

Steady- and transient-state operation of a two-stage bioreactor for the treatment of a gaseous mixture of hydrogen sulphide, methanol and α -pinene

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Abstract

BACKGROUND: A two-stage bioreactor, comprising a biotrickling filter (BTF) as the first stage and a biofilter (BF) as the second stage, operated under steady- and transient-state conditions, was tested to remove gas-phase hydrogen sulphide, methanol and α -pinene.

RESULTS: Hydrogen sulphide and methanol were removed in the first stage, while α -pinene, was removed predominantly in the second-stage fungal BF. The effect of the liquid trickling rate was evaluated in the BTF, while concentration-dependent synergistic and antagonistic interactions in both reactors were assessed by varying the concentration of one pollutant, and by maintaining 100% removal of other pollutants. Increasing the liquid trickling rate decreased methanol removal significantly, from > 93% to 40%. Increasing the concentration of hydrogen sulphide from low to high loading rates did not affect the removal of volatile organic compounds (VOCs), however, the reverse occurred. Under all the conditions tested, α -pinene removal in the second-stage biofilter still remained higher than 80%.

CONCLUSIONS: The results show the maximum elimination capacities achievable for this complex ternary mixture, under a wide range of operating conditions.

INTRODUCTION

The rise in emission levels of toxic pollutants from the pulp and paper industry is a major health concern. Reduced sulphur compounds, such as hydrogen sulphide, methyl mercaptans, dimethyl sulphide and dimethyl disulphide, are emitted from the sulphate process when sodium sulphide reacts with lignin.¹ On the other hand, a typical pulp and paper industry would also emit substantial amounts of volatile organic compounds (VOCs) such as methanol, terpenes, alcohols, phenols, ketones and formaldehyde. Hydrogen sulphide (H_2S), methanol (CH_3OH) and α -pinene ($C_{10}H_{16}$) are major air pollutants in such industries.² Exposure to respiratory irritants such as H_2S among pulp mill workers has shown to increase obstructive lung disorders, prevalence of respiratory symptoms, occupational asthma, reactive airways dysfunction syndrome (RADS) and increased risk for ischemic heart disease.^{3–5} Accidental exposure to methanol through either inhalation or ingestion could result in headache, dizziness, gastric disturbances, visual disturbance, conjunctivitis and severe neurological damage.⁶ α -pinene is released not only during the mechanical treatment of wood, but also during the storage of sawdust and shavings.^{7, 8} It is an irritant to the skin, eyes and mucous membranes and can easily penetrate the different barriers of the body, the gastro intestinal track and intact skin.⁹

Microbial purification of a mixture of waste gases such as hydrogen sulphide, methanol and α -pinene appears to be the most economical and efficient option for emissions of low pollutant concentrations, at moderately high flow rates. Biotrickling filters (BTFs) and biofilters (BFs) are two typical bioreactor configurations that are characterized by the use of packed beds with attached biomass.¹⁰ The applications of BTF and BF have been extended to several industrial facilities, as they appear to be reliable and cost-effective compared with physico-chemical gas treatment technologies.^{11–13} Earlier research developed a series of studies in order to implement bioreactor technologies, specifically using BTF and BF, for the treatment of off-gas emissions containing formaldehyde, methanol, dimethyl ether,¹⁴ α -pinene,² hydrogen sulphide,¹⁵ and hydrogen sulphide and methanol.¹⁶ Most of the previously reported work has focussed on the biofiltration of mixtures of VOCs or mixtures of reduced sulphur compounds.^{17–19} Recently, there have been a few reports concerning the co-treatment of H_2S and a VOC using either BTF or BF.^{16, 20–22} One of the most commonly reported problems during the co-treatment of H_2S and VOC is that the pH of the biofilm drops when H_2S is converted to sulphuric acid, which inhibits the biological activity in the BF and subsequent degradation steps. These include acid attack of the organic media, channelling in some site-specific areas, and filter bed compaction.

In BTFs, the trickling liquid, usually a well-defined nutrient medium, acts as a main agent for oxygen and substrate transport from the gas-phase to the biofilm.²³ As a consequence of increased pollutant transfer limitations from the gas-phase to the biofilm, as a result of the thicker liquid layer than in BFs, removal efficiencies in BTF do sometimes not reach 100%, even at low inlet loads, thereby affecting the maximum elimination capacity (EC) that can be reached.¹⁴ However, this depends on the characteristics of the incoming gaseous stream, whether hydrophilic or hydrophobic. For BTFs treating mixtures of H₂S and other VOCs, the continuous re-circulation of the trickling liquid phase easily eliminates the sulphuric acid produced during the biodegradation step, and helps to control the physiological conditions in the liquid phase, such as nutrient supply to the attached biomass, contaminant absorption, removal of metabolites and toxic byproducts and biofilm moistening.¹⁵ The flow rate of the re-circulation liquid or the liquid trickling rate, a major hydrodynamic parameter in trickle beds, has also been shown to strongly affect the performance of BTFs, and is very critical for full utilization of the filter bed capacity.²⁴

The aim of the present research was to study the biological treatment of a gaseous mixture of H₂S, methanol and α -pinene in a two-stage bioreactor system, consisting of a first-stage BTF followed by a second-stage BF, and to understand the effects of the most important operational parameters. Experiments were carried in continuous mode in the BTF and BF by changing process parameters such as gas flow rate, pollutant concentration and liquid trickling rate, in order to undertake the following studies: (i) dependency of pollutant loading rate on gas flow rate, i.e. the empty bed residence times (EBRTs); (ii) effect of liquid trickling rate in the first-stage BTF; (iii) effect of increasing pollutant peak load on the removal of other pollutants present in the mixture; and (iv) effect of transient-state operating conditions on the performance of the BTF and BF.

MATERIALS AND METHODS

Microorganisms and media composition

The BTF was inoculated with a mixture of autotrophic H₂S-degrading culture and an acid-tolerant methanol degrading yeast (*Candida boidinii*) obtained from previous work on the co-treatment of H₂S and methanol in a low-pH BTF.¹⁶ A *Ophiostoma stenoceras* sp., a well-known sap-wood colonizing fungus, isolated from a biofilter efficiently degrading α -pinene was used to inoculate the second-stage BF.² The composition of the mineral salt medium used in the BTF, was (in g L⁻¹ of de-ionized water); KH₂PO₄: 2; K₂HPO₄: 2; NH₄Cl: 0.4; MgCl₂·6H₂O: 0.2; FeSO₄·7H₂O: 0.01. The medium used in the fungal BF had the following composition (g L⁻¹); K₂HPO₄: 0.5, MgSO₄·7H₂O: 0.1, KH₂PO₄: 4.5, NH₄Cl: 2, and 2 mL trace elements and vitamin solutions.¹⁵ The moisture content of the BF bed was maintained constant by periodic addition (once every 3 days) of fresh mineral salt medium (pH 5.9) from the top.

Experimental setup and operation

The two-stage bioreactor comprising a BTF and a BF is shown in Fig. 1. The first stage is a 2.78 L BTF, 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 m²

m^{-3} . The BF stage consisted of a cylindrical glass column with an inner diameter of 100 mm and a total height of 700 mm. 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% (by weight) of the same polypropylene pall rings as used in the BTF. The bioreactors were provided with four equidistant gas sampling ports, located along the reactor at 50 (outlet), 250, 450, and 650 (inlet) mm from the bottom. Two filter material sampling ports were uniformly distributed on the other side of the column. All fittings, connections and tubings were made of either glass or Teflon.

