Combination of UV-Fenton oxidation process with biological technique for treatment of polycyclic aromatic hydrocarbons using *Pseudomonas pseudoalcaligenes* NRSS3 isolated from petroleum contaminated site

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*Received 14 November 2017; revised 09 January 2018*

Polycyclic aromatic hydrocarbons (PAHs), often from petroleum oil spill, by-product of petroleum refining, incomplete combustion of fossil fuel, leakage in pipeline and underground storage, apart from the effluents of pesticide, dye, pigment, and drug industries, are considered carcinogenic and mutagenic. As the abundance of PAHs in the environment cause adverse effects on humans and ecosystem, the PAHs contamination needs to be monitored and such polluted sites require remediation. Conventional methods available for remediation of PAHs are adsorption, advance oxidation process, electrochemical remediation, solvent extraction, use of synthetic surfactants and photocatalytic remediation. These methods including the alternative Fenton oxidation technology are not only expensive but also produce secondary pollutants. In this study, we evaluated the performance of UV-Fenton-PBBR (Packed bed bioreactor) hybrid system for the treatment of polycyclic aromatic hydrocarbons (naphthalene and fluorene). *Pseudomonas pseudoalcaligenes* NRSS3 isolated from petroleum contaminated site and immobilized on *Sterculia alata* was used as packing media in the PBBR. The naphthalene and fluorene were taken as model polycyclic aromatic hydrocarbon (PAHs) with initial concentration of 400 mg/L. The optimum conditions for UV-Fenton oxidation were (pH: 3, Fe\(^{2+}\): 2.5 g/L, H\(_2\)O\(_2\): 1000 mg/L) for naphthalene and (pH: 3, Fe\(^{2+}\): 3.0 g/L, H\(_2\)O\(_2\): 1200 mg/L) for fluorene. The overall maximum removal efficiency of the combined system was found to be 96 and 94.7% for naphthalene and fluorene, respectively. GC-MS analysis confirmed the formation of catechol, 1-naphthol, salicylic acid and phthalic anhydride as metabolites during degradation process. Biodegradation kinetics of naphthalene and fluorene were studied using Monod model and kinetics constants were found to be \(\mu_{\text{max}}: 0.3057\) per day; \(K_s: 112.87\) mg/L for naphthalene and \(\mu_{\text{max}}: 0.2921\) per day; \(K_s: 114.75\) mg/L for fluorene.

**Keywords:** Biodegradation, Fluorene, GC-MS, Kinetics, Naphthalene, Oil spill, PBBR, Pollution, Remediation, SEM, UV-Fenton

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of soil and wastewater\textsuperscript{19}. The major drawbacks of these methods are high cost and production of secondary pollutants.

Biological treatment involving microorganisms as biocatalyst for degradation of hydrocarbon compounds are not only efficient but also environment friendly and cost effective\textsuperscript{2,17,20-22}. Degradation of high molecular weight compounds (HMW) is slower as compared to low molecular weight (LMW) PAHs\textsuperscript{23}. Microorganisms, such as \textit{Pseudomonas, Acinetobacter, Rhodococcus, Bacillus, Paenibacillus} and \textit{Mycobacterium} are used for bioremediation of PAHs\textsuperscript{24}. As the microbial strains metabolize only limited range of organic compounds, they are unable to provide a sustainable solution for the actual problem (BOD: COD <0.2)\textsuperscript{10,17}. Sakulthaew et al.\textsuperscript{25} attempted combination of chemical and biological treatment of PAHs in the effluents wherein only limited studies are available.

In this direction, here, we designed and fabricated a hybrid system consisting of UV-Fenton oxidation-Packed bed bioreactor (PBBR), and its performance was evaluated under varying process condition for treatment of naphthalene and fluorene. In the PBBR, \textit{Pseudomonas pseudaicaligenes} NRSS3 isolated from petroleum contaminated site and immobilized on \textit{Sterculia alata} was used as packing media.

