

Performance of BTX degraders under substrate versatility conditions

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Abstract

A microbial consortium acclimatized with benzene, toluene or xylene (BTX) was employed to study the degradation pattern of these compounds individually under aerobic conditions. Batch and continuous experiments were conducted to evaluate the adaptability of the enriched cultures under substrate versatility conditions. The bio-kinetic parameters obtained under substrate versatility conditions were compared with those of a single substrate condition. Similar degradation patterns were observed for all the substrates with inhibition occurring at higher concentration (~150 mg/L for benzene and xylene, and ~200 mg/L for toluene). Toluene degradation was highest, followed by benzene and xylene in the aqueous phase. Adaptation to a more toxic compound like benzene and xylene improved the utilization of toluene. On the other hand, microbes grown on a less toxic compound (toluene) grew at a lower rate in the presence of more toxic compounds. Suitable kinetic parameters such as μ_{\max} (maximum specific growth rate per hour), K_s (half saturation constant, mg/L), and K_I (threshold substrate inhibition constant, mg/L) were determined using Haldane and Levenspiel substrate inhibition models. The Haldane equation seems to be an adequate expression for the system. The degradation behavior of pollutants in the gas phase was also evaluated using a toluene acclimatized biotrickling filter operated in continuous mode. The biotrickling filter acclimatized with toluene could degrade benzene and xylene with a lower elimination capacity. But, the system could recover its original efficiency quite fast even after a prolonged shock loading. The degradation was better for toluene, followed by benzene and xylene.

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1. Introduction

Volatile mono aromatic hydrocarbons such as benzene, toluene, and xylene (collectively known as BTX) are key industrial solvents and are frequently required in industrial operations. These compounds are released into the environment during manufacture, transportation, usage, and disposal, leakage in underground storage tanks and pipelines, and through leachate from landfills. The liquid as well as gaseous states of these compounds in the environment pose a significant threat to human health and the environment due to their toxic and carcinogenic properties. The Clean Air Act Amendments of 1990 (CAAA 90) proposed by the US Environmental Protection Agency (EPA) places special emphasis on the handling, usage and treatment of

BTX compounds which are among the 188 hazardous air pollutants (HAPs) linked under this recognized Act. BTX compounds, on the contrary, are hydrophobic volatile organic compounds (VOCs) with maximum solubilities of 1740, 540, and 220 ppm_v, respectively [1].

Among the VOC control technologies practiced worldwide, bioremediation has emerged as the effective, economic and eco-friendly approach to treat VOCs at relatively low concentrations [2–4]. The most common approach to study their degradation potential is by simulating the environmental conditions in a laboratory system [5–9]. The performances of biofilter handling a variety of hydrophilic and hydrophobic compound have been studied by researchers [10–12]. Most of these studies focus on a single pollutant with uniform concentration using the same microbial flora with no special emphasis on its substrate versatility. Most of the individual air emissions, in particular VOCs, have the tendency to fluctuate depending on the process conditions, raw material and operational parameters. In order to with-

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stand these intermediate fluctuations, the mixed population of microorganisms in the biological system should adapt itself to the new pollutant and remove them effectively from the waste gas. However, little research has been carried out to evaluate the substrate versatility behaviour of various continuous biological treatment systems. Hence, there is a need to understand the primary removal pattern of single VOC enriched biological system while exposed to other VOCs.

Most of the liquid batch studies that have been carried out in the past focused primarily on the degradation of individual substrates with a microbial population acclimatized to the same substrate. A few studies have assessed the possibility of degrading a variety of compounds by one particular strain of microbes that was previously acclimatized with a single substrate. Shim and Yang [8] has studied the potential of toluene degrading microorganisms to degrade B, T, E and X at high concentrations, ≤ 1000 mg/L. Gibson et al. [13] isolated strains of *Pseudomonas putida* F1 that was able to grow on two or more aromatic compounds and most of these studies have been carried out at low concentrations (< 70 mg/L) of pollutants. This particular strain used toluene, benzene, ethyl benzene, phenol and other aromatics as the sole carbon source.

The present study is focused on the response of microbes on substrate versatility, in both batch (liquid phase) and continuous (gas phase) systems, and how the acclimatization of microbes with a specific pollutant influenced the degradation and inhibition by other toxic compounds. Shake flask reac-

tors were used for liquid batch studies where as continuous gas phase experiments were carried out using a biotrickling filter fed with various VOCs, viz. benzene, toluene, and xylene.

2. Materials and methods

2.1. Experimental setup

A schematic of the experimental bench-scale biotrickling filter system for treating BTX vapors is shown in Fig. 1. The biotrickling filter was constructed from acrylic hollow cylinders having an internal diameter of 5 cm and filled with 0.177 kg of Fujjino (packing material made of light weight PVC, thin sheets of PVC folded to give more or less a spherical shape) material with an approximate diameter of 8 mm. The resulting reactor had a packed bed height of 50 cm and bed volume of 0.98 L. A 10 cm headspace was left at the top for housing the nutrient feeding arrangement and for venting the treated air. A 10 cm bottom space was designed for the VOC containing inlet air. Leachate was continuously collected at the bottom of the biofilter. The reactor was operated in counter-current mode. The airflow rate was regulated with the aid of a pre-calibrated flow meter (model LGPR 1, Placka Instruments India Pvt. Ltd., Chennai, India). Air was split: one stream was passed through a trough filled with target pollutants and the other was passed through a water filled reservoir, which acted as a humidifier. The desired

