

## **Biofiltration of mixtures of gas-phase styrene and acetone with the fungus *Sporothrix variecibatus***

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### **Abstract**

The biodegradation performance of a biofilter, inoculated with the fungus *Sporothrix variecibatus*, to treat gas-phase styrene and acetone mixtures under steady-state and transient conditions was evaluated. Experiments were carried out by varying the gas-flow rates ( $0.05\text{--}0.4\text{ m}^3\text{ h}^{-1}$ ), leading to empty bed residence times as low as 17.1 s, and by changing the concentrations of gas-phase styrene ( $0.01\text{--}6.3\text{ g m}^{-3}$ ) and acetone ( $0.01\text{--}8.9\text{ g m}^{-3}$ ). The total elimination capacities were as high as  $360\text{ g m}^{-3}\text{ h}^{-1}$ , with nearly 97.5% removal of styrene and 75.6% for acetone. The biodegradation of acetone was inhibited by the presence of styrene, while styrene removal was affected only slightly by the presence of acetone. During transient-state experiments, increasing the overall pollutant load by almost 3-fold, i.e., from 220 to  $600\text{ g m}^{-3}\text{ h}^{-1}$ , resulted in a sudden drop of removal efficiency ( $>90\text{--}70\%$ ), but still high elimination capacities were maintained. Periodic microscopic observations revealed that the originally inoculated *Sporothrix* sp. remained present in the reactor and actively dominant in the biofilm.

### **Keywords**

Biofiltration; Fibre-board industries; *Sporothrix variecibatus*; Styrene–acetone mixture; Maximum elimination capacity; Transient response

## 1. Introduction

The rapid development and proliferation of process industries has made problems associated with air pollution a major concern in industrial areas. The Clean Air Act Amendments (CAAA) of 1990 proposed by the United States Environmental Protection Agency (US-EPA) distinguishes 188 air pollutants based on their inherent toxicity. Styrene ( $C_6H_5CH=CH_2$ ) is an important VOC, which is commonly used as a raw material for the synthesis of plastics, synthetic resins, butadiene-styrene latex, styrene co-polymers and unsaturated polyester resins [1]. On the other hand, acetone ( $CH_3-CO-CH_3$ ) is a manufactured chemical, that is also found naturally in the environment. Styrene and acetone form a major part of the emissions from fibre-reinforced plastics (FRP) industry [2]. Airborne emissions containing both styrene, a hydrophobic VOC, and acetone, a hydrophilic organic compound, have shown significant effects on human health and the environment. This has led to increased attention from the regulatory authorities that have helped industries to undertake continuous modifications/development in the existing control technologies.

Some of the efforts undertaken to control these emissions have often failed due to the inefficiency of conventional treatment systems such as adsorption, absorption, wet scrubbing, ozonation, catalytic oxidation and incineration. Under such situations, biodegradation of these compounds has always yielded promising results, while biofiltration, under continuous mode, extends the application of microbes for waste-gas treatment. Styrene and acetone removal in biofilters and biotrickling filters has been investigated as individual gas-phase pollutants by a few researchers, where pure strains and/or mixed species of bacteria have generally been tested [3] and [4]. Research pertaining to the application of fungal biofilters to treat VOCs was initiated only recently. A styrene degrading fungus, *Sporothrix variecibatus* was isolated from a biofilter treating gas-phase styrene. That fungus remained dominant in the biofilter reaching high elimination capacities [5]. In another previous work, this dominant fungus, when inoculated in a biofilter as a pure strain, showed a maximum elimination capacity of  $336 \text{ g styrene m}^{-3} \text{ h}^{-1}$  at an EBRT of 91.2 s [6]. One of the main advantages of stimulating fungal rather than bacterial growth for the biofiltration of hydrophobic VOCs like styrene is their ability to completely mineralize these compounds under extreme environmental conditions such as acidic pH, low water content and limited nutrient concentration [7]. In fungi, exogenously produced spores are often formed on specialized hyphae and are usually referred to as conidiophores. There are two primary ways in which a conidiophore develops in fungi, i.e., blastic and thallic development. These conidiophores inherently possess the ability to withstand hydrophobic compounds, besides enhancing the absorption and transport of hydrophobic compounds from the contaminated phase to the cell surface [8].

In most of the earlier reported studies on the co-treatment of binary mixtures of gas-phase pollutants, either synergistic or antagonistic type interactions have been reported, i.e., the presence of one compound has led to an increase or decrease in the removal of the other compound and vice-versa. Chang and Lu [9] studied toluene and acetone removal in a trickling biofilter and showed that low acetone loads and medium toluene loads ensured greater than 90% removal for both compounds, and a maximum elimination capacity (EC) of  $160 \text{ g toluene m}^{-3} \text{ h}^{-1}$  and  $20 \text{ g acetone m}^{-3} \text{ h}^{-1}$  was reported. In another study [10], it was observed that toluene removal was inhibited by the presence of high ethyl acetate concentrations in a wood-chip + compost biofilter,

and one possible explanation for the inhibition mechanism was attributed to the failure of the toluene degraders to compete for  $O_2$  that limited total VOC removal at high concentrations. The maximum EC reported in that study was  $300 \text{ g ethyl acetate m}^{-3} \text{ h}^{-1}$  and  $200 \text{ g toluene m}^{-3} \text{ h}^{-1}$ . Liu et al. [11] evaluated two parallel bench-scale biofilters packed with compost, soil and lava rock using indigenous microorganisms and reported that the biodegradation of toluene was suppressed by the presence of ethyl acetate, while the removal of ethyl acetate was not affected by the presence of toluene. At an empty bed residence time (EBRT) of 30 s, the maximum EC were  $450 \text{ g ethyl acetate m}^{-3} \text{ h}^{-1}$  and  $50 \text{ g toluene m}^{-3} \text{ h}^{-1}$ . Dehghanzadeh et al. [12] treated styrene and acrylonitrile mixtures in a biofilter packed with yard compost and shredded high density plastics, inoculated with activated sludge microbes. It was envisaged that, acrylonitrile biodegradation was not affected by the presence of styrene in gas-phase, while styrene removal was enhanced in the lower section of the filter bed when acrylonitrile was completely depleted, suggesting the existence of competition for bio-catalytic activity among different microbial populations within the biofilter. The maximum ECs were  $103 \text{ g acrylonitrile m}^{-3} \text{ h}^{-1}$  and  $45 \text{ g styrene m}^{-3} \text{ h}^{-1}$ , respectively.

In all the above-mentioned studies, either pure bacterial strains or mixed cultures have been used and very little work has been initiated to study fungal biofilters for treating mixtures of pollutants in gas-phase. The present work was carried out, in an effort to understand the removal characteristics of mixtures of gas-phase pollutants in a biofilter, using *S. variecibatus*. Styrene was chosen as the hydrophobic VOC, whose removal characteristics using this fungus were well-established earlier as an individual pollutant [6], while acetone was chosen as the hydrophilic organic compound, owing to its tendency to readily biodegrade under aerobic conditions and its presence, together with styrene, in industrial effluents from fiber-board industries.

Biofilters handling industrial waste-gases often receive sudden fluctuations in pollutant loading rates due to changes in process conditions. An active biofilter should be able to handle such adverse situations in order to provide maximum removal of the target contaminant [13] and [14]. However, little information is known about the shock surviving capacity of fungal biofilters, especially when the gas-phase contains more than one pollutant. Also, in industrial situations, there could be complex interactions among different pollutants, and the microorganisms with respect to biodegradability when biofilters experience a sudden, yet unexpected shock load. To understand this phenomenon better, the transient behaviour of the fungal biofilter was investigated by subjecting the fungal biofilter to short-term fluctuations in inlet loads of both styrene and acetone. A new, batch experiments were conducted to understand the kinetics of the removal process, as well as to ascertain some of the experimental observations from the biofilter.

