Biodegradation of ethyl acetate and toluene mixtures by a peat biofilter: respirometry monitoring and dynamics of living and dead bacterial cells

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ABSTRACT. To investigate the microbial degradation of ethyl acetate and toluene mixtures in biofiltration, three laboratory-scale reactors were operated for a continuous period of 7 months. Each reactor was packed with a fibrous peat, and downflow fed with air contaminated with ethyl acetate, toluene or a 1:1 mixture, respectively, at a constant empty bed residence time of 90 s. Inlet concentration was progressively increased from 0.4 to 4.5 g m^{-3}. The maximum elimination capacity found for toluene as a sole contaminant was 150 gC m^{-3} h^{-1}, but the presence of ethyl acetate decreased the toluene degradation with a maximum elimination capacity of 80 gC m^{-3} h^{-1}. From removal efficiency profiles, stratification in the substrate consumption was observed at high inlet loads: toluene biodegradation mainly occurred when ethyl acetate was mostly metabolized. Biofilters fed with the individual pollutants were used to determine the experimental mass ratio of carbon dioxide produced to the compound removed. Values of 3.19 g CO_2/g-C and 3.06 g CO_2/g-C for ethyl acetate and toluene, respectively, were found, resulting in overall yield coefficients of 0.13 g of dry biomass produced per g of ethyl acetate consumed and 0.28 g of dry biomass produced per g of toluene consumed. By using these coefficients, the carbon dioxide production in the two-component biofilter was successfully simulated. Dynamics of living and dead cells were monitored in four sections of the biofilters. Similar bacterial concentrations were found for the three biofilters, with values ranging between $2.6 \times 10^9 - 3.0 \times 10^{10}$ cells per gram of dry peat for total bacteria, and $2.4 \times 10^9 - 1.6 \times 10^{10}$ cells per gram of dry peat for living bacteria. For the lower inlet loads a greater bacterial density in the top zones of biofilters was observed, and a progressive colonization of the lower zones of biofilters was achieved as increasing inlet load. The high loads applied in the last stages of the operation showed a loss of bacterial density in the upper zones of biofilters, along with an increase in the dead cells percentages up to about 60%.

1 INTRODUCTION

Air streams discharged from industrial sources contain volatile organic compounds (VOCs) for which environmental regulations are being increasingly restrictive. Biofiltration has been proved as an especially attractive technology for treatment of waste gases containing relatively low concentration of VOCs, because of its simplicity, low cost and non-generating hazardous residues. Ethyl acetate and toluene are among the key pollutants in the exhaust air form printing and coating facilities, and paint

manufacturing. The biofiltration of ethyl acetate (Chan and Zheng, 2005; Liu *et al*., 2002) and toluene (Chan and Zheng, 2005; Liu *et al*., 2002; Jorio *et al*., 1998; Delhomenie *et al*., 2001; Mendoza *et al.*, 2003; Rene *et al*., 2005; Gabaldón *et al*., in press) as well as its mixtures (Liu *et al*., 2002; Deshusses *et al*., 1999; Hwang *et al*., 2003) has been investigated by some previous studies. These studies did mainly deal with the influence of the media and operating conditions and the evaluation of elimination capacities. Research has also been performed to determine the qualitative and quantitative characterization of the biofilter microflora showing microbial densities stratification along the biofilter. Microorganism enumeration has been usually performed by indirect plate count methods (Rene *et al*., 2005; Deshusses *et al*., 1999), but recently, some works based on direct cell count (Tresse *et al*., 2003; Khammar *et al*., 2005) reveal greater bacteria concentrations than the plate count technique.

The purpose of this study is to investigate the biofiltration of VOC polluted gas streams in order to first, evaluate the potential of an easy and low-cost technique for monitoring the biofilter performance based on carbon dioxide production and second, determining the dynamics of living and dead cells, in response to the pollutant load to the biofilter. The gas streams were contaminated with two target compounds: toluene and ethyl acetate, as sole pollutants or in mixture. In addition to determining the elimination capacities, experimental results allow to explore the possible substrate competition effects due to the simultaneous presence of both compounds. Results are of particular relevance for better understanding the operational and ecological aspects of the biofiltration, and will also be useful for mathematical modelling.

