

Toluene Biodegradation using a Lab-Scale Biofilter Inoculated with *Pseudomonas Putida* PTCC 1694

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Abstract

Biomanipulation is a reliable method for treating volatile organic compounds (VOCs) in polluted air. The performances of two biofilters in the removal of toluene vapors from air stream were compared. Two identical biofilters designed in parallel configuration were operated in lab-scale for 20 days; one of them was filled by sterilized media (compost and wood charcoal 2:1 v/v) and another was filled by the same media inoculated with *Pseudomonas putida* PTCC 1694, as a native strain. Moreover, batch tests were performed to determine the biodegradation rate of toluene. The results showed that, in comparison with the sterilized BF (89% vs 58%), the inoculated BF could effectively eliminate toluene from air stream. The pressure drop across the inoculated BF and the sterilized BF were 0.66 ± 0.28 and 0.47 ± 0.27 mm water respectively. The batch test results showed that loss of toluene in the control bottles was greater than the blanks. Based on the experimental results, inoculated BFs can effectively treat toluene vapors from gaseous streams.

Introduction

There is a wide range of sources in the industry that emit volatile organic compounds (VOCs). They can directly affect human health and cause environmental pollution problems (Schiavon et al. 2016, Xi J et al. 2014). Traditional physico-chemical mechanisms, which are extensively applied to workplace air contamination (e.g. VOCs), mainly include thermal oxidation, photo-catalytic oxidization, incineration, condensation, scrubbers, and adsorption (Liu L et al. 2009, Pei et al. 2011, Xu Q et al. 2011, Heymes F et al. 2006, Fulazzaky MA et al. 2014). However, none of them provides the final control and each one has specific limitations. In addition, these technologies are expensive due to high energy consumptions and the harmful secondary compounds that need further treatment (An T et al. 2008, Mohamed EF et al. 2016).

The biotechnology processes are based on the microorganisms' activity and pollutants are decomposed (mineralized) into harmless forms. Therefore, these processes overcome many disadvantages of traditional methods (Rojo N et al. 2010). Thanks to this mechanism, microorganisms (bacteria, fungi, molds, mite, yeast) are generally the main removal agents (Hort C et al. 2014, Schiavon M et al. 2016).

Biological processes are suitable for low-medium concentrations of VOCs and they are considered as durable technologies (Hort C et al. 2014). Among the advantages of these technologies are low impact on the environment, small amount of wastes, and negligible environmental consequences. Hence, biological processes are environment friendly compared to the other technologies (Padhi SK et al. 2014, Feizi F et al. 2015). In this category, biofilters can be applied to treat insoluble and recalcitrant compounds (Streese J F et al. 2005, Zou JL et al. 2012).

The presence of some non-active bacterial species, which do not directly take part in pollutant biodegradation, is unavoidable when a bacterial consortium is used in biofilters (Estrada P et al. 2014). In this study, the pure culture of *Pseudomonas putida* was used to examine toluene degradation and

biomass productivity in the biofilter. The reasons for using *Pseudomonas* were its capability to acclimatize to various substrates and possession of several catabolic routes to influence recalcitrant substances (Otenio MH et al. 2005).

Toluene was used as a model pollutant because it is a hydrophobic contaminant and hardly biodegradable. Therefore, it can be a representative compound for VOCs (Wang L et al. 2013, Zhao L et al. 2014). Its biodegradation pathway is well recognized (Kim D-J et al. 2005, Reardon KF L et al. 2000). Furthermore, to integrate global standards for filter testing VOC control, ISO 10121-1 (2014) introduced toluene as a VOCs representative (ISO. 2014). Toluene is the most common VOC, toxic, carcinogenic, mutagenic, and high vapor pressure that is principally produced on an industrial scale from petroleum conversion (Singh K et al. 2010, Mohamed EF et al. 2016).

The purpose of the present study is to investigate the performance of two parallel biofilters for the removal of toluene from a synthetic air stream based on the native strain function. To immobilize a biofilm of *P. putida* PTCC 1694 in the biofilters, compost and wood charcoal (2:1 v/v) were used as the packing medium. The removal efficiencies, elimination capacities, and pressure drops were surveyed by varying some of operating parameters.

Materials And Methods

Experimental set-up and Operational Condition

Two pilot-scale BFs were constructed using a cylindrical PVC columns (10.5cm in diameter and 70cm in height) (Fig. 1). These two columns were arranged in parallel configuration. In this way, one BF (BF-A) was packed with sterilized media (at 121°C for 30 min) and another (BF-B) was inoculated with *P. putida*. Each BF was filled with compost and wood charcoal (2:1 v/v) with an overall height of 8 cm and an effective volume of 0.7 L. An air pump was used to draw air from the biofilters.

