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TOLUENE AND STYRENE REMOVAL FROM AIR IN BIOFILTERS

Mario Zilli, Attilio Converti*, Marco Del Borghi
Department of Chemical and Process Engineering, University of Genoa, Via Opera Pia, 15 – 16145 Genoa, Italy

Abstract. *Two similarly sized laboratory-scale biofilters, inoculated with a toluene-degrading strain of* Acinetobacter sp. *NCIMB 9689 and a styrene-degrading strain of* Rhodococcus rhodochrous AL *NCIMB 13259, are investigated for the purification of toluene and styrene-containing off-gas streams. After a preliminary series of batch tests in water to investigate the intrinsic micro-kinetics of contaminants' degradation, continuous experiments were conducted for both pollutants varying both the inlet pollutant concentration and the superficial gas velocity. Maximum elimination capacities of 242 and 63 g* $m^{-3}_{packing\ material}$ h^{-1} *were recorded for toluene and styrene, respectively. On the other hand, the deodorisations of toluene and styrene were achieved at maximum inlet concentrations of 1.99 and 0.20 g* m^{-3}*, operating at superficial gas velocities of 17.8 and 122 m* h^{-1}*, respectively.*

Keywords: *Biofiltration, Toluene and styrene removal, Elimination capacity, Deodorisation.*

1. INTRODUCTION

BTEX (Benzene, toluene, ethylbenzene and the xylene isomers) and styrene are among the 50 largest-volume industrial chemicals produced in the world. All these compounds are produced at a rate of millions of tons per year [Zilli and Converti, 1999; Smith, 1990]. They are widely used as fuels and solvents and provide starting materials for the production of resins, polymers, plastics, explosives, agrochemicals, and pharmaceuticals. Because of their ubiquitous presence in the environment and their widespread release through industrial and agricultural activities, the biodegradation of these compounds has been widely studied. They are quoted by the US Environmental Protection Agency as priority environmental toxic pollutants, due to their toxicity and their carcinogenic potential, even at low concentrations.

The control of volatile organic air toxics emissions from industrial facilities has become critical and expensive to the chemical industries, particularly the small sized ones, in order to meet the more and more severe quality standards. The biotechnological approach to air pollution is now a promising field of research which can supply reliable, simple and cheap technologies for the prevention of air contamination.

The reliability of biological processes and, in particular, of biofiltration for the treatment of waste gas streams containing volatile organic compounds has been demonstrated by a very large number of experimental studies [Zilli and Converti, 1999; Ottengraf, 1986; Shareefdeen *et al.*, 1993; Zilli *et al.*, 1993; Ottengraf and van den Oever, 1983; Mpanias and Baltzis, 1998]. Biofiltration is particularly suited and cost-effective for the treatment of high volumes of waste gases containing low concentrations of volatile organic compounds (VOCs). Furthermore, it is environmentally friendly because the contaminants are completely converted at low temperature into non-hazardous final products.

Compared with the other biological systems, biofilters have been shown to be more effective for treating some poorly-water-soluble compounds due to the high superficial area available for mass transfer. Optimal removal of aromatic hydrocarbons air pollutants can best

* To whom all correspondence should be addressed

be reached using biofilters rather than bioscrubbers or biotrikling filters [van Groenestijn and Hesselink, 1993].

The aim of this study is to investigate the ability of the selected strains to remove toluene and styrene vapours in biofilters to obtain sufficient data for a future macro-kinetic study as well as useful information for industrial application.

2. MATERIALS AND METHODS

2.1 Microorganisms and culture conditions

The toluene-degrading strain of *Acinetobacter sp*. NCIMB 9689 and the styrene-degrading strain of *Rhodococcus rhodochrous AL* NCIMB 13259 were obtained from the National Collection of Industrial and Marine Bacteria LTD, Aberdeen, Scotland. Both strains were grown on a sterilised nutrient medium containing 1.0 g l^{-1} Lab-Lemco beef extract, 2.0 g l^{-1} yeast extract, 5.0 g l^{-1} peptone, and 5.0 g l^{-1} NaCl. The cultivations were performed at 25°C in 250-ml Erlenmeyer flasks containing 150 ml of medium at pH 6.8 with rotary shaking at 150 rpm and aerated with sterile air. After 24-48 h of growth, the cells were harvested by centrifugation at 5000 rpm for 20 min and re-suspended in fresh medium for the inoculum. Stock cultures of the strains were maintained by periodic subculture on the same nutrient medium and stored at 4°C. The composition of the mineral salt solution used for both batch and continuous tests was 5.8 g l^{-1} KH_2PO_4, 4.5 g l^{-1} K_2HPO_4, 2.0 g l^{-1} $(NH_4)_2SO_4$, 0.34 g l^{-1} $MgCl_2 \cdot 6\ H_2O$, 0.20 g l^{-1} $CaCl_2 \cdot 6\ H_2O$, 2.0 mg l^{-1} $FeSO_4 \cdot 7\ H_2O$, and 1.6 mg l^{-1} $MnCl_2 \cdot 4\ H_2O$.