2 MATERIALS AND METHODS

2.1 Biofilter system

The organic compounds biodegradation was carried out in three identical laboratory-scale biofilters operated in parallel, treating air polluted with ethylacetate, toluene and 1:1 (w:w) ethyl acetate:toluene mixture, respectively. Each biofilter was made of methacrilate, with a total length of 97 cm and an internal diameter of 13.6 cm. A 10 cm head space was used for the waste gas inlet and for nutrient feed, while a 10 cm bottom space was for the treated air outlet and leachate. Each biofilter was equipped with five sampling ports to measure VOC concentrations, located at 0 (inlet port), 25, 50, 75, and 95 (outlet port) cm of column length. Additional ports located at 20, 40, 60 and 80 cm were used for temperature measurement and to recover filter bed samples.

Peat (ProEco Ambiente, Spain), with organic content of 95%, was used as filter material (Gabaldón *et al*., in press). The peat was acidic, so pH adjustment until neutral pH was made by using diluted sodium hydroxide solution. In order to obtain a microbial population adapted to the pollutants, two aerated reactors, seeded with activated sludge from a waste water treatment plant, were continuously fed with ethyl acetate or toluene, respectively, at a rate of 1 mL/h for a period of at least 8 weeks. Three 1 liter microbial suspensions (adapted to ethyl acetate; adapted to toluene; and 1:1 mixture of the previous) were mixed with peat to inoculate the corresponding biofilters depending on the substrate to degrade. Compressed, filtered and dried air was passed through a humidifier to assure a relative humidity value of at least 90%. The empty bed residence time (EBRT) was adjusted by using mass flow controllers (Bronkhorst Hi-Tec). The pollutant was introduced to the air stream by using a syringe pump (New Era, infusion/withdraw NE 1800 model) and then, the contaminated air was flowed downwards into the bed.

2.2 Operation conditions and monitoring of biofilters

The biofilters were operated at a constant EBRT of 90 ± 9 s for a total period of 7 months. During this period, eight increasing inlet loads (ILs) were applied to each biofilter corresponding to concentrations in the inflow gas in the range between 0.4 and 4.5 g m^{-3}. Each IL was maintained during a minimum of 21 days to assure steady state conditions. The strategy adopted to maintain a constant humidity in the biofilter bed was feeding the air previously humidified as well as pouring on top of the biofilters 500 mL of a pH-buffer and nutrient solution (3.84 g/L K_2HPO_4, 1.94 g/L KH_2PO_4, 3.00 g/L NH_4Cl, pH = 6.97) each 4 days.

VOCs concentration profiles were monitored four times per day by using a gas chromatograph (CE Instruments, GC 8000 model) equipped with a 2.5 mL automated gas valve injection system and a flame ionization detector. Temperature, pH of the leachates and pressure drop were monitored daily. Temperature in biofilters varied between 23 and 29 ºC following the ambient temperature. pHs of the leachates were maintained around 5.5 ± 0.2. Pressure drop was less than 15 mm H_2O/m except for the last stage of operation when pressure drops increased up to 60 mm H_2O/m, indicating the clogging of the material after nearly 7 months of operation. Moisture content was measured by the dry weight method once a week at two locations (upper and lower) of each biofilter; and some stratification was observed: values (wet basis) varied between 75% ± 2% on the upper zone and 85% ± 1% on the lower zone. Carbon dioxide profiles measurements were performed at least twice at steady state conditions by using a NDIR carbon dioxide analyzer (Seda, Gaswork NDIR model).