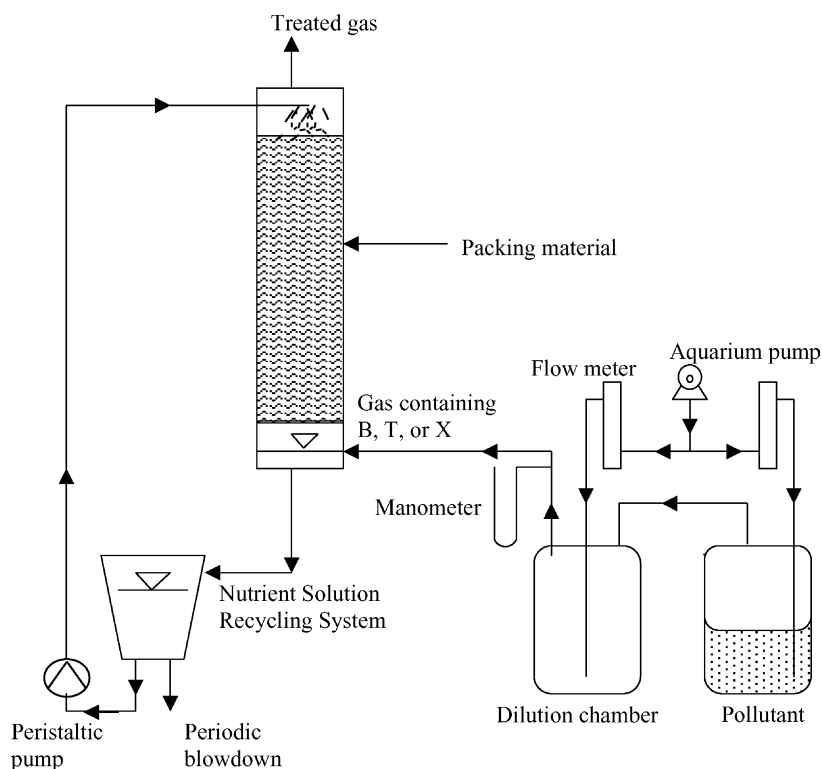


Fig. 1. Schematic diagram of experimental setup used for gas phase continuous studies.

final concentration of air stream was obtained by mixing air from both the troughs in a specific volume ratio by controlling the flows. The biotrickling filter was also facilitated with a nutrient feeding system at the top, which supplied nutrients continuously to the system at the flow rate of 24 L per day ($0.51 \text{ m}^3/\text{m}^2 \text{ h}$). The nutrient solution was controlled by using a peristaltic pump (Miclins, PP20, India). The pH of the nutrient medium was maintained at 7.0 ± 0.2 by adding NaOH or HCl.

2.2. Media composition and microorganism

The composition of the recycled nutrient medium contained per liter was: 1.0 g KH_2PO_4 ; 1.0 g K_2HPO_4 ; 5.0 g KNO_3 ; 1.0 g NaCl; 0.5 g MgSO_4 ; 0.01 g CaCl_2 ; and 1.0 mL of trace elements [14]. Activated sludge from a secondary clarifier of a municipal sewage treatment plant (Nesapakam, Tamil Nadu, India) was used for the preparation of the seed culture. B, T, and X were used as the sole carbon source by the mixed culture. Before going ahead with batch degradation experiments, the culture was properly acclimatized with benzene, toluene, and xylene over a period of 3 weeks through series of repeated transfers. For continuous experiments, the municipal sewage was allowed to settle for a period of 4 h and the supernatant was then discarded to obtain a concentrated sludge. The sludge used for this study had a suspended solids concentration (SSC) of 6200 mg/L, and volatile suspended solids (VSS) of 5240 mg/L. The concentrated sludge along with a previously toluene acclimatized microbial culture was directly fed into the filter bed and re-circulated for 6 h. All the pollutants used in this study were of reagent grade (Ranbaxy, India, toluene—99.5% purity, benzene—99.7% purity, xylene—not less than 92% purity).

3. Analytical methods

3.1. Estimation of biomass

Cell concentration in the batch system was determined by measuring the absorbance of the suspension at 540 nm using a UV–vis spectrophotometer (Shimadzu UV-1601 PC, Japan). Biomass concentration was estimated using a pre-plotted calibration curve between cell density and absorbance.

3.2. GC analysis of gas and liquid phase BTX samples

Initial and residual concentrations of B, T, and X in the aqueous and gaseous samples were measured by gas chromatography (model 5765, nucon gas chromatograph, Nucon Engineering, India) equipped with a flame ionization detector. A chromatopak stainless steel column (6.56 ft, 1/8 in. i.d., liquid—10% FFAP, solid—ch—WIHP, 80/100 mesh) was used. Nitrogen was employed as the carrier gas at a

flow rate of 25 ml/min. The temperatures at the GC injection port, oven and detection port were 150, 120 and 150 °C, respectively.