The airflow entering the BFs was split into two parts (main and minor). The main part was passed through the bubbler to pre-humidifying and the minor part was passed through a 25 ml impinger containing toluene ($\geq 99\%$ pure). Toluene concentrations were set at the desired value by adjusting the micro valves. These two streams were mixed in a mixing chamber and desired concentrations were obtained. Both of the streams were continually monitored. It should be noted that the total airflow in the set up was 2 L/min, so that 1 L/min was supplied to each reactor. The Humidifying column was designed with 60 mm diameter and an effective height of 60 cm. Relative humidity and temperature were monitored both at the upstream and downstream of the BFs. Pressure drop was also measured using digital and U-tube manometers (water fluid) connected to the inlet and outlet of the BFs. The experimental setup is shown in Fig. 1.

The BFs were designed according to a low to medium EBRT (empty bed retention time) of 41.5s defined as follows.

[Please see the supplementary files section to access the equations.] (1)

Where Q_v is the contaminated airflow (m^3/h) and V_b is the packed bed volume (m^3). The main parameters studied were the inlet loading rate (IL; $g/m^3.h$), elimination capacity (EC; $g/m^3.h$), and removal efficiency (RE;%).

[Please see the supplementary files section to access the equations.] (2)

[Please see the supplementary files section to access the equations.] (3)

[Please see the supplementary files section to access the equations.] (4)

Where C_{in} and C_{out} (g/m^3) are toluene concentrations at the inlet and outlet of BFs respectively. The EC ($g/m^3.h$) is the mass of pollutant degraded per unit of time and volume of the filtering bed.

The set-up was made with separable parts that formed an integrated system, so that access to all the points in the system was easily possible. This facilitated the irrigation of filler materials and nutrient solution sprays, the physical mixing of materials, sampling of packed media to measure moisture content, and adjusting pH.

By placing the pump at the downstream of the setup, a partial vacuum was created in the upstream; therefore, toluene vapors leakage from the set up was prevented. This also facilitated handling the operation and adjusting the concentrations. Concentration adjustment in the constant range was made possible by a micro valve. All attempts were made to prevent leakage from the seams and fittings. Therefore, the whole connections were sealed. The sampling ports of brass were embedded at the inlet and outlet of the columns.

It is worth mentioning that in order to prevent the fall of microorganisms in anaerobic state and starvation, aeration ($0.5 L/min$) was applied for 12 hours (overnight) with a small amount of toluene ($2-5 ppm_v$) in all experiments,.

Packing materials and characterization

The mixture of MAG vermi-compost and wood charcoal was used as the media support with a volume ratio of 2:1. Table 1 summarizes the characteristics of the Compost and Wood Charcoal Particles.

Table 1

To prevent bed drying, 30 ml of nutrient solution was sprayed on top of the both columns every three days. A nutrient solution containing the following composition (Table 2) was prepared and sprayed every three days onto the media bed in order to maintain adequate moisture in the filter bed and provide supplemental nutrients to the microbial population. Toluene in contaminated air played the role of sole carbon and energy source during the bacterial degradation.

Table 2

Microorganism, Inoculum

A pure bacterial culture was used to prepare the inoculum. The strain *P. putida* PTCC 1694 was supplied from the Persian Type Culture Collection (PTCC). It was cultured in nutrient broth agar and incubated at 30° C in a rotary shaker (150 rpm, 24 h). The following protocol was developed for the microbial culture in the laboratory: 1) Nutrient broth powder was weighed and dissolved in the 250 ml deionized water in a 1L bottle and then boiled to make it clear. 2) Then it was sterilized in an autoclave for 15 minutes and at 121°C. 3) After sterilizing and cooling, a mono-colony was inoculated in it with the sterile loop and then it was incubated on a rotary shaker (125 rpm, 30°C) overnight.

Gas chromatography analysis

In order to analysis toluene samples, Gas Chromatography equipped with Flame Ionization Detector (GC-FID) (model CP-3800 gas chromatograph and FID detector, Varian Technologies Japan Inc., Japan) focused on the capillary column with a length of 25m and an inner diameter of 0.25 mm was used. The film thickness was 0.25µm and the flow rate of nitrogen carrier gas was 1.8 ml/min. The GC was programmed at 130°C for 3 min and 100 µl of the air sample was injected into the injection port with a split ratio of 5 and injector temperature of 200°C. Moreover, the detector temperature was set at 240°C. Varian workstation software version 6.41 was utilized to determine the peak area. The concentration range of toluene was made in TEDLAR sample bags and injected to GC to sketch the calibration curve ($R^2=0.999$).

Batch Tests

At first, the strain was cultured overnight in Nutrient Broth. Then 10 ml of this medium containing Toluene degrading microorganisms were added to 100 ml filtered (0.45 bio-lab) mineral medium (as described above) in 500-ml bottle. Afterwards, the bottle was capped with aluminum foil and parafilm. For bacteria adaptation, toluene was added to reach a gas-phase concentration of about 6 mg l⁻¹ air. The solution was incubated on a rotary shaker (200 rpm) at 30°C overnight. Then, 10 ml of the solution containing adapted bacteria, along with 90 ml of mineral medium was introduced into three 500ml flasks. Initial toluene concentrations in the flasks were adjusted at 45, 95, 255 ppm_v, and the bottles were incubated on a rotary shaker (250 rpm) at 30°C. The consumption rate and reduction of toluene concentration were measured by taking samples (100 µl) of flask headspaces every hour by gas syringes

using GC-FID. Control tests were conducted to evaluate the loss of toluene from the bottles due to volatilization (Kim D-J et al. 2005, Estévez E et al. 2005).

