

Biodegradation of Toluene and Xylene in a Batch Culture of *Pseudomonas putida*

¹S. Saghafi, ²G. Najafpour, ¹Z. Bakhshi, ¹E. Kariminezhad and N. Rayatdoust

¹School of Civil Engineering, College of Engineering,
Babol Noshirvani University of Technology, Babol, Iran

²School of Chemical Engineering, College of Engineering,
Babol Noshirvani University of Technology, Babol, Iran

Abstract: Removal efficiency of *Pseudomonas putida* on biodegradation of toluene and xylene was investigated. The experiments were operated under batch condition; toluene and xylene with concentrations of 1000, 3000, 5000, 7000 and 10000 mg.L⁻¹ were selected as the sole carbon sources. Optical density and COD removal rate of *Pseudomonas putida* was investigated. With initial organic concentration of 3000 mg.L⁻¹, maximum removal of toluene and xylene were obtained. In the second stage, the kinetic model on utilization of toluene and xylene at a concentration of 3000 mg.L⁻¹ was defined. Monod biokinetic coefficients K_s and μ_{max} were also determined. The values for K_s for toluene and xylene were 6.78 and 6.79 mg.L⁻¹ and for μ_{max} were 0.0018 and 0.0026 h⁻¹, respectively.

Key words: BTEX • Optical density • COD • *Pseudomonas putida* • Toluene • Xylene • Kinetic model

INTRODUCTION

Nowadays, environmental pollution is one of the major concerns of mankind. One of the most common oil based pollutants for water supplies is BTEX compound. Benzene, toluene, ethylbenzene and mixture of xylene (BTEX) are monoaromatic hydrocarbons. Due to their high toxicity, may possess significant health risk and cause profound environmental problems [1-3]. These compounds are known as carcinogenic and mutagenic, also classified as priority environmental pollutants by USEPA [2, 4, 5]. Industrial activities such as, petroleum, oil refineries, plastics, contain high concentrations of monoaromatic compounds. The disposal of effluents contained these compounds without proper treatment causes negative impact on environment, since all of these organic compounds are toxic and are not easily degradable [1].

In comparison to other petroleum hydrocarbons BTEX compounds can be easily transmitted into groundwater [2, 4, 6]. Important sources of pollution originated by monoaromatic compounds are industrial wastes, leakage spills, improper disposal and accidents during oil transportation, storage tanks, gas work sites,

airports, paint manufactures, chemical industries and railway yards [2, 7, 8]. Benzene and toluene are discharged to environment from industrial effluents such as textile manufacture, wood processing, chemical and tobacco products. In addition, ethylbenzene and xylene are involved in manufacture of pesticides, chemicals, detergents, varnishes and paint [1].

One of the most economical and energy sufficient processes for remediation of contaminated water by monoaromatic compounds are biological methods known as bioremediation processes [2, 9]. For biodegradation of monoaromatic compounds some of the important requirements are electron acceptor, nutrients and environmental conditions. The most usual electron acceptors are oxygen, nitrate, Fe (III), sulfate and CO₂. In aerobic respiration, oxygen is utilized as electron acceptor while for anaerobic respiration other electron acceptors are used. Also, within the bioremediation process organic matter is utilized as carbon source. Nutrients, such as nitrogen, phosphorus, sulfur and trace elements are supplied to the medium. Microbial activity depends on several factors, such as, medium composition, temperature, pH and media salinity [10].

In biological treatment, consortia of microorganisms are able to degrade organic pollutants. Among the various microorganisms utilizing BTEX, *Pseudomonas putida* is one of the most distinct organisms in biodegradation of BTEX [11-13]. For treatment of contaminated water by monoaromatic hydrocarbons both aerobic and anaerobic processes are used. In anaerobic biodegradation, the organic matter is degraded into biogas (methane), microbial biomass and residual organic matters [14]. In compare to aerobic processes, anaerobic processes have several advantages including, high capacity to deteriorate concentrated organic pollutants, less production of sludge, low energy consumption and biogas production (methane) which is considered as energy source [9, 14-18].

The purpose of the present study was to investigate the biodegradation of toluene and xylene using pure culture of *Pseudomonas putida*. Also kinetic models for toluene and xylene biodegradation were obtained.