## **2. Materials and methods**

### **2.1. Nutrient medium and fungal inoculum**

The mineral salt medium used for batch and continuous biofilter experiments had the following chemical composition (per liter in distilled water):  $0.5 \text{ g K}_2\text{HPO}_4$ ,  $0.1 \text{ g MgSO}_4 \cdot 7\text{H}_2\text{O}$ ,  $4.5 \text{ g KH}_2\text{PO}_4$ ,  $2 \text{ g NH}_4\text{Cl}$ , and  $2 \text{ ml vitamins and trace minerals}$  [15]. The original pH of that medium was adjusted to 5.9.

The fungus, *S. variecibatus*, isolated from a previously operated biofilter treating styrene vapours [5], was grown and maintained in a sterile atmosphere on 15 g agar l<sup>-1</sup>, under ambient conditions. Typical spores of *Sporothrix* sp., attached to perlite are shown in Fig. 1. The culture medium was autoclaved at 120 °C for 20 min before adding the filter-sterilized solutions of vitamins and trace minerals. 1 g oxytetracycline hydrochloride was aseptically added to 1 l of sterilized agar medium to prevent the growth of bacterial colonies. The petri dishes containing the agar medium and styrene utilizing fungus were incubated in a dessicator in presence of styrene vapours, supplied as the sole carbon source for fungal growth.

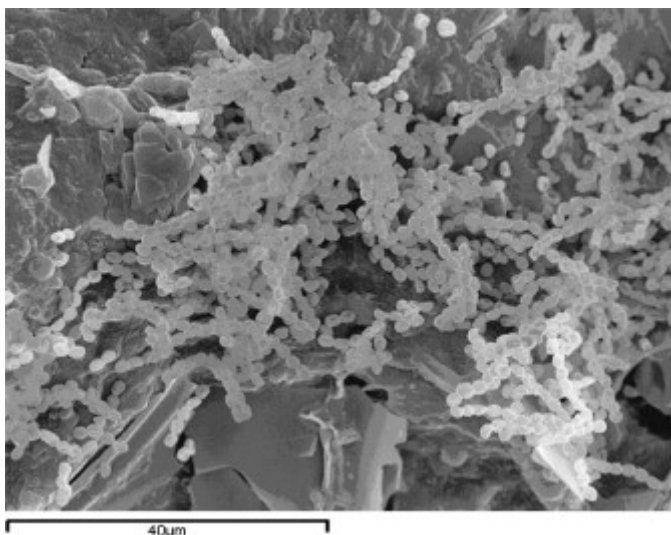


Fig. 1.

SEM photograph of *Sporothrix variecibatus*.

## 2.2. Biofilter setup

The schematic of the experimental setup is illustrated in Fig. 2. The biofilter was made of glass having an internal diameter of 8 cm and 70 cm in height. The packing in the biofilter consisted of sieved perlite beads (4–6 mm), packed to give a total filter bed volume of 0.0019 m<sup>3</sup>. A perforated plate at the bottom provided the support for the packing material while another plate at the top acted as a distributor for the gas flow and mineral salt medium addition. Gas sampling ports sealed with rubber septa were provided at equal intervals along the biofilter height.

- A – Compressor
- B – Flow meter
- C – Humidifier
- D – Mixing unit
- E – Liquid styrene
- F – Liquid acetone
- G – Polluted air mixture
- H – Distributor plate
- I – Port for leachate collection
- J – Treated air
- K – Gas sampling ports
- L – Biofilter packing + microorganism
- M – Port for media addition

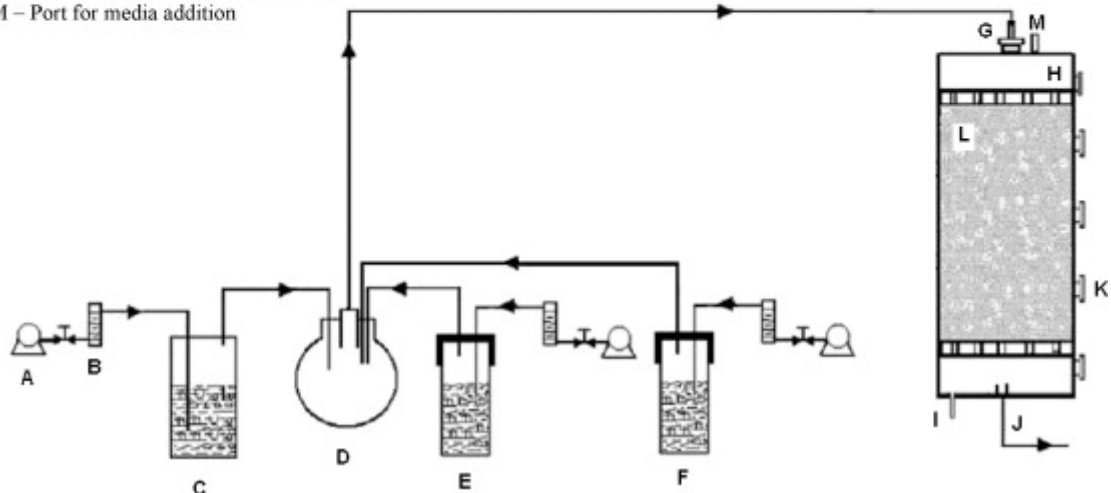


Fig. 2.

Schematic of the experimental setup for styrene and acetone co-treatment.

### 2.3. Biofilter inoculation

*S. variecibatus* was grown under batch conditions in 1000 ml Erlenmeyer flasks, using equal headspace concentrations of both styrene and acetone ( $4 \text{ g m}^{-3}$ ) in the media under shaking conditions (175 rpm) and at  $30^\circ\text{C}$ . After 48–56 h of growth, having reached an optical density (OD) of 1.3 at 600 nm, the cells were harvested by centrifugation at 5000 rpm for 20 min and re-suspended in 2 l fresh nutrient medium to be used as inoculum for the continuous biofilter. This culture was aseptically added to the biofilter from the top, the leachate then drained after 4 h and re-supplied several times till visible biomass was noticed on the perlite surface.

### 2.4. Biofilter operation

Humidified styrene and acetone vapors at constant flow rate and concentration, controlled through valves were passed through the bed in a downflow mode. The bed's moisture content was maintained constant by periodic addition of fresh mineral salt medium from the top (once every 3 d). Experiments were carried out by varying the flow rates of the styrene and acetone vapors and humidified air (>90% relative humidity) independently to get different initial concentrations and residence times in the biofilter, i.e., 17.1, 34.2, 68.5 and 137 s. Gas samples were collected from different ports located at bed heights of 8.5, 23.5, 38.5 and 49 cm from the inlet and were later analyzed for residual styrene and acetone concentrations.

The removal efficiency of the pollutants (RE, %) and the elimination capacity of the filter bed (EC,  $\text{g m}^{-3} \text{h}^{-1}$ ), as well as other key parameters, were estimated by the following equations:

$$\text{Inlet loading rate, ILR} = \frac{QC_i}{V}$$

$$\text{Elimination capacity, EC} = \frac{Q(C_i - C_o)}{V}$$

$$\text{Removal efficiency, RE} = \frac{C_i - C_o}{C_i} \times 100$$

$$\text{Total inlet loading rate, ILR}_{\text{Total}} = \frac{Q [C_{\text{styrene, in}} + C_{\text{acetone, in}}]}{V}$$

$$\begin{aligned} \text{Total elimination capacity, EC}_{\text{Total}} \\ = \frac{Q [(C_{\text{styrene, in}} + C_{\text{acetone, in}}) - (C_{\text{styrene, out}} + C_{\text{acetone, out}})]}{V} \end{aligned}$$

where  $Q$  is the gas flow rate ( $\text{m}^3 \text{h}^{-1}$ ),  $V$  is the volume of the filter bed ( $\text{m}^3$ ) and  $C_i$  and  $C_o$  are the inlet and exit styrene or acetone concentrations ( $\text{g m}^{-3}$ ).

