene (Figures 3–5). The removal efficiency for H<sub>2</sub>S and methanol in the BTF was nearly 100%, while α-pinene removal in the BTF was about 12%. The remaining α-pinene was completely removed in the 2nd stage BF. The complete biodegradation of H<sub>2</sub>S produces sulphuric acid. Since there is no biotransformation consuming the acid, the latter accumulates very fast in the recycling trickling phase, and pH drops significantly to a point where the microbial activity is largely inhibited. In this study, pH was monitored constantly in the recycled liquid, and the values varied between 2.7 and 4.0. Originally it was planned to use only a single-stage acidic bioreactor inoculated with the mixture of acid-tolerant microorganisms, i.e. the H<sub>2</sub>S-degrading culture, *Candida boidinii* and *Ophiostoma stenoceras*. However, the acidophilic fungus (*Ophiostoma* sp.) only tolerated moderately acidic conditions and was hardly active at a pH below 4.0. Conversely, the yeast (*Candida boidinii*) showed a much better tolerance to pH values below 4.0.

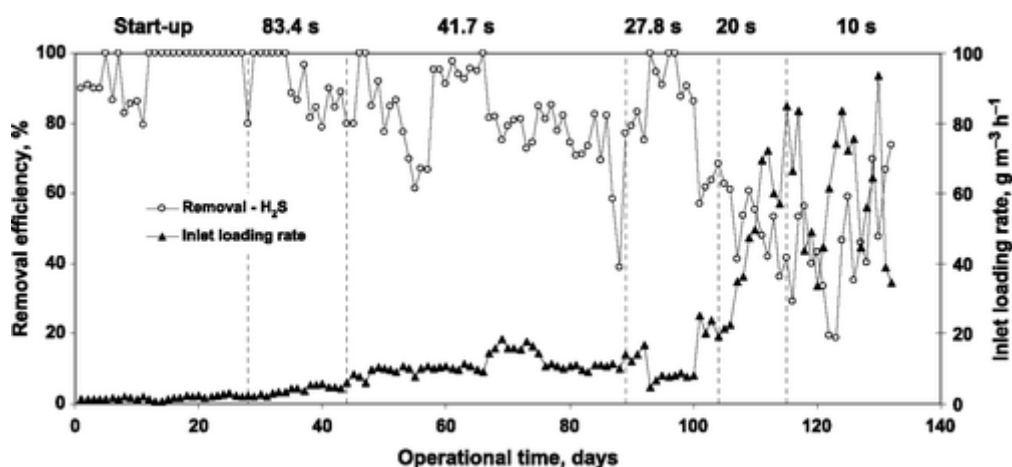


Figure 3 Removal efficiency profile of hydrogen sulphide in the BTF.



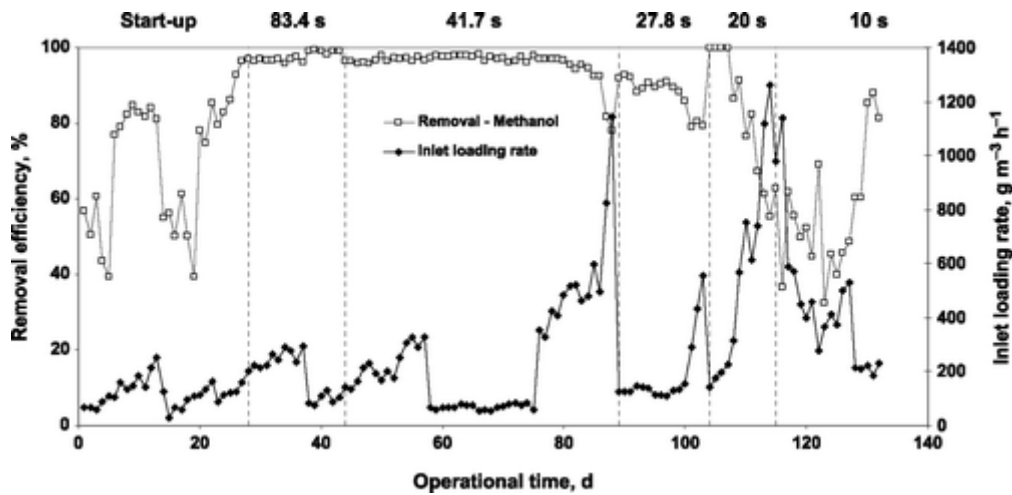


Figure 4 Removal efficiency profile of methanol in the BTF.