MATERIALS AND METHODS

Microorganism and Culture Propagation: *Pseudomonas putida* (PTCC 1694) provided by Iranian research organization for science and technology (IROST) was used throughout the study. In this study the medium used for cell growth contained glucose, yeast extract, K_2HPO_4 and NH_4Cl : 1, 0.3, 0.05 and 0.05, respectively. All media were sterilized in autoclave at 15 psig and 121°C for 20 min. The culture was inoculated with stock culture in a 250 mL flask contained 100 mL medium and then incubated at 32 °C in a shaker (Stuart, England) for 24 h.

Batch Experiments: Batch experiments were performed in 100 mL media contained toluene and xylene in the range of 1000 to 10000 mgL⁻¹. The C/N/P ratio was set at 300/5/1. Anaerobic conditions were secured by evacuating the desiccator by means of vacuum pump. The withdrawn samples from the Erlenmeyer were analyzed for 1, 3, 5 and 7 days. In the second stage, experiments were performed in a larger volume (250 mL Erlenmeyer). The media composition for carbon, nitrogen and phosphorus sources were 3000, 50 and 10 mg.L⁻¹, respectively. The sole carbon sources used for these experiments were toluene [$C_6H_5CH_3$] and xylene [$C_6H_4(CH_3)_2$] [19]. COD was determined by colorimetric method using spectrophotometer (UNICO 2100, USA) as defined in standard method [20]. The batch culture was under anaerobic condition and the media temperature for all batch experiments were set at 27°C.

Optical Density Measurement: In the prepared media, a 5% of the stock culture was used for inoculation. The experiments for determination of optical density were conducted in 20 h. Every 4 h samples were withdrawn from the Erlenmeyer flasks. Moreover, the optical density was determined at 420 nm wave length for each of the samples.

RESULTS AND DISCUSSION

Cell Density: In order to determine the cell density of *Pseudomonas putida* grown on toluene and xylene solution, optical density was measured. Figure 1a presents the optical density obtained at 420 nm wave length. The optimum growth rate of the microorganism was observed with a feed solution contained 3000 mg.L⁻¹ of toluene. Moreover, there are a slightly increase of optical density for an increase in concentrations 3000 to 5000 mg.L⁻¹. Thus, *Pseudomonas putida* had maximum growth rate for organic substances with concentration in the range 3000 to 5000 mg.L⁻¹. The optical density was not well performed for the concentrations higher than 7000 mg.L⁻¹; that was probably due to growth inhibition. The results obtained from Figure 1b shows similar trends for xylene as feed solution. But in comparison with toluene, the optical density for xylene was slightly lower than toluene. Therefore, the growth rate of *Pseudomonas putida* in toluene was slightly higher than xylene.

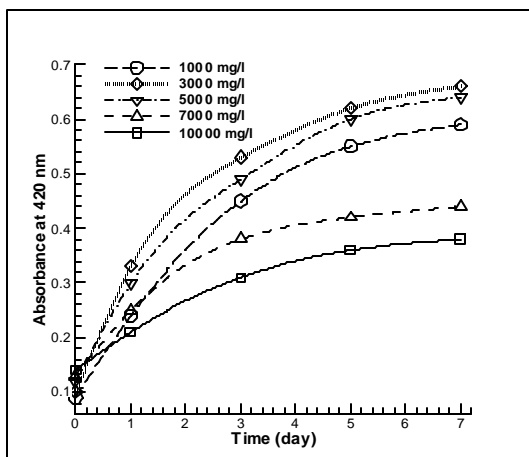
The COD concentration profiles for duration of 7 days and in the range of 1000 to 10000 mg.L⁻¹, are presented in Figure 2a and b. The COD removal efficiency was defined from the following equation:

$$\text{Removal efficiency (\%)} = \frac{100(S_0 - S)}{S_0} \quad (1)$$

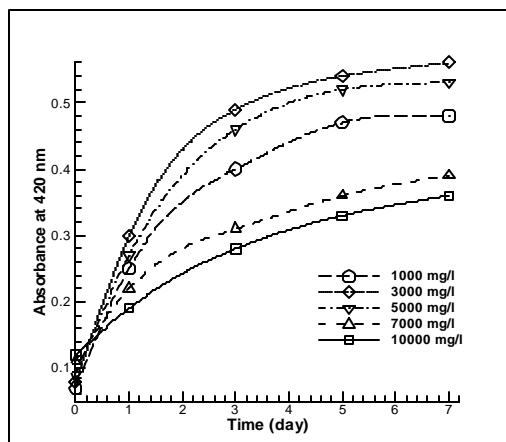
where S_0 (mg.L⁻¹) is the initial COD and S (mg.L⁻¹) is the final COD concentration [21].

