BIOLOGICAL REMOVAL OF TOLUENE VAPOR FROM AIR STREAMS

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1. INTRODUCTION

Highly volatile aromatics, such as benzene, toluene or the isomers of xylene, collectively known as BTX, represent the most common group of air contaminants in many industrialized countries. They are released by the chemical and process industries such as refineries (additive to petroleum products), steel, polystyrene, pharmaceutical, varnish, paint industries (i.e., by degreasing and purging of metals or by handling of varnish, in gum and in printing paint) and during soil remediation. Among the various volatile organic compounds, BTX are classified as priority environmental pollutants by the U.S. Environmental Protection Agency (EPA), due to their substantial toxicity and to the carcinogenic potential of the benzene components.[1,2] Many industrial off-gases have traditionally been treated by physical and/or chemical methods, such as water washing, chemical scrubbing (ozonization and chlorination), activated carbon adsorption, condensation, thermal and catalytic combustion.

Biological technologies are considered as representing a new generation of pollution control systems. These techniques were originally developed in Europe[3,4], although many studies have recently been reported by other groups in United States, Canada and Japan[4,5]. The suitability of these biotechniques for the treatment of various inorganic and organic compounds has been demonstrated over the past, testing the fate of different volatile compounds in bioreactors.[3,6-8] Biofiltration is the major advance in environmental pollution control. Its application at industrial scale has followed an exponentially growing curve, because of an increasing knowledge of biodegradation as well as of its semplicity, low maintenance requests and cost effectiveness. This technology, in fact, has been successfully applied for the control of odorous, toxics as well as volatile organic compounds from a wide range of industrial activities. Biofiltration is particularly suited for the treatment of large volumes of off-gas streams containing readily biodegradable hazardous contaminants in relatively low concentrations. The removal of these contaminants often entails higher costs when the physical and/or chemical methods are applied. In addition, biological processes are more appropriate for the environment because the contaminants are completely destructed into innocuous final products, such as carbon dioxide and water, at low temperature.

The removal of toluene vapor from air streams by biofiltration is investigated at bench-scale in this paper. A preliminary series of batch tests in liquid phase was carried out employing two different

microbial cultures to compare the respective toluene degradation efficiencies. The results of continuous runs performed using a biofilter inoculated with *Acinetobacter sp* are also presented.
Toluene has been selected because of its relative toxicity, its adverse effects on the health being well documented; like most solvents, toluene is hydrophobic, has a high Henry constant (436.8 atm at 30°C)[9] a low solubility in water (0.67 g L^{-1} at 23.5 °C, Merck Index, 1989) and a low transfer rate from the gaseous to the aqueous phase. Because of this, biofiltration can provide higher removal capacity compared to other type of biological processes, such as bioscrubbers, which are advantageous for mass transfer of hydrophilic compounds.

2. MATERIALS AND METHODS

2.1. Microorganisms and media

Toluene-degrading strains used in this work are *Acinetobacter sp* NCIMB 9689 and *Pseudomonas putida* NCIMB 10432. The approximate size range of the cells are 1.0-1.5 x 1.5-2.5 μm and 0.7-1.1 x 2.0-4.0 μm, respectively. Both cultures were grown at 25°C in an aqueous culture media prepared using tap water, supplemented with the following nutrients: 1.0 g L^{-1} Lab-Lemco beef extract, 2.0 g L^{-1} yeast extract, 5.0 g L^{-1} Peptone, 5.0 g L^{-1} NaCl. They were autoclaved for 15 min at 121 °C. After growth on the above medium for about 24-48 h, the cells were harvested by centrifugation at 5,000 rpm for 20 min and resuspended in the same medium to be used as inoculum for biodegradation operations.
The following mineral salts solution was used for batch tests: 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} $MgCl_2 6H_2O$, 0.02 g L^{-1} $CaCl_2$, 0.002 g L^{-1} $FeSO_4$, 0.0016 g L^{-1} $MnCl_2 4H_2O$ (in tap water).

2.2. Analytical techniques

Analysis of toluene in both liquid and gas phases was performed on a Carlo Erba Model HRGC 5160 gas chromatograph equipped with a flame ionization detector connected with a computing integrator and a capillary column (Mega Laboratory). The temperatures of injector and detector were 150°C and 200°C, respectively. Oven temperature was held at 50°C for 2 min and then increased at a rate of 40°C/min up to 250°C, where it remained constant for 5 min. Nitrogen was used as the carrier gas.
Toluene concentration in the liquid phase was determined by mixing 1 mL of the liquid samples with 1 mL of hexane and shaking for 2 min to ensure the quick and effective extraction of toluene. 1 μL of the hexane phase was injected into the gas chromatograph.
Cell concentration in the suspensions was determined by filtering 10 mL of liquid samples through 0.45 μm Millipore filters and drying at 105°C up to constant weight.
The dissolved oxygen concentration in the bottles was monitored at the beginning and the end of each batch experiment by means of an oxygen probe connected with an automatic analyzer.