## 2.5. Batch tests

Batch biodegradation tests were conducted using the pure *Sporothrix* sp., and the *Sporothrix*-like microorganism present in the leachate of the biofilter, in an attempt to evaluate the removal pattern of styrene and acetone. 50 ml of leachate, collected from the biofilter after transient loading conditions, was centrifuged (20 min,  $10,000 \times g$ ,  $25^\circ\text{C}$ ), and the cell-pellets were harvested by several repeated washing with the media. Experiments, with a working volume of 10 ml, were carried out at ambient temperature, by constant shaking at 175 rpm under aerobic conditions in 110 ml glass vials fitted with Viton septa and sealed air-tight with aluminum crimps. Liquid styrene and acetone, corresponding to the desired liquid phase concentrations were added to the mineral salt medium. After attaining equilibrium between the head-space and liquid phase concentrations, the cell-pellets (3% v/v) were suspended, to give similar initial biomass concentrations in the test vial. All batch experiments were carried out in duplicate and the average values were presented. The biodegradation profile was monitored by periodically withdrawing 2 ml of head-space samples and subjecting them to gas chromatograph analysis for residual styrene and acetone concentrations. The concentrations in the liquid-phase was then calculated from Henry's partition coefficient value at  $25^\circ\text{C}$ . The growth of the fungus, monitored as biomass concentration ( $\text{mg l}^{-1}$ ), that was earlier correlated to OD at 600 nm, was estimated by periodically withdrawing 1 ml of sample at different time intervals from the test vial. Head-space samples were also withdrawn periodically for analyzing the  $\text{CO}_2$  generation rate during the biodegradation process.

## 2.6. Analytical methods

Styrene and acetone concentrations in gas samples were measured by gas chromatography on an HP 6890 gas chromatograph, using a 50 m TRACER column and a FID. The flow rates were 30 ml min<sup>-1</sup> for H<sub>2</sub> and 300 ml min<sup>-1</sup> for air. Helium was used as the carrier gas at a flow rate of 2 ml min<sup>-1</sup>. The temperatures at the GC injection, oven and detection ports were 250, 120 and 250 °C respectively. Typical gas elution times in GC were 7.2 and 2.9 min, respectively for styrene and acetone. CO<sub>2</sub> was analyzed with a HP 5890 gas chromatograph, fitted with a Porapak-Q type column and equipped with a TCD. The injection and oven temperatures were 90 and 25 °C, respectively, with the TCD set at 100 °C. For pH measurements, a Crison pH meter (Model-507), connected to an Ingold electrode was used. Fungal growth was quantified by measuring the OD, and a standard calibration line was plotted based on the relationship between cell-dry weight and OD. The absorbance (600 nm) of the liquid cultures, related to the growth of *S. variecibatus* with styrene and acetone as the carbon source, was measured by using a UV-vis spectrophotometer (Hitachi, Model U-200, Pacisa & Giralt, Madrid, Spain). The relative humidity of the incoming air-pollutant mixture, was monitored periodically using a hand held thermo-hygrometer (Model-C210), fitted with a flexible sampling probe (G. Lufft Mess-und Regeltechnik, GmbH, Germany). Pressure drop across the biofilter was measured using a differential U-tube water manometer connected to the top and bottom sections, with the operational range of 0–40 cm H<sub>2</sub>O.

## 3. Results and discussions

### 3.1. Start-up of the biofilter

The performance of the biofilter was evaluated under steady-state conditions by varying the concentrations of styrene and acetone independently, in four different phases of operation, corresponding to gas-flow rates varying between 0.05 and 0.4 m<sup>3</sup> h<sup>-1</sup>. The concentration of each individual pollutant, the EBRT and loading rates tested in these four phases are given in Table 1.

Table 1.

Operational phases in the biofilter.

Phase of operation	Days	Inlet concentration (g m <sup>-3</sup> )		EBRT (s)	Total ILR <sub>max</sub> (g m <sup>-3</sup> h <sup>-1</sup> )	Total EC <sub>max</sub> (g m <sup>-3</sup> h <sup>-1</sup> )
		Styrene	Acetone			
Start-up	1–14	<9	<8	137	361.8	347.4
I	15–35	2.16–6.33	1.81–12.9	137	388.2	363.1
II	36–52	0.38–4.25	0.21–6.75	68.5	481.6	360.0
III	53–66	0.19–3.76	0.11–3.96	34.2	506.9	314.5
IV	67–84	0.01–1.78	0.01–1.13	17.1	608.5	325.9

The biofilter was inoculated with acclimated fungal spores of *S. variecibatus*. During start-up, for the first 3 d, the biofilter was acclimated to low loading rates of both styrene and acetone vapours,  $ILR_{Total} < 50 \text{ g m}^{-3} \text{ h}^{-1}$ , and more than 82% removal was noticed for both compounds. Therefore, for the next 11 d, the inlet concentrations of both styrene and acetone were increased, and the biofilter was able to achieve nearly stable RE of >90% styrene and >80% acetone, when styrene and acetone concentrations were less than 6 and 4  $\text{g m}^{-3}$ , respectively. These concentration ranges for acclimating the fungal biofilter are quite high, as most biofilters generally use only low concentrations, i.e., less than 0.5  $\text{g m}^{-3}$ , during acclimation [8]. This was presumably possible because *Sporothrix* sp. has shown the ability to tolerate high concentrations of styrene (12–14  $\text{g m}^{-3}$ ) [5] and [6] and because acetone, as reported in the literature, is an easily biodegradable compound in both waste-gases and wastewaters [4] and [16]. The relative humidity of the air stream was maintained at 90% or more, during all the experiments by constantly humidifying the incoming pollutant mixture. This also helped in maintaining the necessary moisture conditions specified for biofilter operation [8]. After the start-up period, experiments were conducted by either increasing and/or decreasing the concentration of styrene and/or acetone, in a systematic manner, at different EBRTs, corresponding to  $ILR_{Total}$  varying between 448.9 and 608.5  $\text{g m}^{-3} \text{ h}^{-1}$ .

## 3.2. Performance of the biofilter at different EBRT

### 3.2.1. Phase-I: EBRT of 137 s

The removal and elimination capacity profiles of both styrene and acetone under different initial concentrations and EBRT, corresponding to four different phases of operation are shown in Fig. 3a–c, respectively. It can be seen that the concentrations of styrene varied between 3.5 and 6  $\text{g m}^{-3}$  during the first 5 d of operation, while the acetone concentration was lowered from 4  $\text{g m}^{-3}$  to 2  $\text{g m}^{-3}$ , resulting in 96% RE for styrene and about 90% RE for acetone in all cases. However, when the acetone concentration was increased from 2 to 6.5  $\text{g m}^{-3}$ , the RE of acetone dropped, reaching stable values at around 78%. Subsequently, when the concentration of styrene (2.7–5.7  $\text{g m}^{-3}$ ) and acetone (2.3–5.8  $\text{g m}^{-3}$ ) were maintained between these values from day 28–35, RE > 92% was achieved for styrene, while RE for acetone varied between 67 and 91%. During this phase, the biofilter was subjected to styrene and acetone loading rates varying between 56.8 and 166.5  $\text{g m}^{-3} \text{ h}^{-1}$  and 47.6 and 234.5  $\text{g m}^{-3} \text{ h}^{-1}$ , respectively. As seen from Fig. 4 a and b, maximum ECs of 163  $\text{g m}^{-3} \text{ h}^{-1}$  and 151  $\text{g m}^{-3} \text{ h}^{-1}$  were observed for styrene and acetone, respectively. From these figures, it is also evident that, the EC increased linearly with an increase in the loading rates for both pollutants, but as far as acetone profiles are concerned, these profiles were not linear after a critical loading rate. However, for styrene, the linear relationship between loading rate and EC with a slope of 1 under the tested experimental conditions, i.e., maximum ILR for styrene of 166.5  $\text{g m}^{-3} \text{ h}^{-1}$ , indicates that a maximum overall elimination capacity was not reached at an EBRT of 137 s, and that styrene loading rates could still be increased in the fungal biofilter. ECs exceeding such value of 166.5  $\text{g m}^{-3} \text{ h}^{-1}$  would then be possible.



