

## Biofilters based on the action of fungi

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**Abstract** Traditional biofilters for waste gas treatment are mainly based on the degradation activity of bacteria. The application of fungi in biofilters has several advantages: fungi are more resistant to acidification and drying out, and the aerial mycelia of fungi form a larger surface area in the gas phase than bacterial biofilms, which may facilitate the uptake of hydrophobic volatile compounds. The research described here identifies important conditions for the operation of fungal-based biofilters. Biofilters with perlite packing were operated at different pHs and relative inlet gas humidities. Toluene was used as a model pollutant. It was shown that a low pH is a prerequisite for fungal growth in biofilters. Also, the fungal biofilters were more resistant to drying out and more active than the bacterial biofilters. Fungal biofilters eliminated 80–125 g toluene/m<sup>3</sup> filterbed/h. Several measures that could limit the clogging of fungal biofilters with fungal biomass were investigated. The introduction of mites helped to control excessive fungal growth and pressure drop. The pressure drop of a perlite/fungi/mites filter of 1 m height, loaded with 200 m<sup>3</sup> gas/m<sup>3</sup> filter/h stabilised around 130 Pa. Biofilters based on the action of fungi are cost-effective for the treatment of waste gases containing aromatic compounds, alkenes and other hydrophobic compounds.

**Keywords** Biofilters; fungi; mites; perlite; toluene

### Introduction

Treatment of off-gases from industries is an important measure to reduce atmospheric emissions of volatile organic carbon (VOC). Off-gases can be cleaned by various technologies such as incineration, adsorption, chemical scrubbing and biofiltration. Biofilters are beds packed with biologically active materials, such as compost, through which the gases are ventilated. In the compost, contaminants are absorbed and subsequently biodegraded. A large amount of experience has been gained with biofilters for the reduction of odour from off-gases from municipal wastewater treatment plants and the food industry. Biofiltration was found to be cost-effective for off-gases with low concentrations of VOC (< 3 g/m<sup>3</sup>) (Groenestijn and Hesselink, 1993) and an odour reduction of 99% is possible. However, conventional biofilters, based on compost and bacterial activity, face problems with the elimination of hydrophobic compounds such as aromatic compounds, alkenes and alkanes. Because of the low solubility in water, the compounds are poorly absorbed by the bacterial biofilms. Besides that, biofilter operational stability is often hampered by acidification and drying out of the filter bed.

To overcome these problems, biofilters with fungi on inert packing material have been developed (Cox, 1995; Groenestijn *et al.*, 1995). Fungi are more resistant to acid and dry conditions than bacteria, which is a helpful property when operating biofilters. Moreover, it is hypothesised that the aerial mycelia of fungi, which are in direct contact with the gas, can take up hydrophobic compounds faster than flat aqueous bacterial biofilm surfaces. In previous research, biofilters for the removal of styrene from gases were developed. Different biofilter packing materials to support the growth of fungi were tested. Perlite, which is an inert granular porous ceramic material, was selected as the most suitable support medium for fungal growth as it maintained a high biofilter volumetric elimination capacity. Water amended with the required nutrients has to be added at regular time intervals to support growth.

The biofilter for styrene has been scaled up and was demonstrated on 1.5 m<sup>3</sup> scale at the site of two polyester factories (Kraakman *et al.*, 1997). Preliminary tests have been carried out with mixtures of toluene, ethylbenzene and xylenes, and with ethene, propene, 1,3-butadiene and hexane. Fungal growth was obtained in each case when a low pH was used, except for when hexane was the substrate.

A typical problem connected to high-load biofilters is clogging with biomass, whether it consists of bacteria or fungi. Using packing material with larger pores or introduction of higher organisms (fungi predators) may prevent this. Cocultures of fungi and mites in biofilters may lead to a controlled but active population of fungi in the pores of biofilter packings.