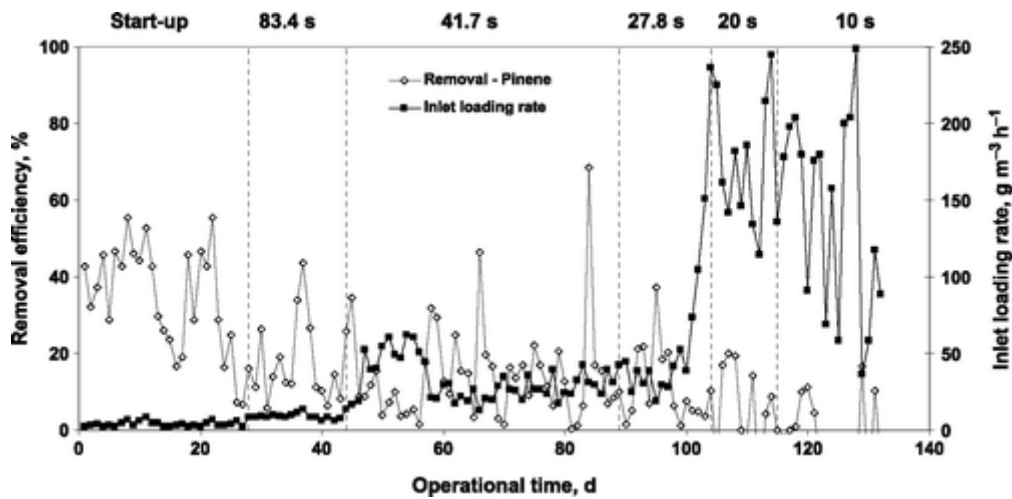


Figure 5 Removal efficiency profile of  $\alpha$ -pinene in the BTF.

## Phase II

At lower EBRTs of 41.7 s in the BTF and 73.2 s in the BF, and for methanol loading rates varying between 135 and 327  $\text{g m}^{-3} \text{h}^{-1}$  in Phase II (days 44–57), almost 98% of methanol was removed in the BTF, while the remaining part was removed in the 2nd stage BF (Figure 6). Under such conditions, though the inlet loading rates of  $\text{H}_2\text{S}$  were low (6–10.8  $\text{g m}^{-3} \text{h}^{-1}$ ), the  $\text{H}_2\text{S}$  removal in the BTF at an EBRT of 41.7 s dropped from 100% to 66.7%. Hence it was decided to reduce the methanol load and increase the  $\text{H}_2\text{S}$  load, thereby enhancing the growth of autotrophic microorganisms and preventing the surplus growth of heterotrophic organisms that could more easily use methanol rather than  $\text{H}_2\text{S}$ . A similar reduction in  $\text{H}_2\text{S}$  removal was observed in previous studies [11] using BTF, where the presence of low concentrations of methanol (18 ppm) had no significant effect on  $\text{H}_2\text{S}$  removal, whereas increasing the concentration of

methanol (>57 ppm) reduced the elimination capacity of H<sub>2</sub>S from 23.8 to 6.4 g m<sup>-3</sup> h<sup>-1</sup>. However in the BF (2nd stage), for α-pinene loading rates up to 33 g m<sup>-3</sup> h<sup>-1</sup>, more than 90% of that pollutant was removed (Figure 7). From the 58th day, the concentration of methanol was reduced and kept constant (~0.7 g m<sup>-3</sup>) for the next 18 days. Though 100% removal of methanol was plausible in the BTF, interestingly more than 90% of H<sub>2</sub>S was also removed in the BTF for loading rates up to 18 g m<sup>-3</sup> h<sup>-1</sup>, whereas just <5% of the remaining H<sub>2</sub>S was removed in the BF (Figure 8). These results suggest that low loading rates of methanol (100 g CH<sub>3</sub>OH m<sup>-3</sup> h<sup>-1</sup>) and moderate loading rates of H<sub>2</sub>S are preferred in the BTF in order to achieve high removal efficiencies without mutual inhibition of both pollutants. Contrary to the present data, it has been observed earlier that the presence of methanol had no significant effect on H<sub>2</sub>S removal rates in a BF packed with porous, inflated glass material, thereby achieving ECs as high as 380 and 95 g m<sup>-3</sup> h<sup>-1</sup> for methanol and H<sub>2</sub>S, respectively [10].