2.2 Analytical procedures

Both toluene and styrene liquid and gaseous samples were analysed with a Carlo Erba Model HRGC 5160 gas chromatograph equipped with a capillary column (25 m x 0.32 mm, Mega Laboratory) and a flame ionisation detector connected to a computing integrator. The injector and the detector were kept at 150°C and 200°C, respectively. Oven temperature was initially maintained at 50°C for 2 min and then increased at a rate of 40°C min^{-1} up to 250°C, where it remained constant for 5 min. Nitrogen was used as carrier gas. Vapour phase pollutant standard curves were obtained by injecting a known amount of the selected compound in calibrated glass bottles following the procedure described by Shareefdeen and Baltzis (1994). The compound concentration in the liquid phase was measured by mixing 1 ml of the samples with 1 ml of *n*-hexane for toluene or 1 ml of carbon disulphide for styrene, respectively, and shaking the solutions for 2 min to ensure the quick and effective extraction of the compound. 1 µl of the *n*-hexane or carbon disulphide phases was injected into the FID gas chromatograph with a 10-µl micro-syringe. Toluene/hexane and styrene/carbon disulphide solutions with known compositions were used as standards for batch experiments.

Cell mass concentration in the liquid phase was determined by filtering 10 ml of broth through tared 0.45 µm-pore filters. After washing with 10 ml 0.9% NaCl solution, the filters were dried at 105°C to constant weight and cooled in a desiccator prior to re-weighing. Filter bed moisture content was determined by the dried weight method [A.P.H.A., 1985].

2.3 Apparatuses for batch experiments

Batch experiments necessary to determine micro-kinetic parameters of both strains were performed aerobically at 25 ± 0.1°C in 1.20-litre glass bottles previously filled with the mineral salts solution. The bottles were closed tightly with teflon-faced silicon septa stoppers, covered with aluminium foil, and autoclaved for 20 min at 121°C. After cooling to ambient

temperature, the selected pollutant (toluene or styrene) was introduced into the bottles using a syringe. The bottles were magnetically stirred for 12 h to ensure homogeneous dispersion of the compound and equilibration of the headspace with the solution. The cells for inoculum were then transferred to the bottles following the same procedure. The headspace volume in the bottles was kept high enough (400 ml) to avoid any oxygen limitation.

2.4 Apparatuses for continuous experiments

Biofiltration experiments were carried out in two identical continuously operating bench-scale biofilters, consisting of two 0.65 m long cylindrical glass columns with an inner diameter of 0.05 m, provided with sampling ports, located at 0.12, 0.25, 0.37, and 0.52 m from the bottom. The height of the filter bed was 0.50 m in both biofilters.

The filter packing material was a mixture of sterilised peat (with a specific surface area of 1.6 $m^2\ g^{-1}$) and glass beads (diameter of 5 mm) in a 4:1 volume ratio, which was supported at the bottom of the column by a perforated plate (diameter of 5 mm) of ceramic (hole diameter of 1 mm). The moisture content of the packing material was kept between 50 and 60%, either by bubbling the influent synthetic waste air in a humidification unit, or by periodically distributing via nozzle at the top of the packing material a mineral salts solution, flowing counter currently with the gas.

The biofilter columns were operated at room temperature (20-21°C). The pH of the packing material was kept around the neutrality as previously described [Zilli *et al.*, 1996]. The biofilters were inoculated with a cell suspension of the selected strain. The suspension was mixed with the dry packing material, previously sterilised by autoclaving at 120°C for 20 min, in the proportion of 25% by volume. Cell density in the inoculum was 6.0 g l^{-1}. During the experiments, an inoculum of 50 ml of cells suspension was provided every 2 months to the columns, in order to guarantee the predominance of the selected strain within the biofilter.