Results

Toluene removal efficiencies

The performances of BF-A and BF-B for toluene removal are shown in Fig. 2 where the inlet toluene concentrations and the removal efficiencies are plotted along with operational time. During the 20 days of operation, the RE of both BFs gradually increased up to the day 13 and then remained stable. This illustrates steady state condition, although this increase was negligible in BF-B.

The maximum RE of the BF-A was 89.5%, which was observed on the last day. However, the maximum efficiency observed in sterilized BF was 57.9% (day 15). It should be noted that the REs on the first day for BF-A and BF-B were 43.99% and 42.67%, respectively. As mentioned, the RE changes in the sterilized BF can be ignored, as there were noticeable decreases on days 16 and 17.

The data of the average removal efficiency (RE), Elimination capacity (EC), inlet concentration (C_{in}), and inlet loading rate (LR) in both BFs during the 20 days is listed in Table 3.

Table 3

The elimination capacity, which indicates the capacity of the BFs to eliminate the pollutants, is plotted as a function of the inlet loading rate of toluene in Fig. 3.

Pressure drop

The media pressure drop is an indicator of bed obstruction. Its variations for BFs are shown in Fig. 4. For 20 days of operation, the mean losses through the BF-A and BF-B were 0.66 ± 0.28 and 0.47 ± 0.27 mm water respectively. The following figure also confirms that BF-A shows an upper limit of losses during the operation.

To ensure that the pressure drop was exclusively related to the media bed, not to the piping and accessories, a control test (empty reactors without packing materials) was also carried out. There was no resistance to airflow in empty reactors.

Batch Tests

A series of experiments were carried out to confirm the bacterial ability in biodegradation of toluene. For this purpose, as noted above, the rate of toluene decomposition in three bottles with different concentrations of gas phase was performed. Three bottles were also considered as blanks. The decreasing trend of toluene concentration in the flasks are discussed separately in the following. These series of experiments lasted six hours and toluene concentration was read every one hour. The final

concentrations of toluene in the first control bottle and for the blank (45 ppm_v) were almost zero (0.73 ppm_v , 6th hour) and 26.4 ppm_v respectively (Fig. 5).

The final concentrations of toluene in the second control bottle (95 ppm_v) and the blank bottle were 2.37 ppm_v (6th hour) and 64.29 ppm_v respectively (Fig. 6).

The final concentrations of toluene in the last control bottle (255 ppm_v) and the blank bottle were 10.43 ppm_v (6th hour) and 139.37 ppm_v respectively (Fig. 7).

Discussion

The results about removal efficiencies of toluene demonstrated that the startup stage lasted 13 days (Fig. 2). The inoculated biofilter's RE curve slop was upward from the first to the 13th days, indicating the *P. putida* gradual adaptation to toluene. In Fig. 3, the converge of LR and EC-A lines indicates a gradual increase in BF-A efficiency by the 13th day. Then, the distance between these two lines remains almost constant until the last day, which reflects the stable state.

Table 3 shows that the toluene loads during the operation were the same for both BFs. As shown in Fig. 3, the maximum EC-A of $12.65 \text{ g/m}^3 \cdot \text{h}$ was observed with the highest inlet loading rate of $14.44 \text{ g/m}^3 \cdot \text{h}$ on the 13th day. Most of the previous studies have also showed higher ECs in higher ILs (Kim D-J et al. 2005, Roy S et al. 2003, Rene ER et al. 2005). Although, the variations of EC-B were similar to those of EC-A, values of the former were much lower than those of the latter. The fact that the biofilters inoculated with *P. putida* species succeeded in removing toluene (VOCs) from the air stream is confirmed by many other studies (Singh K et al. 2010, Park DW et al. 2002, Muñoz R et al. 2008, Mathur AK et al. 2010).

Although, the success achieved in other studies was based on *P. putida*, the effective working volume was much higher than what was observed in the present study. Here, the efficiency of about 90% was achieved with only 0.7 L of packing material, one-third of which was wood charcoal and without any nutrients. For example, S. Roy et al. (2003) applied the same species and media for toluene treat, and the only differences were the effective volume of 18 L and a greater inlet concentration (1500 mg/m^3). They achieved an elimination efficiency of around 80-90%.

Another study with a working volume of 20 L achieved a higher removal efficiency of nearly 100% (Song J et al. 2005). In a study conducted by Estrada et al. (2013) based on IL and EC, with 8.6 L working volume, the ultimate performance was about 88-92% (Estrada JM et al. 2013). Therefore, it seems that the native strain is more efficient than others in eliminating toluene vapors. It is suggested that further studies on the gene sequence similarity of PTCC 1694 strain to the *P. putida* should be conducted.

