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Biodegradation of BTEX: Optimization through Response Surface Methodology

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Abstract: Response Surface Methodology (RSM) was used to optimize the biodegradation of Benzene, Toluene, Ethyl benzene and m-, o-, p-Xylene (BTEX) by *Pseudomonas putida* immobilized in PVA matrices in a specially-designed novel Spouted Bed Bioreactor System (SBBS). A mixture of 60 mg L^{-1} of BTEX compounds in air was contacted with the immobilized bacteria in three spouted bed bioreactors in series. The parameters investigated include the contaminated air flow rate, operating temperature, PVA volume and water pH. Maximum biodegradation efficiency was predicted to occur at an air flow rate of 500 mL min^{-1}, an operating temperature of 33°C, a PVA volume fraction of 30% and a pH of 8.3. Among the evaluated variables, air flow rate had the most significant effect on the efficiency of BTEX biodegradation. At optimum conditions, the novel bioreactor system was able to achieve maximum removal efficiency of 94, 98, 95 and 98% for Benzene, Toluene, Ethylbenzene and Xylene, respectively.

Keywords: Biodegradation, BTEX, Response Surface Methodology, Spouted Bed Bioreactor, *Pseudomonas putida*

Introduction

Benzene, Toluene, Ethylbenzene and Xylene, often referred to as BTEX, are hazardous air pollutants that are commonly found in most industrial gas emissions. Recently, control of these Volatile Organic Compounds (VOC) has become a major concern in air pollution prevention (Tokumura *et al.*, 2008). BTEX compounds are usually found in the vapors or emissions of several sources such as vehicle exhaust, gasoline evaporation, natural gas and liquefied petrochemical gas (Hung-Lung *et al.*, 2007), as well as waste incinerators and composting facilities (Yassaa *et al.*, 2006). They are also emitted through various oil and gas processing activities, such as flaring, venting as well as the dehydration and sweetening of natural gas. Other activities such as exploration, production, refining, storage and transportation of petroleum products may also result in the frequent occurrence of these harmful compounds in air, water and soil (Atlas, 1995).

BTEX are classified as priority pollutants by the U.S. Environmental Protection Agency (EPA), because of their acute toxicity and genotoxicity (Otenio *et al.*, 2005), (CAAALHAP, 2012). They represent a major health hazard to humans and can have detrimental effects on the environment, due to their toxic and cancer-causing properties. Even at low ranges of concentrations, BTEX compounds can cause major harm to human organs such as the liver and kidney and may disable the central nervous system (Jo *et al.*, 2008). The Agency for Toxic Substances and Disease Registry published the names of 11 of these compounds in the top 100 chemicals on the "List of Hazardous Substances" (ATSDR, 2013). In order to follow strict regulations and air quality standards, industrial plants need to reduce the concentrations of VOCs gaseous effluents prior to their release into the atmosphere (Mathur *et al.*, 2007). Therefore, it is essential to treat BTEX compounds in air and prevent deterioration of the ecosystem.

Numerous methods for BTEX removal have been evaluated, including conventional techniques such as adsorption, incineration, absorption, thermal and catalytic oxidation (Kennes and Thalasso, 1998). Compared with physical and chemical methods, biological treatment is becoming more popular since it offers a cost effective alternative and it is usually positively perceived by the general public as environmental friendly (Cox and Deshusses, 1998; Robledo-Ortíz *et al.*, 2011). The principle of biodegradation involves contacting the contaminated air

stream with a bacterial culture that has the ability to utilize the organic pollutant, especially complex hydrocarbons such as BTEX, as a main source of carbon and energy. Successful applications of bioreactors have been mostly found in air pollution control, especially in the treatment of contaminated gas streams for the removal of Volatile Organic Compounds (VOCs) as well as odor control. The volatile or gaseous pollutants can be degraded completely into innocuous or less harmful products such as carbon dioxide and water (Cox and Deshusses, 2002). According to Burgess *et al.* (2001), the transformation process can be expressed simply by:

$$\text{Odorous gas + oxygen} \xrightarrow{\text{via bacteria}} \text{bacterial cells + carbon dioxide + water}$$
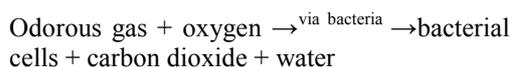
Determining the effectiveness of biotreatment and the availability of microorganisms for degradation of these VOCs have received considerable attention. In recent years many environmental microorganisms can metabolize BTEX and their biodegradative elimination has been heavily developed over the years (Attaway and Schidt, 2002). In this study, a more efficient biodegradation process has been assessed. Bacterial cells, *Pseudomonas putida*, were immobilized in Polyvinyl Alcohol (PVA) pellets and utilized for the biodegradation of BTEX in a spouted bed bioreactor system. As a non-pathogenic bacteria with a saprophytic lifestyle, *Pseudomonas putida* are known to have remarkable metabolic versatility (Krell *et al.*, 2012). *P. putida* is perhaps the best characterized aromatic hydrocarbon-degrading bacteria that can use BTEX and other aromatics as sole source of carbon and energy (Reardon *et al.*, 2000). A large number of studies evaluated the effectiveness of *P. putida* for the degradation of BTEX (Shim and Yang, 1999; Robledo-Ortíz *et al.*, 2011; Krell *et al.*, 2012). Moreover, immobilization of the *P. putida* in PVA matrices protects the bacteria from high substrate concentrations and allows ease of separation and reutilization of the biomass (El-Naas *et al.*, 2009). A laboratory scale Spouted Bed Bioreactor System (SBBS) was specially built for the study. The Spouted Bed Bioreactor provides intense cyclic mixing due to the single air jet injected at the bottom of the reactor (El-Naas *et al.*, 2010a).