The synthetic waste gas was generated by injecting a low flow air stream (1-10 l h^{-1}) into a liquid pollutant reservoir, the stream becoming polluted by the contaminant evaporation. The air containing the pollutant vapour was then adequately mixed with a high-flow rate air stream (25-500 l h^{-1}) previously humidified by bubbling it in a water-containing vessel to obtain the desired concentration of contaminant in the air stream, before entering the base of the biofilter. The system was provided with two flow-meters which allowed the measurement of the total air flow and the streams flowing in the vessels. The pollutant concentration in the influent gas was varied by regulating, by means of the flow-meters, the flows of both contaminated and laboratory air streams in a mixing chamber.

The conversion degree was calculated as the ratio of the difference between inlet and outlet pollutant concentrations (C_{go}-C_{ge}) to the inlet pollutant concentration (C_{go}). The deodorisation tests were carried out varying the inlet pollutant concentration and the superficial gas velocity so as to achieve an outlet pollutant concentration below the olfactory threshold values, that are $8.8 \cdot 10^{-3}$ g m^{-3} and $0.2 \cdot 10^{-3}$ g m^{-3} for toluene and styrene, respectively.

3. RESULTS

3.1 Degradation of toluene and styrene in batch operation

A series of batch tests with a starting cell concentration of about 0.5 $g_{\text{dry-cell weight}}\ l^{-1}$ was conducted aerobically at 25°C using the toluene-degrading strain of *Acinetobacter sp*. at starting toluene concentration in the liquid phase varying from 0.020 to 0.210 g l^{-1}. The batch tests for styrene degradation were carried out aerobically at 30°C using the styrene-degrading strain of *R. rhodochrous* at a starting cell concentration of about 0.65 $g_{\text{dry-cell weight}}\ l^{-1}$ and at

initial styrene concentration in the liquid phase ranging between 0.051 and 0.140 g l^{-1}. The experimental results of these two series of tests are illustrated in Fig. 1.

In both cases, the pH of the solution, initially regulated at 6.8, kept nearly constant during the experiments, thus indicating negligible acidification of the medium. All tests were made in duplicate and abiotic losses of the target compound were substracted by sampling an identical sterile bottle. Biomass growth was nearly negligible under the selected conditions.

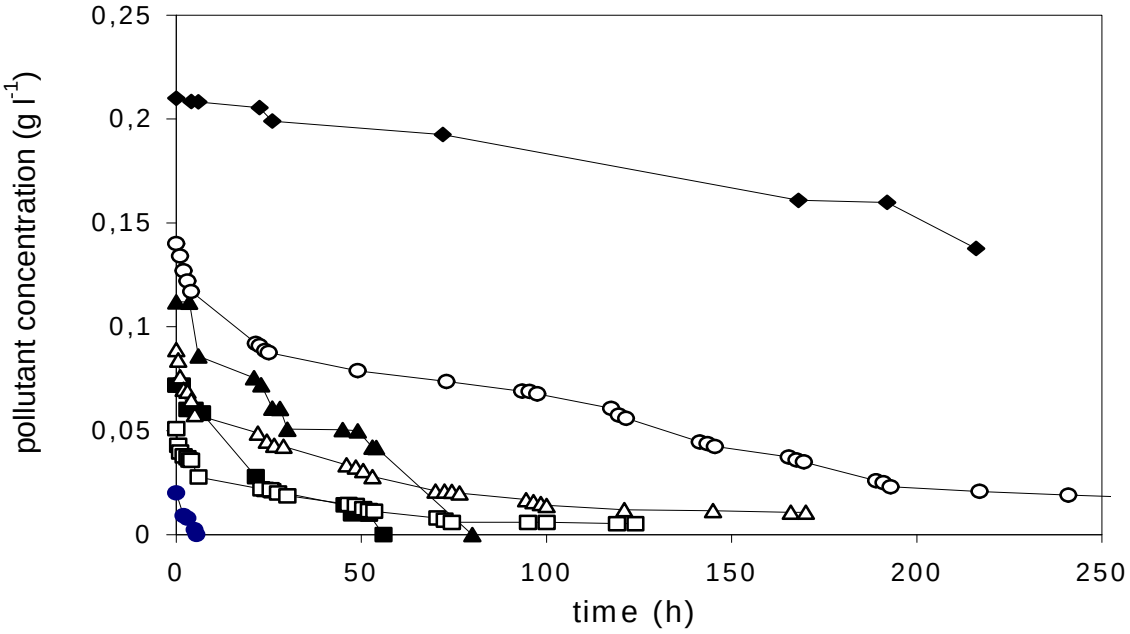

Figure 1. Batch tests of toluene and styrene degradation at different starting pollutant concentrations, S_o (g l^{-1}). Toluene: (●) 0.020; (■) 0.072; (▲) 0.112; (◆) 0.210. Styrene: (❒) 0.051; (Δ) 0.089; (❍) 0.141.