3.3. Analysis of gas samples

A 3–5 mL of the gas sample (benzene, toluene or xylene) from the reactor headspace was collected into a gas-tight syringe and immediately injected into the gas chromatograph. The sample volume injected into the gas chromatograph was 2 mL. Three to four injections were used for each gas sample. The average concentrations of the three injections were used for data analysis. The standard curve for benzene, toluene and xylene were prepared individually by injecting known amounts of the respective compound into a sealed bottle equipped with teflon septum according to a standard procedure [15]. The injected solvent was allowed to evaporate in the air space within the flask at room temperature. The air sample from the flask were drawn by a gas-tight syringe (Hamilton Co. Reno, Nev.) and analyzed by gas chromatograph.

3.4. Analysis of the liquid samples

Using a micropipette, 3 mL of samples were collected from the shake flask (250 ml erlenmeyer flasks) at regular intervals. A 1.0 mL sample was then transferred to a gas-tight centrifuge tube (Eppendorf, Germany) and centrifuged at 10,000 rpm for 15 min below room temperature and the supernatant was used for residual substrate analysis. The remaining 2 mL sample was used for cell density estimation. The concentration of benzene, toluene, and xylene in the liquid samples was calculated from their peak area on GC/FID spectra based on standard calibration curves. The injected volume of the liquid sample was 2 μL . The analysis of leachate from the biotrickling filter was also carried out in a similar manner.

4. Results and discussion

4.1. Biodegradation kinetics—single substrate

Batch studies were carried out under aerobic conditions using a mixed microbial culture acclimatized with single substrate to evaluate the biodegradation potential of pollutants like benzene, toluene, and xylene and the growth profile of the acclimatized microbes in the respective pollutants.

Using the experimental data, specific growth rate (μ) and the specific substrate utilization rate were calculated and were plotted against the substrate concentration (Fig. 2a and b). From the figures, it is clear that as the substrate concentration increased, the specific growth rate also increased. However, after reaching a particular concentration, the specific growth rate started declining with increase in substrate concentration, thus expressing substrate inhibition, a widely noticeable phenomenon while studying the degradation of

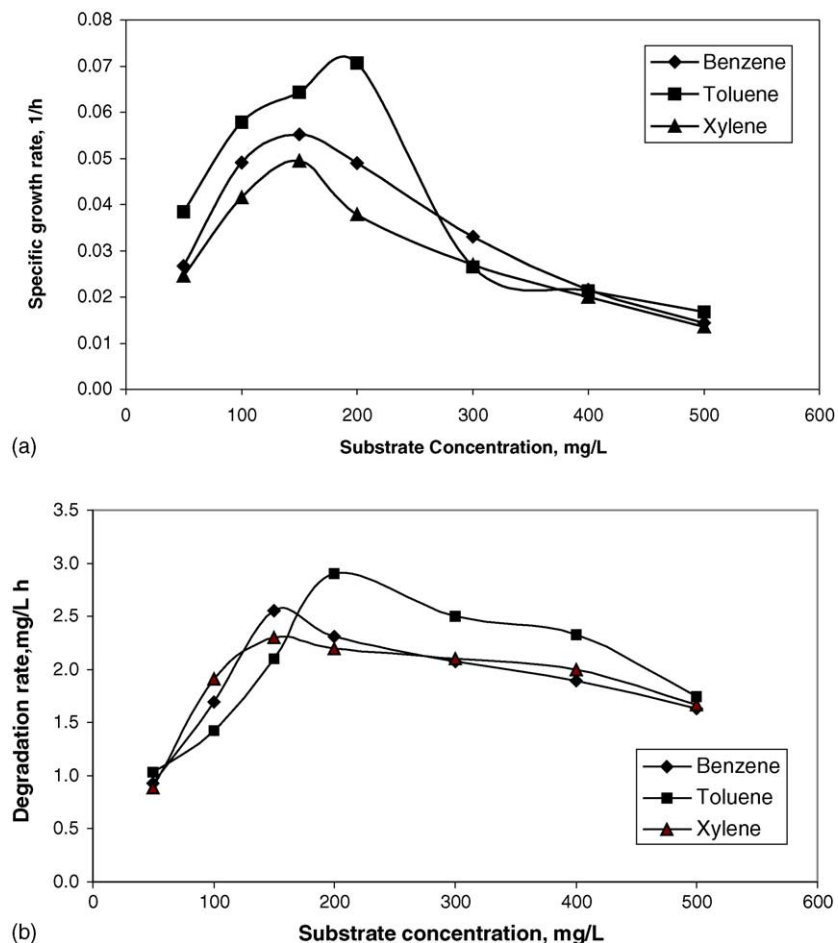


Fig. 2. (a) Specific growth rate profile of the mixed microbial culture using B, T, and X as a sole carbon source. (b) Degradation rate profile of B,T, and X compounds by mixed microbial culture as a sole carbon source.

toxic compounds. The observed substrate inhibition concentrations were 156.2 mg/L, 147.4 mg/L, and 222 mg/L for benzene, xylene and toluene, respectively. Yang has reported substrate inhibition in this concentration range while studying toluene degradation using toluene-enriched culture. Many researchers have also reported inhibition by B, T, and X compounds at relatively low concentrations

[8,9,16]. However, the focus of their research was primarily on developing new models and validating them accordingly with their experimental data.