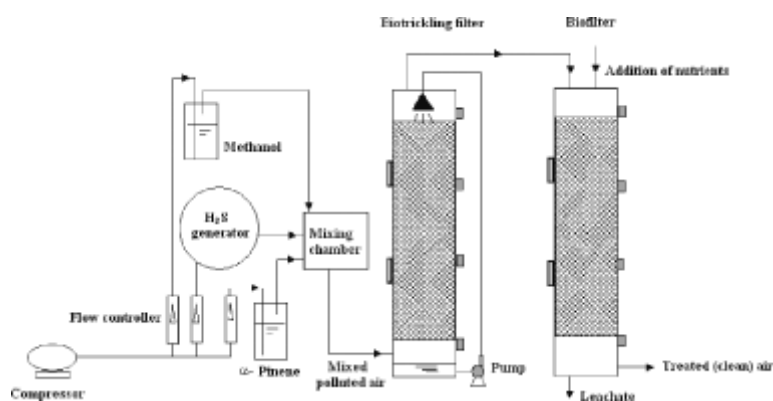


Figure 1. Schematic of the two-stage, BTF and BF connected in series.

A compressed air stream was split into three flows. H_2S was generated by passing the major portion of the air stream over a H_2SO_4 solution into which a solution of Na_2S was dripped. Different gas phase H_2S concentrations were obtained by changing the Na_2S concentration and/or dripping rate. The other two minor air streams were bubbled through flasks containing either liquid methanol or α -pinene separately. The three streams were combined in a mixing chamber, and fed to the bottom of the BTF column in a counter-current flow mode (Fig. 1). The aqueous mineral medium described above was continuously recirculated over the packed bed using a peristaltic pump (323E/D, Watson-Marlow Ltd, Falmouth Cornwall, England). During start-up and for experiments undertaken to ascertain the maximum elimination capacity, the liquid trickling rate was held constant at 50 mL min^{-1} , but varying its flow rate between 50 and 150 mL min^{-1} for certain experiments, depending on the study condition. 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.²⁴

Analytical methods

Hydrogen sulphide concentration was determined using a handheld sensor (Dräger Sensor XSEC H_2S HC6809180, Madrid, Spain). Inlet and outlet gas-phase concentrations of methanol and α -pinene were measured via gas chromatographic (GC) analysis using a Hewlett-Packard 5890 series II GC (Agilent Technologies, Madrid, Spain). The GC was equipped with a flame ionization detector (FID). The following flow rates were used; H_2 : 30 mL min^{-1} , air: 300 mL min^{-1} . The GC was equipped with a 50 m TRACER column (TR-WAX, ID: 0.32 mm, film thickness: $1.2 \mu\text{m}$) and helium was used as the carrier gas (flow rate: 2.0 mL min^{-1}). The temperatures at the GC

injection, oven and detection ports were 150, 150 and 150 °C, respectively. Similarly, CO₂ concentrations were measured using another Hewlett-Packard 5890 GC fitted with a thermal conductivity detector (TCD). The CO₂ concentrations were determined at an injection temperature of 90 °C, an oven temperature of 25 °C and using a TCD at 100 °C. pH was measured with a (Crison, Alella, Spain) pH-meter 507, using a combined glass electrode. A glass U-tube water manometer was used to measure the pressure drop across the filter bed height. At different time intervals, packing samples were gently removed from the upper and lower port of the second-stage BF. Dry biomass weight was measured by placing around 2 g of perlite sample with biomass in an oven at 90 °C for 12 h until reaching constant weight. After drying, moisture content (MC) in the packing material was determined by measuring the weight loss. The dried samples were later placed in a muffle furnace at 550 °C for 2 h, and the dry biomass content was determined by measuring the weight loss. After each biomass sampling, the same quantity of fresh perlite grains was added to compensate for the withdrawn samples. Pall ring and perlite samples, immobilized with biomass and exposed to methanol, H₂S and α -pinene vapours were prepared for observations under a scanning electron microscope (SEM). Examinations were performed with a JOEL JSM-6400 SEM (Izasa, Madrid, Spain) working at a voltage of 20 kV and a working distance of 15 mm, and with Oxford Instruments EDX equipment, (Izasa, Madrid, Spain) and quantitative elemental composition analysis, to confirm the accumulation of crystalline-sulphur particles, was done with the same equipment. Before SEM analysis, the samples were dried for 24 h, placed on a metallic stub and covered with gold by means of a Balzers SCD-004 sputter coater (Izasa, Madrid, Spain).

RESULTS AND DISCUSSIONS

Start-up of the bioreactors

The first-stage BTF and second-stage BF were acclimated with low concentrations of mixture of H₂S, methanol and α -pinene at a flow rate of 0.12 m³ h⁻¹, corresponding to an EBRT of 83.4 s in the first-stage BTF and 146.4 s in the second-stage BF. The inlet loading rates during start-up were as follows: 1.5–2.8 g H₂S m⁻³ h⁻¹, 28.9–205.1 g_{methanol} m⁻³ h⁻¹ and 0.7–3.9 g _{α -pinene} m⁻³ h⁻¹. The removal efficiency of H₂S and methanol in the BTF improved slowly reaching an EC of 1.9 g m⁻³ h⁻¹ for H₂S and 197 g m⁻³ h⁻¹ for methanol. The much higher EC reached for methanol than H₂S was presumably due to the much faster growth and activity of the heterotrophic *Candida boidinii* strain. The nutrient medium was changed once every 4 days or when its pH dropped below 2.7. On the other hand, the BF (second stage), although fed with low concentrations of α -pinene (<0.2 g m⁻³), showed steady and increasing removal performance, where nearly 100% of the incoming α -pinene was removed at the end of 28th day, corresponding to an EC of 3.9 g m⁻³ h⁻¹. After this start-up period (28 days), both the BTF and BF were ready to be tested for their performance under different operating conditions according to the operational schedule shown in Table 1.

Table 1. Operational schedule of the two-stage bioreactor

Nature of study	Range of inlet loading rates, g m ⁻³ h ⁻¹			Operating time
	H ₂ S1	Methanol1	α -pinene2	
1. Acclimation	1.5–2.8	29–205	0.7–3.9	28 d
2. Effect of pollutant load on RE and EC	0.6–45	28–1260	0.7–161	132 d
3. Effect of liquid tricking rate	28–48	308–489	69–126	20 d

Nature of study	Range of inlet loading rates, g m ⁻³ h ⁻¹			
	H ₂ S1	Methanol1	α -pinene2	Operating time
4. Effect of increasing concentrations	4–20	37–775	3–36	23 d
5. Effect of transient loads				
–Long–term shock loads	10–38	60–254	11–70	66 h
–Short–term shock loads	8–106	95–1220	17–176	12 h

- 1, 2 Pollutant loading rates to the BTF, BF, respectively.