3.2 Continuous biofiltration tests

In order to test the elimination capacity of the system, different sets of continuous experiments were made over a period of 10 months by changing both the superficial gas velocity and the toluene concentration in the influent air stream. In particular, four series of tests were performed at 17.8, 35.7, 127.5, and 255 m h^{-1}, and testing, for each superficial gas velocity, five different inlet toluene concentrations, namely 0.1-0.2, 0.4-0.5, 1.0, 2.0, and 4.0 g m^{-3}. All tests were performed under non-sterile conditions. During the start-up phase, a control column was used in order to determine the starting abiotic removal due to the physical adsorption by the packing material. Toluene breakthrough occurred after 3 h of operation.

Figure 2 shows the results of continuous tests carried out during the whole experimental investigation. Each test, under a given set of operative conditions, lasted a period of about 10-12 days. In order to prevent a shock to the microorganisms due to excess substrate, the first set of experiments was performed at the lowest superficial gas velocity (17.8 m h^{-1}) as well as at the lowest range of inlet toluene concentration ($0.1<C_{go}<0.2$ g m^{-3}). During this period, a conversion degree close to 1.00 was initially observed, which was followed by a gradual decrease and a final restoration of the high starting values. This behaviour is the result of the preliminary adsorption of the pollutant by the bed and of the subsequent biological action.

Nearly constant values of the conversion degree were reached after about 3 days of continuous operation at the lowest concentration range and the highest residence time, which

demonstrated the achievement of steady state. This short start-up period was likely due to the biofilter inoculation with specific and adapted microorganisms. A final average conversion degree close to 1.00 has been assured, which indicated biofiltration as an efficient technique in the control of waste gases containing toluene at low concentrations (up to 0.4 g m^{-3}).

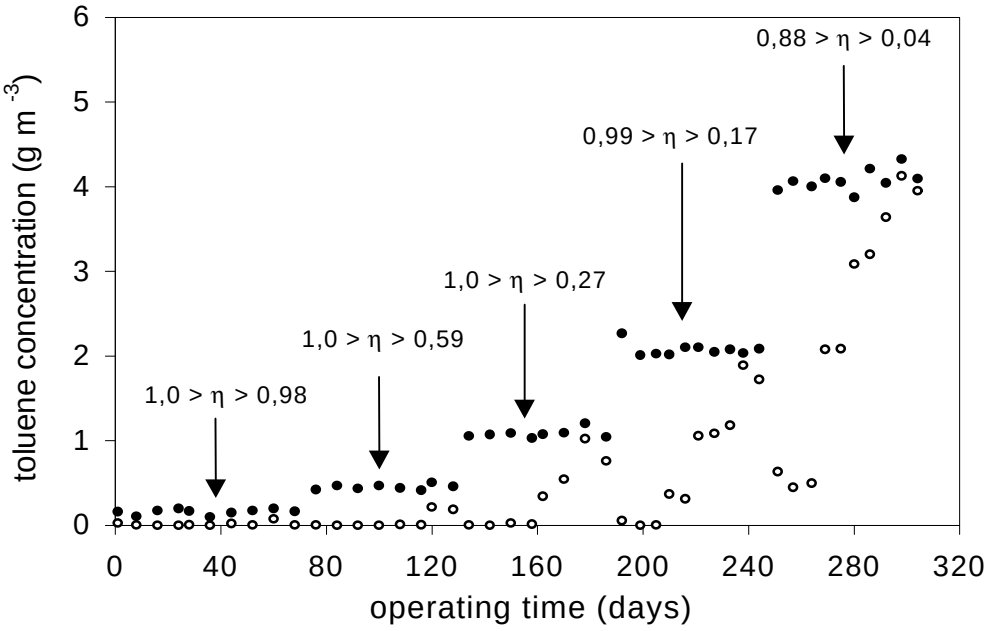

Figure 2. Biofilter behaviour during continuous experiments of toluene removal. Toluene concentration (g m^{-3}): (●) inlet; (❍) outlet. η = conversion degree

After the efficiency of the system had been tested at low pollutant levels, the inlet toluene concentration was progressively increased to 0.4-0.5, 1.0, 2.0, and 4.0 g m^{-3}, while the residence time was decreased from 202 to 14 s, with the aim of evaluating the actual possibility of employing this system in the presence of variable and high toluene loads.