As shown in Fig. 4, the slightly higher average pressure drop in the BF-A indicates the formation of the biomass in the packed media bed and as a result, the partial obstruction of the bed pores. Due to clogging, higher pressure drops are possible. Similar results were obtained in previous studies (Schiavon

M et al. 2016, Padhi SK et al. 2014, Dorado A et al. 2012). Clearly, pressure drop changes are in saw shape associated with the irrigation of the media bed every three days and accumulation of water in it. After three days, the decreasing trend continues until the next irrigation time. The curve peaks are related to the days of irrigation. Similar results have been reported by some previous studies (Dorado AD et al. 2010, Amin MM et al. 2014, Kawasea Y et al. 2014).

As shown in Figs. 5, 6, and 7, the decreasing trend of toluene concentration in the control was more considerable. Previous studies and the present one ours show that *P. putida* is a bacterial species that is continuously present in BFs devoted to VOC degradation. The ability of this bacterium in toluene biodegradation has also been confirmed in other studies (Kim D-J et al. 2005, Roy S et al. 2003, Park DW et al. 2002). The blanks in batch tests showed that toluene degradation from initial gaseous concentrations was due to microbial activity (Fig. 5-7). These results are consistent with the results of other studies with the same procedure and different concentrations of the gaseous phase (Kim D-J et al. 2005).

Toluene was successfully biodegraded. Elimination capacity increased linearly to reach its maximum value of 12.65 g/m³.h at inlet loading of 14.44 g/m³.h and on the 13th day. Toluene removal efficiencies close to 90% were achieved throughout a steady state condition. This indicated that the biomass was well formed and had a good stability. It appears that *P. putida* PTCC 1694, a native strain, was more effective than others alternatives in toluene vapors removal. The slight difference in pressure drop across two BFs is another advantage to be noted. In addition, the slightly higher pressure drop and its constant changes in the BF-A during operation were indicative of stable biofilms with a fixed thickness. The low-pressure drop indicates a lower energy requirement and this study showed that there was a negligible difference in pressure drop between the two BFs. During batch experiments, it was found that toluene biodegradation rate was higher in the control bottles compared to the blanks.

Declarations

Authors' contributions

F.G, M.J.J supervised the project. R.N designed the experiments. R.Gh performed the experiments, analyzed the data and wrote the manuscript. MR.P, S.R and E.M performed the bacterial and fungal experiments. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All data are presented in figures and tables within this article. Any material used in this study will be available for research purposes upon request.

Consent for publication

Not applicable.

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

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Tables

Table 1.

Item	Compost	Wood Charcoal
Particle mean diameter (mm)	2-4	15±0.5
Void fraction (%)	55	50
Wet bulk density (Kg/L)	0.628	0.8
Dry bulk density (Kg/L)	0.302	0.44
Moisture Content (%)	48 - 70	43-67
Moisture-Holding Capacity (%)	72	78

Table 2.

Trace element		Macro element	
compounds	Concentration(mg ^l ⁻¹)	compounds	Concentration(g ^l ⁻¹)
CaCl ₂ .2H ₂ O	26	KH ₂ PO ₄	1
EDTA Na ₄ (H ₂ O) ₂	5.5	K ₂ HPO ₄	1
FeCl ₃ .4H ₂ O	1.3	MgSO ₄	0.2
CoCl ₂ .6H ₂ O	0.12	NaCl	1
MnCl ₂ .2H ₂ O	100	KNO ₃	1
ZnCl ₂	0.07		
H ₃ BO ₃	0.06		
NiCl ₂ .6H ₂ O	0.025		
NaMoO ₄ .2H ₂ O	0.025		
CuCl ₂ . 2H ₂ O	0.015		

Table 3.

Part	Day	C _{in} (mg/m ³)	LR (g/m ³ .h)	EC (g/m ³ .h)	RE (%)
Sterilized BF (A)	1-20	134.5±26.2	11.56±2.26	6.22±1.45	52.96±4.12
Inoculated BF (B)	1-20	134.5±26.2	11.65±2.26	9.2±2.65	77.21±13.55