Fig. 3 shows the growth profile of B, T, and X compounds at their respective inhibition concentration using a mixed microbial consortium enriched in respective pollutants. It is clear from this figure that the culture utilized B, T, and

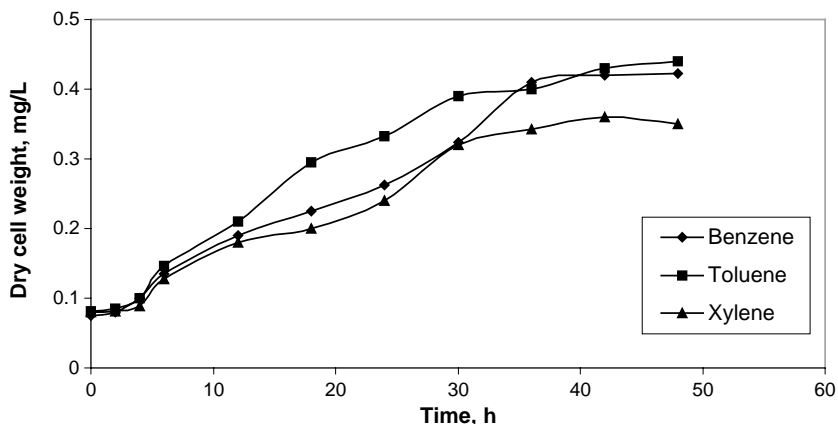


Fig. 3. Growth kinetics of mixed cultures at inhibition concentrations of B, T, and X.

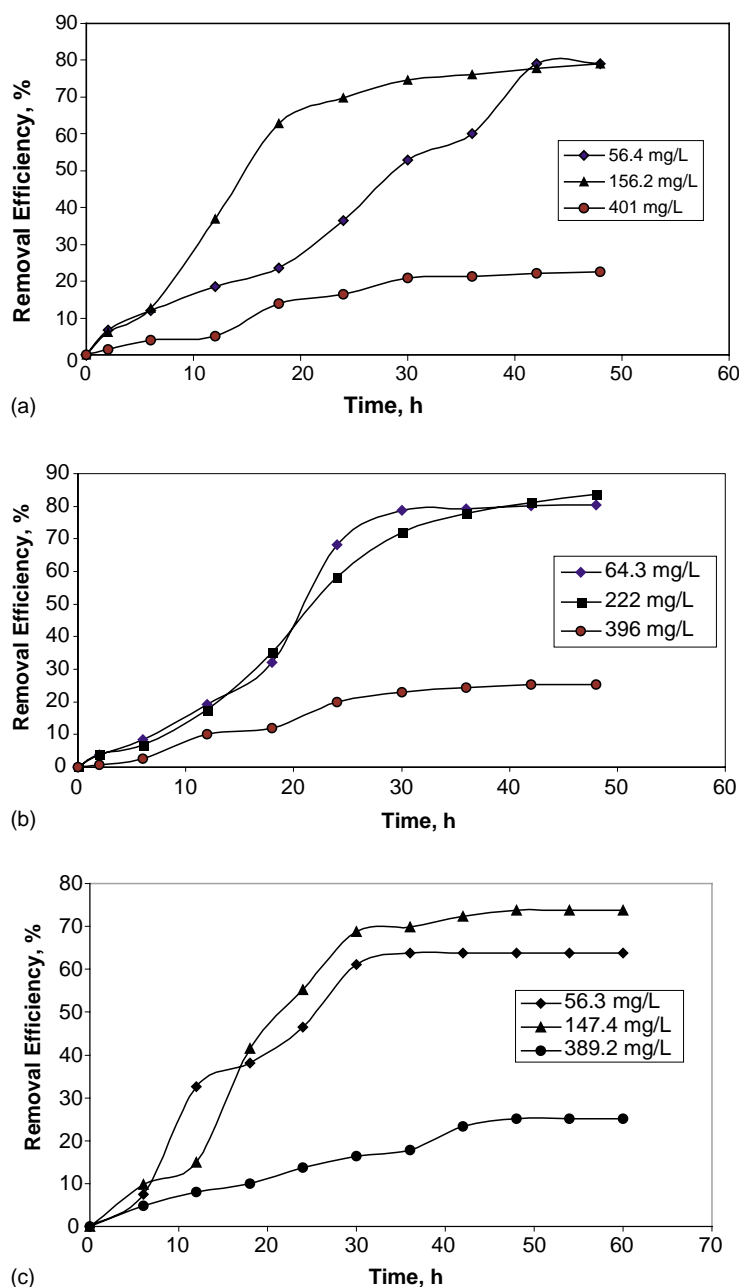


Fig. 4. (a) Degradation rate profile of benzene in terms of removal efficiency. (b) Degradation rate profile of toluene in terms of removal efficiency. (c) Degradation rate profile of xylene in terms of removal efficiency.

X as the sole carbon source and was able to degrade them quite effectively. Figs. 4(a–c) depict the degradation profiles of benzene, toluene and xylene in terms of its removal efficiency at three significant concentration ranges (other data not shown). The trend in degradation was quite similar for all the three compounds after its threshold inhibition concentration. Oxygen was not the limiting factor in these degradation experiments as proper headspace (~150 ml) with continuous shaking ensured transfer of O_2 from the headspace to the liquid substrate.

Table 1 shows the degradation rate and maximum specific growth rate (μ_{max}) observed for benzene, toluene and

xylene at various initial concentrations. The table also presents the approximate concentrations at which the specific growth rates were observed. The degradation rate followed a similar trend as that of specific growth rate, where the increasing and declining phase explicitly displays substrate inhibitions. However, toluene growth and degradation rates were higher compared to those of benzene and xylene. This may be due to the higher toxicity of these compounds compared to toluene.