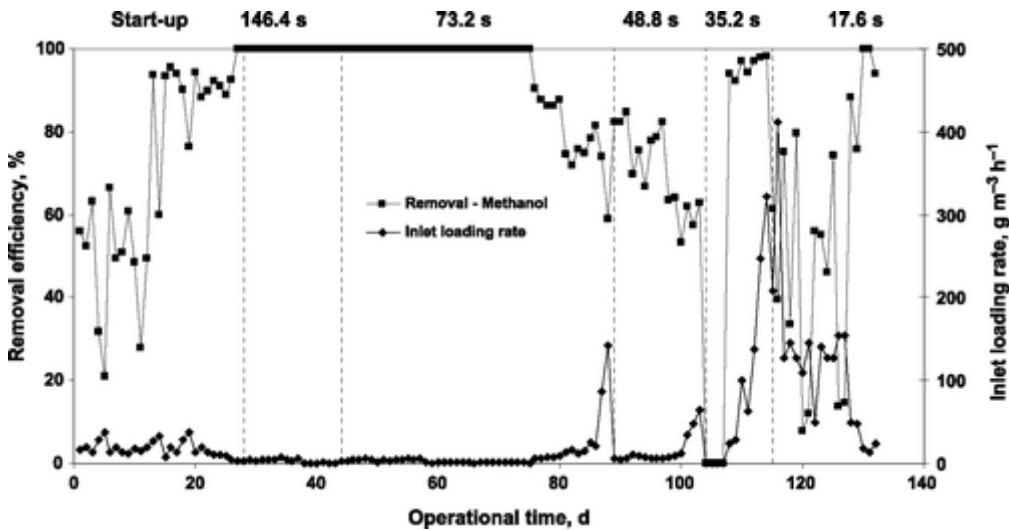


Figure 6 Removal efficiency profile of methanol in the BF.

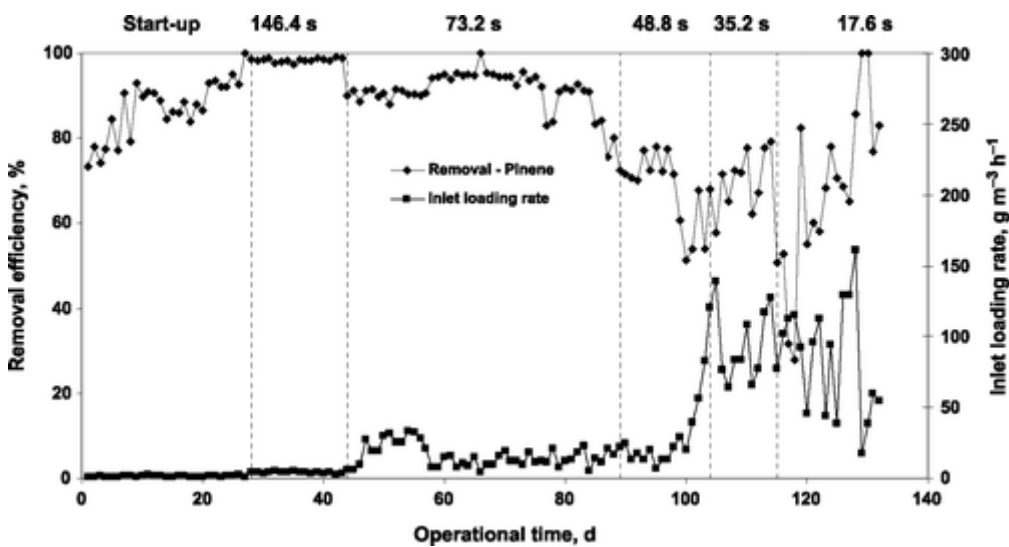


Figure 7 Removal efficiency profile of α-pinene in the BF.