Figures

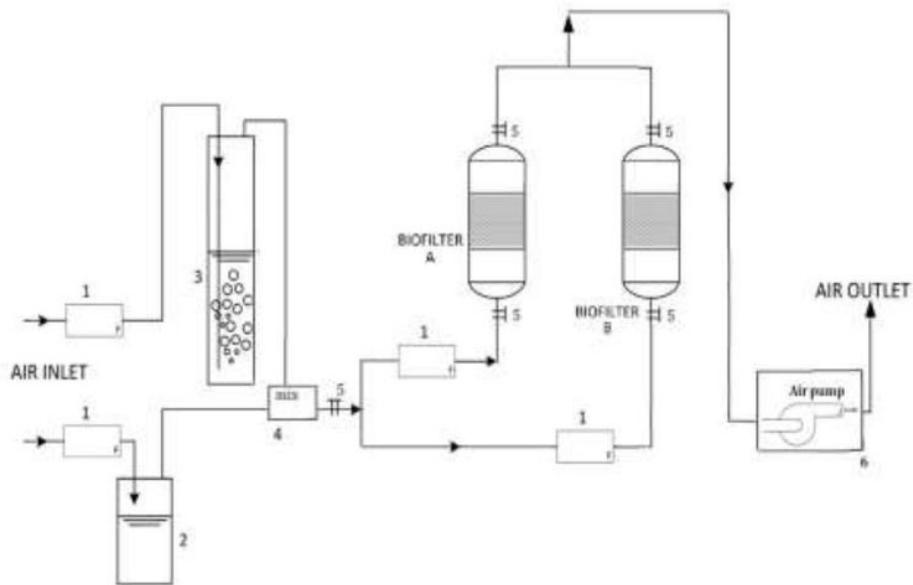


Figure 1

Experimental set-up of the biofilter system: 1. Gas flow controllers, 2. Toluene evaporation chamber, 3. Humidifying column, 4. Mix chamber, 5. Gas sampling ports, 6. Air pump.

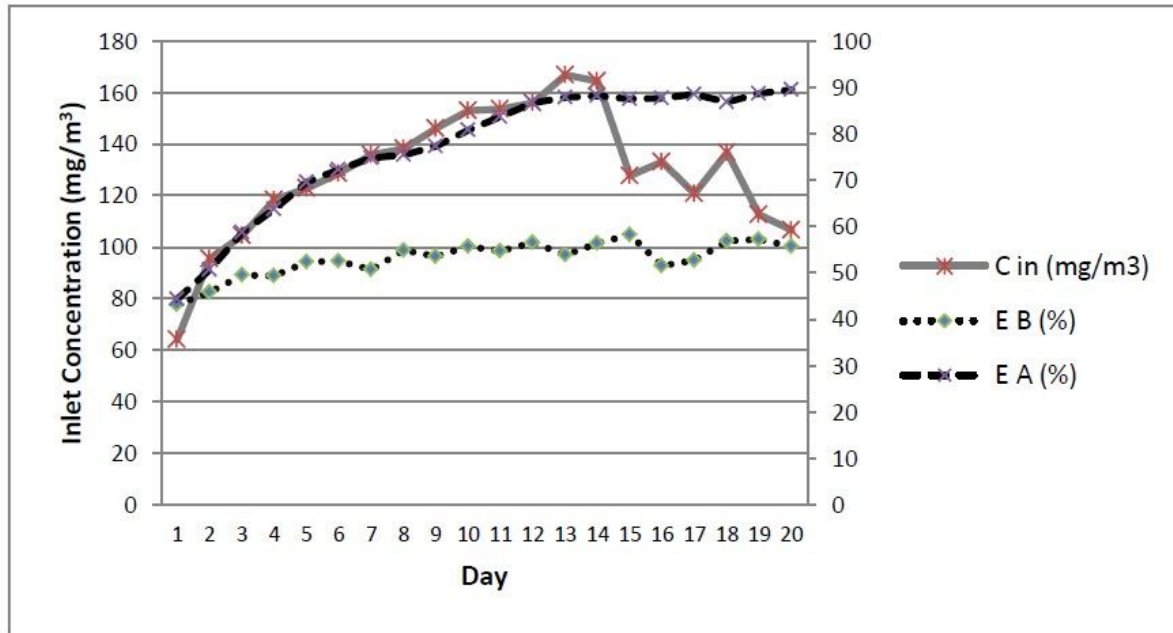


Figure 2

Influence of inlet toluene concentration on the removal efficiency of the BFs (BF-A, BF-B) as a function of time