The presence of a single methyl group in toluene initiates the growth of free cells. As reported in the literature, this is normally initiated by side chain oxidation where

Table 1
Specific growth rate and degradation rates observed from experiments

Inoculum precultured with	Substrate	μ_{\max}^a (h^{-1})	Change in μ_{\max}^b (%)	D_{\max}^c (mg/L/h)	Inhibition observed (mg/L)
Benzene	Benzene	0.0553	–	2.55	~150
	Toluene	0.0892	38.0 \uparrow	3.66	~250
	Xylene	0.0486	12.12 \downarrow	2.43	~150
Toluene	Benzene	0.0427	39.6 \downarrow	1.95	~100
	Toluene	0.0707	40.59 \downarrow	2.9	~200
	Xylene	0.0420	–	1.9	~100
Xylene	Benzene	0.0574	13.76 \uparrow	3.05	~200
	Toluene	0.0966	48.75 \uparrow	4.25	~250
	Xylene	0.0495	–	2.3	~150

^a μ_{\max} : Maximum μ observed.

^b \uparrow : Percentage increase in μ_{\max} values, \downarrow : percentage decrease in μ_{\max} values.

^c D_{\max} : Maximum observed degradation rate.

a mono-oxygenase catalyzed reaction converts benzene to benzyl alcohol, which could be further transformed to benzoic acid. Destabilization of benzene is more complex compared to toluene breakdown. The ring cleavage and the bacterial metabolism of benzene alone require that the aromatic ring to be made more reactive. A dioxygenase catalyzed reaction with molecular O_2 results in the production of benzene dihydrodiol that subsequently oxidizes to catechol. On the contrary, xylene breakdown is more complex depending on its isomers. The microbes oxidize a single methyl group first, which results in the formation of complex intermediates. Ring cleavage becomes more difficult once these intermediate compounds are formed, as it becomes more stable. The degradation pattern of xylene is clearly distinguished in this study as being more toxic and inhibitory to the mixed cells. However, more and elaborate explanation for its structural changes and transformation are given elsewhere [17–19].

4.2. Biodegradation kinetics—substrate versatility studies

Batch experiments were also carried out in an aqueous system to evaluate the viability of a microbial species that was initially acclimatized with a single pollutant to degrade

other pollutants having toxicities at higher or lower levels than the pollutant used for acclimatization.

In this work, benzene, toluene, and xylene biodegradation pattern was obtained individually, in all six possible combinations, using inocula pre-cultured with benzene, toluene and xylene. The initial concentration of the B, T, and X was varied between ~50 and 500 mg/L. To understand the effect of the other pollutant on the microbial system, the growth kinetic parameters thus established were compared with those obtained from the experimental study involving single substrate degradation using microbial cultures enriched in the same pollutants. The results obtained from the experiment are given in Table 1.

The maximum growth observed for benzene during one of the experiments with toluene and xylene enriched inocula is shown in Fig. 5. Growth was visible after a short lag period for the toluene-enriched culture, which could be easily characterized by its benzene consumption. However, the degradation of benzene by toluene-enriched culture was relatively less in the concentration range studied. The inhibition concentration of benzene was observed to be ~150 mg/L when an inoculum enriched in benzene was used. The xylene enriched inoculum showed a positive shift by 50 mg/L (i.e.197.5 mg/L). This specifically indicates the

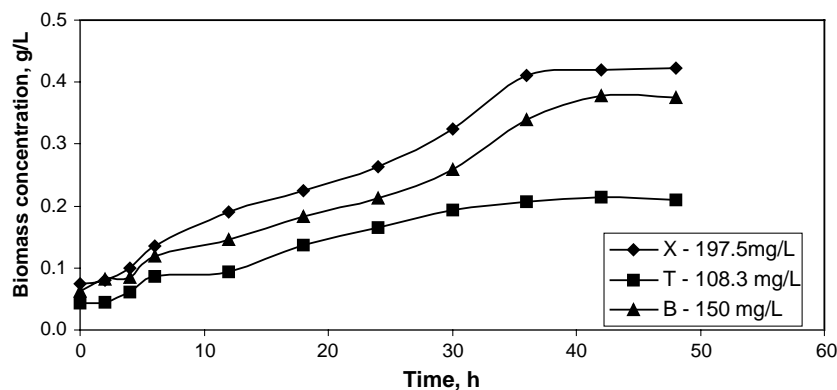


Fig. 5. Growth profile of the mixed culture enriched by individual BTX compounds when benzene was used as the sole carbon source. B, T, and X refer to benzene, toluene and xylene enriched cultures; the concentration mentioned depicts the inhibition concentration observed for the respective substrate studied.