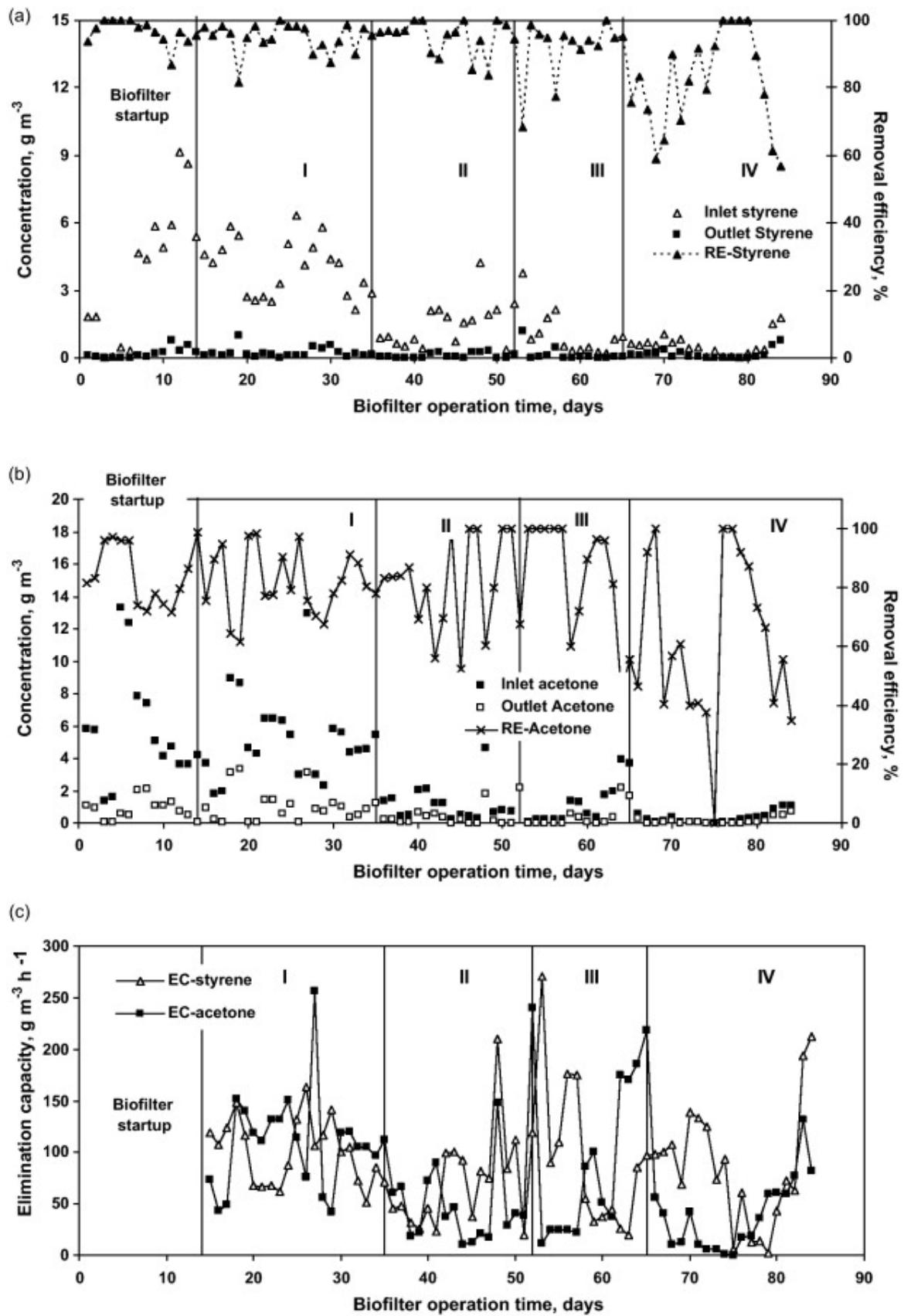


Fig. 3.

Profiles of (a) styrene removal, (b) acetone removal, and (c) elimination capacity of the fungal biofilter during different phases of biofilter operation.

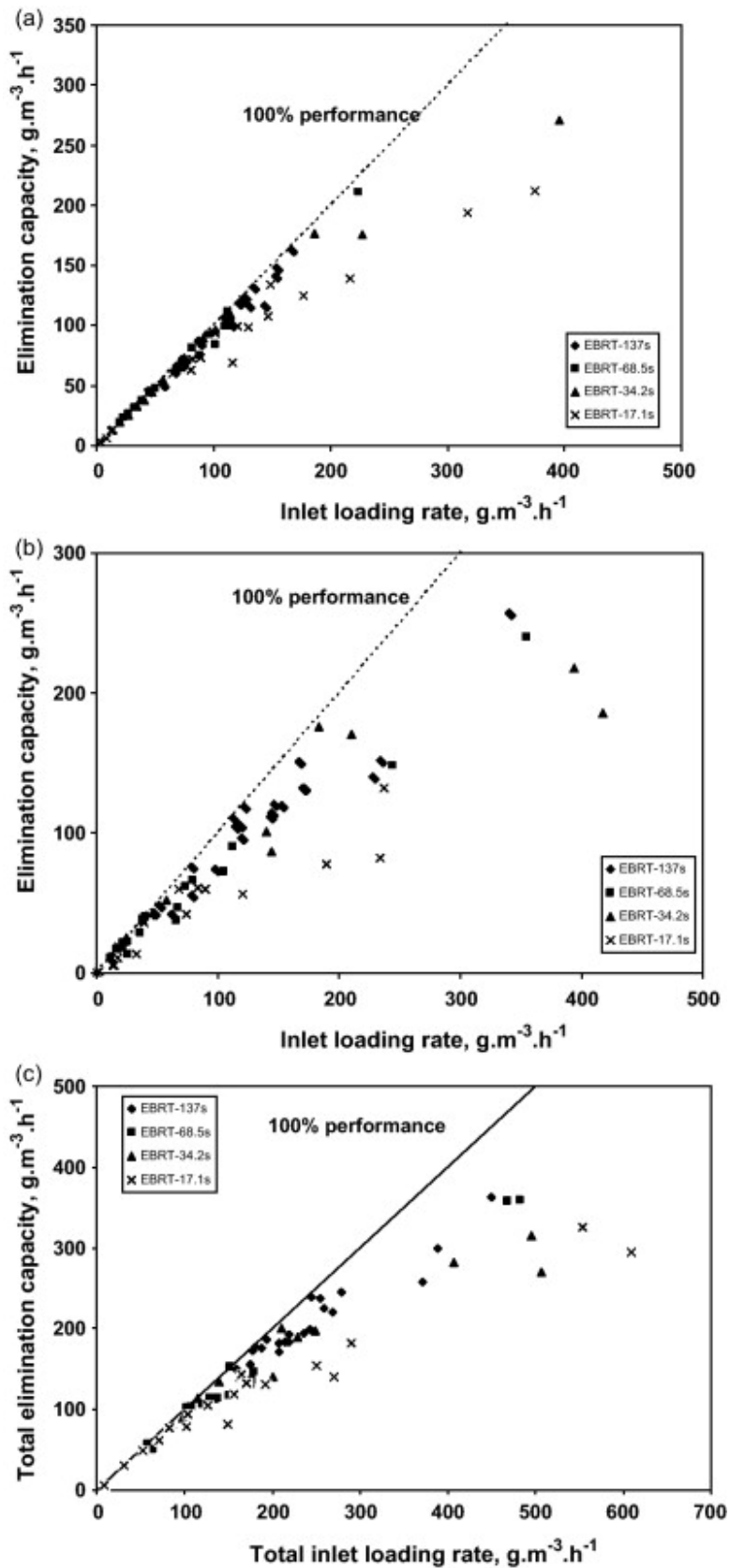


Fig. 4.

Profiles of inlet loading rate vs. elimination capacity at different EBRTs, (a) styrene, (b) acetone, and (c) total pollutant load.