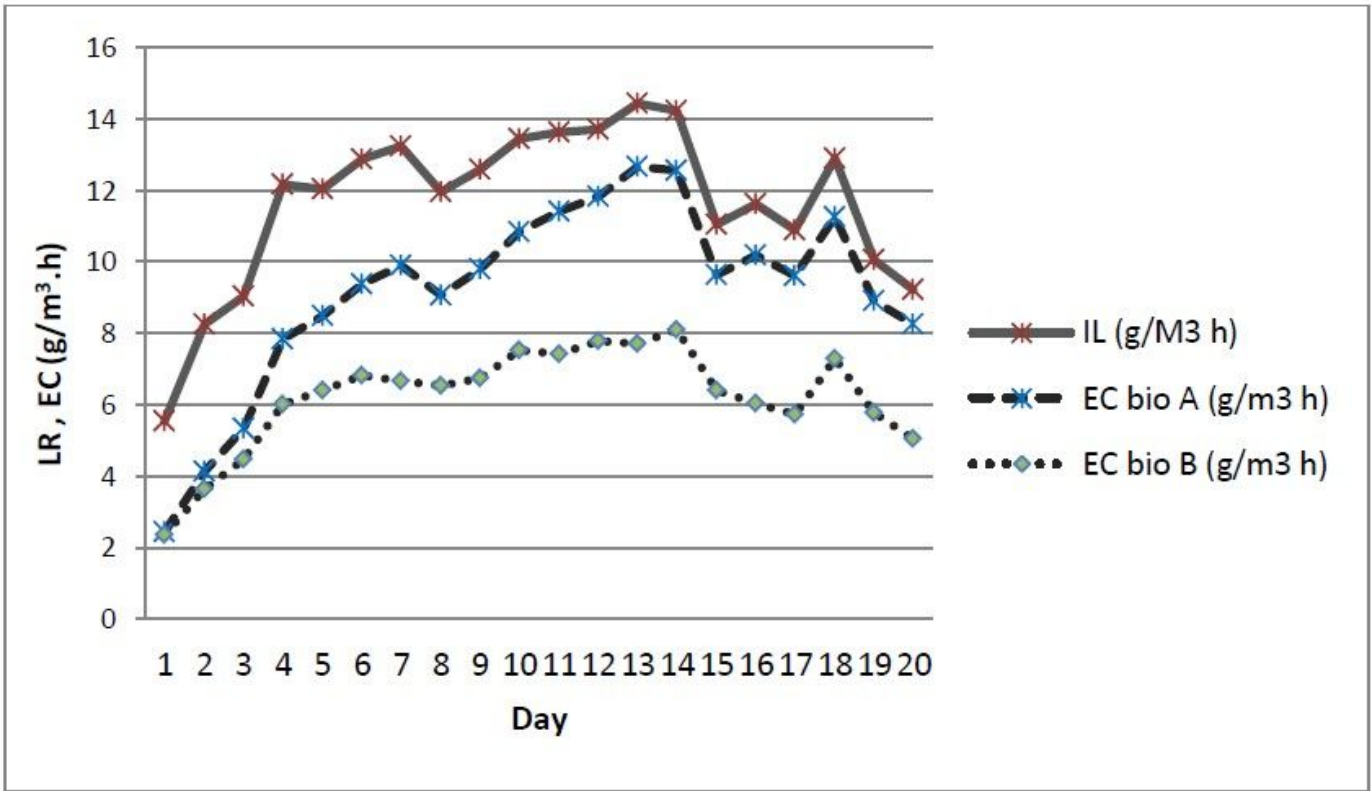


Figure 3

Influence of inlet toluene loading on the elimination capacity of the BF's (BF-A, BF-B) as a function of time

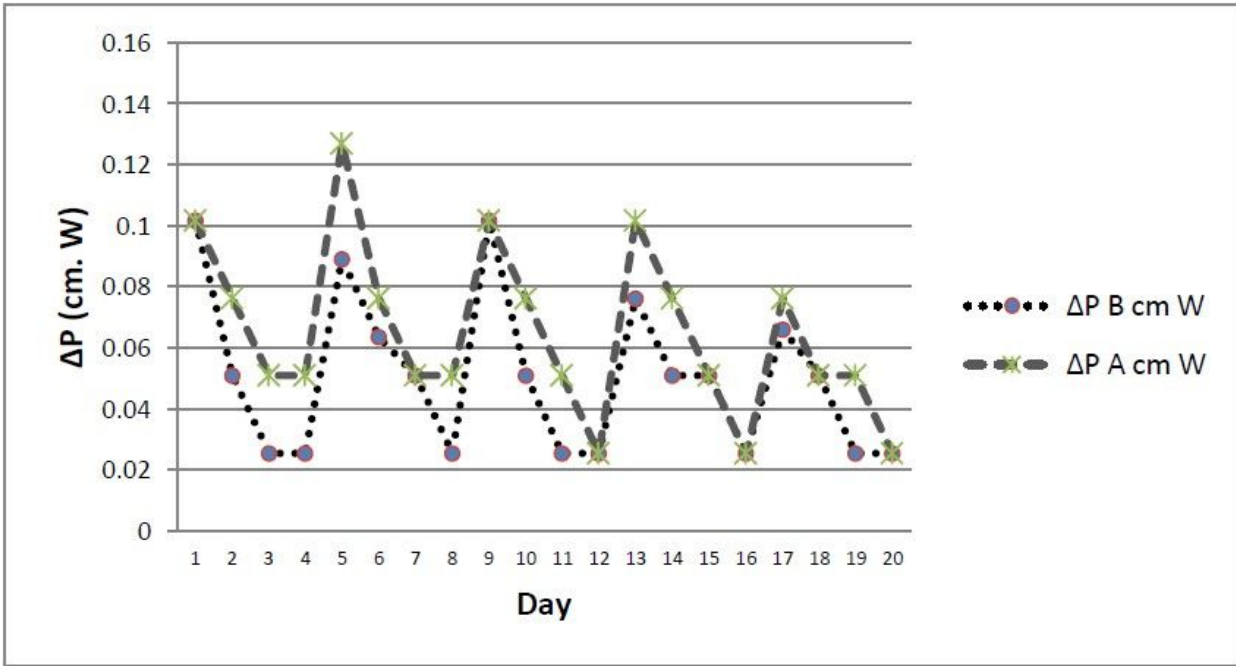


Figure 4

pressure drop as a function of time for the two parallel biofilters (BF-A, BF-B)