Since the biodegradation of BTEX by microorganisms is strongly affected by many parameters, it is important to determine the key factors that can significant effect on the degradation process. Common experimental approaches such as "one-factor-at-a-time" are often incapable of determining optimum conditions and cannot detect any synergistic effects or interactions between two or more experimental parameters. Thus, through the use Response Surface Method (RSM), one can optimize all the major parameters and eliminate the limitations of one-

parameter optimization (Choudhury and El-Baradie, 1999). RSM has been used in various fields such as machining techniques (Choudhury and El-Baradie, 1999), chemical reactions (Sultania *et al.*, 2011; Setiabudi *et al.*, 2013) enzyme and catalytic reactions (Hosseinpour *et al.*, 2011; Thouchprasitchai *et al.*, 2011; Yüksel and Yeşilçubuk, 2012) and with recent broader application extended to the optimization of water and water treatment processes (Güven *et al.*, 2008; Körbahti and Tanyolaç, 2008; Trinh and Kang, 2011; Kumar and Pal, 2012; Yuliwati *et al.*, 2012), in biochemistry (Wang *et al.*, 2012) toxicology studies (Carter *et al.*, 1985; Kiran and Thanasekaran, 2011; Jeong *et al.*, 2012) and in biotechnology for studying biodegradation of phenol (Sheeja and Murugesan, 2002). However, a limited number of studies (Jo *et al.*, 2008; Jin *et al.*, 2013) were reported on the optimization of BTEX's removal efficiency. Therefore, the prime objective of this study is to employ RSM to determine and optimize the key parameters that effect the biodegradation of BTEX in a Spouted Bed Bioreactor System (SBBS) specially-designed for the study.

Materials and Methodology

Extraction and Immobilization of Bacterial Culture

The bacteria were obtained in a cereal form (Cleveland Biotech, UK), which contained a consortium of microorganisms, with *P. putida* as the dominate strain. Bacterial cells were extracted from the cereal through two consecutive steps of centrifugation using IEC CL31R Multispeed Centrifuge, Thermo Electron Cooperation, USA. The extracted bacterial cells were collected in a glass jar and stored in a refrigerator for the next step, which is the immobilization in PVA gel pellets. More details about the bacterial extraction and immobilization were reported in a previous publication (El-Naas *et al.*, 2010b).

Biomass Acclimatization

The immobilized bacteria were slowly acclimatized to Toluene over a period of 5 days by placing them in a nutrient mineral solution with 300 mg L^{-1} of glucose as an easily biodegrade source of carbon. The concentration of glucose was gradually reduced to zero, while the toluene concentration was gradually increased to 300 mg L^{-1}. At this stage, the bacteria were considered fully acclimatized to toluene as a representative of the BTEX compounds. A similar procedure was reported for the acclimatization of the same biomass to phenol (El-Naas *et al.*, 2010b).

Spouted Bed Bioreactor System (SBBS)

The patent-pending SBBS consisted of three one-liter spouted bed bioreactors that were made of Plexiglas and equipped with surrounding jackets for temperature

control. At the start of the experiment, each one of the three reactors was filled with the standard nutrient media solution, in addition to the PVA gel cubes with the immobilized bacteria, which occupied 30% of the reactor volume (El-Naas *et al.*, 2010a). The three bioreactors were operated in series; the gas leaving from the top of the first reactor was injected into the bottom of the second reactor and then from the second to the third reactor. The good mixing inside the bioreactor provided good contact between the substrate and the bacterial cells which led to good pollutant removal. Mass flow meters (Sierra Side-Tank) and controller (Auto-Tank) were used for accurate measurement and control of the gas flow rates (BTEX in Air). The standard output for the transducers was 0-5 V, corresponding to a gas flow rate of 0 to 5000 mL min^{-1}. The feed gas mixture contained 10 mg L^{-1} of each BTEX compound in air and was injected into the bottom of the bed for good mixing and to provide the oxygen needed for the biodegradation.

Analytical Method

The treated air stream, leaving the reactor, was analyzed for BTEX using a Gas Chromatograph (Varian 3800), which was equipped with a flame ionization detector. Gas samples were withdrawn at 7 min time intervals, using an automatic sampling system and the separation was carried out on a CP-sil 8CB 30 m ×0.32 mm ID, 1 μm film thickness fused silica capillary column with Helium as carrier at 18 psi. The column temperature was programmed at a heating rate of 40°C min^{-1} for 1 min, then 35°C min^{-1} up to 90°C; it is held for 1 min and then raised to 114°C at 8°C min^{-1}. The temperatures of the injector and detector were maintained constant at 200°C and 250°C, respectively.