Effect of inlet loading rate on the elimination capacity and removal efficiency

The effect of H₂S, methanol and α -pinene load on the removal efficiency of these compounds in the BTF and BF was evaluated at different gas flow rates (0.12 to 1 m³ h⁻¹), for 132 days, corresponding to EBRTs varying between 83.4 and 10 s in the first-stage BTF and 146.4 and 17.6 s in the second-stage BF (Table 2). It was observed that the maximum EC (EC_{max}) achieved at different EBRT, for each pollutant in either the BTF or BF, was highly dependent on the inlet loading rate (ILR). The removal efficiencies of each pollutant at each step of operation, corresponding to different EBRTs, depended not only on the concentration of individual pollutant, but also on the concentrations of other pollutants in the mixture, suggesting interaction effects could play a major role in determining the removal of a given compound in the mixture, as also shown in later experiments. As seen from Table 2, the EC_{max} of methanol decreased from 894 g m⁻³ h⁻¹ to 695 g m⁻³ h⁻¹, when α -pinene load was increased from 61 to 245 g m⁻³ h⁻¹, in the first-stage BTF, which occurred due to a decrease in the EBRT from 41.7 to 20 s, as well as the combined effect of the hydrophobic and hydrophilic VOC concentrations. On the other hand, the critical H₂S and methanol load in the BTF, dominantly removing these two pollutants, decreased with an increase in the gas flow rate. For instance, at an EBRT of 83.4 s in the BTF, the critical H₂S load to achieve > 90% removal was 11 g m⁻³ h⁻¹, while for the same pollutant, at an EBRT of 27.8 s, the critical load decreased to 7.9 g m⁻³ h⁻¹. A maximum EC of 45 g H₂S m⁻³ h⁻¹ was achieved with 70% removal in the BTF. Similarly, the critical methanol loads to reach > 90% removal decreased from 497 to 186 g m⁻³ h⁻¹, when the gas flow rate was increased from 0.36 (27.8 s) to 1 m³ h⁻¹ (10 s). For a load of almost 1200 g m⁻³ h⁻¹, the maximum methanol EC decreased when decreasing the EBRT from 41.7 to 20 and 10 s (Table 2). However, this dependency of critical load on the gas flow rate was not clearly evident in the second stage BF, where α -pinene was predominantly removed by the *Ophiostoma* sp., followed by the removal of residual methanol and H₂S entering from the first-stage BTF. Batch experiments with attached microbes taken from the perlite, from different ports along the bed height, confirmed that the heterotrophic microbial population in the BF and the predominantly available *Ophiostoma* sp. was able to remove both methanol and mixtures of methanol and α -pinene in aqueous phase (data not shown). The maximum EC achieved in the BF, for each step of operation, for α -pinene was a strong function of the inlet loading rate. The high EC achieved in the BF, 138 g m⁻³ h⁻¹, for a hydrophobic VOC, α -pinene, was plausible due to the inoculation of that reactor with the fungus *Ophiostoma stenoceras*. It was later confirmed through SEM analysis results and by means of periodically examining the perlite particle under an optical microscope, that the second-stage BF showed a predominant presence of the original fungus. Besides, the maximum EC of hydrogen sulphide and methanol in the

BTF were $16.8 \text{ g m}^{-3} \text{ h}^{-1}$ and $315 \text{ g m}^{-3} \text{ h}^{-1}$, respectively. It has been shown that fungal dominant systems could offer contaminant removal rates greater than those observed for bacterial systems.² For biofiltration of hydrophobic compounds like α -pinene, fungal growth is preferred over bacterial growth owing to their ability to degrade the substrates under extreme operating conditions regardless of pH and water content fluctuations, and limiting nutrient concentrations.²⁵

Table 2. Maximum elimination capacity in the first-stage BTF and second-stage BF

EBRT (s)	Hydrogen sulphide		Methanol		α -pinene	
	ILR _{max}	EC _{max}	ILR _{max}	EC _{max}	ILR _{max}	EC _{max}
First-stage BTF						
83.4	5.7	4.5	294	282	13.3	5.8
41.7	18.4	13.9	1145	894	61.8	21.4
27.8	25	15.1	553	439	150.7	8.2
20	72	33.2	1260	695	244.5	35
10	93.5	45	1139	613	248.5	18.1
Second-stage BF						
146.7	0.68	0.68	7.16	7.16	4.9	4.8
73.2	3.5	1.98	142.8	84.1	33.7	30.5
48.8	6.16	2.54	64.9	40.9	82.7	44.6
35.2	23.8	8.2	321.7	315.9	138.7	100.7
17.6	34.2	16.8	411.2	161.9	161.1	138.1

1. ILR_{max} and EC_{max} are in $\text{g m}^{-3} \text{ h}^{-1}$.

Sulphur accumulation in the filter bed

Pall rings with attached biomass from the BTF were collected after 44 days of continuous operation and subjected to SEM and elemental composition analysis. As seen from the SEM micrograph in Fig. 2, the rod-shaped particles formed on the surface of the pall rings was mainly condensed crystal-like elemental sulphur, which was also confirmed by elemental analysis as well as by the change in colour (colourless to pale yellow in less than 6 h and to dark yellow in less than 24 h) of the nutrient medium collected in the reservoir of the recirculation liquid. The sulphur content in the attached biomass, on the 44th day of the experiment was about 60% (wt basis), in a condition where nearly 80% of the incoming H_2S vapour was removed at an inlet loading rate of $6.1 \text{ g m}^{-3} \text{ h}^{-1}$. On the same day, methanol removal efficiency was about 96% at an inlet loading rate of $146 \text{ g m}^{-3} \text{ h}^{-1}$. Sulphur formation during the partial oxidation of sulphide instead of the complete oxidation to sulphate, as shown in Equations (1) and (2), could be due to oxygen limitation caused by preferential utilization of methanol by the acid tolerant yeast, because of its good biodegradability.¹⁶

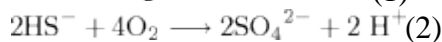
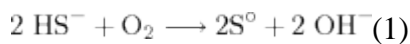




Figure 2.

SEM image showing the accumulation of elemental sulphur crystals on the pall rings.

Similar observation has been reported extensively in the literature, and this has environmental implications as elemental sulphur can easily be removed by sedimentation. During biodegradation, the incomplete oxidation of H_2S is generally reflected in high values of SO_3^{2-} and S^{2-} . The accumulation of elemental sulphur or ammonium sulphate have also been observed in biofilters packed with wood chips and granular activated carbon, treating H_2S and NH_3 as single compounds.²⁶ Such periodic accumulation led to a rapid decrease in the performance of those systems, from 99% to 75% and 30%, respectively. Elias *et al.*²⁷ showed that at a loading rate of $45 \text{ g H}_2\text{S m}^{-3} \text{ h}^{-1}$, the conversion products in biofilters were mainly sulphur (82%), followed by sulphates and thiosulphates (<18%). Buisman *et al.*²⁸ reported that, at sulphide concentrations below 20 mg L^{-1} , the oxygen concentration should be kept sufficiently low, below 1 mg L^{-1} , to limit sulphur oxidation to sulphate. The accumulation of sulphur in the packing material of a BTF, as well as in the re-circulation liquid has shown to decrease the removal efficiency of H_2S , additionally causing operational problems such as blockage of pores and channelling.¹⁵ However, in this study, as the recirculation liquid was changed once every 4 days or when the pH dropped to values below 2.7, it was presumed that consistent accumulation of sulphur and other metabolic end-products was minimized, thereby reducing some major operational problems.