2.3. Operating conditions

Batch runs were done to determine kinetic data for both bacterial strains, using different initial toluene concentrations. They were carried out in 1.0 L glass bottles previously sterilized at 120°C for 20 min and, after cooling, tightly sealed with teflon-coated silicon stoppers to prevent the release of toluene by evaporation.
Continuous experiments have been carried out in a bench-scale biofilter consisting of a cylindrical glass column with a cross sectional area of 19.6 cm^2 and a total height of 50 cm. The column was filled with 590 mL of a mixture of peat and glass beads (2:1) as packing material, corresponding to

a height of 30 cm. The biofilter was operated at room temperature, ranging from 20 and 24°C, and the moisture level of the filter material was kept between 60 and 70% either by humidifying the inlet waste gas or spraying, by means of a nozzle, the mineral salts solution on the top of the bed. The pH of the filter bed was maintained around the neutrality by addition of a NaOH solution. The biofilter was fed with an airstream containing toluene vapor produced artificially as follows. Ambient air was divided in two streams. The first high flow rate-stream was injected into a bubbler containing water for humidification. The second low flow rate-stream was passed through a storage vessel containing toluene. The stream became saturated by evaporation of toluene. The polluted and humidified streams were mixed in a ratio suited to obtain the desired inlet concentration and then introduced at the bottom of the biofilter.

3. RESULTS AND DISCUSSION

3.1. Toluene degradation in batch culture

Batch toluene degradation experiments for both microbial cultures were carried out aerobically at 30°C in 1.0 L glass bottles with a 1:2 headspace/mineral salts solution ratio, capped with teflon-coated silicon stoppers and continuously stirred. Before each test, the solution was oxygenated in order to sustain the aerobic biodegradation of toluene. After oxygenation, toluene was added into the bottles using a tight syringe. The bottles were then closed and stirred for 12 hours to allow the complete dissolution of toluene and the equilibrium between the liquid and the gaseous phases in the headspace. Cells for inoculum were then transferred into the bottles. Both microbial strains were tested as inoculum in order to evaluate and compare the related kinetic parameters.
Parallel series of experiments were carried out using about the same initial biomass concentration (X_0), 0.4-0.6 g L^{-1}, and different starting toluene concentrations in the liquid phase. The pH of the solution was initially 6.7 and kept nearly unvaried during the tests. Toluene degradation was followed by sampling approximately every 2 hours. Biomass growth associated to the utilization of this recalcitrant substrate was nearly negligible. The initial toluene concentrations (S_0) selected for batch runs are listed in Table 1 for both microbial strains.

Table 1 – Initial toluene concentrations tested in batch degradation tests using both microorganisms.

Acinetobacter sp.		*Pseudomonas putida*	
Test n.1	S_0 : 20 mg L^{-1}	Test n.1	S_0 : 35 mg L^{-1}
Test n.2	S_0 : 72 mg L^{-1}	Test n.2	S_0 : 68 mg L^{-1}
Test n.3	S_0 : 95 mg L^{-1}	Test n.3	S_0 : 119 mg L^{-1}
Test n.4	S_0 : 210 mg L^{-1}	Test n.4	S_0 : 141 mg L^{-1}
		Test n.5	S_0 : 193 mg L^{-1}

The results of Figs.1 and 2 show the inhibitory effect of starting substrate concentration on the biological degradation of toluene by both microorganisms.
The kinetic constants of toluene utilization under different conditions were estimated using a Monod-type expression and calculated by Lineweaver-Burk plots. The data of Table 2 indicate that the saturation constant (K_s) increases while the maximum specific degradation rate (r_{max}) decreases with increasing initial substrate concentration (S_0), due to an evident substrate inhibitory effect.
It can be observed that, in the case of *Pseudomonas putida*, K_s increases slowly with increasing initial toluene up to 141 mg L^{-1}, whereas the same inhibitory effect seems to cause an increase of K_s more significant for *Acinetobacter sp.* With regards to r_{max}, both series of experiments show a quite similar behavior for initial concentrations of toluene ranging between 20 and 119 mg L^{-1}.