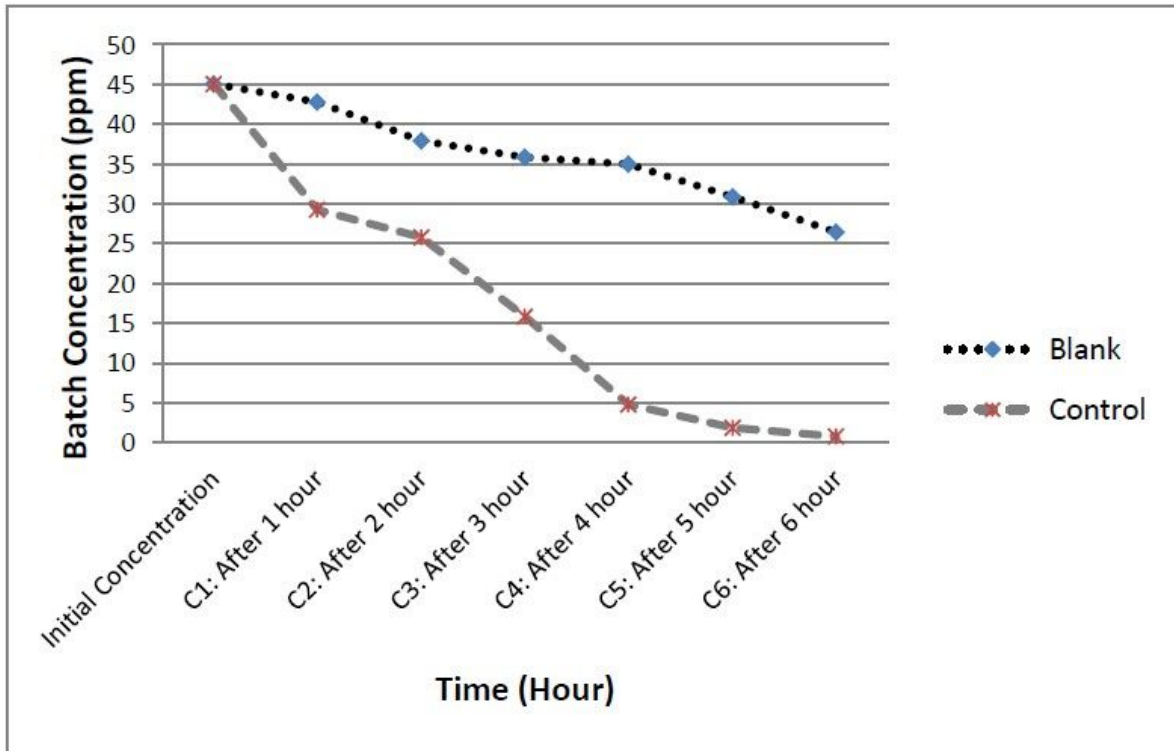


Figure 5

Biodegradation of toluene by *Pseudomonas putida*, control (x) and blank (⊠) for 45 ppmv