### 3.2.2. Phase-II: EBRT of 68.5 s

In this phase (days 36–52), the concentrations of both styrene and acetone were reduced in comparison to Phase-I, and varied between 0.38 and 4.25 g styrene m<sup>-3</sup> and 0.21 and 6.8 g acetone m<sup>-3</sup>, respectively (Fig. 3). By doing so, it was observed that, low concentrations of styrene led to high RE (>85%) of that compound, irrespective of the concentration of acetone in the gas-phase mixture. For instance, during days 36–41, when styrene concentration was less than 1 g m<sup>-3</sup>, and when acetone concentrations were varied between 0.4 and 2.1 g m<sup>-3</sup>, styrene RE > 96% was easily maintained in the biofilter, while increasing the acetone concentration in that concentration range decreased the acetone RE from 90% to nearly 70%. This trend clearly indicates that the system would suffer from some interactive effects among these pollutants, leading to a decreased performance to a certain level, depending on the individual pollutant concentration and the gas-flow rate. Nevertheless, though the system readapted quickly to changes in loading rates during this phase, an increase in pollutant load did not lead to any drastic performance drop, and the RE for styrene and acetone did not drop below 85% and 60% during this phase. The ILR varied between 20 and 224 g styrene m<sup>-3</sup> h<sup>-1</sup> and 11 and 355 g acetone m<sup>-3</sup> h<sup>-1</sup>, and EC<sub>max</sub> of 210 and 148 g m<sup>-3</sup> h<sup>-1</sup> were achieved for styrene and acetone, respectively (Fig. 4a and b). Higher EC could have been reached for styrene since the critical load was not reached in this experiment.

### 3.2.3. Phase-III: EBRT of 34.2 s

During days 53–65, the biofilter was subjected to a much lower residence time (34.2 s), and the inlet styrene and acetone concentrations were also maintained low for most part of the experiment (<1 g m<sup>-3</sup>), although on certain occasions, they were increased up to about 4 g m<sup>-3</sup>, to identify the interaction effects at high concentration of one pollutant on the other. As seen from Fig. 3, when the concentrations of both pollutants were relatively low in Phase-III, i.e., less than 0.5 g m<sup>-3</sup>, REs were greater than 90% for both styrene and acetone. This indicates that the biofilter was subjected to loads that were less than the critical loading rate to achieve more than 90% RE. On the other hand, low concentrations of acetone and moderately high concentrations of styrene (2.2 g m<sup>-3</sup>), did not affect acetone removal much. However, the reverse occurred when the biofilter was fed low concentrations of styrene and moderately high concentrations of acetone (3.5–4 g m<sup>-3</sup>). The removal of acetone reduced significantly and varied between 45 and 55%. At this residence time, increasing acetone concentrations, by holding styrene levels at constant values, appeared to have a drastic effect on its own removal. The EC<sub>max</sub> at this EBRT were nearly 170 g m<sup>-3</sup> h<sup>-1</sup> for both styrene and acetone (Fig. 4a and b) with RE of about 90% and 85%, respectively.

### 3.2.4. Phase-IV: EBRT of 17.1 s

Phase-IV, corresponding to the lowest EBRT tested in this study, i.e., 17.1 s, was investigated at relatively low concentrations of the compounds, corresponding to ILR varying between up to 374 g m<sup>-3</sup> h<sup>-1</sup> for styrene and 237 g m<sup>-3</sup> h<sup>-1</sup> for acetone. Once again, the performance of the system depended on the concentration of these two compounds in mixture. For styrene and acetone concentrations less than 0.8 and 0.3 g m<sup>-3</sup>, nearly 80% styrene and 70% acetone was removed in the biofilter. However, for some days, when the concentrations of these two compounds were increased, thereby increasing the ILR<sub>Total</sub> to the biofilter, an abrupt decline in the RE of both the

compounds was noticed. For instance, for styrene concentrations  $>1.7 \text{ g m}^{-3}$  and acetone concentration  $>1 \text{ g m}^{-3}$ , their RE were just 56% and 34%, respectively. During this phase, the biofilter was tested for styrene and acetone ILR up to  $374 \text{ g m}^{-3} \text{ h}^{-1}$  and  $237 \text{ g m}^{-3} \text{ h}^{-1}$ , while their  $\text{EC}_{\text{max}}$  values were  $212 \text{ g m}^{-3} \text{ h}^{-1}$  and  $82 \text{ g m}^{-3} \text{ h}^{-1}$  (Fig. 4a and b). At this EBRT, the total maximum EC achieved was  $326 \text{ g m}^{-3} \text{ h}^{-1}$  at an  $\text{ILR}_{\text{Total}}$  of  $554 \text{ g m}^{-3} \text{ h}^{-1}$ , which corresponds to about 41.2% removal of the total pollutant load (Fig. 4c).

### 3.2.5. Maximum EC of the biofilter

The maximum EC for styrene was  $212 \text{ g m}^{-3} \text{ h}^{-1}$  with 58% removal, at an EBRT of 17.1 s and when acetone loads were comparatively low in mixture ( $235 \text{ g m}^{-3} \text{ h}^{-1}$ ). On the other hand,  $\text{EC}_{\text{max}}$  for acetone was  $151 \text{ g m}^{-3} \text{ h}^{-1}$ , at an EBRT of 137 s, when styrene ILR was  $87 \text{ g m}^{-3} \text{ h}^{-1}$ . Some of the higher EC values observed for both styrene and acetone under non-anticipated peak load condition (S:  $270 \text{ g m}^{-3} \text{ h}^{-1}$  and A:  $257 \text{ g m}^{-3} \text{ h}^{-1}$ ), were not considered to be steady-state (Fig. 4a and b). As mentioned earlier, and from Fig. 4a and b, it is quite evident that the EC for styrene could have been still higher if the biofilter was fed with higher loads of styrene, at an EBRT of 137 and 68.5 s, while that was obviously not the case for acetone, which clearly shows the existence of a critical load at these EBRTs. This clearly indicates that the *Sporothrix* sp., isolated from a styrene operated biofilter [5] has the ability to achieve higher ECs for styrene, although the biofilter was co-supplied with varying loads of acetone. Nevertheless, the fungal biofilter performance, in terms of the maximum EC for acetone, decreased significantly when the EBRT was decreased from 34.2 s to 17.1 s, though such a difference in the  $\text{EC}_{\text{max}}$  values were not observed at higher EBRTs, i.e., 68.5 s and 137 s. At the lowest EBRT, i.e., 17.1 s, the fungus gets less contact time for the degradation of gas-phase styrene and acetone, resulting in reduced biofilter performance. Usually, it has been observed in BFs that the EC or  $\text{EC}_{\text{max}}$  decreases when increasing the gas flow rate or space velocity, i.e., decreasing the EBRT [14], [17] and [18].

The pH of the leachate occasionally dropped to values ranging between 2.3 and 4.7, depending on the  $\text{ILR}_{\text{Total}}$  to the biofilter. On days 48, 64–66 and 82–84, when the  $\text{ILR}_{\text{Total}}$  was greater than  $400 \text{ g m}^{-3} \text{ h}^{-1}$ , the EC of the fungal biofilter was greater than  $300 \text{ g m}^{-3} \text{ h}^{-1}$ , despite the variation in pH values between 2.3 and 2.9. Besides, the periodic sprinkling of nutrient medium from the top helped to maintain the biological activity of the fungus, even though the pH dropped occasionally. The pressure drop values were less than 1.3 cm  $\text{H}_2\text{O}$  during the entire experimental period. In comparison, these values of pressure drop are somewhat lower than the values reported in literature for a mixed culture inoculated biofilter (0.5–4.2 cm  $\text{H}_2\text{O}$ ) and a fungal biofilter (3 cm  $\text{H}_2\text{O}$ ) treating gas-phase styrene [5] and [6].