Sierra Side-Tank^{TM} and Auto-Tank^{TM} mass flow meters and controllers were used for accurately measuring and controlling gas flow rates. Standard output for the transducers was 0-5 V DC signal, which corresponded to 0 to 5000 mL min^{-1} mass flow.

Response Surface Methodology

Response Surface Methodology (RSM) is a statistical approach that is implemented to determine the relationship between a certain response and a group of factors, which are of importance and interest to the investigator. A model is built to describe the response over specific ranges of the factors of interest. The response surface is referred to as the fitted model in many industrial applications, because the response can then be graphed as a line (curve) for one factor of interest or a surface for two factors of interest

(Mason *et al.*, 2003; Jo *et al.*, 2008). RSM is often used to identify the range of operating conditions that generate the optimum response and satisfy certain operating or process specifications. It may also be used to identify new operating conditions that would improve product quality, while correlating between the response and the main quantitative factors (Cutright and Meza, 2007).

To evaluate the contribution of the solubility of BTEX in water and the role of biodegradation, blank runs were carried out using Toluene as representative of BTEX compounds. Toluene removal (without bacteria) was tested for two reactors in series as shown in Fig. 1. After 50 min, only 15% of the Toluene was removed by the water as compared to the system with the immobilized bacteria, which achieved 65% removal. Clearly, the difference in the percentage removal between the two cases is due to the biodegradation of Toluene by the immobilized bacteria. It is important to note here that at the beginning of the experiment, the solubility of toluene in water contributes to the reduction of Toluene until the water in the reactor reaches the saturation limit. Figure 1 also shows the biodegradation efficiency for one, two and three bioreactors in series. The results indicated that the biodegradation of Toluene increasing by about 25% with the addition of each reactor. However, any improvement in the biodegradation rate beyond the third reactor was not worth the addition of an extra reactor and therefore, only three bioreactors in series were used.

A few experiments were carried to screen out the operating parameters and determine the optimal range of operation. One factor-at-a-time was employed in the screening step to determine the significant factors affecting BTEX degradation (El Telib, 2014). One factor-at-a time implies that only one factor is varied while keeping others constant (Mohapatra *et al.*, 2011).

When the ranges of the optimum values were determined, a full Central Composite Design (CCD) was utilized to find the optimum conditions of the four factors (X_1, X_2, X_3 and X_4). In this study, a total of 31 experiments were conducted in a randomized order as required in many design procedures and 7 center points, giving six degrees of freedom for estimating errors in the experiments (Jo *et al.*, 2008) and verifying any change in the estimation procedure as a measure of the precision property (Mohan *et al.*, 2012). Seven axial points and 17 points equivalent to a matrix of 24 which incorporate 17 experiments (+1, 0, -1) including four variables were carried out. It is worth mentioning here that 4 more points' equivalents to a matrix of 24 were added in order to cover the optimal domain for the flow rate. Table 1 shows the levels and codes of each factor as well as the values of experimental designs.

Table 1. Range and level of independent variables for biodegradation of BTEX

Independent variable	Symbol	Level				
		$-\alpha$	-1	0	$+1$	$+\alpha$
Temperature (°C)	X_1	27.5	30	32.5	35	37.5
Flow rate (ml/min)	X_2	500	700	850	1000	1150
PVA volume (%)	X_3	15	20	25	30	35
pH	X_4	5	6	7	8	9
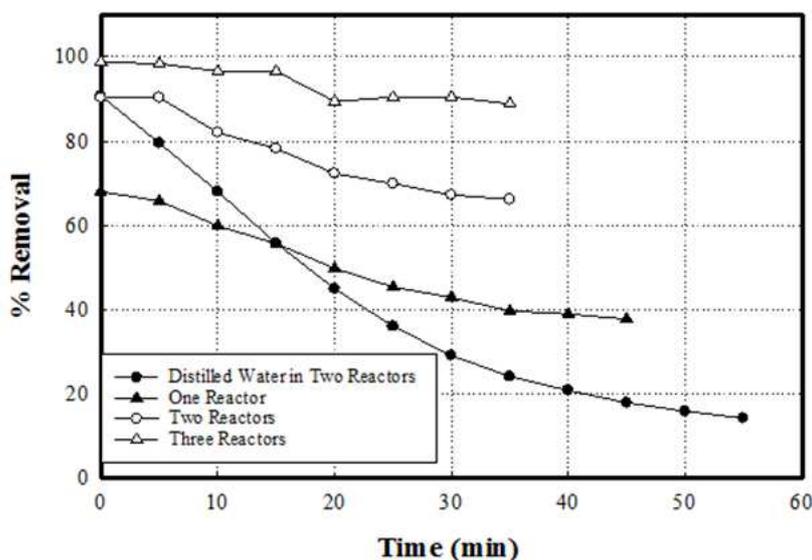
Fig. 1. Biodegradation of toluene at T = 30°C; initial toluene concentration = 50 mg L^{-1}; air flow rate = 500 mL min^{-1}