\textbf{Materials and Methods}

\textbf{Chemicals and composition of mineral salt medium}

Analytical grade fluorene and naphthalene with purity more than 98.0\% (Sigma-Aldrich, India) was used in the experiment. Hydrogen peroxide (30\%, v/v) and ferrous sulphate (FeSO\textsubscript{4}·7H\textsubscript{2}O) were purchased from Merck, India. Sulfuric acid (1N-H\textsubscript{2}SO\textsubscript{4}) and sodium hydroxide (1N-NaOH) (Merck, India) were used to adjust the pH during UV-Fenton oxidation process. A non-ionic surfactant TritanX-100 (Sigma-Aldrich, India) was used to enhance the solubility of PAHs in distilled water. The enrichment of bacteria was done in mineral salt medium (MSM) containing 1.0 g of K\textsubscript{2}HPO\textsubscript{4}, 2H\textsubscript{2}O, 1.0 g KH\textsubscript{2}PO\textsubscript{4}, 0.5 g NaCl, 0.3 g MgSO\textsubscript{4}·7H\textsubscript{2}O and 0.3 g (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} per litre of distilled water is used as source nutrient for the growth of microorganism. The trace elements solution containing 0.02 mg CaCl\textsubscript{2}, 0.0023 g FeCl\textsubscript{3}, 0.005 g MnSO\textsubscript{4}·H\textsubscript{2}O, 0.005 g ZnSO\textsubscript{4} and 0.001 g (NH\textsubscript{4})\textsubscript{6}Mo7O\textsubscript{24} per litre of solution was also prepared as a source of micronutrient for microorganism. Final pH of the MSM was adjusted to 7.0±0.2 and sterilized by autoclave at 121°C for 15 min.

\textbf{Isolation and identification of PAHs degrading bacterial species}

PAHs contaminated soil and wastewater samples were collected from IOCL refinery, Mathura (UP) India (27°30'12" N, 77°40'19" E and 181 m elevation above sea level) and stored at 4°C for further use. Soil sample (10 g) was added to flask (250 mL) which containing 100 mL MSM and PAHs ranges from 25 to 300 mg/L. These flasks were incubated at 150 rpm and at 37°C for one week. After one week, 20 mL sample aliquot was transfer to 100 mL fresh MSM containing 25-300 mg/L of PAHs and again incubated. This process was repeated to thrice for acclimation of microorganism in PAHs environment. The pure species was obtained by diluting one mL of culture by diluting factor (1000) and spreading a 200 µL of aliquot on MSM-agar plates. Single colony was harvested from the plate based on difference in morphology. Finally, this colony was transferred to fresh agar-MSM plate and retained for further use.

\textbf{UV-Fenton oxidation process (Batch process)}

The UV-Fenton oxidation experiments were conducted in the batch mode. Experimental set up consisted a cylindrical glass reactor with working volume of 2 litres and coupled with UV light (Mineral light V41, Ultraviolet Prod. Inc) at room temperature (25±2°C) with 150 rpm (Fig. 1). Tubing in the reactor assembly was made of silicon and peristaltic pumps (Miclins Peristaltic pump PP 10) were used for feeding the input during experiments. The experiments were carried out to optimize the process parameter for PAHs and COD reduction. The parameters, such as pH, ferrous ions (Fe\textsuperscript{2+}) and dose of hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) were optimised. Optimisation experiment work carried out keeping the PAHs concentration fixed at 100 mg/L in UV-Fenton reactor. For the pH optimization, all other parameters were kept fixed and pH was varied in the range (1.0-4.0) and similarly, effect of Fe\textsuperscript{2+} (0.5-4.0 g/L) and H\textsubscript{2}O\textsubscript{2} (200-1600 mg/L) were optimized at optimum pH. Finally, the optimum process time for PAHs removal was calculated at optimum pH, Fe\textsuperscript{2+} ions, and H\textsubscript{2}O\textsubscript{2}. The experiments were performed in triplicates to minimize experimental error.