### 3.3. Pollutant interactions

During the treatment of gas-phase mixtures, synergistic and/or antagonistic effects may appear. In this study, at a given EBRT, the following interactions were observed: (i) low concentrations of both pollutants did not affect their removal and  $\text{RE} > 90\%$  was easily maintained, (ii) styrene concentrations  $< 1 \text{ g m}^{-3}$ , and moderately high concentrations of acetone around  $2 \text{ g m}^{-3}$  affected acetone removal by 20%, while styrene RE was  $>96\%$ , (iii) low concentrations of acetone and a high concentration of styrene ( $4 \text{ g m}^{-3}$ ) did not

affect styrene nor acetone removal, and (iv) high concentrations of both acetone and styrene ( $>4.5 \text{ g m}^{-3}$ ) affected acetone removal more severely than styrene that was affected only slightly (2–6%). A recent short study shed more light on the type of interactions observed in a bacterial biofilter, during the co-treatment of styrene and acetone [19]. In that study, the concentration of gas-phase styrene was kept constant ( $50 \text{ mg m}^{-3}$ ), while acetone concentration was increased from 47 to  $1057 \text{ mg m}^{-3}$ , and it was reported that, the varying concentrations of acetone not only affected its own removal, but also that of styrene. In some cases, the removal of one compound in mixture consistently remains unaffected despite the varying concentration levels of the other compound. In a study involving methanol and toluene as the co-substrate, it has been reported that toluene produced higher inhibitory effects than methanol in a biofilter, and that toluene, a non-polar compound, could easily be transported across the hydrophobic cell membrane and is able to leak essential metabolites and co-factors that could kill the cells [20].

### 3.4. Substrate stratification along the bed height

The dynamics of pollutant removal along the bed height was envisaged by measuring the concentration profiles at different bed depths, during Phase-I, and Phase-IV (Fig. 5 a and b). The inlet concentrations of styrene and acetone during these experiments were as follows;  $6.4, 5.8 \text{ g m}^{-3}$  and  $1.02, 1.28 \text{ g m}^{-3}$ , corresponding to ILR of 169.2 and  $215.5 \text{ g styrene m}^{-3} \text{ h}^{-1}$  and 153.2 and  $269.4 \text{ g acetone m}^{-3} \text{ h}^{-1}$ , respectively. It can be observed that, substrate stratification occurred in terms of styrene and acetone removal. At low gas-flow rates, i.e., Phase-I, acetone removal was almost linear across the filter bed, the first half of the filter bed (23.5 cm) removed nearly 40% and the later half (26 cm) removed 40% of gas-phase acetone. However, 30% styrene was removed in the first half of the filter bed, while 60% was removed in the later half. During phase-IV (EBRT-17.1 s) and at low inlet concentrations of these pollutants ( $<1.3 \text{ g m}^{-3}$ , high ILR), a major fraction of the styrene and acetone removed (74% and 66%), occurred in the lower half of the filter bed, while the first 23.5 cm removed only about 37% styrene and 22% acetone. These profiles suggest that the filter bed was colonized completely with the fungal population, and it was able to utilize styrene and acetone simultaneously. Besides, at higher loading rates (Phase-IV), a major portion of the biodegradation occurred in the lower half of the filter bed ( $\text{ILR}_{\text{Total}}=484.9 \text{ g m}^{-3} \text{ h}^{-1}$ ). From Fig. 5a, it is also evident that high concentrations of acetone and styrene to the biofilter affected acetone removal more severely than styrene removal.

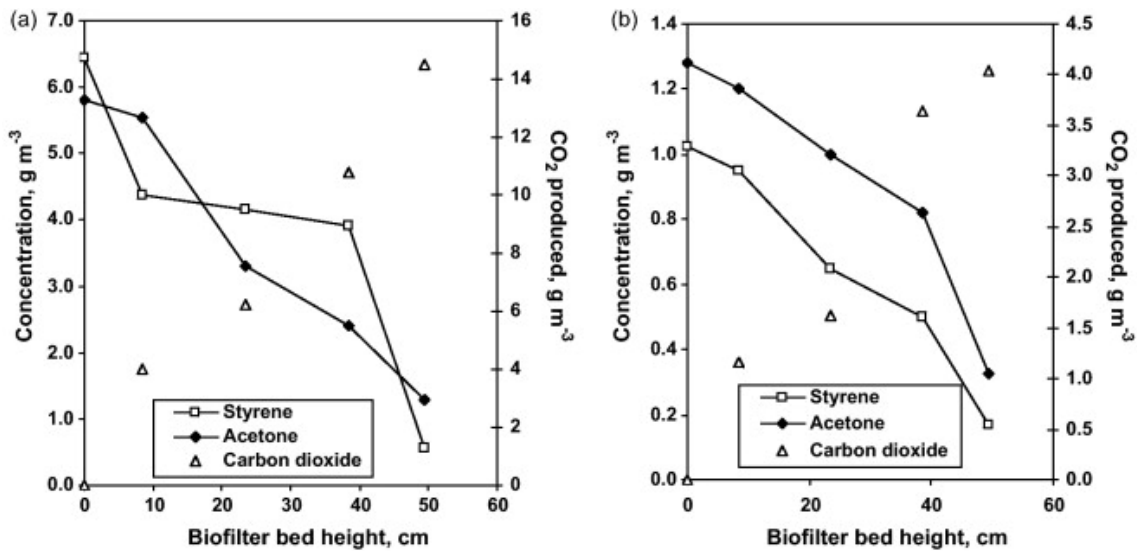


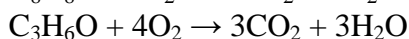
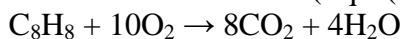
Fig. 5.

Styrene, acetone and carbon dioxide concentration profiles along the biofilter height; (a) EBRT: 137 s; CS: 6.4 g m<sup>-3</sup>; CA: 5.8 g m<sup>-3</sup>; and (b) EBRT: 17.1 s; CS: 1.02 g m<sup>-3</sup>; CA: 1.28 g m<sup>-3</sup>.

Perlite samples were intermittently removed through the gas-sampling ports and periodically examined under an optical microscope, in order to confirm the presence of the original fungus in the biofilter. The cells on the perlite samples clearly looked like the original *Sporothrix* sp. based on its morphological characteristics. Several reports on bacterial or mixed culture biofilters have also reported substrate stratification along the bed height, during the treatment of two or more gas-phase pollutants. Ergas et al. [21] studied toluene and DCM removal in a biofilter and observed that, toluene removal occurred in the top section of the filter bed, while DCM removal mainly occurred in the lower section, and this stratification was attributed to the possible presence of two different microbial communities within the filter bed, each one having preference for one compound over the other. Also, it has been well-established that, in biofilters treating mixture of VOCs, specific microorganisms have the ability to preferentially choose and degrade the less toxic compound rather than the ones that are more toxic and inhibitory to its growth. Similarly, concentration profiles in a biofilter inoculated with the fungus *Cladosporium sphaerospermum* treating a complex mixture of MPK, MEK, toluene and *n*-butyl acetate, showed that the removal profiles of these compounds differed largely depending on the substrate utilization potential of that fungus. In that study, *n*-butyl acetate was readily removed followed by MEK, MPK and toluene [22].