Fig 1 -Batch tests of toluene degradation by Acinetobacter sp.

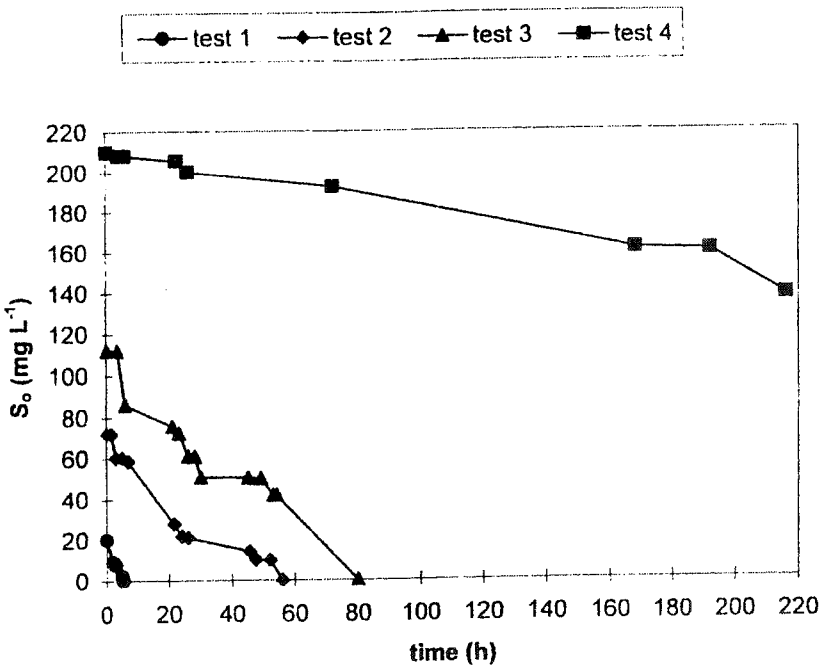


Fig 2 -Batch tests of toluene degradation by Pseudomonas putida

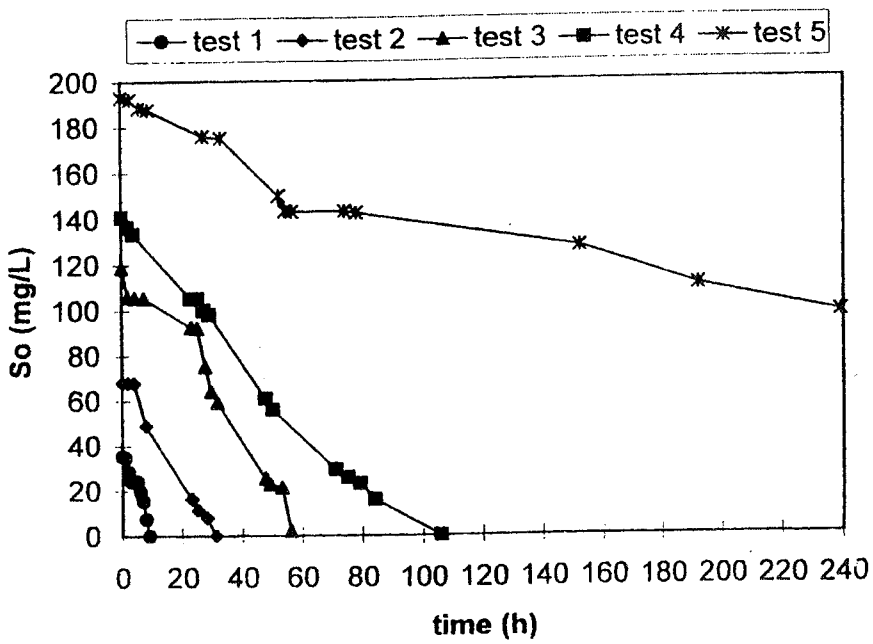


Table 2 - Kinetic parameters calculated for toluene utilization in batch culture.

Acinetobacter sp			*Pseudomonas putida*		
S_0 ($mg_S\ L^{-1}$)	K_s ($mg_S\ L^{-1}$)	r_{max} ($mg_S\ g_X^{-1}\ h^{-1}$)	S_0 ($mg_S\ L^{-1}$)	K_s ($mg_S\ L^{-1}$)	r_{max} ($mg_S\ g_X^{-1}\ h^{-1}$)
20	7.0	0.010	35	3.9	0.0081
72	27.3	0.0074	68	6.9	0.0077
112	57.0	0.0059	119	47.1	0.0059
210	397	0.0014	141	47.6	0.0057
			193	333	0.0036

The run carried out using *Acinetobacter sp* at a starting toluene concentration of 210 mg L^{-1} is, on the contrary, characterized by a value of r_{max} significantly lower than that obtained using *Pseudomonas putida* at a comparable initial concentration of 193 mg L^{-1}.