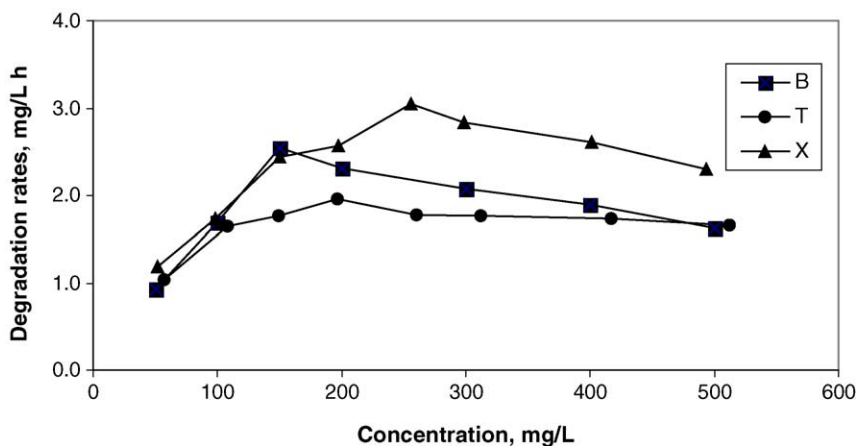


Fig. 6. Effect of substrate concentration and acclimatization on the degradation rate of mixed cultures grown using benzene as the sole carbon source. B, T, and X refer to benzene, toluene and xylene acclimatized cultures.

ability of the culture to degrade a relatively less toxic compound at a higher rate than the compound used for the enrichment. Studies were carried out with all other possible combinations. As a representative result, benzene degradation at three significant concentrations using toluene, xylene and benzene acclimatized cultures is shown in Fig. 6.

Similarly, toluene degradation and xylene degradation were carried out using all three benzene, xylene and toluene enriched cultures. The utilization of toluene was more rapid with benzene and xylene enriched cultures as inoculum. Here, also a significant shift in the threshold inhibition concentration was observed for toluene degradation using benzene and xylene enriched cultures. Xylene degradation using toluene and benzene enriched cultures reveals that xylene was consumed at a slower rate as compared to benzene and toluene. To understand the effect of toxicity better, the specific growth rate value observed for a mixed culture acclimatized with a particular pollutant, say benzene in benzene acclimatized culture, is compared with those observed for the same culture grown in other pollutants like xylene and toluene in terms of percentage variation. The percentage variation in specific growth rates are shown in Table 1.

4.3. Quantification of substrate inhibition kinetics

In order to predict the microbial kinetics at substrate versatility condition, an attempt was made to fit the kinetic rate data to appropriate kinetic models. Having experimentally observed substrate inhibition, kinetic data were fitted with the most widely accepted Haldane model (1968) and Levenspiel (1980) inhibition models. The kinetics coefficient, μ_{\max} (maximum specific growth rate per hour), K_s (half saturation constant, mg/L), and K_I (threshold substrate inhibition constant, mg/L) were estimated by the method of least-squares as in the mathematical problem solver package Matlab 5.1. Values of K_s indicate the ability of microbes to grow at low substrate levels [9] and K_I values indicate the sensitiveness of the culture to substrate inhibition [8].

The higher K_I value physically means, the culture is less sensitive to substrate inhibition and vice versa. A comparison of predicted values of μ_{\max} , K_s and K_I for B, T, and X degradation using pre-culture cells of B, T and X is given in Table 2. Values of K_I from Haldane model for BTX using three different culture varied between 142.1 and 211.8 mg/L, 209.4 and 269.5 mg/L and 134.3 and 175.6 mg/L for benzene, toluene, and xylene degradation respectively. As seen from the table, K_I values obtained from the Levenspiel model fit were found to be on the higher side (>500 mg/L), which is not in the concentration range studied. K_s values varied between 30.3 and 72.3 mg/L for various experimental observations. On close observation of the μ_{\max} , K_s and K_I values predicted from these models, the models predicted values were slightly higher than the experimental observations. But, on the other hand, the Haldane model predicted parameters were closer to the experimental values and also well simulated the experimental data. A plot of various experimental and model predicted specific growth rate values for B, T, and X compounds grown in benzene, toluene and xylene enriched cells are shown in Fig. 7a–c respectively. Even though the Levenspiel model fitted the specific growth rates well based on their standard deviation from the experimental, the values predicted for K_I is about three times higher than the experimentally observed substrate inhibition. The results from Haldanes models were hence found to be more illustrative of the experimental behaviour of mixed culture degradation studies.

4.4. Substrate versatility studies-continuous mode (gaseous phase)

The biotrickling filter was inoculated with a sludge collected from a sewage treatment plant, fed with low concentration of toluene (40 ppm_v) and nutrient solution (pH 7.0 ± 0.2) at a constant flow rate (24 L per day) for a period of 3 weeks. The growth of the microbial film on the packing material could be visually distinguished (onset of

Table 2
Kinetic parameters evaluated from substrate inhibition models

Inoculum precultured with	Substrate	μ_{\max}^a (mg/L)	K_s^b (mg/L)	K_i^c (mg/L)	Standard deviation
Haldane's model					
Benzene	Benzene	0.0973	64.3	170.2	0.0093
	Toluene	0.1311	49.2	269.5	0.0250
	Xylene	0.0976	51.1	154.8	0.0113
Toluene	Benzene	0.0826	43.8	142.1	0.0058
	Toluene	0.1117	56.6	29.4	0.0152
	Xylene	0.0879	52.0	134.3	0.0065
Xylene	Benzene	0.0629	30.3	211.8	0.0119
	Toluene	0.1229	72.3	252.3	0.0252
	Xylene	0.0811	58.2	175.6	0.0065
Levenspiel's model					
Benzene	Benzene	0.0953	86	555	0.0059
	Toluene	0.1624	77.4	582	0.0186
	Xylene	0.0861	40.3	536.2	0.0061
Toluene	Benzene	0.0684	70.9	592.1	0.0043
	Toluene	0.1166	125	570	0.0069
	Xylene	0.0743	54.8	543.7	0.0029
Xylene	Benzene	0.0860	88.0	515.9	0.0086
	Toluene	0.1358	98.2	636.6	0.0208
	Xylene	0.0865	85	561.7	0.0048

^a μ_{\max} : Maximum specific growth rate evaluated from substrate inhibition model per hour.