\textbf{Biological process}

The Packed bed bioreactor (PBBR) was fabricated using a cylindrical borosilicate glass having internal diameter and height of 6 and 65 cm, respectively with total reactor volume of 1.837 L and working volume of one litre Fig. 1. Agrowaste biomaterial (\textit{Sterculia alata})
was collected from IIT (BHU) campus, Varanasi, India and used as a packing media in bioreactor for PAHs degradation. *Sterculia alata* were cut into small pieces, dried overnight at 40°C, rinsed with 70% (v/v) ethanol and then washed thoroughly with distilled water and sterilized. In a 1000 mL flask, 500 mL of MSM media, 50 g of packing material, 10 mL inoculum was added and kept for 20 days in order to immobilize the bacterial cells properly on the surface of the packing material. To perform the biological experiments, bacteria were cultured in mineral salt media supplemented with PAHs to an optical density (OD600) of approximately 2.0±0.2. The effect of pH (5.0, 6.0, 7.0, 8.0, and 9.0), DO (4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5 mg/L) were studied for their influence on growth of isolated strain in the biodegradation of PAHs.

**Coupled UV-Fenton-packed bed bioreactor**

The coupled UV-Fenton-PBBR system used for degradation of PAHs compounds which has been designed and constructed in the laboratory as shown in Fig. 1. The experimental set-up consisted of three (1<sup>st</sup> UV-Fenton, 2<sup>nd</sup> stabilization and 3<sup>rd</sup> Biological) stages. First stage experiments were performed in a 2L reactor filled with 500 mL of PAHs sample mixed at 150 rpm using magnetic stirrer (ELICO; GI 631) for 2 h at room temperature 25±2°C. The pH of solution was pre-adjusted to 2.5 using 1N H<sub>2</sub>SO<sub>4</sub> and 1N NaOH. Then Fe<sup>3+</sup> (2.5 g/L) and H<sub>2</sub>O<sub>2</sub> (1000 mg/L) were added into the reactor at the beginning of the process. After Fenton experiment, effluent was filtered using filter paper (0.22 µm GSWP, Merck Millipore Ltd.) with filtration unit. UV-Fenton pretreated effluent was fed to 1L stabilization reactor (2<sup>nd</sup> stage) and adjusts the pH adjusted at 7.0±0.2 suitable for the microbial growth.

The effluent from the stabilization reactor was filtered (0.22 µm) and fed to the PBBR (3<sup>rd</sup> stage) with the peristaltic pump. PBBR fabricated of a cylindrical borosilicate column and packed with equal size, autoclaved, small cubical pieces (1 cm) of *Sterculia alata*. The sampling ports were provided for collect inlet sample (5 cm from bottom) and outlet sample (20 cm from top) in PBBR. A valve was provided at the bottom of the reactor to collect the leachate. All sampling ports of the reactor were close by silicon tubing with pinch cork. The biological degradation of PAHs in the PBBR was performed at room temperature (25.0±0.2). The air filtered through 0.22 µm filtration unit was supplied using a compressor (XP-AC-24L, Xtra Power) to keep the aerobic environment in the PBBR. A calibrated rotameter (SRS-MG5, Eureka, Pune) was used to control the flow rate of air (0.2-0.5 L/min) in the bioreactor.

**Morphological analyses of immobilised microbes on Sterculia alata**

Developed uniform biofilm of *P. pseudoalcaligenes* on the *Sterculia alata* was analyzed by SEM (EVO-18, ZEISS) during the degradation of PAHs. To obtain the morphology of immobilised *Sterculia alata* samples were filtered (0.22 µm GSWP, Merck Millipore Ltd) and obtained on filter paper. Further, the *S. alata* samples were dried for 24 h at 37°C in an oven (NSW-151, India). To obtain effective resolution, samples were coated with fine carbon and
Chemical oxygen demand, gas chromatography, gas chromatography-mass spectrometry analysis