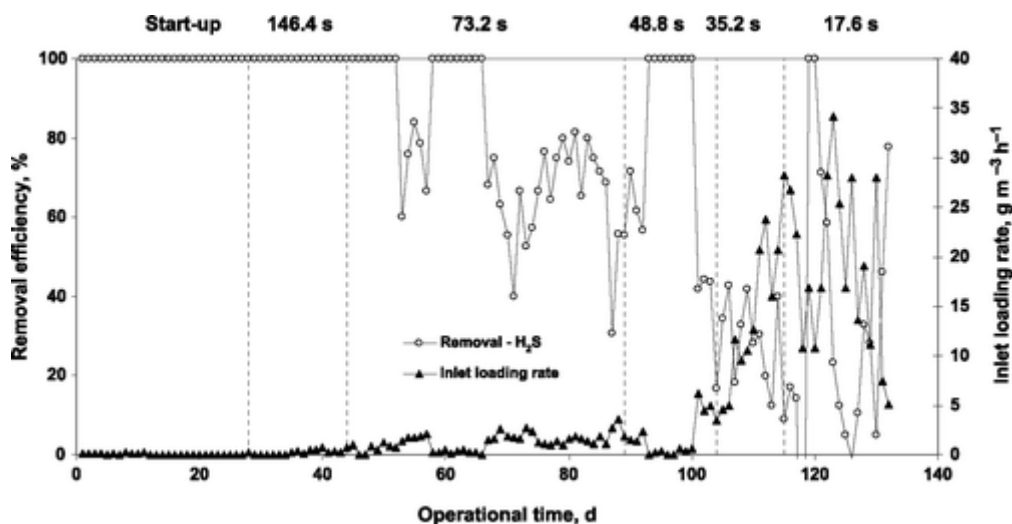


Figure 8 Removal efficiency profile of hydrogen sulphide in the BF.

In the later steps of operation, from the 77th day, the methanol concentration was increased steadily ( $3.2$  to  $13.2 \text{ g m}^{-3}$ ) to estimate its maximum elimination capacity. The loading rates were increased from  $327 \text{ g m}^{-3} \text{ h}^{-1}$  to  $1145.2 \text{ g m}^{-3} \text{ h}^{-1}$ , where a maximum EC of  $894.5 \text{ g m}^{-3} \text{ h}^{-1}$  was noticed with 78.1% removal in the BTF. The BF in the second stage (Figure 6) was able to remove nearly 60% of the remaining methanol when fed at a loading rate of  $142.8 \text{ g m}^{-3} \text{ h}^{-1}$ . Though the loading rate of  $\text{H}_2\text{S}$  in the BTF was maintained at around  $10 \text{ g m}^{-3} \text{ h}^{-1}$  during this phase, the removal of  $\text{H}_2\text{S}$  dropped by about 30–60% (Figure 3) depending on the applied methanol load. However, this stark increase in the methanol load did not affect  $\alpha$ -pinene removal in the BF and its removal was consistently greater than 90%, irrespective of the load (Figure 7).

### Phase III

In phase III, at a flow rate of  $0.36 \text{ m}^3 \text{ h}^{-1}$  and EBRTs of 27.8 s in the BTF and 48.8 s in the BF, the loading rates were varied between 4.6 and  $25.2 \text{ g m}^{-3} \text{ h}^{-1}$ , 109 and  $554 \text{ g m}^{-3} \text{ h}^{-1}$ , and 18.7 and  $150.8 \text{ g m}^{-3} \text{ h}^{-1}$  for  $\text{H}_2\text{S}$ , methanol and  $\alpha$ -pinene, respectively. In the BTF, for loading rates less than  $10 \text{ g m}^{-3} \text{ h}^{-1}$ ,  $\text{H}_2\text{S}$  removal was greater than 95%, whereas this value dropped to 65% when the load was increased beyond  $20 \text{ g m}^{-3} \text{ h}^{-1}$ . This could possibly be due to an increase in the methanol concentration (days 100–103) from  $1.2$  to  $4.3 \text{ g m}^{-3}$ . The results clearly indicate severe antagonistic effects on  $\text{H}_2\text{S}$  removal due to the presence of an easily biodegradable VOC – methanol. The BTF was also able to remove nearly 10% of the incoming  $\alpha$ -pinene vapour, though most  $\alpha$ -pinene removal occurred in the second stage (Figure 7). A maximum EC of  $44.6 \text{ g } \alpha\text{-pinene m}^{-3} \text{ h}^{-1}$  was observed in the BF at an inlet loading rate of  $82.7 \text{ g m}^{-3} \text{ h}^{-1}$ .

