Biodegradation of Polyaromatic Hydrocarbons by Acclimatized Mixed Culture Using Shake Flask and Roller Bioreactors

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Abstract

The ability of acclimatized mixed culture from sewage waste sludge was tested to biodegrade (PAHs):

mechanisms, preventing the e f cient biodegradation of solid PAHs. T e use of a roller bioreactor partially overrides such a problem [11,12].

In this study sewage waste sludge was investigated as a mixed bacterial culture for the biodegradation of naphthalene and phenanthrene which are commonly used as model compounds for PAH biodegradation with two system types: shake fask and roller slurry bioreactors. All the experimental data were fted to logistic and second order inhibition models, in order to better characterize the biodegradation process.

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In the present study, the used naphthalene and phenanthrene, are of analytical grade (CDH India). T e mineral salt medium (MSM) is of analytical grade also (CDH and Merck, India). T e nutrient broth was purchased from HIMEDIA India.

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For microorganisms' growth, McKinney's modifed medium mineral salt medium (MSM) was employed. Tables 1 and 2 show composition and trace elements in one liter of MSM, respectively. Preparation of the medium involved the mixing of inorganic chemicals with distilled water to make a bu fered solution of pH of 6.5-6.7.

Substance	Mass or Volume
Trace element, ml	1
$Fe(NH_4)_2SO_4$, mg	10
$MgSO_4$, mg	30
$CaCl_2$, mg	30
NaCl, mg	30
$(NH_4)_2SO_4$, mg	237
K_2HPO_4, mg	375
KH_2PO_4, mg	420

Table 1: Modi fed McKinney's medium in 1 liter of distilled water.

Substance	Mass (mg)
$CuCl_2$	10
$NiCl_2$	20
Na_2MoO_4	2O5

Giardia lambihia (CFU/ml)	90000

Table 3: Physical, chemical and biological characteristic of sewage sludge.

T e physical, chemical and biological characteristics of the sewage sludge are shown in Table 3. T e prevailing bacteria in the microbial consortium was

mixer. The procedures of centrifuging, decanting and rinsing were repeated three times. Finally, this biomass suspension was transferred to pre-weighed aluminum boats and dried at 105°C for 24 hours. The original broth was also subjected to a series of dilutions and analyzed for OD. These dilutions provided a range of known concentrations with measured ODs and were used to plot the dry-weight calibration curve.

The relation: (Biomass concentration (g/L)=0.9 × OD) consequently from the previous procedure.

np on dh pmd

d b od a c oc c oc d nc f flask d o

The effect of using acclimatized mix culture of microorganisms for the biodegradation of PAHs using shake flask bioreactor were investigated in these experiments. Figure 2 shows that the PAHs concentrations decreased continuously with time until depletion, indicating that the acclimatized mixed culture from sewage waste sludge could effectively degrade these compounds. Naphthalene was degraded completely after 13 days while phenanthrene was degraded completely after 14 days. The proximity of the biodegradation of these two compounds in spite of their significantly different chemical structures (two benzene rings in naphthalene vs. three fused rings in phenanthrene) and water solubilities (32 mg/L for naphthalene vs. 1.6 mg/L for phenanthrene at 25°C) is not eworthly. It may be due to the fact that the microorganisms were efficiently acclimatized on PAHs although phenanthrene was thought to be the more difficult compound to biodegrade.

Figure 2: PAH biodegradation in shake flask bioreactor.

Romero et al. [7] isolated *Pseudomonas aeruginosa* from a stream heavily polluted by a petroleum refinery. Complete removal of high dosages of phenanthrene (200 mg/L) in a period of 30 days was found.

Nasrollahzadeh et al. [10] studied the biodegradation of phenanthrene using mixed consortia of microorganisms from the effluents of a local industrial zone. The biodegradation data of phenanthrene indicate about 100%, 100% and 85% degradation at concentrations of 20, 50 and 100 mg/L, respectively within 6 days.

Also, Janbandhu and Fulekar [8] reported that the biodegradation data of phenanthrene indicated to about 100%, 56.9% and 25.8% degradation at concentrations of 100, 250 and 500 mg/L, respectively within 14 days by using adapted microbial consortium from an old petrochemical refinery field.

The results obtained in the present research, in which high dosage of phenanthrene (300 mg/L) was completely degraded after 14 days, are in line with previous results. This indicates that the microbial consortium from sewage waste sludge have a promising application in bioremediation of PAH contaminated environments.