The overall degradation of PAHs compound was evaluated on the basis of variation in chemical oxygen demand (COD) using UNIPHOS COD digester and analyser. During COD analysis, pH of the treated sample was adjusted at 10 to eliminate the interference of ferrous sulphate and hydrogen peroxide. The samples were centrifuged at 8000 × for 15 min to settle down bacterial cell mass and supernatant (containing PAHs) extracted twice with 5 mL of n-hexane. The extracts were dried over sodium sulphate (Na₂SO₄) for GC analysis. The concentration of naphthalene and fluorene in samples were measured by using a gas chromatograph (GC) with flame-ionization detector (FID) (Thermo scientific Trace 1110) and TG-5MS column (30 m × 0.25 mm × 0.25 µm) with N₂ as carrier gas. The temperature of column, injector and detector were kept at 250, 260 and 300°C, respectively for GC analysis of naphthalene. The temperature of column, injector and detector were kept at 280, 310 and 320°C, respectively for GC analysis of fluorene.

Two equal volumes of ethyl acetate were used to extract the metabolites of naphthalene and fluorene for GC-MS analysis. The analysis of metabolites was carried out at JNU, New Delhi using GC-MS (QP2010) with Rxi-5 Sil MS column (30 m x 0.25 mm i.d. X 0.25 µm film thickness). The MS measurements were performed at a temperature of 230 for ion source and 270 for interference with 3.5 min solvent delay.

Growth kinetics of polycyclic aromatic hydrocarbon biodegradation

Separate set of batch experiments were performed for naphthalene and fluorene in concentration range 20-400 mg/l for kinetic growth study. The kinetics of P. Pseudoalcaligenes growth and utilization of substrate is described using Monod model Eq. (1).

\[ \frac{dC}{dt} = \mu_{\text{max}} \left( \frac{X}{J_s + X} \right) \]

where \( C_o \) & \( C \) are biomass concentration (mg/L) between time t=0 and any time t; \( X \) is the substrate concentration (mg/l); \( \mu \) is specific growth rate (per day), \( \mu_{\text{max}} \) is maximum specific growth rate (per day), \( J_s \) is half rate constant (mg/l).\(^{16,27}\) The equation is converts into linear form as given below (eq. 2).

\[ \frac{1}{\mu} = \left( \frac{J_s}{\mu_{\text{max}} X} \right) + \frac{1}{\mu_{\text{max}}} \]

The value of \( 1/\mu \) and \( 1/X \) were calculated and plotted to obtain the values of \( \mu_{\text{max}} \) and \( K_s \).

Results and Discussion

Molecular characterization and phylogenetic analysis of isolated microorganism

The molecular characterization of species nucleotide sequences was carried out at Bioraj Laboratories, Nagpur, India and a sequence was submitted to NCBI. The Gene bank database (NCBI) was used to search for 16S rRNA sequences homologous to the 16S rRNA of the isolated species. The result shows that the P. pseudoalcaligenes NRSS3 sp. was most abundant having accession numbers (MF992192) and the phylogenetic evolutionary was performed by Neighbour-Joining method as shown in Fig. 2. Similar results for Pseudomonas sp. was reported by various researcher.\(^{27,29}\)

UV-Fenton’s oxidation process: Parameter optimisation

pH is one of the important aspect in oxidation of PAHs compound in the UV-Fenton oxidation process. The experiments were conducted at the pH range of 1.0-4.0 for 120 min by dosing Fe²⁺ (2.5 g/L) and H₂O₂ (600 mg/L). The % removal of COD was found to be 41.5, 52.7, 57.5, 64.3 and 68.6% at the pH 1.0, 1.5, 2.0, 2.5 and 3.0, respectively for naphthalene degradation. Further increased in the pH from 3.5 to 4.0 resulted in the decrease in % COD removal from 58.6 to 50.3. Similarly, the percentage removal of COD for fluorene was found to be 38, 42.7, 47, 54 and 57.8% at the pH 1.0, 1.5, 2.0, 2.5 and 3.0, respectively.