^b K_s : Half saturation constant (mg/L).

^c K_i : Threshold substrate inhibition constant (mg/L).

whitish biofilm) from the third day onward. On the 10th day, the microbial culture could degrade about 90% of the toluene at that particular concentration. Once the system had been stabilized, it was used for substrate versatility studies in continuous flow mode.

Experiments carried out in a continuous up-flow mode operation were divided into five distinct phases to evaluate the adaptability of the biosystem for various toxic compounds like toluene, benzene and xylene. In the first phase of the study, inlet toluene concentration was varied between 0.163 and 0.247 g/m³ at an empty bed residence time (EBRT) of 42 s for 5 days to achieve steady state conditions. The operational schedule of the biotrickling filter

is given in Table 3. At an EBRT of 72 s and a loading rate of 0.55 g/m³ of toluene, the elimination capacities of the system was 24.33–29.13 g/m³ h. This was reduced to 13.96–21.13 g/m³ h when EBRT was changed to 42 s. To examine the adaptability of the bio system to a different pollutant, the target pollutant was changed to xylene. The elimination capacity was only 8.784–19.087 g/m³ h. This corresponds to a removal efficiency of 82%. The reason for lower efficiency may be due to the toxicity of xylene compared to that of toluene and microorganisms were not acclimatized to this particular pollutant during the study in biotrickling filter. The system was again exposed to toluene to evaluate how fast it can recuperate from the shock

Table 3
Operational schedule of the bio-trickling filter

Phase of operation	EBRT ^a (S)	Target pollutant	Days of operation	Maximum ILR ^b (g/m ³ h)	EC ^c (g/m ³ h)
1	42	Toluene	5	30.519	29.193
	72		10		
2	42	Xylene	10	25.708	19.087
	72		5		
3	42	Toluene	10	31.734	28.844
	72		5		
4	42	Benzene	15	37.791	27.480
	72				
5	42	Toluene	10	62.279	52.422

^a EBRT: Empty bed residence time.

^b ILR: inlet loading rate.

^c EC: elimination capacity.

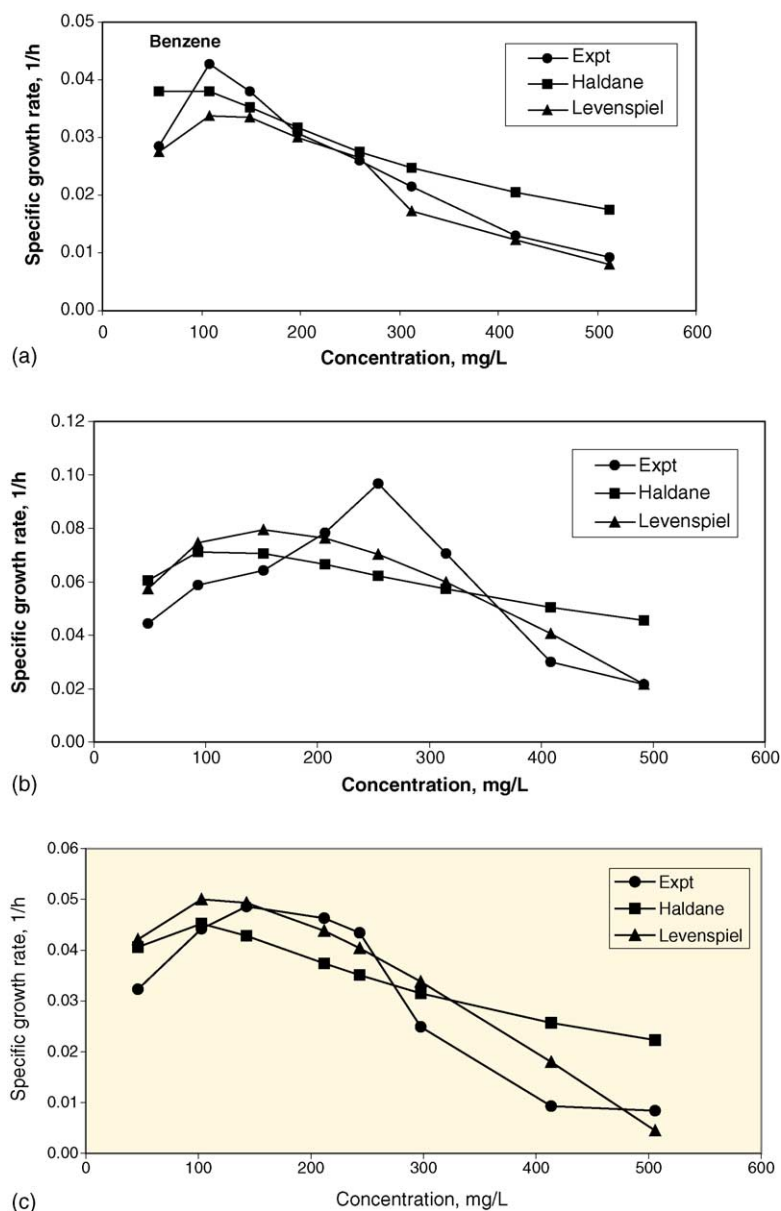


Fig. 7. (a) Experimental and model predicted profiles of specific growth rate for benzene biodegradation using toluene-acclimatized inoculum. (b) Experimental and model predicted profiles of specific growth rate for toluene biodegradation using xylene-acclimatized inoculum. (c) Experimental and model predicted profiles of specific growth rate for xylene biodegradation using benzene-acclimatized inoculum.