### Phases IV and V

In the subsequent two stages of operation (Stages IV and V, days 104–131), the bioreactors were operated at their lowest EBRTs, namely 20 s and 10 s in the 1<sup>st</sup> stage

and 35.2 s and 17.6 s in the 2nd stage, and subjected to very high loading rates of individual pollutants. The H<sub>2</sub>S loading rates were varied between 19 and 93.5 g m<sup>-3</sup> h<sup>-1</sup>, while methanol and α-pinene loading rates were varied between 140 and 1260 g m<sup>-3</sup> h<sup>-1</sup>, and 36.3 and 248.6 g m<sup>-3</sup> h<sup>-1</sup>, respectively. At the lowest EBRT of 10 s, the BTF was not able to remove any α-pinene from the contaminated mixture, whereas both H<sub>2</sub>S and methanol were removed moderately under the tested conditions. For instance, on the 130th day, at H<sub>2</sub>S loading rate of 93.5 g m<sup>-3</sup> h<sup>-1</sup> and methanol loading rate of 221 g m<sup>-3</sup> h<sup>-1</sup>, the removal efficiencies of H<sub>2</sub>S and methanol were 47.6% and 85.2%, respectively, in the BTF, followed by 100% removal of the remaining methanol and a mere 5.1% removal of the remaining H<sub>2</sub>S in the second stage (BF). Even under such extreme conditions, during the final two phases of operation, the maximum EC of α-pinene in the BF was 138.1 g m<sup>-3</sup> h<sup>-1</sup> with 85.7% removal, which is highly similar to our previous studies on α-pinene removal, as a single pollutant, in a fungal biofilter inoculated with *Ophiostoma* [20]. However, the EBRT was quite low here, i.e. 17.6 s, to be compared to an EBRT of 72s used in the fungal biofilter.

### **Pressure drop variations**

The pressure drop in the BTF was initially very low at less than 0.8 cm H<sub>2</sub>O, and increased to a maximum of about 4.6 cm H<sub>2</sub>O at the end of the experiments, when the flow rate was 1 m<sup>3</sup> h<sup>-1</sup>. On the other hand, the fungal BF (2nd stage) showed no increase in pressure drop during the experimental period, and these values fluctuated moderately from 0.3 to 0.9 cm H<sub>2</sub>O, irrespective of the flow rate, despite the fact that fungal biofilters are often expected to become clogged faster than other systems because of the filamentous morphology of fungi [21].

### **Relationship between inlet loading rate and elimination capacity in the bioreactors**

To establish suitable operating criteria necessary for scaling up the bioreactors, the relationship between the inlet loading rates and the elimination capacities has to be identified. Such relationships are shown in Figure 9, by comparing the elimination capacity of the bioreactors in stage I (BTF) and stage II (BF) for individual pollutants. The elimination capacity is defined as the amount of pollutant degraded per unit time, normalized to the volume of packed bed [22]. As shown in Figure 9, the relationship between the inlet load and elimination capacity is almost linear for all the pollutants up to a critical load after which the linear trend tends to either decrease slightly or become nearly constant. Such a pattern for elimination capacity profiles are quite common for biological treatment systems such as BTFs and BFs, where the maximum performance is affected by a number of factors that include pollutant load and toxicity, microbial growth rate and activity, physico-chemical parameters, O<sub>2</sub> consumption rates and kinetics of the removal process [1,22,23].