![Phylogenetic tree based on 16S rRNA gene sequences for Pseudomonas pseudoalcaligenes NRSS3 (MF992192)](image)
respectively and decreased to 38.8% with increase in the pH to 4.0 as presented in Fig. 3A. The decreased in COD removal above pH 3.0 may be due to scavenging of OH* with H+ ions as well as conversion of Fe2+ into Fe3+ which further combines with OH* to produce Fe(OH)3 and precipitates.

The Fe2+ ion concentration for COD removal of naphthalene and fluorene was varied in the range of 0.5-4.0 g/L. The H2O2 dose, process time and optimum pH were maintained at 600 mg/L, 120 min and 3.0 respectively, during this experiment. The percentage COD removal increase with Fe2+ ion concentration and reached to maximum value of 71.8% at ferrous ion concentration of 2.5 g/L above this increase in the concentration of Fe2+ did not affect the COD removal. Similarly, in the case of fluorene, maximum 67.6 % COD removal was obtained at 3.0 g/L Fe2+ as shown in Fig. 3B. The decreased in percentage removal of COD at increased Fe2+ ion concentration presumably due to direct reaction of hydroxyl radical (OH*) with ferrous ion at high concentration to form ferric ion. In a similar study, Lin et al. reported the effect of Fe2+ ion on removal of 16 PAHs from textile effluent.

The effect of H2O2 during optimisation was varied from 200–1600 mg/L for both naphthalene and fluorene at optimum value of pH (3.0), Fe2+ ion (2.5 and 3.0 g/L, respectively) and contact time 120 min. The effect of H2O2 concentrations on % COD removal of naphthalene and fluorene in UV-Fenton reactor is presented in Fig. 3C. The results showed 67.1% COD removal for H2O2 dose of 1000 mg/L. Similarly for fluorene, optimum COD removal was found to be 63.2% at H2O2 dose of 1200 mg/L (Fig. 3C). The increase in concentration of H2O2 above the optimum limit did not result in any further COD removal. Ebraheim et al. have reported degradation of organic pollutants using Photo-Fenton process in which degradation rate of organic compounds increased with increasing H2O2 concentration until a critical limit, and above this limit H2O2 reacted with the OH* competing with organic pollutants and consequently decreased the efficiency of the process.

**Bioreactor: Parameter optimisation**

The effect of pH (5.0–9.0) and DO (4.0-7.5 mg/L) on the bioremediation (in terms of % COD removal) of naphthalene and fluorene were studied at concentration of 100 mg/L. As shown in Fig. 4A, the maximum COD removal (70.2% naphthalene and 65.6% fluorene) was obtained at neutral pH (7.0). The removal efficiency significantly declined when the pH...
was less than 6 or more than 8. When pH was decreased from 7 to 5, the removal was also found decreased to 44.8 and 40.9%, for naphthalene and fluorine respectively. Similar result was obtained when pH was increased from 7 to 9. The pH range of 6.0 to 8.0 was found favourable for microorganism’s growth by various researchers as well\textsuperscript{23,33}. In a similar study, Lin et al.\textsuperscript{28} reported maximum removal of naphthalene at 7 pH while at acidic or alkaline conditions inhibit bacterial activity or growth.

The maximum COD removal of 66 and 61.4% for naphthalene and fluorine, respectively was obtained at DO level 6.5 mg/L as given in Fig. 4B. Higher loading rate of substrate consumption by microorganism is reported to be affected by DO level\textsuperscript{34}. The low concentration of oxygen due to low DO level in the bioreactor could be a reason for decrease in the growth rate of microorganism\textsuperscript{34}.

**Study of Coupled UV-Fenton-packed bed bioreactor**

The schematic of UV-Fenton-packed bed bioreactor as described in Fig. 1 is coupled system consist of three stages treatment process. These are UV-Fenton (advanced oxidation process), pH stabilisation and PBBR (biological) treatment. In the first stage process, naphthalene and fluorene samples were subjected to UV-Fenton reactor as a pre-treatment option before biodegradation. Naphthalene and fluorene were treated in two separate reactors. The naphthalene was treated under optimized conditions: Fe\textsuperscript{2+}: 2.5 g/L, H\textsubscript{2}O\textsubscript{2}:1000 mg/L, pH: 3 and contact time 120 min. The results demonstrated 67.1% naphthalene removal was obtained at 400 mg/L naphthalene concentration (Fig. 5). The fluorene removal was performed under the optimized conditions: Fe\textsuperscript{3+}:3.0 g/L, H\textsubscript{2}O\textsubscript{2}:1200 mg/L, pH: 3.0 and process time of 120 min. The results indicated 63.7% fluorene removal with an initial concentration of 400 mg/L.