2.3 Quantitative determination of bacterial distribution

Bacteria were enumerated by fluorescence microscopy using LIVE/DEAD® BacLight™ Bacterial Viability Kit by Molecular Probes, thus co-staining was performed with SYTO 9 to define the number of living cells and with propidium iodine to define the number of dead cells. Under steady state conditions, one sample was taken from each sampling port of the biofilters. Microorganisms were dispersed from peat with an ultra-turrax as follows: 0.5 g of peat was placed in 20 mL of phosphate buffer and crushed 1 min at 14000 rpm and 3 min at 24000 under refrigeration. Then, a sample of 1 mL was collected from the supernatant and centrifuged 1 min at 800 rpm. Three serial decimal dilutions were performed. The resulting suspension was incubated with 3 µL/mL of 1:1 dye mixture for 15 min in the dark at an agitation speed of 1000 rpm. Stained bacteria were recovered by vacuum filtration through 0.2 µm black polycarbonate membrane microfilters (Millipore GTBP) and washed under vacuum suction 3 times with 5 mL of distilled sterilized water. The filters were mounted on microscope slides in mounting media (Molecular Probes) and examined using an epifluorescence microscope (Nikon Eclipse E800) equipped with a blue excitation filter (B-2A; ex 450-490 nm, dm 505 nm, ba 520 nm) and a green excitation filter (G-2A; ex 510-560 nm, dm 575 nm, ba 590 nm). Living and dead cells were enumerated counting ten random microscopic fields three times, with standard deviations ranging from 2.5 to 30.2 % (mean value of 11 %).

3 RESULTS AND DISCUSSION

3.1 Elimination capacity

The performance of the biofilters was evaluated in terms of the elimination capacity (EC). In order to facilitate comparison between experiments with the two different compounds, IL and EC have been expressed as grams of carbon by cubic meter by hour

(gC m^{-3} h^{-1}). ECs have been investigated for the global biofilter and for the first quarter of the biofilter, where ethyl acetate was mostly removed. The maximum EC for these compounds has been estimated from first section data, calculated related to the volume of this section. Results shown in Figure 1 are consistent with the biodegradability of the compounds, greater EC for ethyl acetate than for toluene. For the biodegradation of pure ethyl acetate, EC was directly proportional to the inlet load up to 165 gC m^{-3} h^{-1}. Maximum EC was 190 gC m^{-3} h^{-1} (365 ethyl acetate g m^{-3} h^{-1}) at an IL of 250 gC m^{-3} h^{-1}. In case of toluene as single pollutant, a near linear relation between the IL and the EC was observed until an IL of 135 gC m^{-3} h^{-1}, and a maximum EC of 150 gC m^{-3} h^{-1} (165 toluene g m^{-3} h^{-1}) was obtained for an IL of 250 gC m^{-3} h^{-1}. These maximum ECs are comparative to those previously reported for high loads studies (Liu *et al*., 2002; Rene *et al*., 2005). Results of the 1:1 mixture experiment show a negligible influence of the presence of toluene for ethyl acetate removal at inlet loads up to 200 gC m^{-3} h^{-1}. However, toluene removal in the mixture showed that the proportionality between IL and EC was reached only for inlet loads below 60 gC m^{-3} h^{-1}. The maximum EC (80 gC m^{-3} h^{-1}) was also lower than that obtained with pure toluene. These data indicate an inhibition of the toluene biodegradation in the presence of moderate to high loads of ethyl acetate, particularly in the first quarter of the biofilter, when applying the highest inlet concentrations (> 2.0 g m^{-3} of each compound). Similar inhibition effects have been previously reported (Liu *et al*., 2002; Hwang *et al*., 2003; Tresse *et al*., 2003).

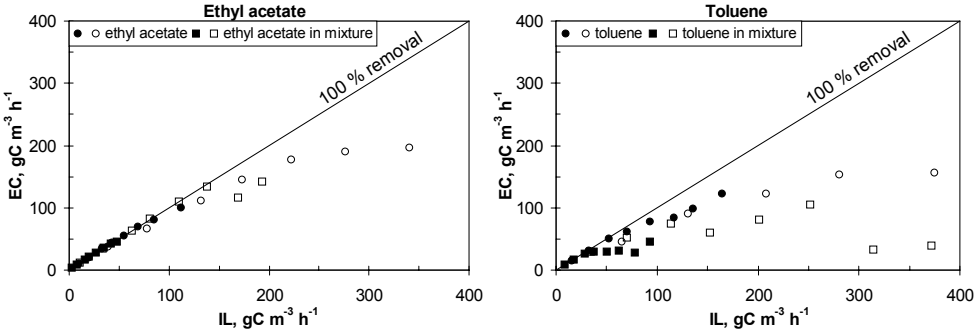

Figure 1. EC of the global biofilter (solid symbols) and in the first quarter of the biofilter (open symbols) vs IL for ethyl acetate and toluene as sole pollutants or in mixture.