The aim of the present research is to show that a low pH is a prerequisite for fungal development in biofilters, coinciding with a high toluene elimination capacity. Furthermore, the effect of filter drying out, size of the packing material and presence of mites on the pressure drop across the filter bed was tested. Toluene was chosen as a model compound because it is hydrophobic and is still emitted in large quantities in the atmosphere.

## Materials and methods

### Humidity and pH experiments

Four laboratory scale glass columns were used as biofilters. Columns 1 and 2 had a filter bed volume of 1 l and filters 3 and 4 were 2 l. Perlite (AGRA 4 from Pull BV, Rhenen, the Netherlands; 4 mm diameter granules) and soil from a fuel station were used as packing material and inoculant, respectively. Before packing, the perlite was soaked in a phosphate buffered mineral medium (pH 4 or 8) and mixed with the soil (50 g per l perlite). The filters were placed in a temperature controlled room (25°C) and connected to a gas supply system. A large stream of compressed air was humidified up to 97% RH (relative humidity) by passing it through vials with water (placed in a 37°C water bath). A small stream of air was bubbled through a vial containing pure toluene and was mixed with the larger humidified gas stream, resulting in an inlet gas with a concentration of about 1000 mg toluene/m<sup>3</sup>. Two filters were supplied with a gas stream with a RH (relative humidity) of 92%, which was created by blending the above gas stream with 5% dry air. The other two filters were supplied with the 97% RH gas stream. The respective gas streams were introduced into the top section of each biofilter (down stream operation), and the flow was adjusted using rotameters. The RH of the influent gas was checked regularly.

- *Filter 1*: Medium pH 4.0; gas RH 92%
- *Filter 2*: Medium pH 8.0; gas RH 92%
- *Filter 3*: Medium pH 4.0; gas RH 97%
- *Filter 4*: Medium pH 8.0; gas RH 97%

The gas loading rates applied ranged from 90 m<sup>3</sup> gas/m<sup>3</sup> filter bed/h to 220 m<sup>3</sup>/m<sup>3</sup>/h. The biofilter gas inlet and outlet were connected (by a tube) to a flame ionisation detector (FID) with automatic sampling (each channel once every two hours) and to a carbon dioxide analyser with automatic sampling. During the first few weeks of operation, the filters were completely filled on a weekly basis with fresh medium and drained to add depleted nutrients and remove accumulated compounds. The pH of the drained water was measured. After two weeks, the wetting frequency depended on the activity in the filter. After a decline in activity, a new medium was added, which usually resulted in an increased activity.

### Packing material experiment

Seven 2 l biofilters with a packing height of 60 cm were operated similarly to those described above. The gas loading rate was 200 m<sup>3</sup>/m<sup>3</sup> filter bed/h, and the toluene concentration ranged from 400 to 700 mg/m<sup>3</sup> influent gas. Three biofilters were filled with perlite

and four biofilters with lava stones, each with a different diameter: (1) 4.5–6.5 mm; (2) 6.5–8 mm; (3) 8–10 mm; and (4) 10–15 mm. The filters were inoculated with fungi isolated from the pH experiment biofilters. Biomass protein in the biofilter drainage water and in the biofilter was determined according to Lowry. In these determinations the whole content of biofilters were boiled in alkali.

#### Mite experiment

Two 10 l biofilters of 1 m height were loaded with gas containing toluene ( $200 \text{ m}^3/\text{m}^3/\text{h}$ ; toluene concentrations up to  $700 \text{ mg}/\text{m}^3$ ;  $\text{RH} > 95\%$ ). Two months after inoculation, a culture of fungi-consuming mites was introduced. The effect on toluene elimination and gas pressure drop was measured.