After running the CCD experiments, the response is then represented by the following second-degree quadratic polynomial:

$$Y = \beta_0 + \sum_{i=1} \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum_{i=1} \sum_{j=i+1} \beta_{ij} X_i X_j \qquad (1)$$

where, Y is the predicted response, β_o the offset term, β_i the coefficient of the linear effect, β_{ii} the coefficient of squared effect, X_i is the coded value of variable i, X_j is the coded value of variable j and β_{ij} is the coefficient of interaction effect. Temperature, gas flow rate, pH and PVA volume fraction were chosen as the factors for this study, while the BETX removals were considered as the response variables. Analysis of Variance (ANOVA) was conducted to determine the effects of the three main variables (X_1, X_2, X_3 and X_4) and their interaction on the biodegradation of BTEX as well as the significance of the model and regression coefficients. The feature of polynomial equation was evaluated by determination coefficient (R^2) and P-value of the lack-of-Fit. The statistical adequacy was evaluated by Fischer's F-test and the significance of regression coefficient was assessed by t-test. Calculations and analysis were carried out using MINITAB Statistical software (Version 16.2, Hewlett-Packard, US).

Results and Discussion

Optimization of Degradation Conditions using RSM

The experimental results utilized CCD for studying the effect of temperature (X_1), gas flow rate (X_2), PVA volume fraction % (X_3) and pH (X_4) on the biodegradation of each BTEX compound. The experimental results and theoretically predicted responses are presented in Table 2. The experimental results were then analyzed to find an empirical model for the best response. From the analysis of variance for each response, regression equations were obtained, which were then used to explain the mathematical relationship between the factors and responses. These equations are given below in terms of coded factors with all terms regardless of their significance. Using response optimizer, the optimum BTEX removal was found to be at a temperature of 33°C; a flow rate of 500 ml/min; a PVA volume fraction of 30 % and a pH of 8.3.

$$
\begin{aligned}
Y_B = {}& 82.95 - 1.79 X_1 - 5.97 X_2 + 0.30 X_3 - 0.75 X_4 \\
& -1.09 X_1^2 - 0.45 X_2^2 - 1.06 X_3^2 - 0.19 X_4^2 \\
& -0.44 X_1 X_2 - 0.39 X_1 X_3 - 0.02 X_1 X_4 \\
& -0.58 X_2 X_3 + 0.8 X_2 X_4 + 0.98 X_3 X_4
\end{aligned} \qquad (2)
$$

$$Y_T = 89.65 - 1.32X_1 - 4.54X_2 - 0.39X_3 - 0.03X_4$$
$$- 0.65X_1^2 - 0.6X_2^2 - 1.55X_3^2 - 0.49X_4^2$$
$$+ 0.13X_1X_2 + 0.38X_1X_3 - 0.38X_1X_4$$
$$- 0.09X_2X_3 - 0.91X_2X_4 - 0.25X_3X_4$$

(3)

$$Y_{m-pX} = 92.02 - 0.07X_1 - 3.38X_2 - 0.2X_3$$
$$+ 0.3X_4 - 0.65X_1^2 - 0.91X_2^2 - 1.1X_3^2$$
$$- 0.35X_4^2 - 0.16X_1X_2 + 0.34X_1X_3$$
$$- 0.04X_1X_4 - 0.5X_2X_3 - 0.94X_2X_4 - 0.54X_3X_4$$

(5)

$$Y_E = 90.53 - 0.53X_1 - 4.46X_2 + 0.56X_3$$
$$+ 0.44X_4 - 0.23X_1^2 - 1.12X_2^2 - 1.8X_3^2 - X_4^2$$
$$+ 0.06X_1X_2 - 0.21X_1X_3 - 0.03X_1X_4$$
$$- 0.22X_2X_3 - 0.70X_2X_4 - 1.03X_3X_4$$

(4)

$$Y_{O-X} = 91.88 - 0.001X_1 - 2.91X_2 - 0.08X_3$$
$$+ 0.33X_4 - 0.15X_1^2 - 0.4X_2^2 - 1.61X_3^2 - 0.09X_4^2$$
$$- 0.22X_1X_2 + 0.18X_1X_3 + 0.18X_1X_4$$
$$- 0.17X_2X_3 - 0.92X_2X_4 - 0.57X_3X_4$$

(6)