3.2 Carbon dioxide production

Carbon dioxide released has been measured at the five gas sampling ports at each biofilter. For the experiments with the pure compounds, the evolution of this production as a function of the EC is presented in Figure 2. The plots of CO_2 production throughout the whole biofilters (from inlet to outlet) are well aligned (solid symbols), which shows that a proportionality ratio exists between EC and CO_2 production. Linear regressions for these data resulted in values of 3.19 g CO_2/g-C and 3.06 g CO_2/g-C for ethyl acetate and toluene, respectively. Assuming a general biomass composition formula as $C_5H_7NO_2$, overall yield coefficients of 0.13 g of dry biomass produced per g of ethyl acetate consumed and 0.28 g of dry biomass produced per g of toluene consumed were ontained. As can be observed in the figure, the CO_2 production of the biofilters sections (open symbols), calculated from inlet to each intermediate sampling port, followed the tendency of the global biofilters, indicating similar microbial behavior throughout the

bed. As a conclusion, CO_2 production, as indicator of the intensity of the microbial activity in the biofilter, is an easy-measurement and reliable parameter for monitoring the biofilter performance.

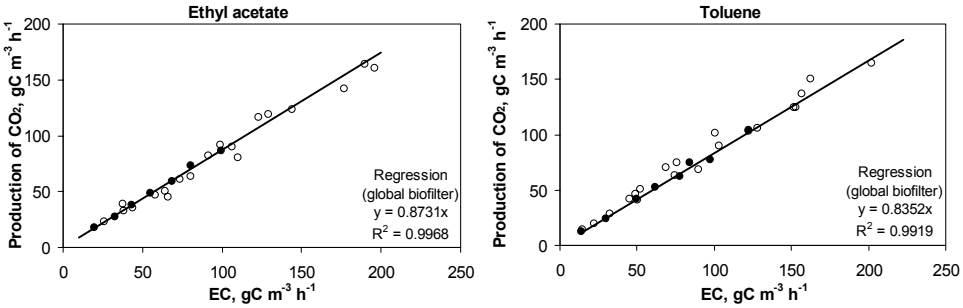

Figure 2. Relationship between elimination capacity and production of carbon dioxide in single ethyl acetate and toluene biofiltrations. ● Global biofilter; ○ First quarter, half and three quarter of the biofilter.

The CO_2 production in the mixture biofiltration experiment has been estimated by using CO_2 production coefficients for the biofiltration of pure ethyl acetate and pure toluene. Comparison between measured and estimated data is shown in Figure 3, where total EC represents the sum of the ethyl acetate and toluene ECs. Mean absolute deviations between experimental and calculated values was 4.4 gC m^{-3} h^{-1}, with a mean relative deviation of 5.0%. The good correlation between experimental and simulated values shows that microbial metabolism for degrading ethyl acetate or toluene is independent on the simultaneous presence of both compounds.

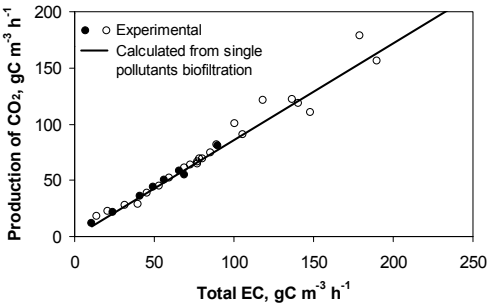

Figure 3. Comparison between experimental and calculated CO_2 production in the mixture biofiltration. ● Global biofilter; ○ First quarter, half and three quarter of the biofilter.

3.3 Removal efficiency profiles

The contribution of the upper half and the lower half of the biofilter columns to the overall removal efficiency is presented in Figure 4. The removal efficiency at each half-part has been calculated as the ratio of the difference between the inlet and outlet concentrations of pollutant in this section to the concentration at the entrance of the biofilter. For single experiments, 85% RE were reached at ILs below 85 gC $m^{-3}h^{-1}$ (inlet