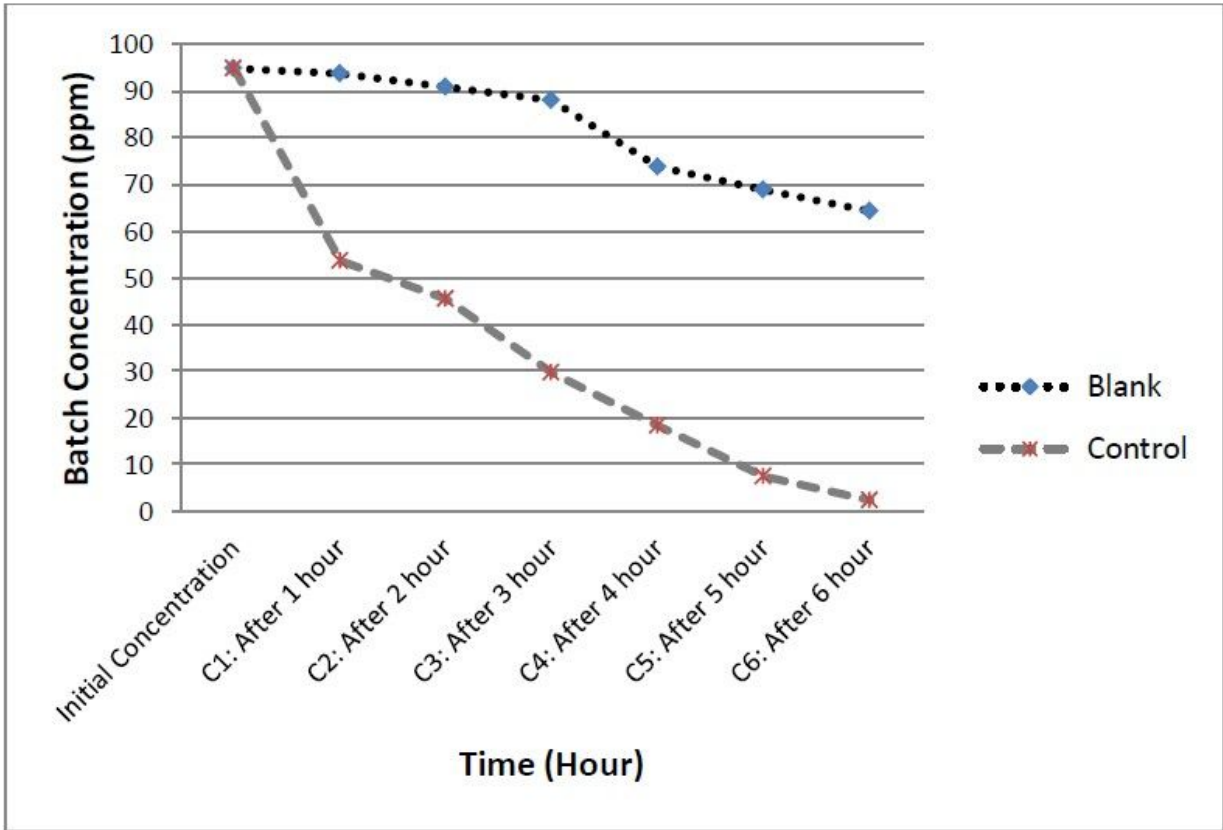


Figure 6

Biodegradation of toluene by *pseudomonas putida*, control (x) and blank (◊) for 95 ppmv

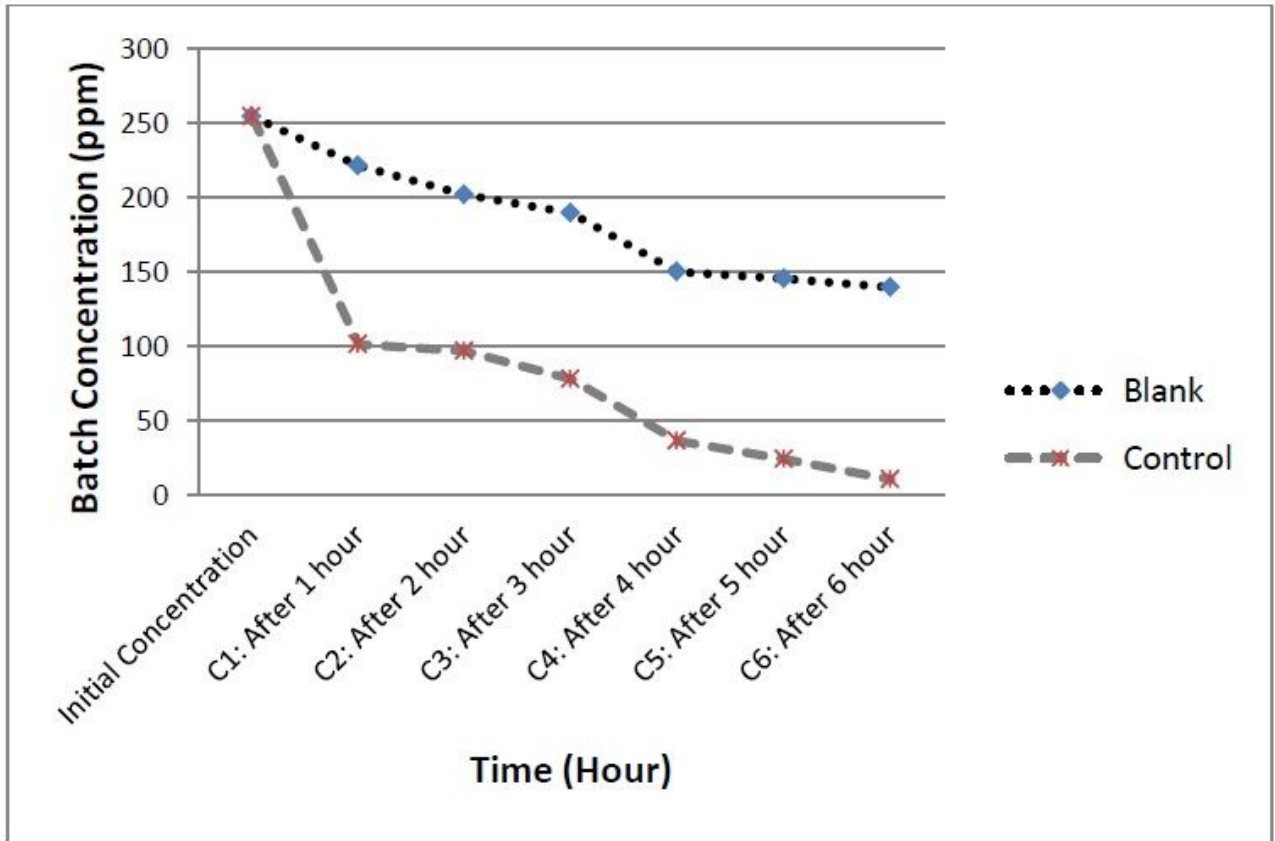


Figure 7

Biodegradation of toluene by pseudomonas putida, control (×) and blank (⊠) for 255 ppmv

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Equations.docx](#)