The satisfactory average degree of conversion calculated in these subsequent phases (0.65) indicates the high capacity of the microorganism to adapt itself to large variations of pollutant concentrations as well as an unexpected ability to survive relatively concentrated feeds.

The laboratory-scale biofilter used for styrene vapours removal was operated for a period of six months with styrene volumetric loading rates varying from 6.14 to 588 g $m^{-3}_{\text{packing material}}$ h^{-1}, corresponding to superficial gas velocities from 61.2 to 245 m h^{-1} and inlet styrene concentrations from 0.05 to 1.2 g m^{-3}, which approximately corresponds to the concentration range of styrene in industrial emissions [Tossavainen, 1978].

The experimental procedure followed for the continuous tests of styrene removal was similar to that followed for toluene. So, also in this case, in order to prevent a shock to the selected microorganism, the first series of runs was conducted at the lowest superficial gas velocity (61.2 m h^{-1}) as well as at the lowest styrene content in the inlet gas (0.05 g m^{-3}). The adaptation period of the biofilter lasted 2 days, during which the styrene conversion degree increased nearly to 1.00, evidencing the achievement of the system stabilisation.

After 20 days, gas velocity was progressively increased to 122 and 245 m h^{-1}, while the inlet styrene concentration (0.05 g m^{-3}) was maintained constant. Every series of experiments lasted 10 days, during which a new steady state appeared normally within 10 h. The same experimental procedure was followed for the other inlet concentrations investigated (0.2, 0.4,

0.8, and 1.2 g m^{-3}), in order to study the influence of both parameters on the elimination capacity. The performance of the biofilter over a period of six months is shown in Fig. 3.

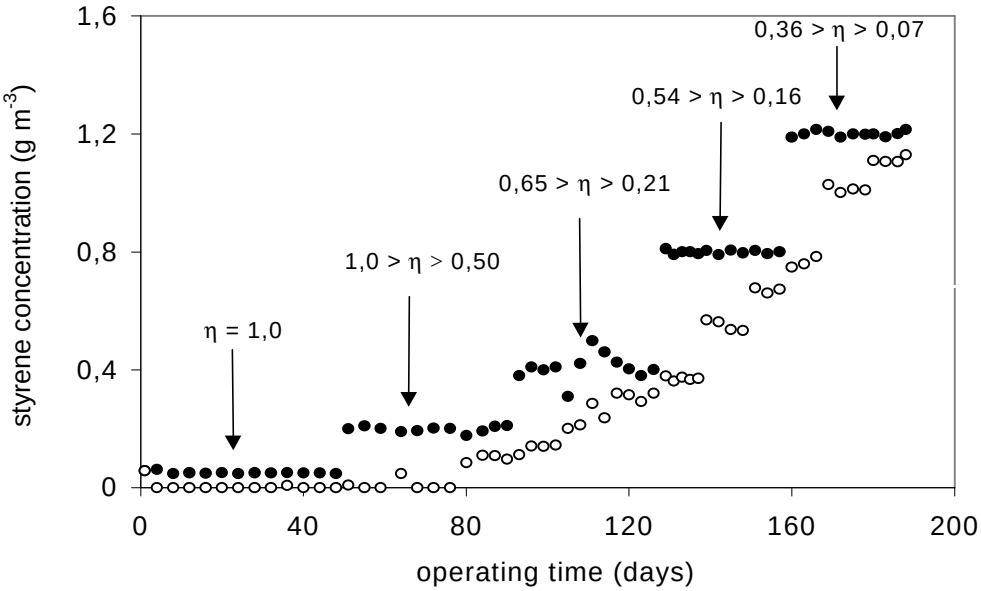

Figure 3. Biofilter behaviour during continuous experiments of styrene removal. Styrene concentration (g m^{-3}):(●) inlet; (❍) outlet.

3.3 Deodorisation tests

In the last part of this study, the deodorisation of both toluene and styrene waste-gases was considered, whose main results are presented in Table 1. In particular, the influence of the superficial gas velocity and the inlet pollutant concentration on the biofilters deodorisation capacity was investigated.