Table 2. Full Factorial central composite design (CCD) for BTEX removal

Run Order	Temp	Flow	PVA	pH	Experimental value %					Predicted value %				
					B	T	E	m-pX	O-X	B	T	E	m-pX	O-X
1	32.5	850	25	7	83	92	95	96	96	83	89	90	92	92
2	32.5	1150	25	7	68	78	77	80	84	69	78	77	81	84
3	30	700	20	8	81	94	95	96	96	83	94	92	94	93
4	32.5	850	25	7	77	91	94	97	95	83	89	90	92	92
5	32.5	850	35	7	77	78	84	86	85	80	84	85	88	86
6	32.5	850	25	7	85	91	93	95	94	83	89	90	92	92
7	35	1000	30	6	72	84	84	87	88	70	81	83	86	87
8	35	700	20	8	81	90	93	93	96	82	90	91	93	94
9	37.5	850	25	7	69	82	87	90	90	75	84	89	89	91
10	32.5	850	15	7	80	88	80	89	82	78	85	83	88	86
11	30	700	20	6	86	90	88	91	93	88	91	88	91	90
12	32.5	850	25	7	81	84	87	89	91	83	89	90	92	92
13	32.5	850	25	7	86	90	89	91	93	83	89	90	92	92
14	32.5	550	25	7	90	92	93	92	94	93	96	94	96	95
15	35	1000	20	6	75	81	78	84	87	74	82	80	86	87
16	35	1000	20	8	72	78	84	87	88	71	79	81	86	87
17	30	1000	30	8	76	82	82	85	86	77	82	81	83	85
18	30	1000	20	8	73	81	82	85	87	75	84	84	87	88
19	32.5	850	25	9	82	89	85	90	91	81	88	87	91	92
20	32.5	850	25	7	84	89	88	90	87	83	89	90	92	92
21	32.5	850	25	7	84	92	87	89	86	83	89	90	92	92
22	30	700	30	6	90	92	91	92	92	89	90	91	92	92
23	30	1000	20	6	76	84	84	87	89	78	85	83	87	88
24	32.5	850	25	5	82	86	86	91	89	84	88	86	90	91
25	30	1000	30	6	75	84	83	85	88	75	83	84	85	88
26	35	1000	30	8	76	81	81	83	85	73	79	80	83	85
27	27.5	850	25	7	88	91	90	88	89	82	90	91	89	91
28	35	700	20	6	88	89	89	90	91	86	89	87	91	90
29	35	700	30	6	89	91	92	92	93	86	90	92	94	93
30	30	700	30	8	87	93	94	94	94	89	93	92	93	93
31	32.5	850	20	7	85	89	89	90	92	81	89	88	91	90
32	32.5	500	25	7	95	97	95	96	95	92	99	96	97	97
33	32.5	500	25	8	94	98	95	98	98	93	96	95	95	96
34	35.0	500	25	7	94	98	94	98	98	95	96	95	96	95
35	32.5	500	30	7	94	96	95	97	96	94	95	93	96	95

Table 3. ANOVA of quadratic model for Benzene

Source	Sum of squares	Degrees of freedom	Mean square	F-value	P-value
Model	1614.98	14	115.36	10.15	0.000
Residual	227.30	20	12.415		
Lack of fit	172.45	14	14.2	1.35	0.375
Pure error	54.86	6	9.143		
Total	1842.29	34			

*Std dev = 3.37, Mean = 82.95

Table 4. ANOVA of quadratic model for Toluene

Source	Sum of squares	Degrees of freedom	Mean square	F-value	P-value
Model	958.35	14	68.454	9.13	0.00
Residual	149.93	20	7.687		
Lack of fit	103.07	14	7.62	0.98	0.542
Pure error	46.86	6	7.81		
Total	1108.29	34			

*Std dev = 2.74, Mean = 89.65

Table 5. ANOVA of quadratic model for Ethylbenzene

Source	Sum of squares	Degrees of freedom	Mean square	F-value	P-value
Model	844.416	14	60.315	9.55	0.000
Residual	126.327	20	6.316		
Lack of fit	54.612	14	3.901	0.33	0.960
Pure error	71.714	6	11.952		
Total	970.743	34			

*Std dev = 2.51, Mean = 90.53

Table 6. ANOVA of quadratic model for m-pXylene

Source	Sum of squares	Degrees of freedom	Mean square	F-value	P-value
Model	582.034	14	41.574	6.81	0.000
Residual	122.138	20	6.107		
Lack of fit	50.423	14	3.602	0.3	0.970
Pure error	71.714	6	11.952		
Total	704.171	34			

*Std dev = 2.47, Mean = 92.02

Table 7. ANOVA of quadratic model for o-Xylene

Source	Sum of squares	Degrees of freedom	Mean square	F-value	P-value
Model	461.975	14	32.998	4.47	0.001
Residual	147.625	20	7.381		
Lack of fit	56.196	14	4.014	0.26	0.981
Pure error	91.429	6	15.238		
Total	609.600	34			

*Std dev = 2.72, Mean = 91.88

Analysis of Variance (ANOVA)

The results of Analysis of Variance (ANOVA) are shown in Table 3-7 and indicate that the model can predict the experimental data within the 95% confidence interval. From a statistical point view, the p-value is a good indicator of the statistical significance and adequacy of the model can be inferred from the p-value of the lack-of-fit. If this value is greater than 0.05, there is no statistical significance of the lack-of-fit at the 95% confidence level and the model is considered adequate. The p-values of the lack-of-fit for the results, as shown in Table 3-7, are clearly more than 0.05, which confirms that the model is adequate.