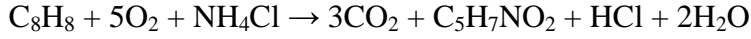
### 3.5. Carbon dioxide evolution in the biofilter

During these experiments, carbon dioxide concentrations were also monitored along the bed height, by calculating the difference between the inlet and outlet CO<sub>2</sub> concentrations (secondary 'y-axis' in Fig. 5a and b). For complete oxidation of styrene and acetone to CO<sub>2</sub> and water [23], the mass-ratio of CO<sub>2</sub> produced to the amount of styrene and/or acetone degraded should be 3.4 and 2.3, according to the following stoichiometric reaction (Eqs. (6) and (7)):



However, when biomass growth is taken into account with ammonium chloride as nitrogen source, 1.27 g CO<sub>2</sub> should be generated per g styrene degraded, while 0.378 g CO<sub>2</sub> should be produced for 1 g acetone degraded, and the stoichiometric equations can be written as follows (Eqs. (8) and (9));

equation(8)



equation(9)



In this study, the ratio of CO<sub>2</sub> produced to the amount of pollutant that was collectively removed in the biofilter, was less than the theoretical amount of CO<sub>2</sub> that was expected to be produced in the biofilter, i.e., 1.648 g CO<sub>2</sub> should theoretically be produced for every one g styrene and one g acetone that is simultaneously removed in the fungal biofilter. In this study, 6.4 and 5.8 g m<sup>-3</sup> of styrene and acetone was fed to the biofilter during Phase-I (EBRT-137 s), and RE greater than 80% was noticed for both compounds. Discrepancies between theoretical and observed CO<sub>2</sub> profiles are common and have often been reported in biofilters, both fungal and bacterial populations, especially when subjected to high loads of individual or mixtures of gas-phase pollutants. Woertz et al. [24] reported CO<sub>2</sub> recoveries around 57% for *Exophiala leccanii-corni* grown on toluene. Cox et al. [25] reported CO<sub>2</sub> recoveries ranging from 40 to 100% over a 300 d period for styrene removal using the fungus *Exophiala jeanselmei*. Besides, CO<sub>2</sub> production exceeding 100% is also not unusual in biofilters when endogenous respiration becomes a relevant process.

### 3.6. Transient-state response of the biofilter

Non-steady state or transient operations in a biofilter are frequently encountered in the form of a sudden pollutant shock-loads, and/or as unexpected shut-down of the system. Since biofilters include the presence of active microorganisms, they are sensitive to these sudden variations, i.e., flow rate or concentration or a combination of both at once, and the absence of gas-phase pollutant during shut-down. The microorganisms could sustain sudden, yet medium shock loads well, but high shock loads are harder to tolerate over a long period of time, especially for hydrophobic VOCs [5] and [13]. Though such operations have been stimulated under lab conditions in many previously reported studies, most of them address the biofilter response for individual hydrophobic or hydrophilic compounds. Only a few papers have mentioned the events associated with mixtures of two or more pollutants, especially in mixed-culture or bacteria - inoculated biofilters [26], [27] and [28], while fungal biofilter response to handle transient shock-loads has been reported for  $\alpha$ -pinene removal [14]. During this experimental phase, the fungal biofilter was subjected to a short term-12 h shock load by suddenly increasing the concentrations from 0.9 to 1.7 g m<sup>-3</sup> for styrene and from 0.1 to 1 g m<sup>-3</sup> for acetone, respectively, while maintaining the gas-flow rate constant at 0.4 m<sup>3</sup> h<sup>-1</sup>, i.e., at an EBRT of 17.1 s. The onset of shock-loads was the 4th hour after taking measurements in the biofilter, that was stabilized overnight and subjected to low pollutant concentrations. The response of the fungal biofilter is shown in Fig. 6. It can be seen that, the RE of both styrene and acetone dropped, from nearly 90 to 100% before the shock, to about 70% when their concentrations were increased suddenly. Though their concentrations were low during the shock loading step, high gas-flow rates imparted high loading rates of these pollutants to the biofilter, viz. as 360 g styrene m<sup>3</sup> h<sup>-1</sup> and 240 g acetone m<sup>3</sup> h<sup>-1</sup>. Though the removal efficiencies of individual compounds dropped during the shock load, the fungal biofilter was able to withstand an ILR<sub>Total</sub> of up to 647 g m<sup>-3</sup> h<sup>-1</sup>,

showing  $EC_{Total}$  as high as  $360 \text{ g m}^{-3} \text{ h}^{-1}$  (Fig. 6-inset). Anew, after the shock load and when the concentrations of styrene and acetone were brought down to previously applied low values, the biofilter showed good tendency to recover and reached stable RE almost instantaneously. In a previous study, a fungus belonging to the *Ophiostoma* sp. was shown to withstand periodic shock loads of gas-phase  $\alpha$ -pinene for more than one month of transient-state operations, where EC and RE were found to be  $60 \text{ g m}^{-3} \text{ h}^{-1}$  and  $>90\%$  respectively. Besides, the biofilter recovery time was less than 4 h and the fungus showed good activity despite being supplied with high shock-loads [14]. The results from this study suggest that the *Sporothrix* sp. was able to maintain its cells in an active state and has the ability to develop its own survival strategy during transient-state conditions.

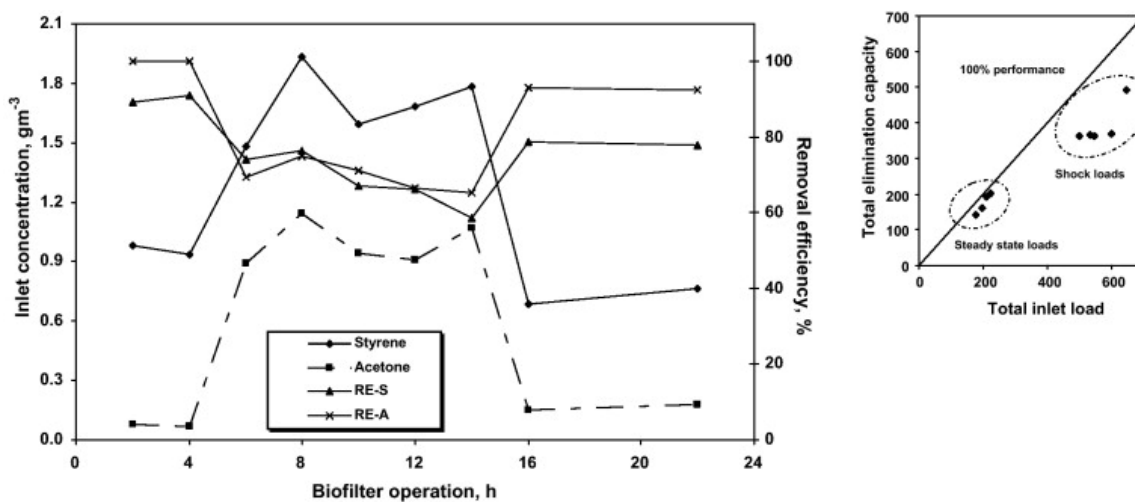


Fig. 6.

Transient response of the fungal biofilter to changes in concentration of both styrene and acetone and total inlet load vs. total elimination capacity ( $\text{g m}^{-3} \text{ h}^{-1}$ ) profiles during short-term shock loads (inset), RE-Removal efficiency, S-Styrene and A-acetone.

### 3.7. Batch confirmation tests for styrene and acetone removal

After the completion of steady and transient-state biofilter experiments, four sets of batch experiments were carried out by using the leachate-borne *Sporothrix* sp., viz., in vials with styrene, vials with acetone and vials with mixtures of styrene and acetone. Besides, two additional vials inoculated with the pure *Sporothrix* sp., were also tested at different initial concentrations of styrene and acetone as mixtures. The objective of this test was to check whether the microbes were able to mineralize styrene and acetone completely, as well as to observe the pattern of complete removal during the co-treatment of mixtures. It is known that interactions may appear in the biodegradation process when several pollutants are present simultaneously [9], [10], [11], [12] and [29]. The results illustrated in Fig. 7a–f, show the biodegradation profiles of styrene and acetone, as individual compounds ( Fig. 7(a) and (b)), as mixtures ( Fig. 7(c) and (d)) in leachate inoculated vials, while Fig. 7(e) and (f) show the biodegradation profile of styrene and acetone as mixtures in the pure culture inoculated vials. From these profiles, it is evident that, biomass growth occurred almost instantaneously, followed by steady utilization of the substrate. When 2.411 mg acetone was added to the test vial (liquid phase concentration of  $235 \text{ mg l}^{-1}$ ), complete mineralization occurred in about 84 h by



the mixed culture (leachate), while 2.607 mg styrene (concentration=105 mg l<sup>-1</sup>) was removed completely in 235 h ( Fig. 7b). Biomass growth and CO<sub>2</sub> generation profiles were also constantly monitored during the experiments, and by comparing Fig. 7(a) and (b), it was observed that biomass growth was higher during styrene degradation than with acetone.