load. The reactor could maintain an elimination capacity ($20.97\text{--}26.69\text{ g/m}^3\text{ h}$), which is almost equal to the initial performance of the system. In third phase, the reactor was again subjected to benzene and the system could maintain a good elimination capacity ($5.928\text{--}24.4\text{ g/m}^3\text{ h}$). To monitor the recuperation ability of the system, toluene was fed to the system again. However, the elimination capacity was reduced slightly. Fig. 8 shows the performance of the various phases of steady state biotrickling filter operation for substrate versatility studies.

This study is indicative of the capability of toluene degrading microbial population to degrade variety of other VOCs. The consistency of this culture to degrade toluene

over a good range of loading conditions has been studied. The removal efficiency obtained in this work also confirms some of the batch results. Among the VOCs studied, xylene appears to be more toxic than benzene and toluene. Toluene is readily degradable compare to benzene and xylene. In this study, toluene has been degraded completely in the concentration range studied, where as the maximum benzene degradation was about 86% and xylene degradation was much lower compared to toluene and benzene. Further, the removal of the highly toxic compounds like xylene more likely depends on the acclimatization of microorganism to these toxic compounds prior to treatment. As this study has used a toluene degrading microbial culture to degrade

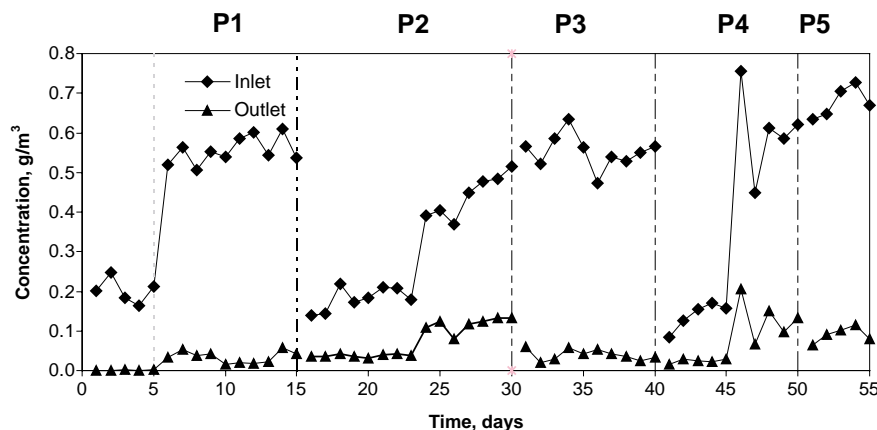


Fig. 8. Various phases of steady state biotrickling filter operation for substrate versatility studies (P1: toluene-42 and 72 s; P2: xylene-42 and 72 s; P3: toluene-72 s; P4: benzene-42 and 72 s; P5: toluene-42 s). (*) Indicates phase-pollutant treated-empty bed residence time.

benzene and xylene, the removal capacities were slightly reduced. As evident from batch studies, if the culture was enriched with a high toxic pollutant and being used to treat a less toxic compound, the efficiency might have been higher.

Benzene, toluene, and xylene degradation studies were carried out in liquid as well as gaseous phase using acclimatized inocula. The kinetic parameters obtained from batch experiments using shake flask reactors would give an idea about the order and extent of biodegradability of these pollutants. The same can be utilized for gaseous phase also. However, mass transfer limitations plays an important role for facilitating pollutant transport across the biofilm, which affects the performance in the biotrickling filter. Once the concentration in gas phase is known, corresponding concentration in liquid phase can be calculated using well-known equations.

As the waste gas treatment system employed in the study was a biotrickling filter, the exposure of the pollutant gas to the microorganisms are insignificant, mostly it has to pass through the liquid phase and then to the biofilm. Transfer of the pollutants from gas phase to liquid phase is comparatively easier, which mainly depends on its solubility and concentration. Hence an understanding of the kinetics of the B, T, and X biodegradation in batch system would provide some meaningful insight for modeling the performance of biotrickling filters.

5. Conclusion

In this work, biodegradation of relatively high concentrations of BTX compounds was carried out individually in batch liquid and continuous (gas) phase. An attempt has been made successfully to study the substrate versatility and inhibition kinetics of mixed cultures capable of degrading individual BTX compounds in suspended and attached growth systems. All cultures were able to grow

well using BTX as the sole carbon source. Growth rates of xylene and benzene pre-cultured cells in batch cultures were much faster when toluene was used as the sole carbon source. However, growth rates of toluene pre-cultured cells were much slower when benzene and xylene were employed as the sole carbon source. This study clearly reveals that inocula pre-cultured with comparatively high toxic compounds can degrade the compound with relatively less toxicity. In continuous mode study, the biotrickling filter acclimatized with toluene degraded benzene and xylene with a lower elimination capacity. However, the system could recover its original efficiency quite fast even after a prolonged shock loading. The degradation was better for toluene, followed by benzene and then xylene.

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