concentration <3.7 g m^{-3}) and 94 gC $m^{-3}h^{-1}$ (inlet concentration < 1.8 g m^{-3}) for ethyl acetate and toluene, respectively. It can be observed that the contribution of the last part of the biofilter became more relevant as IL increased. More uniform removal along the biofilter was observed for toluene, the least biodegradable of both compounds. The effect of multiple substrate treatment can be observed by comparison of single experiment and mixture experiment for each compound at similar total ILs. As an example, at an ethyl acetate IL in the mixture of about 42 gC m^{-3} h^{-1} (120 gC m^{-3} h^{-1} total IL), with global RE of practically 100%, the distribution of ethyl acetate removal between the two half-parts is comparable to that obtained for pure ethyl acetate biofiltration (112 gC m^{-3} h^{-1} IL). However, for toluene ILs in the mixture greater than 38 gC m^{-3} h^{-1} (58 gC m^{-3} h^{-1} total IL), the RE efficiency is reduced in presence of ethyl acetate mostly because of a decrease of the RE in the first half-part of the biofilter. It seems to indicate that competition among the two pollutants defers toluene removal to the last sections of the biofilter, where ethyl acetate concentration has decreased enough. The effect is more patent for the higher ILs, with an inversion in the contribution of both half-parts to the toluene removal.

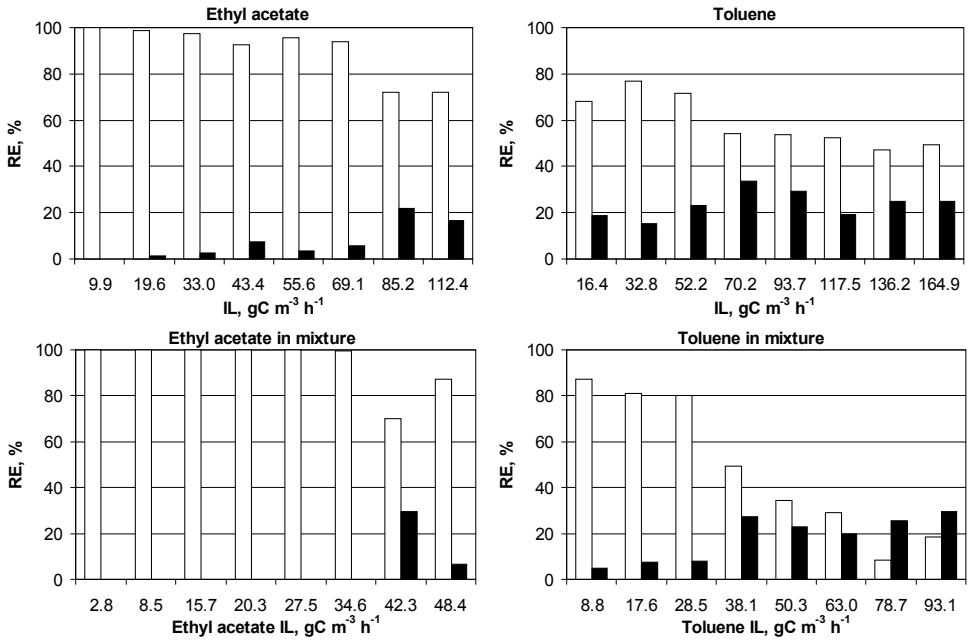

Figure 4. Removal efficiency profiles in the biofilters vs. IL.
□ Upper half of the biofilter; ■ Lower half of the biofilter.

3.4 Dynamics of living and dead cells

The colonization dynamics were monitored in the four sections of the biofilters. Results of total and living bacterial concentration (expressed as bacteria g^{-1} dry peat) are presented in Table 1. Total bacterial concentration has been determined as the sum of measured living and dead cells. Similar bacterial concentrations were found for the three biofilters, with values ranging between $2.6 \times 10^9 - 3.0 \times 10^{10}$ cells per gram of dry

peat for total bacteria, and $2.4 \times 10^9 - 1.6 \times 10^{10}$ cells per gram of dry peat for living bacteria. Similar tendency of dead cells evolution has also been observed for the three biofilters, and it is presented in Figure 5, as an example, for the mixture biofiltration. The bacterial concentrations depend on the pollutant load applied. In the first three ILs of the operation, a greater density of living bacteria was observed in the top zones of biofilters, and the percentage of dead cells remained approximately constant in values around 5-15%. As inlet load increased, a change in biomass distribution occurred (4^{th} to 6^{th} ILs): a progressive colonization of the lower zones, but a reduction in living cells on the top of the biofilters, yielding a more uniform distribution of biomass throughout the bed. The reduction of living cells on the top sections can be explained on the basis of the inlet concentration increase over 1.5 g of pollutant m^{-3}. For the last two ILs of operation, the living bacterial density in the two upper zones of biofilters was stabilized around $3.3 \times 10^9 - 6.4 \times 10^9$ cells per gram of dry peat, while the values in the lower sections remained still higher. At these high inlet loads, homogeneous distribution of dead cell percentage was reached, remaining in a stable value of about 60%.