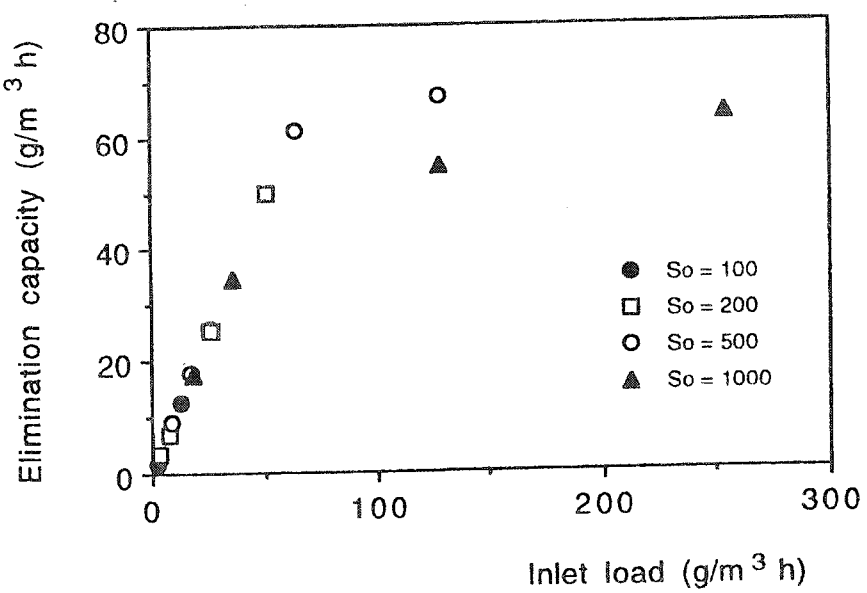
3.2. **Continuous biofiltration of toluene**

Even though *Acinetobacter sp* did not show a better ability in degrading toluene in water if compared to *Pseudomonas putida*, it was employed in the biofiltration runs because of the lack of information about its application either in the removal of toluene vapors or in biofiltration systems. Continuous experiments at various inlet toluene concentrations in the air stream and superficial gas flow rates were then performed using *Acinetobacter sp* as inoculum, in order to test the elimination capacity of the system under different conditions. Inlet concentrations of 100, 200, 500 and 1,000 mg m^{-3} and superficial gas flow rates of 17.9, 35.7, 127.5 and 255 m h^{-1}, corresponding to residence times of 3.22 min, 1.40 min, 28 sec and 14 sec were investigated. In this way, the elimination capacity was measured for each gas flow rate, operating at four different inlet concentrations.
Excellent degrees of conversion (0.964-1.000) have been ensured at inlet concentrations not exceeding 500 mg m^{-3} and residence times no less than 28 sec. Remarkably higher residence times were necessary (100-200 sec), on the other hand, to ensure degrees of conversion in the same range when higher inlet toluene concentrations were tested (up to S_0 = 1,000 mg m^{-3}).
The elimination capacity is plotted in Figure 3 versus the organic load, at the different inlet toluene concentrations. A gradual and linear increase of the elimination capacity occurs for values of the organic load ranging from 0 to 60 g m^{-3} h^{-1}, whilst, beyond this value, the elimination capacity increases more slowly and reaches a maximum threshold of about 68 grams per m^3 of packing material per hour. Such a behavior of the elimination capacity indicates that, at low load values there is a linear relationship between the removal rate and the inlet load and that toluene is nearly completely degraded. With further increasing the load, on the contrary, the elimination rate increases more slowly up to a critical load at which it keeps constant, indicating that the maximum elimination capacity of the biofilter (about 68 g m^{-3} h^{-1}) is achieved. Under these conditions, the limiting step of the process is the biological reaction.

4. CONCLUSIONS

Summarizing, the results obtained in this study reveal that toluene vapors can be effectively removed from waste gases by means of a biofilter inoculated with *Acinetobacter sp.* In fact, the system proved capable of ensuring removal capacities comparable with those reported in the literature for other microorganisms degrading similar pollutants.

Fig 3 - Toluene elimination capacity versus inlet load. Microorganism: Acinetobacter sp.



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