The best COD removal efficiency was obtained for xylene and toluene at a concentration of 3000 mg.L⁻¹, as feed solution. Figure 3a and b shows the COD removal with respect to time. Maximum removal of COD for toluene and xylene were 58 and 45%, respectively.

Figure 4 shows cell dry weight and COD concentration profiles for toluene and xylene for 20 h incubation time. The sharp reductions of toluene and xylene concentrations were accompanied with rapid

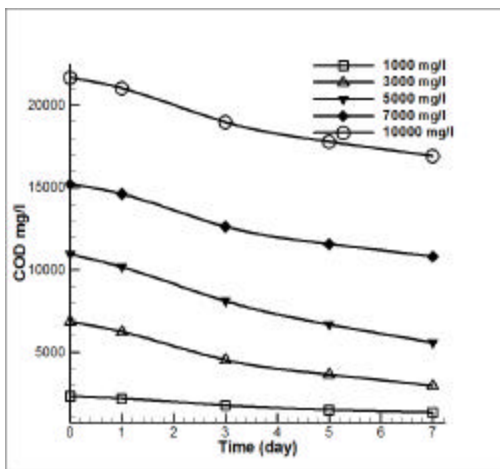


a.

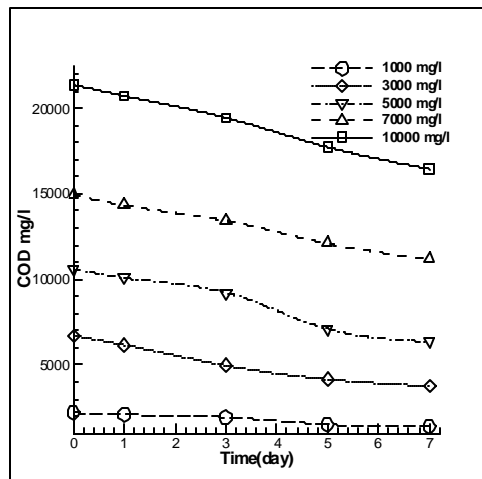


b.

Fig. 1: Optical density of *Pseudomonas putida*, a. toluene b. xylene

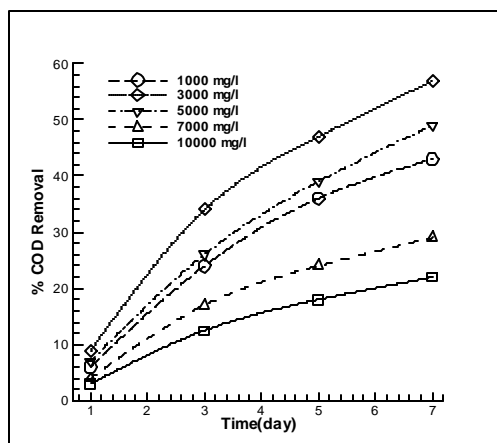


a.

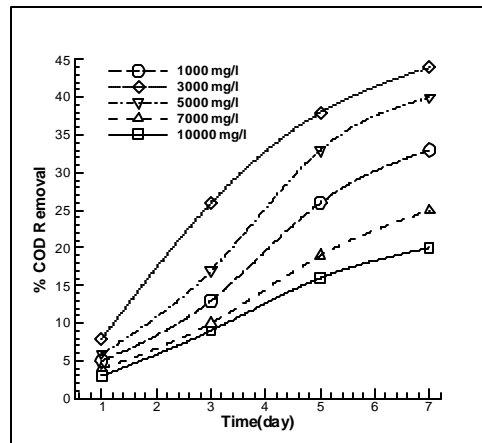


b.