Table 1. Distribution of the bacterial concentrations (bacteria $\times 10^{-10}$ g^{-1} dry peat) detected along the biofilters at different inlet loads, under steady-state conditions.

Single ethyl acetate

	IL (gC m^{-3} h^{-1})															
	9.9		19.6		33.0		43.4		55.6		69.1		85.2		112.4	
h/H	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T
0.2	0.80	0.91	0.91	1.11	1.20	1.52	0.66	1.00	0.75	0.92	0.59	1.04	0.47	1.30	0.41	1.08
0.4	0.67	0.88	0.70	0.82	1.05	1.30	0.55	0.74	0.59	0.98	0.53	1.22	0.58	1.71	0.64	1.36
0.6	0.25	0.31	0.41	0.44	0.64	0.85	0.52	0.65	0.89	1.06	0.89	1.67	1.07	2.12	0.48	1.06
0.8	0.35	0.47	0.24	0.26	0.50	0.55	0.31	0.39	1.32	1.42	1.26	1.59	0.41	0.81	0.27	0.65

Single toluene

	IL (gC m^{-3} h^{-1})															
	16.4		32.8		52.2		70.2		93.7		117.5		136.2		164.9	
h/H	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T
0.2	0.49	0.53	0.97	1.15	1.45	1.84	0.77	1.13	0.97	1.45	0.47	1.06	0.40	1.46	0.33	1.38
0.4	0.46	0.50	0.81	0.97	1.36	1.59	0.99	1.27	0.68	1.08	0.61	1.04	0.47	1.27	0.64	1.99
0.6	0.70	0.77	0.43	0.51	0.58	0.65	0.61	0.79	0.31	0.38	0.83	1.08	1.53	2.54	1.45	2.98
0.8	0.70	0.77	0.26	0.30	0.54	0.61	0.64	0.77	0.40	0.69	0.71	0.87	0.81	1.28	1.48	2.38

Ethyl acetate/ toluene mixture

	Total IL (gC m^{-3} h^{-1})															
	11.6		26.1		44.2		58.4		77.8		97.6		121.0		141.5	
h/H	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T
0.2	0.47	0.50	0.91	1.07	1.91	2.19	0.56	0.90	0.34	0.69	0.30	0.95	0.33	0.96	0.42	0.91
0.4	0.57	0.62	0.60	0.69	1.77	1.98	1.08	1.39	1.01	1.32	0.43	1.42	0.43	1.23	0.34	0.87
0.6	0.34	0.36	0.67	0.75	0.92	1.04	0.62	0.82	1.63	1.94	1.45	2.18	1.25	2.29	0.82	2.33
0.8	0.39	0.41	0.62	0.68	0.39	0.43	0.61	0.71	1.44	1.58	1.04	1.61	0.99	1.95	0.90	1.89

L: living cells determined by fluoro-microscopic count method.

T: total cells: sum of living and dead cells, determined by fluoro-microscopic count method.

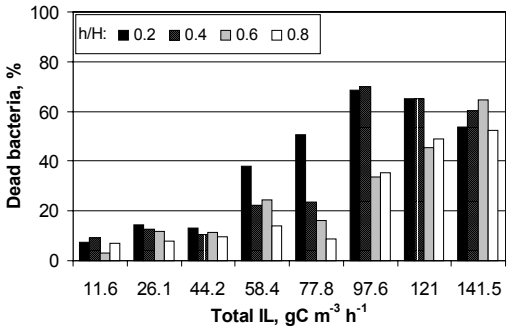

Figure 5. Dead bacteria percentage on the dimensionless filter height, h/H, at different ILs in the mixture biofiltration.

4 ACKNOWLEDGEMENT

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