Substrate stratification along the bed height of the BTF and BF

In order to understand the dynamics of pollutant removal within the BTF and BF, their concentration profiles were measured at different heights in the column. Figure 3(a) and (b) shows the normalized concentration profiles of H_2S , methanol and α -pinene along the length of the BTF and BF, measured on the 57th and 126th day of operation, when gas flow rates were 0.24 and $1 \text{ m}^3 \text{ h}^{-1}$, respectively (EBRT: in BTF-83.4, 10 s; in BF-146.7, 17.6 s). From Fig. 3(a), it is evident that for different flow rates and at methanol concentrations of 3.70 and 4.12 g m^{-3} the first one-third section of the filter bed (BTF) removed most of the alcohol (nearly 78% and 56%, respectively). On the other hand, H_2S was predominantly removed linearly over the next two-third section of the filter bed, suggesting that methanol was biodegraded before H_2S . The first one-third section of the filter bed was only able to remove < 10% H_2S , while the second and third section

removed more than 50% of H₂S under the tested conditions. As expected, removal of α -pinene did not occur in the BTF. Therefore, it could be envisaged that, the acid-tolerant, heterotrophic, yeast *Candida boidinii* colonized predominantly in the inlet section of the filter bed, while the autotrophic H₂S degraders prevailed in the later two sections of the column. An obvious explanation for this is that the fast growing heterotrophic methanol-degrading culture overgrows the slower H₂S-degrading organisms, at neutral to slightly acidic pH, close to the inlet of the BTF. Conversely, a slow pH decline takes place in the lower part of the reactor as a result of H₂S removal. The pH of the leachate reaches a minimum value of 2.7 at the outlet of the BTF, which becomes inhibitory for the methanol degraders overgrown by the H₂S degraders in the bottom section of the reactor. A similar behaviour was observed in another study on the removal of H₂S and methanol in a BTF. In that study, more than 75% of methanol was degraded in the first one-third section of the column, while H₂S, at low concentrations, was removed at a constant rate over the bed height.¹⁶

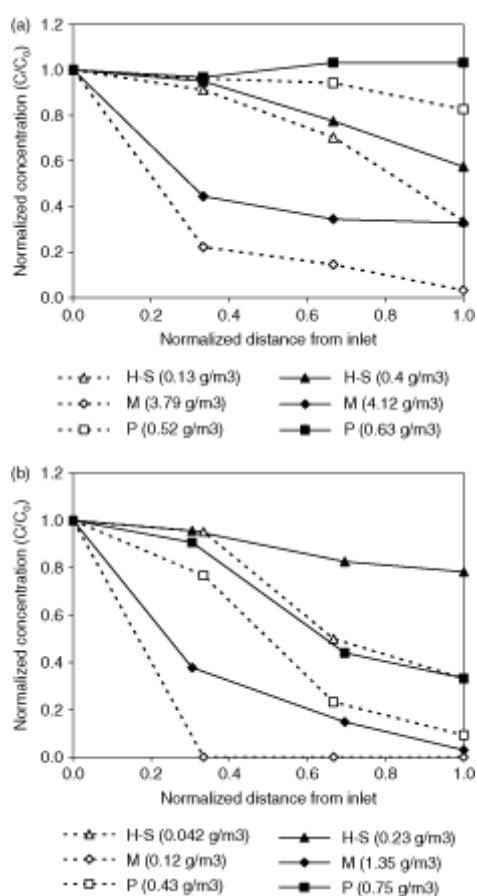


Figure 3.

Normalized concentration profile along bioreactor bed height at different flow rates: (a) BTF and (b) BF [--- dotted line: 0.24 m³ h⁻¹; — dashed line: 1 m³ h⁻¹; H—S: hydrogen sulphide, M: methanol and P: α -pinene].

For the second-stage BF, expected to remove α -pinene linearly over the bed height by *Ophiostoma sp.*, clear stratification in terms of methanol and α -pinene removal was noticed (Fig. 3(b)). At gas flow rates of 0.24 and 1 m³ h⁻¹, corresponding to EBRTs of 73.2 and 17.6 s in the BF, methanol was removed in the first one-third section of the

column, while the other sections predominantly removed α -pinene. When low concentrations of methanol (0.12 g m^{-3}) entered the BF, nearly 100% was removed in the first section of the filter bed, while more than 60% of the methanol was also removed in the first section when methanol concentrations were high (1.35 g m^{-3}). The other two sections contributed to more than 80% of the α -pinene removed in the BF, irrespective of the concentrations in the gas phase ($0.43\text{--}0.75 \text{ g m}^{-3}$). At low concentrations (0.042 g m^{-3}), nearly 60% of the non-treated H_2S entering the BF from the first-stage BTF was removed in the second and third section of the column, suggesting the presence of H_2S degraders that would have developed in the BF. Presumably, air from BTF carrying methanol and H_2S degraders reached the inlet of the second-stage BF. The fast, heterotrophic, methanol degraders colonized the inlet section of that reactor, while H_2S degraders colonized deeper sections of the system.

Biomass concentration, measured as dry biomass weight per gram of perlite is shown in Fig. 4. During the start-up step, biomass concentration was initially low (0.9 g g^{-1} perlite) and this value then gradually increased to a maximum of 1.6 g g^{-1} perlite over a period of 102 days. However, a minor increase in these values was noticed when the EBRT was decreased beyond 48.8 s after 102 days, where the pollutant load to the two-phase bioreactor, both BTF and BF, was linearly increased to observe the maximum EC. The moisture content was also monitored periodically by collecting known amounts of samples, taken 2 days after medium addition, from the two sampling ports (Fig. 5). It was found that the moisture levels across the biofilter height varied somewhat depending on the gas flow rate, but remained within an optimal moisture range, i.e. 53–64%, for biofilters.¹⁰ The lowest moisture content (MC) was found at the highest gas flow rate of $1 \text{ m}^3 \text{ h}^{-1}$, while the highest MC was attained at a gas flow rate of $0.12 \text{ m}^3 \text{ h}^{-1}$.

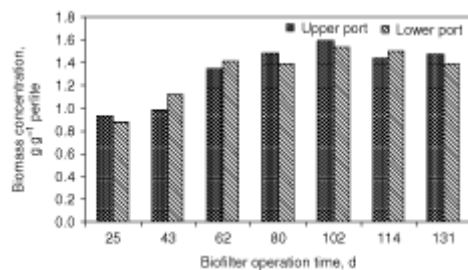


Figure 4.

Variation of biomass concentration in the second phase BF.

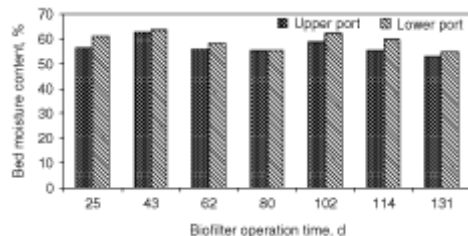


Figure 5.

Variation of bed moisture content in the second phase BF.