Fig. 2: COD concentration profiles; a. toluene b. xylene



a.



b.

Fig. 3: COD removal; a. toluene b. xylene

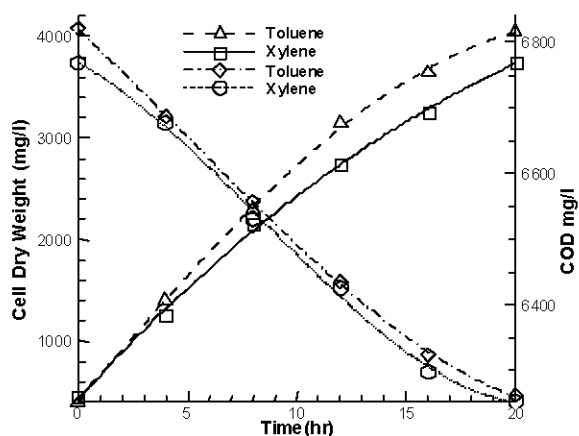


Fig. 4: COD concentration and cell dry weight for toluene and xylene

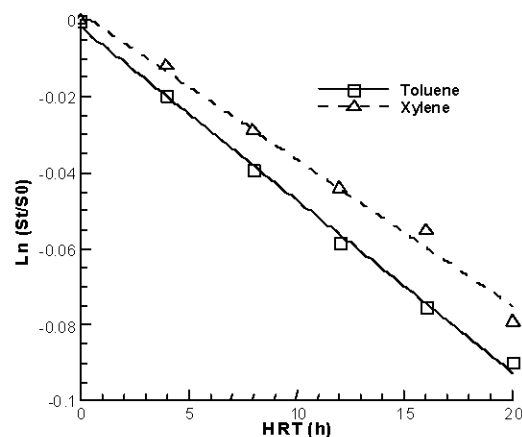


Fig. 5: Logarithmic model for substrate utilization

biomass formation. This means the organic chemicals were biologically degraded and biomass was also generated. The yield of biomass formation was 0.88 mg cell/ mg substrate.

Substrate Consumption Rate: One of the common models for the limited substrate consumption is the first-order reaction which is defined by the following equation [22]:

$$-\frac{ds}{dt} = k_s S \quad (2)$$

Where k_s is the rate constant. After characterization of equation (2), the substrate concentration and exponential growth profile with respect to HRT is defined as follows:

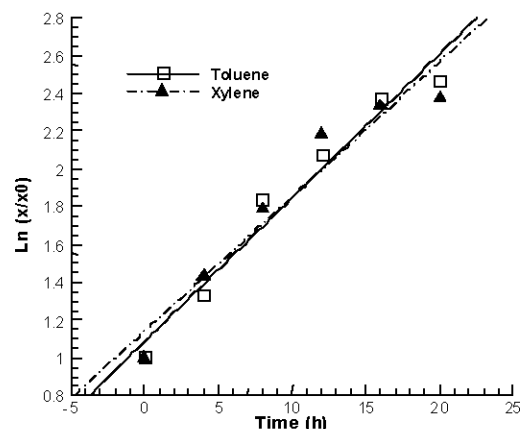


Fig. 6: Malthus model for toluene and xylene as feed solution

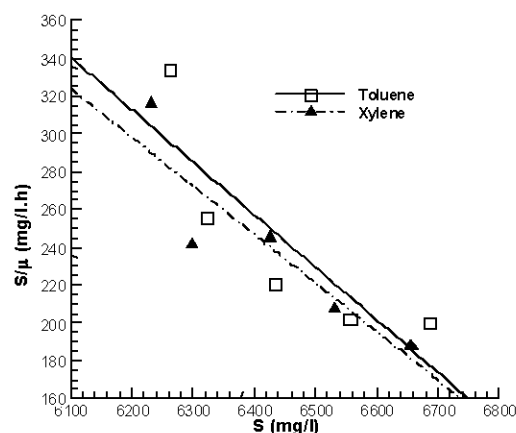


Fig. 7: Hanes-Woolf plot for toluene and xylene as feed solution

$$S = S_0 e^{-k_s t} \quad (3)$$

Where S is substrate concentration and S_0 is the initial substrate concentration.