Figure 2 shows the variation of biomass concentration with time. Biomass concentration increases with time which indicates that the microbes utilized the PAHs as energy and carbon source. An

Romero et al. [7] reported that the specifc growth rates for *Pseudomonas aeruginosa* isolated from a contaminated stream were equal to 0.041 and 0.037 h^{-1} for phenanthrene concentrations of 100 and 200 mg/L, respectively. Also, Lei et al. [14] found that the specifc growth rate of *Pseudomonas mendocina* was equal to 0.033 h^{-1} for 100 mg/L phenanthrene.

In the present research the specifc growth of phenanthrene is equal to 0.016 h^{-1} only, which is less than the corresponding values

$$= \frac{max}{1 + \left[\ln\left(\frac{max}{0} - 1\right) - \right]} \quad (4)$$

D is the naphthalene or phenanthrene removal e f ciency (%) at time t, D_0 and D_{max} are the initial and maximum PAHs removal e f ciencies (%), and μ_D is the speci f c degradation rate (h^{-1}).

T e model parameters with their values are shown in Table 4. Figure 6 shows a successful f tting between the theoretical and the experimental results.

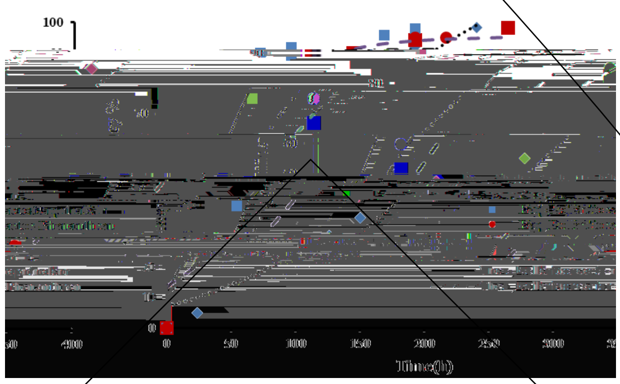

Figure 6: Simulated PAH removal e f ciency f tted with Logistic model in shake and roller bioreactors.

Figure 9: Simulated cell concentration for PAH fitted with Logistic model in roller bioreactor.

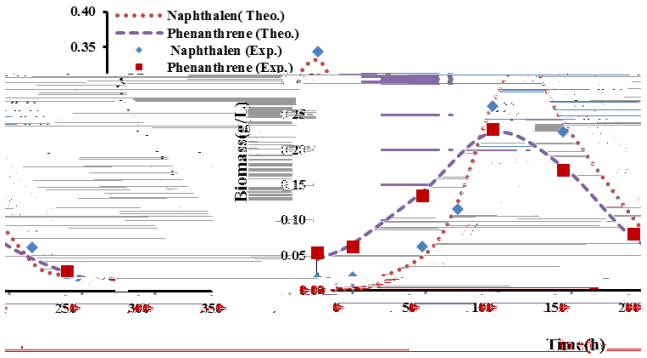

Figure 10: Simulated cell concentrations for PAH fitted second order inhibition model in roller bioreactor.

It was observed that the experimental data were well represented by the proposed models. The parameters defining the logistic models and second order inhibition model for the roller bioreactor are presented in Table 3, with R^2 of more than 97%. The results for the biodegradation of naphthalene and phenanthrene in the roller bioreactor were fitted to the Logistic equation (4). A fair fitting was obtained as shown in Figure 6. The model parameters with their values are shown in Table 4, with R^2 of 97%.

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The biodegradation of naphthalene and phenanthrene by an acclimatized mixed culture from sewage waste sludge was investigated in two types of reactors: shake flask and roller bioreactors. The results show that complete biodegradation of naphthalene and phenanthrene was achieved after 13 and 14 days, respectively in the shake flask bioreactor. While in the roller bioreactor complete biodegradation was achieved after 11 and 12 days, respectively. The specific growth rate was observed to be 0.014 and 0.016 h^{-1} for naphthalene and phenanthrene, respectively, in the shake flask bioreactor while the corresponding values were 0.022 and 0.012 h^{-1} in the roller bioreactor.

The results indicate that the roller bioreactor enhanced the biodegradation of naphthalene due to its efficient mixing which enhanced the dissolution of naphthalene particles to the aqueous phase making it more bioavailable to the microorganisms. The roller bioreactor didn't show any enhancement to the biodegradation of phenanthrene, this may be due to the very low solubility of this polyaromatic compound in water and the need to use a surfactant to enhance its solubility.

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