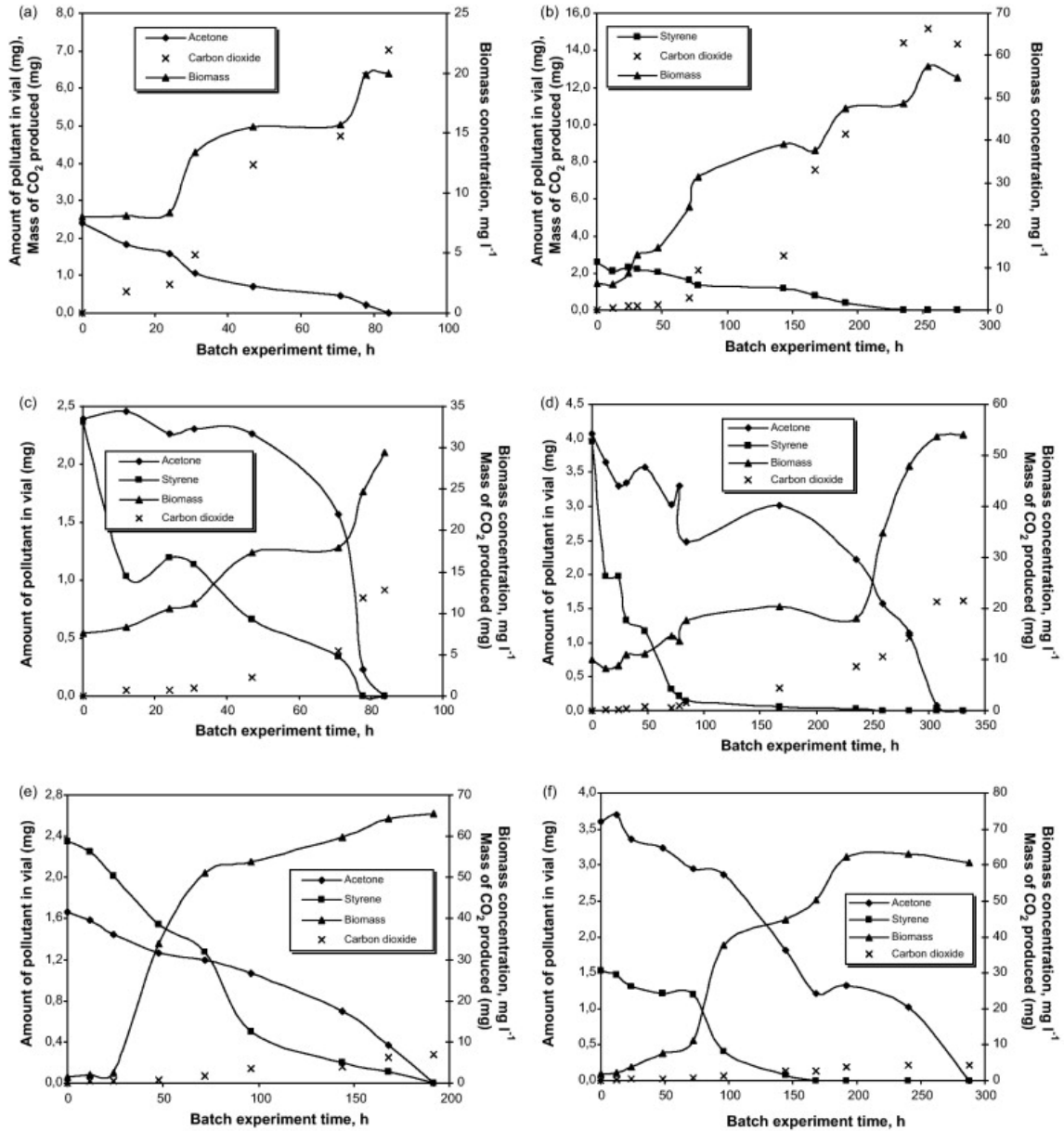


Fig. 7.

Biodegradation, biomass growth and CO<sub>2</sub> generation profile in batch test vials inoculated with *Sporothrix* sp., present in the leachate; (a)acetone alone, (b) styrene alone, (c and d)mixtures of styrene and acetone, and (e and f)styrene and acetone removal profiles by the pure strain of *Sporothrix* sp.

The biodegradation profile of these two compounds, when present as mixtures is shown in Fig. 7(c) and (d). It can clearly be seen that, though both compounds were utilized by the microorganisms, they definitely had different biodegradation rates, indicating possible chances of preferential utilization of the fungus. When low amounts of styrene

and acetone were present (S–2.367 mg, and A–2.393 mg in mixture), both compounds were removed completely in less than 84 h (Fig. 7c), compared to the longer times taken for their degradation at high initial concentrations (3.949 mg styrene and 4.072 mg acetone, Fig. 7d). However, in both these cases, biodegradation of styrene occurred faster than acetone, and acetone degradation rates were higher when styrene was not present in the test vial, 4.63 and 4.2 mg acetone  $\text{mg biomass}^{-1} \text{h}^{-1}$ , respectively. Irrespective of the initial concentrations, it seems that acetone biodegradation takes place mainly after or at the end of styrene removal (Fig. 7(c) and (d)). Similarly, when the pure strain was tested at two different initial concentrations of styrene and acetone as mixtures (S - 2.348 mg and A - 1.663 mg and S - 1.527 mg and A - 3.6 mg), the removal of styrene occurred more rapidly ( $< 192$  h) than for acetone and acetone started being removed at a faster rate only when styrene was completely depleted in the test vial (Fig. 7(e) and (f)). The pH of the medium also dropped from an initial value of 5.9 to 4.5 and 3.7 in the mixture vials (Fig. 7(c) and (d)), due to the formation of acidic intermediates like HCl during the biodegradation process (Eqs. (8) and (9)). The results of these simple tests confirm the fact that *Sporothrix* sp. can degrade both styrene and acetone. It showed preferential utilization of styrene and corroborates the high removals,  $>60\%$  in all four phases of operation, noticed in the biofilter for that compound. Nevertheless, further batch studies using the pure strain of *S. variecibatus* are warranted at different process conditions, i.e., different initial concentrations of styrene and acetone, pH, temperature and nutrient concentrations, to constitute the kinetics of the process and estimate different bio-kinetic parameters like maximum specific growth rate, half-velocity and inhibition constants and specific substrate utilization rate.

#### 4. Conclusions

The results from this study showed that co-treatment of gas-phase styrene and acetone can be performed in a biofilter with high elimination capacities ( $212 \text{ g styrene m}^{-3} \text{ h}^{-1}$  and  $151 \text{ g acetone m}^{-3} \text{ h}^{-1}$ ) using the fungus *S. variecibatus*.

The fungus was also able to resist high total loading rates of styrene and acetone. At an EBRT of 17.1 s and an  $\text{ILR}_{\text{Total}}$  of  $553.6 \text{ g m}^{-3} \text{ h}^{-1}$ , nearly 41.2% of the incoming gas-phase pollutant was removed.

When the incoming air contained high concentrations of both acetone and styrene ( $>4.5 \text{ g m}^{-3}$ ), acetone removal decreased in comparison to styrene that was affected only slightly ( $<6\%$ ) and the degree of antagonism depended highly on the styrene loading rate.

Substrate stratification occurred along the biofilter height in terms of styrene and acetone removal that confirmed fungal activity in all sections of the biofilter.

Transient operations were performed as short-term tests, by increasing the concentrations of both styrene and acetone suddenly, to ascertain the response of the biofilter to fluctuating pollutant loads. This shock load ( $\text{ILR}_{\text{Total}}=647.2 \text{ g m}^{-3} \text{ h}^{-1}$ ) led to a decline in the RE by about 20–30%, though high  $\text{EC}_{\text{Total}}$  were maintained ( $>350 \text{ g m}^{-3} \text{ h}^{-1}$ ), and complete recovery to original performance occurred almost instantaneously, when previous loading conditions were restored.

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