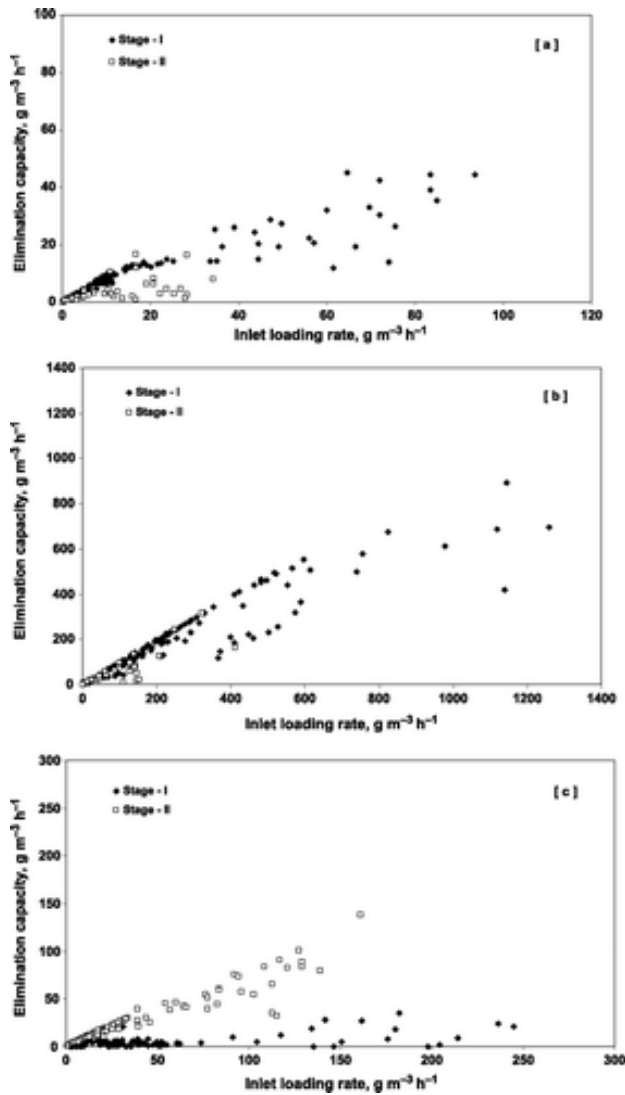


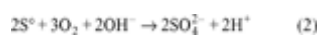
Figure 9 Elimination capacity of stage I BTF (◆) and stage II BF (□): (a) hydrogen sulphide (b) methanol (c)  $\alpha$ -pinene.

## Hydrogen sulphide

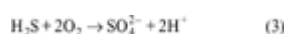
Hydrogen sulphide loads applied to the BTF (1st stage) varied from 1.5 to 93.5  $\text{g m}^{-3} \text{h}^{-1}$ , whereas the non-degraded  $\text{H}_2\text{S}$  entering the second stage BF had loads varying between 0.27 and 34.2  $\text{g m}^{-3} \text{h}^{-1}$ , for the entire operation time of 132 days. A maximum EC of 45.1  $\text{g m}^{-3} \text{h}^{-1}$  was achieved in the first stage, followed by a maximum EC of 16.8  $\text{g m}^{-3} \text{h}^{-1}$  in the second stage (Figure 9a). Under aerobic conditions, oxidation of  $\text{H}_2\text{S}$  by chemotrophs occurs with  $\text{O}_2$  as an electron acceptor, while the electron donors could be  $\text{S}^0$ ,  $\text{H}_2\text{S}$  or  $\text{S}_2\text{O}_3^{2-}$  [24,25]. Under oxygen-limiting conditions, sulphur is the major end product. Otherwise, sulphate is formed as well. This can be represented by the following equations:



(1)

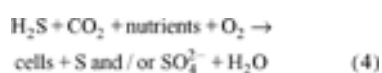


(2)



(3)

In aerobic autotrophic oxidation of sulphide [26], the following reaction would occur:

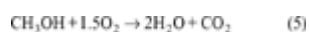


(4)

According to the stoichiometry of the aerobic biological oxidation, oxygen is the key parameter that controls the level of oxidation. The sulphate formed during the process could be later removed through precipitation in a settling tank coupled to the BTF, rather than being treated and eventually converted again to H<sub>2</sub>S in a wastewater treatment plant [17].

### Methanol

The maximum EC of methanol was 894.4 g m<sup>-3</sup> h<sup>-1</sup> in the first stage followed by 315 g m<sup>-3</sup> h<sup>-1</sup> in the second stage (Figure 9b). These values for methanol are significantly higher than the results of our previous studies, where the reported maximum EC was 236 g m<sup>-3</sup> h<sup>-1</sup> in a BTF [11] and 280 g m<sup>-3</sup> h<sup>-1</sup> in a lava rock BF [27]. Ideally, if the entire methanol supplied to the reactor is oxidized to carbon dioxide, the removal of 1 g of methanol will result in the production of 1.375 g of CO<sub>2</sub> according to Equation (5), neglecting biomass growth. Such a complete conversion was confirmed through mass balance calculations.