Effect of Temperature

Figure 2 represents the maximum Benzene removal selected here as an example of the BTEX and plotted against temperature (X_1) and PVA (X_3) while keeping the flow rate (X_2) and pH (X_4) constant. A small increase in Benzene removal % could be achieved when the value of temperature increased from 32.5 to 35°C, beyond this the

removal % is decreased. The maximum percentage of Toluene, Ethylbenzene, m-pXylene and o-Xylene degradation was found to occur at 30, 37, 33, 37.5°C, respectively. The removal % increased as the temperature increased from 30 to 35 and then decreased. It is believed that sudden exposure to temperature higher than 35°C might have detrimental effects on the bacterial enzymes that are often responsible for the main step in the biodegradation process (El-Naas *et al.*, 2009). In addition, low temperature slows down the bacterial activity. The optimum temperature for the maximum specific growth rate of *Pseudomonas putida* falls between 30 and 35°C (Alagappan and Cowan, 2004). This is clearly reflected in the biodegradation rate of Benzene as an example of the BTEX, with 33°C as the optimum temperature. Moreover, the operating temperature can have major effects on mass transfer within the reactor, which could influence the reactor performance (Oh *et al.*, 1994).

Effect of Gas Flow rate

The interactions between the flow rate (X_2) and temperature (X_1) were analyzed using 3D response

surface plots. Figure 3 clearly shows that biodegradation efficiency of Benzene decreased with increasing the gas flow rate from 500 to 1150 mL min-. Maximum removal of approximately 94, 98, 95, 98 and 98% for Benzene, Toluene, Ethylbenzen, m-pXylene and o-Xylene, respectively, were obtained at the lowest flow rate of 500 ml/min. This is expected since longer gas residence time results in higher biodegradation rate. As the gas bubbles through the liquid in the bioreactor, its solubility in water limits its accessibility to the biomass.

Effect of pH

The initial pH of the mineral nutrient solution plays a significant role in the microbial growth and enzyme activity and it is often considered to be a key parameter for the development of biological treatment processes (El Hajjouji *et al.*, 2008; Yus Azila *et al.*, 2008). The effects of the initial pH of the mineral nutrient solution (from 5 to 9) on the BTEX degradation rate were determined and the results are shown in Fig. 4 for Benzene. The initial pH seemed to have significant effect on the biodegradation of BTEX, which seemed to increase with increasing pH, reaching the maximum at a pH value of 8. When the initial pH in the medium was lower than 6, the BTEX degradation rate significantly decreased, but declined slightly for pH values higher than 8 as shown in the figure for Benzene. The maximum percentage of Toluene, Ethylbenzen, m-pXylene and o-Xylene degradation was found to occur with pH 9, 8, 9 and 9, respectively. However, Benzene has maximum removal % at 5 pH. The physiological effect of pH on microbial activity is similar to that of temperature, as almost all biological species seem to have optimal pH conditions. Although some species are more tolerant to a wider pH range, others prefer a narrow pH range. Some species are good at high pH and some at low pH. For the biodegradation of BTEX mixtures by *Pseudomonas putida*, a study by You *et al.* (2013) revealed that biomass activity was fully inhibited at pH 5, 9 and 10 and the optimum was at pH 6- 8. Most bacteria are neutrophils, so the optimum pH at which the highest degree of BTEX biodegradation is achieved is usually around 7.5 (Słomińska *et al.*, 2012).

Effect of PVA Volume %

Three-dimensional plot representing removal percentage of Benzene degradation against PVA volume fraction (15-35%) and pH (5-9) is shown in Fig. 5, while keeping the gas flow rate and temperature constant. The volume fraction of PVA pellets is directly related to the number of active biomass cells in the bioreactor and hence plays an important role in determining the rate of BTEX degradation. The maximum removal efficiency for