The exponential growth of the organism can be converted to logarithmic rate model for substrate utilization is also defined as follows:

$$\ln \frac{S}{S_0} = -k_s t \quad (4)$$

Figure 5 shows the experimental data were well fitted with the projected model as the data for $\ln S/S_0$ vs. HRT had R^2 of 0.99.

Growth Kinetic in Batch Culture: Malthus model is a simple model which projects the biomass growth that follows the exponential pattern. The following equation describes unstructured Malthus model [23]:

Table 1: The Monod kinetic parameters, K_s and μ_{max} , for toluene and xylene

Parameter	μ_{max} (h^{-1})	K_s ($mg.L^{-1}$)
Toluene	0.0018	6.78
Xylene	0.0026	6.79

$$\frac{dX}{dt} = f(x) = \mu X \quad (5)$$

Where μ is the specific growth rate and X represents the biomass concentration.

The experimental data for biomass concentration with respect to time are shown in Figure 6. The data are well fitted with the proposed model. Biomass was grown on toluene and xylene as carbon sources.

The specific growth and the essential substrate utilization are shown with the Monod growth kinetic model which is given by the following equation [22]:

$$\mu = \frac{\mu_{max} s}{K_s + s} \quad (6)$$

Where μ_{max} is the maximum specific growth rate and K_s is the Monod rate constant.

The Monod model was linearized and modified by Hanes-Woolf plot (S/μ vs. S) as the data are presented in

The Monod kinetic parameters based on substrate consumption, K_s and μ_{max} , for toluene and xylene are summarized in Table 1.

CONCLUSIONS

BTEX are toxic compounds; they usually cause profound problems for human health and environment. From the results determined, it was demonstrated that by biodegradation of *Pseudomonas putida*, treatment of contaminated water supplies by monoaromatic compounds is possible. Moreover, the bacteria have quite satisfactory growth rate in a medium contained concentration below 5000 $mg.L^{-1}$. While, for concentrations higher than 7000 $mg.L^{-1}$ the removal efficiency of BTEX compounds did not have a good performance. The Monod biokinetic coefficients; K_s and μ_{max} were determined. The values presented for K_s for toluene and xylene were 6.78 and 6.79 $mg.L^{-1}$ and for μ_{max} were 0.0018 and 0.0026 h^{-1} , respectively.

Nomenclature

S	substrate concentration at t time ($mg.L^{-1}$)
S_0	substrate concentration at t_0 time ($mg.L^{-1}$)
K_s	first order rate constant (h^{-1})
i	specific growth rate (h^{-1})
i_{max}	maximum specific growth rate (h^{-1})
X	cell dry weight at t time ($mg.L^{-1}$)
k_s	Monod constant (h^{-1})
t	time, h

REFERENCES

1. Chakraborty R. And J.D. Coates, 2004. Anaerobic degradation of monoaromatic hydrocarbons. Appl. Microbiol. Biotechnol., 64: 437-446.
2. Farhadian, M., D. Duchez, C. Vachelard and C. Larroche, 2008. Monoaromatics removal from polluted water through bioreactors-A review. Water Research, 42: 1325-1341.
3. Ribeiro de navadi, I., R. ribeiro, M. Zaiat and E. Foresti, 2005. Anaerobic packed-bed reactor for bioremediation of gasoline contaminated aquifers. Process Biochemistry, 40(2): 587-592.
4. Chang, S.W., H.J. La and S.J. Lee, 2001. Microbial degradation of benzene, toluene, ethylbenzene and xylene isomers (BTEX) contaminated groundwater in Korea. Water Sci. Technol., 44(7): 165-171.
5. Martinez, A.B., D. Patureau, J.P. Delgenès and H. Carrère, 2009. Removal of polycyclic aromatic hydrocarbons (PAH) during anaerobic digestion with recirculation of ozonated digested sludge. J. Hazardous Materials, 162: 1145-1150.
6. Garoma, T., M.D. Gurol, O. Osibodu and L. Thotakura, 2008. Treatment of groundwater contaminated with gasoline components by an ozone/UV process. Chemosphere, 73(5): 825-831.
7. Souza, D.A., F.A. Chinalia, E. Foresti and M. Zaiat, 2009. Bioremediation of gasoline-contaminated groundwater in a pilot-scale packed-bed anaerobic reactor. International Biodeterioration and Biodegradation, 63(6): 747-751.
8. deNardi, I.R., M. Zaiat and E. Foresti, 2007. Kinetics of BTEX degradation in a packed-bed anaerobic reactor. Biodegradation, 18(1): 83-90.
9. Khelifi, E., H. Bouallagui, M.L. Fardeau, Y. Touhami, J.J. Godon, J.L. Cayol, B. Ollivier and M. Hamdi, 2009. Fermentative and sulphate-reducing bacteria associated with treatment of industrial dye effluent in an upflow anaerobic fixed bed bioreactor. Biochemical Engineering J., 45(2): 136-144.