Effect of liquid trickling rate

In order to understand the effect of liquid trickling rate on the removal of H₂S, methanol and α -pinene in the BTF and BF, experiments were carried out at constant gas flow rate (0.5 m³ h⁻¹) corresponding to EBRTs of 20 s in the BTF and 35.2 s in the BF. The liquid trickling rate or liquid recirculation rate was varied in 4 steps at equal intervals of 25 mL min⁻¹, from 50 mL min⁻¹ to 150 mL min⁻¹, while the corresponding loading rates to the BTF ranged from 28–48 g H₂S m³ h⁻¹, 308–489 g_{methanol} m⁻³ h⁻¹ and 121–247 g _{α -pinene} m⁻³ h⁻¹. At each of the aforementioned trickling rates, experiments were carried out for 4–5 days. The nutrient medium was changed at the end of every step-change in liquid trickling rate to remove the acidic metabolites formed (pH ~ 2.7) and to start each experiment under the same conditions. Only a few data have been published in the literature on the influence of this parameter,²⁴ with sometimes different conclusions, showing the importance of such aspects as the type of pollutant or the range of liquid flow rate. The results from this study are shown in Fig. 6(a)–(c). In the BTF, at a liquid trickling rate of 50 mL min⁻¹, and for an ILR up to 40 g H₂S m⁻³ h⁻¹, the removal of H₂S was about 40%. However, although this value of RE seemed to increase slightly at a higher liquid trickling rate of 75 mL min⁻¹, it can be considered that it actually remained almost constant, considering the somewhat fluctuating behaviour of the inlet load (36–51% removal). On the other hand, stepwise increase in the liquid trickling rate from 50–150 mL min⁻¹ showed a clear decreasing trend in the removal profile for methanol. Initially, even at methanol loading rates greater than 350 g m⁻³ h⁻¹, high RE (>95%) were noticed at 75 mL min⁻¹, nevertheless, at ILR of 407 g m⁻³ h⁻¹ and at a liquid trickling rate of 150 mL min⁻¹, the removal efficiency dropped significantly, to 22%. The influence of liquid trickling rate on BTF performance has been reported in the literature. Liquid flow rates have shown to have higher impact on the removal of the pollutant at higher inlet loads than at lower ones, such as for H₂S observed in this study.²⁴ At near constant loads, the high removal of methanol at 75 mL min⁻¹ and the subsequent decrease in removal at high liquid trickling rates, suggests that high liquid trickling rates and moderate pollutant loading rates may not favour better removal of gaseous mixtures of H₂S and methanol. Under operational conditions such as batch recirculation modes with continuous recycling of medium, although liquid residence time per pass decreased with increase in liquid trickling rate, the number of passes increased.²⁹ Hence, during all the 20 days of operation in this study, irrespective of the liquid flow, the overall liquid residence time inside the BTF remained the same over the duration of the experiment. Changes in liquid trickling rate did not appear to have any effect on the removal of α -pinene in the first-stage BTF. Under all the tested conditions, α -pinene removal was less, and in some instance even slightly negative due to improper residence times of that pollutant in the BTF, and this can be attributed to the low solubility of α -pinene in the liquid phase, which eventually shows that mass transfer resistance was present in the trickling liquid (Fig. 6(c)). Another explanation for observing a negative RE profile for α -pinene is given in a later section. Some possible explanations for the reduction in methanol removal, a hydrophilic VOC, at high liquid trickling rates and high pollutant loads are given here: (i) an increase in the thickness of the liquid phase at higher liquid flow rate causes mass transfer limitation, due to high methanol concentrations, between gas-phase and the liquid/biofilm phase;¹⁵ (ii) high liquid flow rates could cause liquid channelling in the centre region of the column, which in-turn affects the liquid–gas–biomass contact; (iii) the high gas velocity (0.5 m³ h⁻¹) used in this study could have affected methanol removal, in addition to the liquid trickling rate, as it has been reported that, at high gas flow rates, the external mass

transfer resistance becomes negligible, but transfer from the liquid to the biofilm becomes limiting;30 and (iv) changes in flow pattern at high liquid trickling rates, as the liquid trickling rate increases, the rivulets will grow in size and an enlargement of the existing channels or the formation of an additional channel would occur. However, in order to ascertain the exact reason and to find a proper trickling rate that ensures higher removal at high pollutant loads, more studies on the hydrodynamic critical factors such as property of support matrix, amount of biomass present, bed void fraction, pressure drop, liquid hold-up, liquid distribution, partial wetting and stagnant water, are needed. In the BF, for ILR up to $88 \text{ g m}^{-3} \text{ h}^{-1}$, $> 95\%$ of α -pinene was removed, irrespective of the loading rate of H_2S and methanol, reaching a maximum EC as high as $83.4 \text{ g m}^{-3} \text{ h}^{-1}$. Methanol loading rate to the BF varied between 69 and $126 \text{ g m}^{-3} \text{ h}^{-1}$ depending on its removal in the first-stage BTF, and the BF was able to maintain high RE for this compound (83–86%).

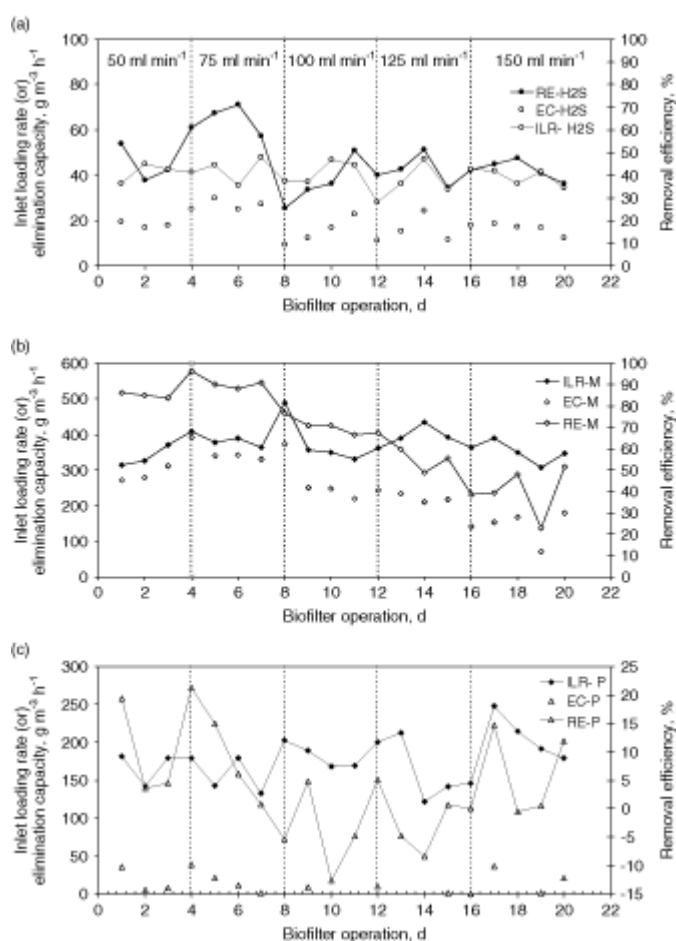


Figure 6.

Effect of liquid trickling rate on the removal efficiency of (a) hydrogen sulphide (b) methanol and (c) α -pinene in the BTF.