Table 1. Operating conditions at which toluene and styrene deodorisations were obtained

C_{go} (g m^{-3})	U_g (m h^{-1})	Loading rate (g m^{-3} h^{-1})	Removal rate (g m^{-3} h^{-1})
Toluene deodorisation			
1.99	17.8	70.8	70.5
1.02	35.7	72.8	72.2
0.31	127.5	79.0	76.8
0.25	255.0	127.5	123.0
Styrene deodorisation			
0.05	245.0	24.5	24.5
0.20	122.0	48.8	48.8

4. DISCUSSION

4.1 Degradation of toluene and styrene in batch operation

Figure 1 shows the experimental data of toluene and styrene batch degradations obtained at different initial substrate concentrations (S_o) by the *Acinetobacter sp*. and *Rhodococcus rhodochrous* strains, respectively.

The kinetic results listed in Table 2 indicate that the maximum specific degradation rate (r_{max}) decreases in both cases with increasing the initial substrate concentration, due to an evident substrate inhibitory effect at higher concentrations similar to that observed for alcohol fermentation by Ciftci *et al.* (1983) and Converti *et al.* (1986). It is also evident from these data that *R. rhodochrous* starts to utilise styrene as sole carbon source more rapidly than *Acinetobacter sp.* does with toluene. In fact, maximum specific rates for styrene degradation were about 3-8 times higher than those estimated for toluene at comparable S_o values. In addition, these microorganisms show opposite behaviours with regard to the dependence of the Monod's saturation constant (K_s) on starting substrate concentration. The increase of K_s with S_o for toluene degradation by *Acinetobacter sp.* is consistent with that observed for other bioprocesses [Ciftci *et al.*, 1983; Converti *et al.*, 1986] and, thus, it can be considered as an additional effect of substrate saturation. The decrease of K_s observed with increasing S_o for styrene consumption by *R. rhodochrous* can only be justified, on the other hand, by a sort of mixed inhibition due to excess substrate.

Table 2. Kinetic parameters determined in batch culture for toluene and styrene degradation

Microorganism	S_o ($g_S\ l^{-1}$)	$r_{max} \cdot 10^3$ ($g_S\ g_X^{-1}\ h^{-1}$)	K_s ($g_S\ l^{-1}$)
Acinetobacter sp.	Toluene concentration		
	0.020	10.0	0.007
	0.072	7.41	0.027
	0.112	5.88	0.057
	0.210	1.37	0.397
R. rhodochrous	Styrene concentration		
	0.051	71.4	1.36
	0.089	24.4	0.59
	0.140	9.6	0.31

Most kinetic studies reported in the literature were carried out mainly using the Monod's and Haldane's models to calculate the specific growth rate and the kinetic constants for different microorganisms able to degrade toluene and styrene in the liquid phase under different conditions. A wide variability of data can be observed for species belonging to the genus *Pseudomonas* while very little has been reported on *Acinetobacter sp.* [Acuña *et al.*, 1999] and *Rhodococcus sp.* [Arnold *et al.*, 1997]. This wide variability demonstrates that it is hard to compare the kinetics of different strains and microbial systems.

However, in this work, attention has mainly been paid to toluene and styrene specific uptake rate, which is the most important parameter for biofiltration. A relative lack of data exists in the literature on this parameter, most of the work available reporting the volumetric uptake rate in liquid phase, with insufficient indication of biomass concentration.

4.2 Continuous tests of toluene and styrene biofiltration

The combined effects of the superficial gas velocity (U_g) and of the inlet pollutant concentration (C_{go}) on the biofilter conversion degree are evident in Fig. 4 for both toluene and styrene degradations. The ratios between the outlet and inlet pollutant concentrations are reported, for different ranges of inlet concentrations, as a function of the ratio between the minimum superficial gas velocity (17.8 and 61.2 m h^{-1} for toluene and styrene, respectively) and the one considered in each experiment ($U_{g\ min}/U_g$), that is a dimensionless superficial gas

velocity. Each experimental value corresponds to the average of about 10 experimental runs with inlet concentration equal to the reported nominal value ± 5%.

As expected, these results show that the biofilter conversion degree ($1-C_{ge}/C_{go}$) decreases with increasing either U_g or C_{go}. In addition, they evidence a greater ability of *Acinetobacter sp.* in toluene removal if compared with *R. rhodochrous* capacity to utilise styrene.