Benzene, Toluene, Ethylbenzen, m-pXylene and o-Xylene were found at PVA volume fraction of 25, 25, 27.5, 26.5 and 26.5%, respectively. As the PVA volume fraction increases, the % BTEX removal increases. However, PVA volume fraction 25 and 30% seem to have almost the same removal efficiency. This may be due to the fact that the 30% has more biomass, while the 25% has better mixing. It is expected that increasing the amount of PVA volume fraction beyond 30% results in less mixing, consequently, lower degradation efficiency.
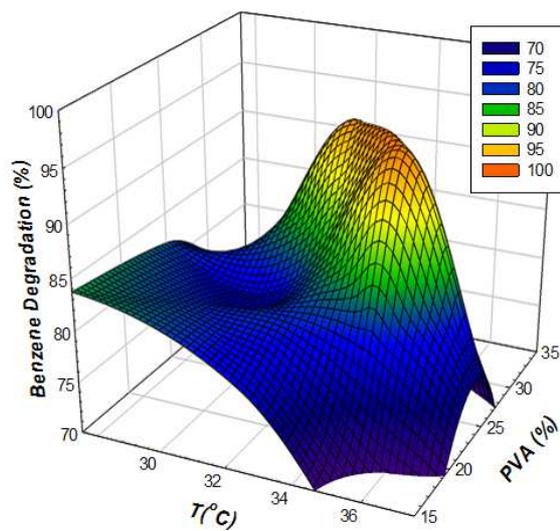
Fig. 2. Surface response optimization of Benzene degradation versus temperature and PVA volume
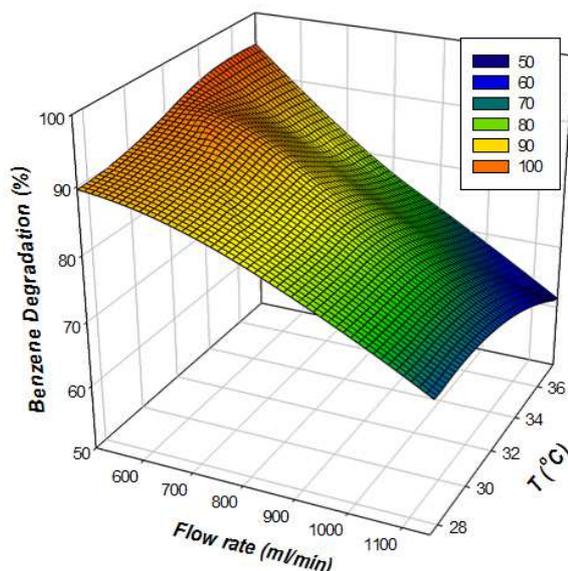
Fig. 3. Surface response optimization of Benzene degradation versus temperature and gas flow rate
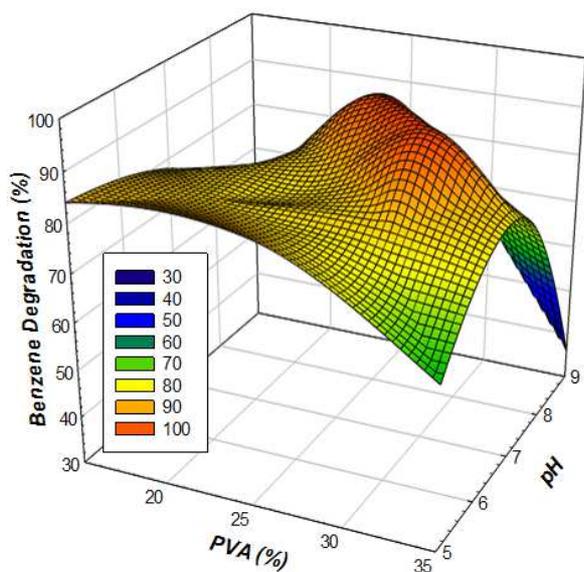
Fig. 4. Surface response optimization of Benzene degradation versus pH and PVA volume
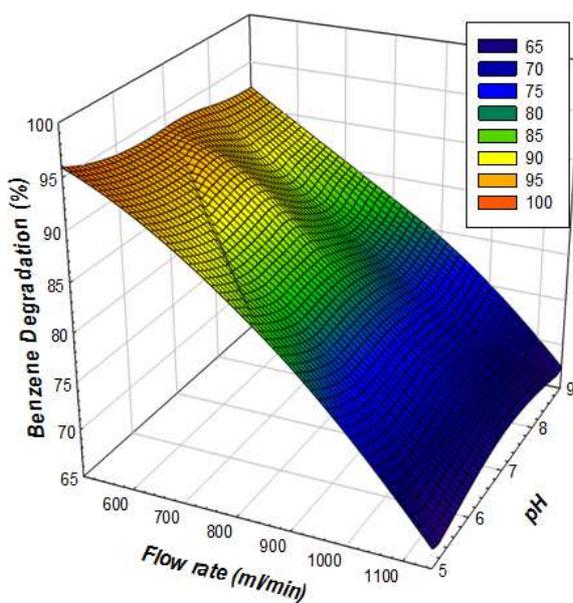
Fig. 5. Surface response optimization of Benzene degradation versus pH and gas flow rate

Desirability

Using Response Optimizer, the effect of the presence or absence of an individual compound on the biodegradation rate was predicted. Maximum removals of 94, 98, 95 and 98% were achieved for Benzene, Toluene, Ethylbenzene and m,p,o-Xylene at the optimal conditions determined by the response surface analysis.

In a mixture of BTEX, the isomers of Xylene were consumed first due to: (i) Different enzymatic systems catabolize the ring fission products of diverse catechols (Duggleby and Williams, 1986) and (ii) Xylene degradation may lead to accumulation and polymerization of intermediates such as toluic acid, tolualdehyde and methyl salicylic acid (Davey and Gibson, 1974; Chang *et al.*, 1993). Without the presence of Benzene, the removal can reach up to 99%, for Toluene, 97% for Ethylbenzene and 98% for the Xylenes. The absence of Benzene and Ethylbenzene enhanced the biodegradation of Toluene and Xylenes reaching up to 99 and 98%, respectively. It was observed that the presence of m-pXylene slows down the utilization of Toluene and Benzene which agrees with the findings reported by Oh *et al.* (1994).