Effect of high concentrations of one pollutant on the removal of mixtures

In general, multi-component gas-phase mixtures having different biodegradation rates and characteristics tend to show interactive effects, i.e. the presence of one pollutant can increase or decrease the removal of the other pollutant in biological systems. In order to

understand the synergistic and antagonistic interactions during the removal of H₂S, methanol and α -pinene, experiments were conducted for 23 days, by varying the concentration of one pollutant from low to high values at a constant gas flow rate of 0.24 m³ h⁻¹, EBRT-41.7 s in the BTF and 73.2 s in the BF, and by maintaining the concentrations of the other pollutants constant so as to achieve near 100% removal (Figs 7 and 8). The reduction in removal performance of the individual pollutant due to the presence of other pollutants was then calculated from the original 100% removal achieved at constant, yet low loading rates. On days 1–9, in the BTF, the concentration of H₂S was increased from 0.05–0.23 g m⁻³ (i.e. loading rate of 4.8–20.4 g H₂S m⁻³ h⁻¹), while the loading of methanol and α -pinene were kept constant at 50 g m⁻³ h⁻¹ and 12 g m⁻³ h⁻¹, respectively. The removal of methanol in the BTF was > 98%, and the RE of H₂S dropped from 100% to 71%, while as usual, α -pinene removal in the BTF was not significant (Fig. 7(a)–(b)). In the next step (days 10–18), an increase in α -pinene concentrations from 0.11 to 0.73 g m⁻³ (i.e. loading rate of 9.7 to 63.3 g m⁻³ h⁻¹) rapidly decreased the removal of H₂S by 23% and slowly decreased methanol removal by 10% in the BTF, when their loading rates were 8 and 42 g m⁻³ h⁻¹. However, due to an increase in the concentration of α -pinene in the first-stage BTF, the α -pinene load to the second-stage BF also increased from 3 to 35 g m⁻³ h⁻¹, and its removal decreased by about 15% in that reactor (Fig. 8). In the BF, H₂S and methanol ECs were 1.2 and 3.8 g m⁻³ h⁻¹, respectively, and these values were low, as most of the H₂S and methanol were removed in the first-stage BTF. On days 19–23, when methanol concentration was increased stepwise from 1.4 to 9 g m⁻³, corresponding to loading rates as high as 775 g m⁻³ h⁻¹ to the BTF, its removal in the BTF decreased from 95% to 71%, the removal of H₂S decreased by 25% and α -pinene removal was not altered. A maximum EC of 554 g m⁻³ h⁻¹ was noticed for methanol in the BTF. However, in the BF, the remaining non-treated methanol caused an increase in the inlet load from 3–125 g methanol m⁻³ h⁻¹, where the RE of methanol also decreased from 88 to 78%. An increase in the concentration of methanol in the second-stage BF also caused an average decrease in the removal of α -pinene in that reactor of 14% (Fig. 8). In other words, the biocatalyst had a preference for methanol biodegradation in some zones of the biofilter. In a compost biofilter fed with equal proportions of gas-phase methanol and ethanol, the presence of ethanol decreased methanol removal by 32%, while ethanol removal was also reduced by 30%.³¹ This phenomenon, mutual inhibition, was later explained by the presence of two different groups of microorganisms, namely methanol utilizers and ethanol utilizers, while the methanol utilizers showed the ability to switch to ethanol utilization in the presence of ethanol, the ethanol utilizers appeared to be inhibited by the presence of methanol.

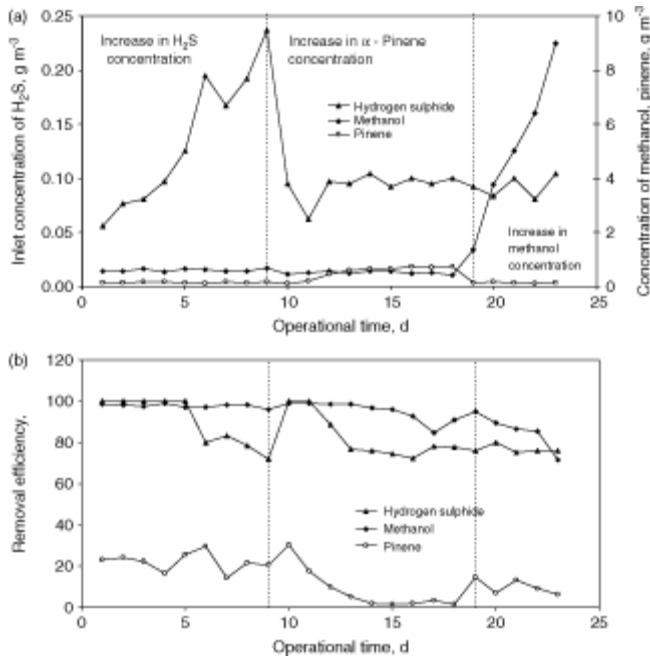


Figure 7.

Effect of changing pollutant concentration on the removal efficiency of hydrogen sulphide, methanol and α -pinene in the BTf: (a) concentration profile; (b) removal efficiency.

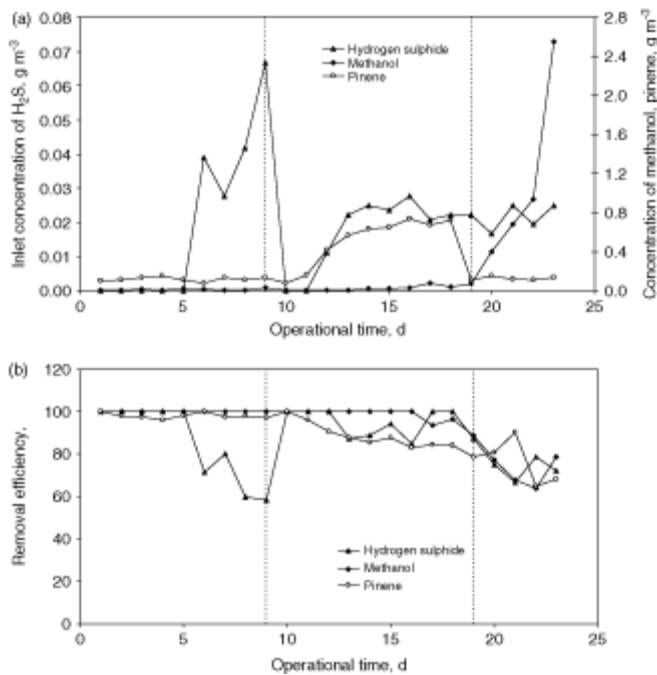


Figure 8.

Effect of changing pollutant concentration on the removal efficiency of hydrogen sulphide, methanol and α -pinene in the BF: (a) concentration profile; (b) removal efficiency.

Overall, increasing the H₂S concentrations from low to high values, did not affect the removal of VOC in both reactor configurations, however, increasing the concentration of either methanol or α -pinene reduced the removal of H₂S by almost 25% in the BTF. Increasing the concentration of methanol in the BTF, indirectly also had an effect on α -pinene and methanol removal in the second-stage BF. An increase in the α -pinene concentrations from low to high values, did not significantly decrease methanol removal (10%) in the first and second-stage bioreactors, however H₂S removal was affected strongly in the first-stage BTF, and α -pinene removal decreased slightly in the second-stage BF, depending on the loading rate. In a two-stage biofilter, BTF followed by BF, treating a gaseous mixture of formaldehyde and methanol, RE as high as 88% and 72% in the first-stage BTF followed by > 95% removal of both compounds in the second-stage BF was observed.¹⁴ However, no cross-inhibition effects were reported in that work. In a biofilter packed with inert glass beads, the removal of methanol in the presence and absence of H₂S was found to be more stable than H₂S biofiltration alone, reaching EC as high as 480 g m⁻³ h⁻¹.²¹ No mutual cross-inhibition occurred during biofiltration of H₂S and toluene in two BTFs operated in parallel at pH 7 or 4.5.²⁰ This was attributed to the possible presence of two different types of microbes within the BTF, namely autotrophic microorganisms for H₂S degradation and heterotrophic microorganisms for toluene degradation. Apparently, these behaviours are clearly different from the results observed in our study and some of the other literature. During the co-treatment of methanol and H₂S, the presence of methanol significantly affected the removal of H₂S, while the removal of methanol was not affected by the presence of H₂S, despite low-pH values.¹⁶ Greater than 90% removal of H₂S in a two-stage BF, with a maximum EC of 1.46 g m⁻³ h⁻¹, in the first-stage acid-gas biofilter and moderate removal (73%) in the second-stage BF, has been reported during the treatment of H₂S and a wide range of VOCs present in the waste gas.³² The low pH might have caused poor VOC removal in some of the studies using mixtures of H₂S and VOC. Jeong *et al.*³³ studied the removal of ethanol, acetaldehyde and toluene in single-and two-stage biofilters and envisaged that the two-stage BF indeed improved the performance with no cross-inhibition patterns occurring among pollutants. Mohseni and Allen³⁴ studied the removal pattern of gas-phase methanol and α -pinene in a biofilter packed with a mixture of wood-chips and spent mushroom compost. The presence of pinene did not affect methanol removal, which indicated that α -pinene did not interfere with the activity of the methanol degrading microorganisms, however maximum EC of α -pinene depended strongly on the concentration of the incoming vapour and a value of 45 g α -pinene m⁻³ h⁻¹ was observed when methanol was not present in the air stream. Jianwei *et al.*³⁵ attributed multi-substrate inhibition or cross-substrate interactions effects to low ECs of H₂S, ethyl mercaptans, ammonia, styrene and butyric acid (1.7–3.1 g m⁻³ h⁻¹) in low and neutral pH biofilters.