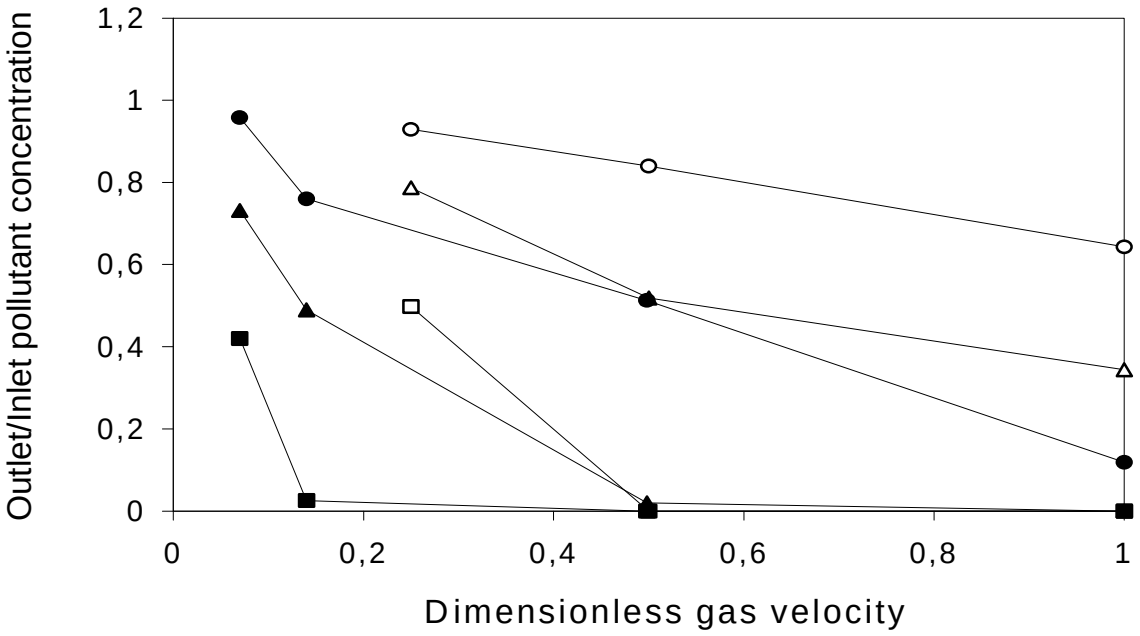

Figure 4. Profiles of pollutant gas concentration as a function of the dimensionless superficial gas velocity, obtained at different inlet pollutant concentrations, C_{go} (g m^{-3}). Toluene: (■) 0.4-0.5; (▲) 1.0; (●) 4.0. Styrene: (❑) 0.2; (Δ) 0.4; (❍) 1.2.

This result, which appears to be in disagreement with the previous kinetic results of batch tests, can be justified with a faster but incomplete styrene degradation by *R. rhodochrous*.

The satisfactory removal capacity of the biofilter is demonstrated in Fig. 5, where the elimination capacity of both toluene and styrene, obtained at different inlet concentrations of these pollutants, are plotted versus the organic load. From these results, it is also evident that this parameter increases regularly with the organic load, with scarce relevance whether such a dependence is the result of an increase in C_{go} or of a decrease in residence time. In particular, a gradual and linear increase of the elimination capacity occurs up to a value of the organic load corresponding to about 100 g m^{-3} h^{-1} for toluene and 50 g m^{-3} h^{-1} for styrene. Beyond these values, the elimination capacity increases more slowly and reaches maximum values around 230-240 and 63 g $m^{-3}_{\text{packing material}}$ h^{-1} for toluene and styrene, respectively (results not shown). These thresholds were obtained for toluene at an inlet concentration of 4.0 g m^{-3} and at a superficial gas velocity of 127.5 m h^{-1} and for styrene at 0.8 g m^{-3} and 245.0 m h^{-1}.

Such a behaviour of the elimination capacity indicates that, at low organic loads, there is a linear relationship between the removal rate and the inlet load, and that the pollutants are nearly completely removed. In this range of the organic load, the system performance is only limited by pollutant availability, that is by the diffusion. With further increase of the load, on the contrary, the elimination rate increases more slowly up to a critical load (characteristic for each pollutant) at which it keeps constant, indicating that the maximum elimination capacity of the biofilter is achieved. Under these last conditions, the limiting step of the process is the biological reaction, the elimination rate being lower than the pollutant feed rate.