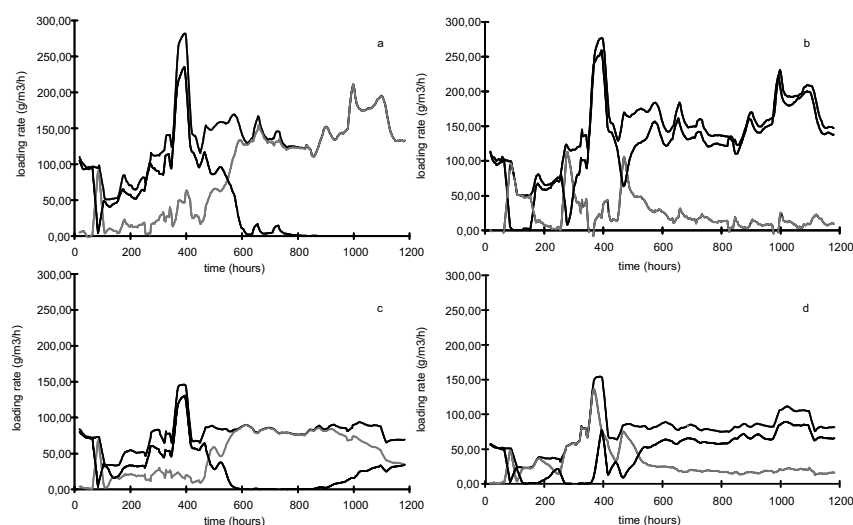
## Results and discussion

### The effect of pH and humidity

The elimination of toluene in the four filters is presented in Figure 1. In this figure the performance is expressed in terms of volumetric elimination capacity ( $\text{g}$  toluene removed per  $\text{m}^3$  filter bed per hour). This capacity is the difference between the toluene loading rate (influent) and the rate of toluene released via the effluent.

After four days (100 hours) of operation toluene removal activity had developed in all filters. This was supported by carbon dioxide production (not shown). However, after 400 hours, a difference in performance appeared: the filters with the low pH were able to remove more toluene from the gas than the filters with the high pH. The colour of the biofilters with the high pH had turned from white to yellow, and microscopic analysis of the water from the filters revealed that mainly bacteria were present. In the biofilters with low pH a dark green mycelium began forming. The water from these filters contained mycelium and spores.

After 500 hours the addition of medium was stopped in all filters in order to simulate drying out (which is one of the main problems in full scale biofilter practice). The different reaction of the low pH filters compared with the high pH filters was striking: in the latter a



**Figure 1** Toluene loading rate (thin line), effluent release rate (dotted line) and volumetric elimination capacity (bold line) (all expressed in grams of toluene per  $\text{m}^3$  filterbed per hour) of: (a) biofilter 1 (pH 4; RH 92%); (b) biofilter 2 (pH 8; RH 92%); (c) biofilter 3 (pH 4; RH 97%); (d) biofilter 4 (pH 8; RH 97%)

rapid activity decline occurred, while the first maintained their activity at a high level for a long time. Filter 1 maintained a volumetric activity of about  $125 \text{ g/m}^3/\text{h}$  up to 1000 hours running time, after which the run was stopped due to clogging of the filter with fungal biomass. Filter 3 had a capacity of  $80 \text{ g/m}^3/\text{h}$ , but after 400 hours of drying out, this activity gradually declined (over many hundreds of hours). The bacterial biofilters had a capacity of not more than  $20 \text{ g/m}^3/\text{h}$  during this drying period. The fungal biofilters eliminated up to 99% of the loaded toluene from passing gas.

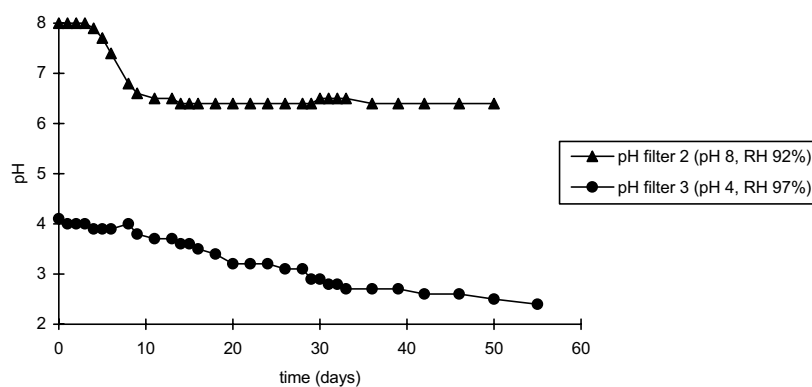
The results indicate that toluene can be degraded in biofilters by bacteria as well as fungi, and at low pH fungi are enriched (preferentially selected over bacteria). Relatively better growth of fungi at low pH compared with the average bacterial species is a well known fact. This study was a clear manifestation of this phenomenon. The other expected property of fungi, a better resistance against dry conditions, was also demonstrated by the month-long maintenance of a high substrate consumption rate in fungal biofilters during extended drying out with unsaturated air. The bacterial filters were not able to maintain their maximum activity for more than a week.