Effect of transient loads on bioreactor performance

The effect of transient-state conditions, such as shock loads, on biofilters in practical situations was simulated in two steps: as long-term shock loads (66 h experiment, low to medium loads) and short term shock loads (12 h experiment, low to high loads), by increasing the concentration of all three compounds, H₂S, methanol and α -pinene, during the applied shocks.

Long-term shock loads

In this study, the gas flow rate was set to the maximum, $1 \text{ m}^3 \text{ h}^{-1}$, and experiments were carried out for 66 h, including a shock load period of 52 h for the BTF and BF. Initially for 8 h, the loading rates of H_2S , methanol and α -pinene were maintained low at 10, 60 and $18 \text{ g m}^{-3} \text{ h}^{-1}$, respectively. The bioreactors were subjected to shock loads from 8 h and the concentrations of pollutants were measured after 12 h, allowing the BTF and BF to respond to the shock load. Figure 9 shows the shock loading and pollutant removal pattern observed in the BTF. As can be seen, the removals of H_2S decreased from 100% to as low as 50%, at a shock load of $38 \text{ g m}^{-3} \text{ h}^{-1}$, while methanol removal decreased slightly by 30%, when the load was $250 \text{ g m}^{-3} \text{ h}^{-1}$. However, no removal of α -pinene was noticed in the BTF. Barona *et al.*³⁶ investigated the response of a biofilter to abrupt changes in flow rate and concentration and showed that an increase in concentration of H_2S from low to high levels (0.59 g m^{-3}) reduced the RE significantly. When the concentration was decreased to 0.07 g m^{-3} the biofilter performance fully recovered to 100% efficiency. The fraction of the non-treated H_2S and methanol, as well as the incoming pinene vapours induced a shock load to the second-stage BF, where the EBRT of the pollutants was 17.6 s (Fig. 10). Though the ILR of H_2S and methanol were low in the BF (<9.9 and $<41.9 \text{ g m}^{-3} \text{ h}^{-1}$), the α -pinene loads increased from 11 to $70 \text{ g m}^{-3} \text{ h}^{-1}$. It was observed that the removal of α -pinene in the BF was not affected much by this medium shock load, RE reducing by just 10%.

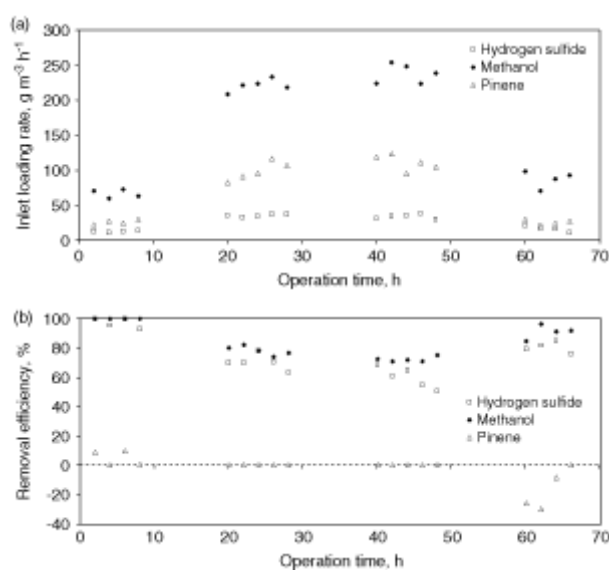


Figure 9.

Effect of long-term, low to medium shock loads on the first-stage BTF: (a) inlet loading rate and (b) removal efficiency.

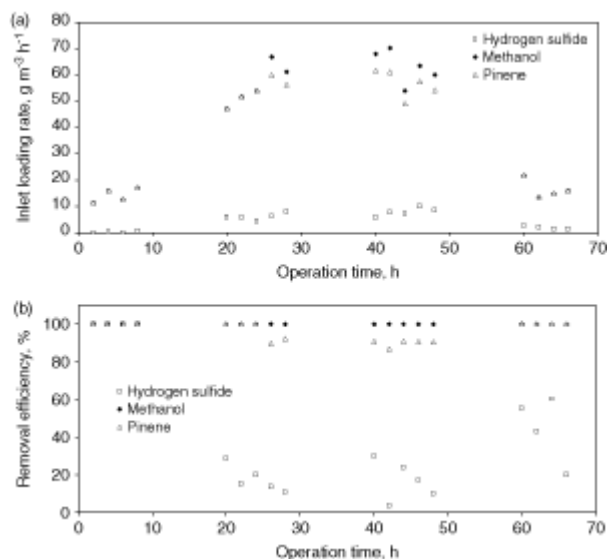


Figure 10.

Effect of long-term, low to medium shock loads on the second-stage BF: (a) inlet loading rate and (b) removal efficiency.

Short-term shock loads

During this experimental step, the BTF and BF were submitted to short-term high shock loads for 8 h. The concentrations of individual pollutants were increased from low values to very high values during the shock loading step, corresponding to increases from 8.5 to 106 $\text{g H}_2\text{S m}^{-3} \text{h}^{-1}$, 95 to 1220 $\text{g methanol m}^{-3} \text{h}^{-1}$ and 30 to 305 $\text{g } \alpha\text{-pinene m}^{-3} \text{h}^{-1}$, respectively. Earlier, the BTF and BF were adjusted to receive low loads of H_2S , methanol and α -pinene overnight, and measurements were taken the next day for 2 h initially at these low loads, before subjecting both bioreactors to the shock loads, as shown in Figs 11 and 12. It was observed that the high shock loads instantaneously and severely affected the removal of both H_2S and methanol in the BTF, although nearly 100% removal was noted for all pollutants before the shock load. From the 3 h, H_2S removal was just 31%, when the H_2S load was 106 $\text{g m}^{-3} \text{h}^{-1}$, while at a loading rate of 1220 $\text{g methanol m}^{-3} \text{h}^{-1}$, methanol removal decreased by 50% initially and occasionally dropped to 33%. On the other hand, as observed during the previous shock loading study (Fig. 9), α -pinene was not removed in the first-stage BTF during the shock load, irrespective of the applied load, whether medium or high. Instead, just after the shock load, when the concentrations were brought down to previously applied low values, α -pinene concentrations were even somewhat higher, by 0.03 to 0.12 g m^{-3} , in the outlet of the first-stage BTF than the inlet section, giving rise to short, transient, negative removal profiles, as shown in Figs 9 and 11, a phenomenon that was noted earlier (Fig. 6(c)). It has been reported that organic pollutants tend to adsorb to the surface of the solid surfaces, packing material and biomass related compounds, and also partially absorb into the liquid film present over the packing material. In such cases, the pollutant would be retained for a somewhat longer period in the BTF than the mean residence time of the pollutant. Similarly, Mendoza *et al.*³⁷ observed through tracer studies that, in a biofilter operated at a calculated EBRT of 56 s, the mean residence time of styrene was 185 s during start-up, while during the second year of continuous biofilter operation, that value increased 5.35 fold. The lower volatility and higher

density of styrene, as well as heavy biomass growth during the experimental period was considered the main reason for such a major change in residence time of the pollutant.