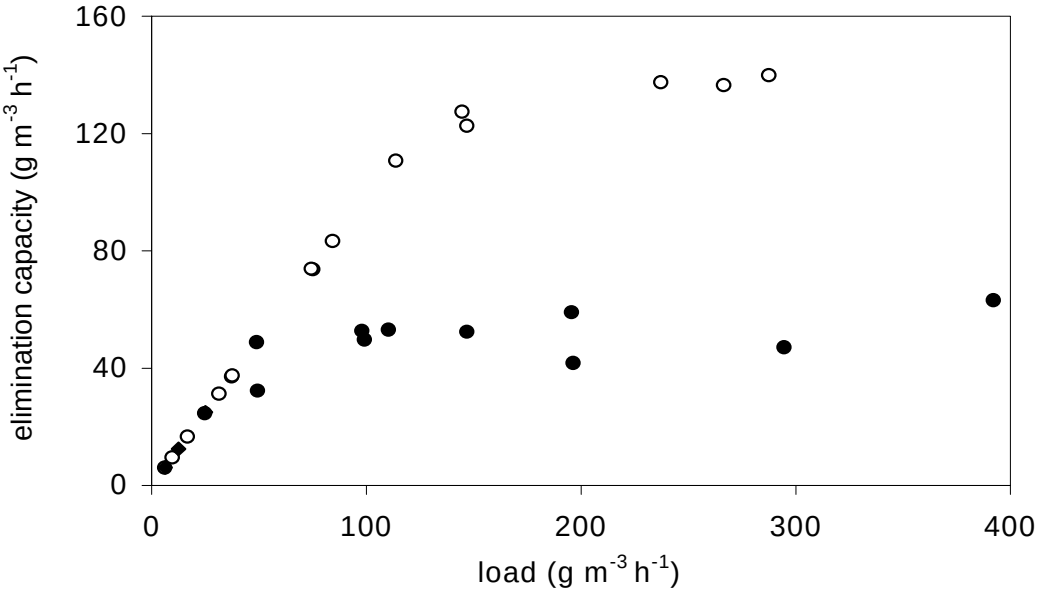

Figure 5. Elimination capacity versus the organic load. Toluene: (❍); Styrene (●).

The maximum toluene elimination capacity obtained in this work through continuous tests in biofilter is about 10% higher than the best values reported in the literature (190 - 215 g m^{-3} h^{-1}) [Acuña *et al.*, 1999; Morales *et al.*, 1998]. A higher toluene elimination capacity (275 g m^{-3} h^{-1}) was obtained in a biotrickling filter [Laurenzis *et al.*, 1998]. The good results obtained in this work could be due to *Acinetobacter sp.* capability to predominate in heterogeneous consortia, as the result of its peculiar adaptability to the conditions present within the biofilter.

As far as the styrene degradation ability of *R. rhodochrous* in biofilter is concerned, the elimination capacity obtained in this work is about 100% higher than that (30 g m^{-1} h^{-1}) reported by Arnold *et al.* (1997) for a peat biofilter inoculated with activated sludge and nearly coincident with that (62 g m^{-1} h^{-1}) observed by Cox *et al.* (1997) for a biofilter operated in a downflow mode inoculated with the yeast *Exophiala jeanselmei*.

4.3 Deodorisation tests

The average results of toluene and styrene deodorisation obtained under different conditions (Table 1) showed the effects of superficial gas velocity on the maximum inlet pollutant concentration, at which the gas deodorisation is obtained, as well as the removal rates calculated at the different superficial gas velocities. It can be observed, from those results, that the value of the maximum inlet pollutant concentration at which the gas deodorisation is ensured, sharply decreases with increasing superficial gas velocity, as a consequence of the decrease of the removal efficiency of the biofilter with decreasing residence time. The maximum concentrations at which the outlet pollutant concentration was reduced below the olfactory threshold values ($8.8 \cdot 10^{-3}$ g m^{-3} for toluene and $0.2 \cdot 10^{-3}$ g m^{-3} for styrene) were 1.99 g m^{-3} and 0.2 g m^{-3}, and were obtained at superficial gas velocities of 17.8 m h^{-1} for toluene and 122.0 m h^{-1} for styrene, respectively. They seem to be interesting results if one considers that biofiltration is usually considered an effective technique for treating emissions containing contaminants only in relatively low concentrations (< 1.0 g m^{-3}).

Nomenclature

C_{ge} pollutant concentration in the effluent gas (g m^{-3})
C_{go} pollutant concentration in the influent gas (g m^{-3})
K_s saturation constant of Monod's equation (g $_{pollutant}$ l^{-1})
r_{max} maximum specific elimination rate (g $_{pollutant}$ g^{-1} $_{biomass}$ h^{-1})
S pollutant concentration (g l^{-1})
S_0 starting pollutant concentration (g l^{-1})
U_g superficial gas velocity (m h^{-1})
$U_{g\,min}$ minimum superficial gas velocity (m h^{-1})
η degree of conversion (dimensionless)

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