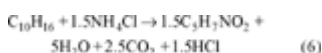
(5)

### α-Pinene

The effect of the α-pinene load on the elimination capacity is plotted in Figure 9c, both for the BTF and the fungal BF. The relationship was nearly linear up to 160 g m<sup>-3</sup> h<sup>-1</sup> in the BF, and, within the operating loading range, it did not tend to approach stationary conditions. This behaviour tends to confirm that still higher elimination capacities for α-pinene are plausible, though this would primarily depend on the loading rates of methanol, which is an easily degradable VOC. The removal efficiencies in the BF were greater than 80% for loading rates less than 128 g m<sup>-3</sup> h<sup>-1</sup>, and the maximum EC achieved was 138.2 g m<sup>-3</sup> h<sup>-1</sup> at a loading rate of 161.2 g m<sup>-3</sup> h<sup>-1</sup>. These values are

comparable to the results of our previous studies ( $146 \text{ g m}^{-3} \text{ h}^{-1}$ ), where pinene was treated individually in a lava rock BF in the presence of *Ophiostoma* sp. [20]. In the BTF (1st stage), a maximum EC of  $35.4 \text{ g } \alpha\text{-pinene m}^{-3} \text{ h}^{-1}$  was attained, though the majority of the  $\alpha$ -pinene was removed in the BF. Leachate samples collected periodically from the BF were analysed for pH, and these values were found to vary from 3.5 to 4.9 depending on the applied pollutant loading rates. The pH drop here was mainly due to the use of  $\text{NH}_4\text{Cl}$  as the nitrogen source.

The presence of filamentous fungi, such as *Ophiostoma stenoceras*, offers some advantages in the BF. Fungi develop hyphae, which could provide a large surface area in contact with the gas phase so that a direct efficient mass transfer from the gas phase to the biofilm phase is possible. This allows a faster uptake of hydrophobic compounds ( $\alpha$ -pinene) than in flat aqueous bacterial biofilms [21,28]. Carbon dioxide was identified as the end product ( $\text{CO}_2$  data not shown), the rest being converted to biomass. From previous shake flask experiments with *Ophiostoma stenoceras*, the yield coefficient of biomass was determined to reach  $1.25 \text{ kg dry biomass per kg } \alpha\text{-pinene consumed}$  [20]. The following stoichiometry was derived considering the yield coefficient value and the fact that  $\text{NH}_4\text{Cl}$  was used as the nitrogen source and that the typical cellular composition could be denoted as  $\text{C}_5\text{H}_7\text{NO}_2$  [1].



(6)

## Conclusions

The methanol degrader (a yeast) appeared to tolerate very low pHs (below 3.0), but the  $\alpha$ -pinene degrader (a fungus) tolerated only mild acidification (around pH 4.0). The pH dropped below 3.0 in the first-stage reactor removing  $\text{H}_2\text{S}$  and methanol.

In the BTF, the presence of methanol had a significant effect on  $\text{H}_2\text{S}$  removal, at high inlet loading rates of methanol. For methanol loading rates less than  $66 \text{ g m}^{-3} \text{ h}^{-1}$ , although 100% methanol was removed, more than 90%  $\text{H}_2\text{S}$  was removed for  $\text{H}_2\text{S}$  loads less than  $18 \text{ g m}^{-3} \text{ h}^{-1}$ . The presence of  $\alpha$ -pinene in the waste gas stream did not appear to show any antagonistic or synergistic effects on  $\text{H}_2\text{S}$  and methanol removals in the biotrickling filter. The maximum EC of methanol and  $\text{H}_2\text{S}$  reached as high as  $894.4 \text{ g m}^{-3} \text{ h}^{-1}$  and  $45.1 \text{ g m}^{-3} \text{ h}^{-1}$ , respectively.

In the BF,  $\alpha$ -pinene removal was not affected by the presence of non-degraded methanol from the first stage. Maximum ECs in the BF were as high as  $138.3$  and  $315.9 \text{ g m}^{-3} \text{ h}^{-1}$  and maximum removal efficiencies were 85.7% and 98.2% for  $\alpha$ -pinene and methanol, respectively. The high EC achieved in the BF can be attributed to the dominant presence of the filamentous fungus *Ophiostoma stenoceras* which acts as a

biological catalyst for rapid mass transfer of the hydrophobic pollutant,  $\alpha$ -pinene, from the gas phase to the aqueous biofilm.

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