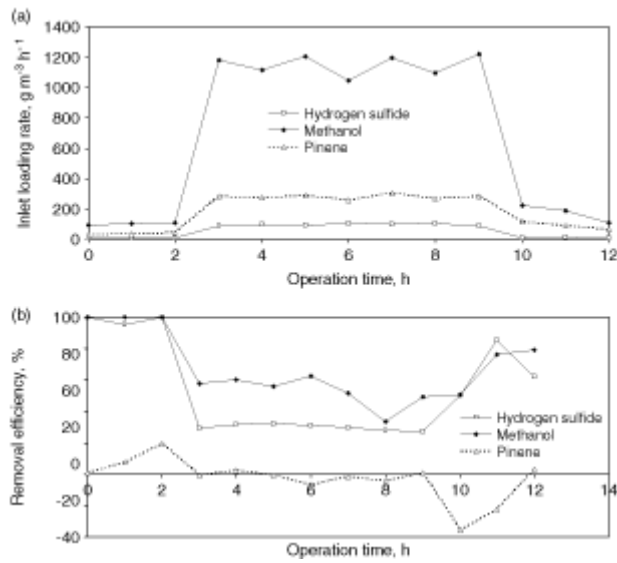


Figure 11.

Effect of short-term, low to high shock loads on the first-stage BTF (a) inlet loading rate and (b) removal efficiency.

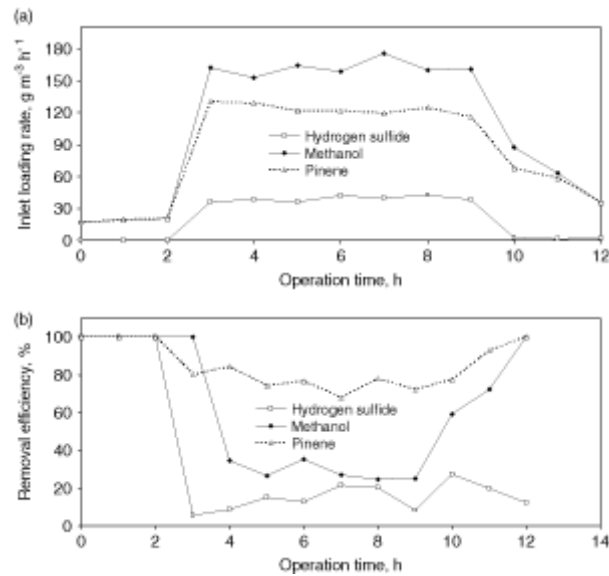


Figure 12.

Effect of short-term, low to high shock loads on the second-stage BF: (a) inlet loading rate and (b) removal efficiency.

However, as a result of this high shock load, and the lower removal of both H₂S and methanol in the BTF, the second-stage BF received loads of 42.4, 414 and 176 g m⁻³ h⁻¹ of H₂S, methanol and α -pinene, respectively (Fig. 12). H₂S and methanol removal efficiencies were lower, about 8.5% and 25%, while high removals (75%) of α -pinene were maintained in the BTF giving rise to a maximum EC of 130.1 g m⁻³ h⁻¹. H₂S and

methanol EC in the BF were 8.6 and 101.7 g m⁻³ h⁻¹, respectively. The consistently high performance of the second-stage BF during shock loads is not surprising, as results from a previous study have shown the ability of the *Ophiostoma sp.* to withstand periodic shock loads of gas-phase pinene, for more than 1 month of transient-state operations, where EC and RE were found to be 60 g m⁻³ h⁻¹ and > 90%, respectively.² Moreover, the presence of filamentous fungi as *Ophiostoma stenoceras* in the BF offers some advantages for the treatment of hydrophobic compounds such as α -pinene. Fungi develop hyphae which provide a large surface area in contact with the gas phase so that direct, efficient mass transfer from the gas phase to the biofilm phase is possible. This allows a faster uptake of hydrophobic compounds (α -pinene), than in flat aqueous bacterial biofilms.¹⁰

CONCLUSIONS

Biological treatment of a complex gas-phase mixture of hydrogen sulphide, methanol and α -pinene has shown promising results in a two-stage bioreactor system, composed of a first-stage BTF inoculated with autotrophic H₂S degraders and an acid-tolerant yeast (*Candida boidinii*) and a second-stage BF inoculated with the fungus *Ophiostoma stenoceras*, at different inlet loading rates and process conditions.

The first-stage BTF showed maximum elimination capacities of 45 g m⁻³ h⁻¹ for hydrogen sulphide and 894 g m⁻³ h⁻¹ for methanol. In the second-stage BF, when the gas flow rate was increased two-fold, the EC_{max} for α -pinene removal increased from 100 to 138 g m⁻³ h⁻¹ at an ILR of 127 and 161 g m⁻³ h⁻¹.

Stratification in terms of biodegradation of pollutants along the bed height was observed in both reactor configurations. The first one-third section of the BTF was able to remove nearly 78% of methanol, while H₂S was removed linearly over the next two-sections of the filter bed.

The effect of the liquid recirculation rate on pollutant removal characteristics in the BTF was determined by changing the liquid trickling rate from 50 to 150 mL min⁻¹. The results showed that, due to mass transfer limitations, high liquid trickling rates and moderately high pollutant loading rates might not favour better, simultaneous removal of gaseous mixture of H₂S, methanol and α -pinene.

Increasing the concentration of the hydrophilic VOC, methanol, or the hydrophobic VOC, α -pinene to the BTF, led to a decline in the RE of H₂S by about 25%, however, a stepwise increase in H₂S concentration did not appear to affect the removal of VOC in both the BTF and BF. Being an easily biodegradable compound, methanol was also removed in the second-stage BF, which could have been possible due to the preferential utilization of methanol as carbon and energy source by the original biocatalyst in the BF.

The commonly reported practical problems in continuous bioreactor operation, such as prevalence of unexpected shock loads, either medium and/or high loads, were simulated in the present study. H₂S removal was affected strongly in the BTF, while methanol removals were not much affected when the applied shock load was less than their critical load. During high shock loads, H₂S and methanol removal was less in the BTF, while high removals (75%) of α -pinene were maintained with an EC of 130.1 g m⁻³ h⁻¹.

This shows that the two-phase bioreactor system was sensitive to changes in loading rates.

The results from this study provide sufficient information on the antagonistic and synergistic effects occurring during the biological treatment of a complex gaseous mixture containing organic, inorganic, hydrophilic and hydrophobic pollutants.

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