The volumetric toluene elimination capacities of our fungal biofilters were high as compared to results obtained with compost-based biofilters ( $10\text{--}20 \text{ g/m}^3/\text{h}$ ) and biotrickling filters ( $20\text{--}38 \text{ g/m}^3/\text{h}$ ) (Weber, 1995). Other research on fungal-based biofilters indicated higher capacities; García *et al.* (2000) even attained capacities as high as  $200 \text{ g toluene/m}^3/\text{h}$ . Our results are very much in line with observations made by Sanchez-Peña *et al.* (2000) that biofilters based on bacteria develop higher toluene elimination capacities after fungal invasion.

Although the media used had a pH of 4 or 8, the actual pH of the aqueous phase of the biofilters was lower, as shown in Figure 2. In filter 2 the pH stabilised at a steady state value of 6.4, while in filter 3 the pH gradually decreased to 2.3 within 56 days. From earlier experience with fungal biofilters for styrene removal, in which the pH dropped from 5.6 to 2–2.5, it is known that the pH decrease is caused by the uptake of  $\text{NH}_3$  from the medium for incorporation into cellular constituents. If  $\text{NH}_4\text{Cl}$  is replaced by  $\text{NaNO}_3$  as the nitrogen source in these styrene biofilters, the pH increases in time.

#### The effect of the size of granules in the packing

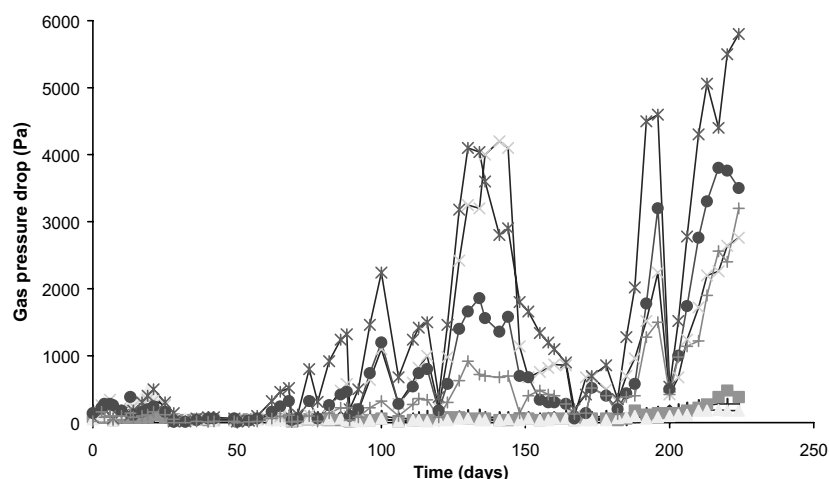
All seven biofilters developed fungi and removed toluene from the passing gas. The volumetric toluene elimination capacities obtained in the second half of the 220 day experimental period are summarised in Table 1. A negative effect of the larger packing granule size can be observed (with exception of filter 2), which indicates that packing surface area is important in fungal biofilters. This fact was already established for bacterial based systems.