The presence of Ethylbenzene inhibits BTX degradation (Reardon *et al.*, 2000). It was also reported that for the degradation of a binary mixture of Toluene and Benzene, the biomass *P. putida* F1 utilized Toluene better than Benzene. The presence of Toluene was also reported to inhibit the degradation of Benzene (Baggi, 2000; Jo *et al.*, 2008; Song *et al.*, 2012) {JIHYEON SONG, 2012, Kinetics and simulations of substrate interactions during the biodegradation of benzene`, toluene`, pxylene and styrene}. One can therefore predict that the presence of both Benzene and Ethylbenzene in the system inhibits the biodegradation of other compounds. The severe antagonistic effects during the biodegradation of mixtures of BTEX compounds can be caused by competitive inhibition, toxicity and the formation of toxic intermediates by nonspecific enzymes (Jo *et al.*, 2008).

Validation of the model

Experimental validation is an essential step for confirming the adequacy of the statistical model and this was performed through running experiments at the optimum conditions: 500 mL min^{-1}, 30% PVA, pH = 8 and maintaining the temperature at 33°C. The predicted and experimental response for Benzene degradation was exactly the same at 94%. For Toluene, Ethyl benzene and Xylenes the experimental value were all 2% lower than the predicted values at 97, 94 and 95%, respectively but still within the 95% Confidence Interval (95% CI). Therefore, all the experimental values were close to the predicted values. Moreover, response optimizer was used and compared with the experimental results for dual components (Benzene-Toluene). The response optimizer predicted that working with temperature = 32°C, flow rate = 500 mL min^{-1}, pH = 8 and PVA = 30% will give 95% removal for Benzene and 98% removal for Toluene, which are very close to the experimental results as showing in Fig. 6.
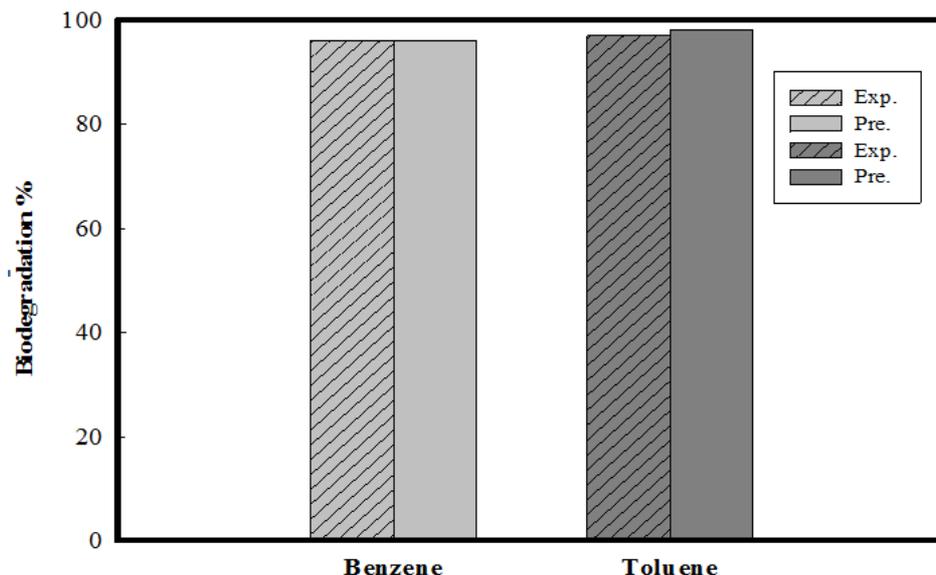
Fig. 6. A comparison of predicted and experimental results for the biodegradation of dual components (Benzene-Toluene) at T = 32°C; PVA volume = 30%; air flow rate at 500 mL min^{-1}

Conclusion

The biodegradation of BTEX was evaluated in a special spouted bed bioreactor system and the optimum values for the operating conditions were determined. The study revealed that second order polynomial was adequate in predicting the BTEX degradation within four independent variables: Temperature, gas flow rate, pH and PVA volume %. All four variables indicated significant effect on the biodegradation of BTEX and decreasing the gas flow rate played a major role in the removal efficiency. Analysis of Variance (ANOVA) for the biodegradation of BTEX provided a coefficient of determination (R^2) of 82.2%, which indicated that the model had a good fit. Desirability study revealed that the presence of Benzene and Ethylbenzene decreased the removal of other components. The overall optimum conditions predicted by the response surface model in order to get maximum removal of 94, 98, 95, 98% for BTEX were: A gas flow rate of 500 mL min^{-1}; an operating temperature of 33°C; a PVA volume fraction of 30% and a pH of 8.3.

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Author's Contributions

Ayat El Telib: She made considerable contributions including carrying out the experimental work, analyzing and modeling the experimental data. She also prepared the initial draft of the manuscript.

Muftah El-Naas: He made considerable contributions including designing the research plan and experimental procedure. In addition, he supervised the experimental work and revised the draft of the manuscript.

Janice Acio: She helped with carrying out the experimental work and data analysis.

Ethics

This paper is original and includes unpublished materials. The corresponding author authorizes that all other authors have read and agreed the manuscript and there is no ethical issues involved.

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