10. Chakraborty, R., S.M. O'Connor, E. Chan and J.D. Coates, 2005. Anaerobic Degradation of Benzene, Toluene, Ethylbenzene and Xylene Compounds by *Dechloromonas* Strain RCB. *Appl. Environ. Microbiol.*, 71(12): 8649-8655.
11. Nikolova, N. And V. Nenor, 2005. BTEX degradation by fungi. *Water Sci. Technol.*, 51(11): 87-93.
12. Duetz, W.A., B. Wind, J.G. van Andel, M.R. Barnes, P.A. Williams and M. Rutgers, 1998. Biodegradation kinetics of toluene, *m*-xylene, *p*-xylene and their intermediates through the upper TOL pathway in *Pseudomonas putida* (pWWO). *Microbiol.*, 144: 1669-1675.
13. Das, K. And A.K. Mukherjee, 2007. Crude petroleum-oil biodegradation efficiency of *Bacillus subtilis* and *Pseudomonas aeruginosa* strains isolated from a petroleum-oil contaminated soil from North-East India, *Bioresource Technol.*, 98: 1339-1345.
14. Escudé, R., T. Conte, J.P. Steyer and J.P. Delgenès, 2005. Hydrodynamic and biokinetic models of an anaerobic fixed bed reactor. *Process Biochemistry*, 40(7): 2311-2323.
15. Bertini, L., S. Berselli, F. Fava, M. Petrangelipapini and L. Marchetti, 2004. Anaerobic digestion of olive mill wastewaters in biofilm reactors packed with granular activated carbon and Manville silica beads. *Water Research*, 38(14-15): 3167-3178.
16. Chaisri, R., P. Boonsawang, P. Prasertsan and S. Chaiprapat, 2007. Effect of organic loading rate on methane and volatile fatty acids productions from anaerobic treatment of palm oil mill effluent in UASB and UFAF reactors. *Songklanakarin J. Sci. Technol.*, 2: 311-323.
17. Ayati, B. And H. Ganjidoust, 2006. Comparing the efficiency of UAFF and UASB with hybrid reactor in treating wood fiber wastewater. *Iran. J. Environ. Health. Sci. Eng.*, 3(1): 39-44.
18. Chang, W., Y.S. Um and T.R. Pulliam Holoman, 2006. Poly aromatic hydrocarbon (PAH) degradation coupled to methanogenesis. *Biotechnol. Lett.*, 28(6): 425-430.
19. Aldrich, 1988-1989. Catalog hand book of fine chemicals. D.H. Hill.
20. Clesceri, L.S., A.E. Greenberg and R.R. Trussel, 1992. American Water Works Association, Water Pollution Control Federation, Standard Methods for The Examination of Water and Wastewater. 18th ed. APHA, American Public Health Association.
21. Metcalf and Eddy, 2003. Wastewater engineering treatment and reuse. 4th Edition, McGraw Hill, New York.
22. Najafpour, G.D., M. Tjallipour, M. Komeili and M. Mohammadi, 2009. Kinetics model for an up-flow anaerobic packed bed bioreactor: Dairy wastewater treatment. *African J. Biotechnol.*, 8(15): 3590-3595.
23. Najafpour, G., 2007. *Biochemical Engineering and Biotechnology*, Elsevier, Amsterdam, pp: 199-227.