**Figure 2** Course of the pH in biofilter 2 (pH 8, RH 92%) and 3 (pH 4, RH 97%) over time

**Table 1** Average volumetric toluene elimination capacity in 2 l biofilters filled with different types and sizes of packing material, in the period of 150–220 days after inoculation

Filter number	Packing material	Granule diameter (mm)	Toluene elimination capacity (g/m <sup>3</sup> filter bed/h)
1	Perlite	4	100
2	Perlite	4	60
3	Perlite	4	100
4	Lava	4.5–6.5	100
5	Lava	6.5–8	80
6	Lava	8–10	80
7	Lava	10–15	50



**Figure 3** Development of gas pressure drop in 60 cm high biofilter columns loaded with 200 m<sup>3</sup> gas/m<sup>3</sup> filter bed/h and packed with granules of different sizes: ◆, □, △: perlite (4 mm); x: 4.5–6.5 mm lava; ★: 6.5–8 mm lava; ●: 8–10 mm lava; and +: 10–15 mm lava

The development of the gas pressure drop is given in Figure 3. Surprisingly, the pressure drop in the biofilters with lava stones was much higher than that of the biofilters with perlite, despite their larger size. The reason is that the pores between the lava stones were completely filled with aerial mycelium. Apparently lava stimulates the development of long hyphae, more than perlite. Every time after the biofilter packing was wetted with fresh medium (by temporary submerging), the pressure drop decreased. To give an indication of the amounts of biomass that are washed out from the biofilters, the protein concentrations in the drainage water and in the biofilters were measured in filters 1, 4, 5, 6 and 7 at the end of the experimental period. Amounts ranging from 0.5 to 3 mg biomass protein were washed out, while the 2 l biofilters contained 0.8 to 1 g biomass protein. It can be concluded that washing with medium did not remove much fungal biomass. Presumably, the decrease of the pressure drop after washing was caused by compaction of the hyphae. The highly loaded perlite biofilters developed relatively low pressure drops, but a tendency for the pressure drop to increase was observed. Additional measures, such as the introduction of mites, are therefore required for long-term stable operation.

#### Co-cultures of fungi and mites

The mites developed, multiplied and spread over the biofilter packing. Their presence coincided with the appearance of shorter aerial hyphae in the biofilter pores. In both filters the elimination capacity ranged between 50 and 100 g toluene/m<sup>3</sup> filterbed/h, but the filter with

the mites showed slightly higher capacities. After 100 days of operation the biofilter without mites had a gas pressure drop of 400 Pa with a tendency to increase. In the biofilter with mites, however, the pressure drop was only 130 Pa and it was decreasing with time. The experiments will be continued and repeated, but the use of mites to control fungi growth and pressure drop seems promising.

#### Costs

A high volumetric capacity and low pressure drop increases the cost-effectiveness of biofilters. However, the use of perlite, mites and nutrient solutions increases the costs of perlite/fungi biofilters compared to compost/bacteria systems. Before our optimisation studies, the costs of perlite/fungi biofilters for styrene removal were estimated by Kraakman *et al.* (1997): €5 – €8 per m<sup>3</sup> gas/h investment costs and €0.5 – €1 per 1000 m<sup>3</sup> gas treated operational costs including capital costs. It is expected that these costs will be further decreased by implementing the results of the research work presented in this paper.

#### Conclusions

- Biofilters for elimination of toluene can be started up within a week using soil from a fuel station as an inoculant.
- In biofilters with a perlite packing, a fungal population is developed when a pH between 2.5 and 4 is used, whereas bacteria develop if the aqueous phase of the biofilters has a pH of 6.5–8.
- Fungal biofilters are more resistant against drying out than bacterial biofilters.
- High volumetric toluene elimination capacities of 80–125 g/m<sup>3</sup>/h can be attained in biofilters with fungi.
- 99% of the loaded toluene can be eliminated from the gas by biofilters containing fungi.
- The use of lava stones with larger sizes than perlite granules does not lead to reduced gas pressure drops.
- Introduction of mites in biofilters containing fungi may be an interesting tool for the prevention of filter bed clogging while maintaining high elimination capacities.

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