In the second stage, the effluent from the UV-Fenton reactor was filtered through filtration unit (0.22 µm) and fed to equalisation tank to maintain the pH 7±0.2. The pH is one of the vital parameter for microbial treatment of naphthalene and fluorene. The pH of effluent was adjusted (by 1N H\textsubscript{2}SO\textsubscript{4}/NaOH) nearly neutral (7±0.2) and fed to the PBBR for microbial treatment\textsuperscript{23}.

In the third stage, remaining naphthalene (131 mg/L) and fluorene (145 mg/L) were treated by biological process (PBBR). *P. pseudoalcaligenes* immobilized on *Sterculia alata* was used as packing material and the biofilm that formed as a result is shown in Fig. 5. The naphthalene was treated under the optimized conditions (pH 7 and DO 6.5 mg/L). The concentration of naphthalene and fluorene was monitored day to day during the biological treatment. Naphthalene removal was increased to 84% on the 4\textsuperscript{th} day and maximum removal of 96% was obtained on 9\textsuperscript{th} day. Similarly, fluorene removal was increased to 76% on the 4\textsuperscript{th} day and maximum removal of 94.7% was obtained on 10\textsuperscript{th} day. After this, no significant removal was obtained during the treatment. In the coupling system, the removal efficiency enhanced from 67.1 to 96% for naphthalene, and 63.7 to 94.7% for fluorene. The coupled system gave better result than the individual treatment. The coupled system was used for the treatment of different substrates\textsuperscript{35,36}.

**Scanning electron microscopy analysis of Sterculia alata shell before and after treatment**

The micrographs of SEM correspond to before (0\textsuperscript{th} day) and after inoculation (20\textsuperscript{th} day) in the presence of naphthalene and fluorene. Pores of different size and shape could be observed before

![Fig. 5 — Three stage coupling process for naphthalene and fluorene removal](image-url)
inoculation as shown in Fig. 6A. These small pores provide immense surface area for formation of biofilm and better percolation of effluent through/across the pores. Before inoculation, no biofilm formed, while uniform population densities of *P. pseudoalcaligenes* NRSS3 were observed after 20th day of inoculation on packing media. The formation of biofilm confirmed the affluent acclimatization of *P. pseudoalcaligenes* on *S. alata*. The formation of biofilm depends up on many factors, such as nutrient availability, PAHs concentration, pH, and temperature in the bioreactor. SEM result obtained on 30th day [end of process; Fig. 6 (B-C)], was similar to the result taken on 20th day which indicates that *P. pseudoalcaligenes* was capable of withstanding during degradation. Tam et al. reported a mixture of PAHs (phenanthrene, fluoranthene and pyrene) were successfully biodegraded by alginate-immobilized media.

**Residual metabolites analysis**

Degradation metabolites of naphthalene and fluorene were identified by GC–MS analysis. The standard peak (Fig. 7A) of pure naphthalene (C10H8) was obtained at the retention time of 9.98 min with molecular weight 128 g/mol. The results of the treated sample showed the presence of metabolites catechol (C6H6O2) and 1-naphthol (C10H8O) (Fig. 7B) at retention time of 10.515 and 16.304 min, respectively, during degradation. The metabolites produced during biodegradation of naphthalene were reported by previous researchers. In the case of fluorene (C13H10), the standard peak (Fig. 7C) was obtained at the retention time of 16.726 min with molecular weight 166 g/mol. The metabolites formed during fluorene degradation show the presence of catechol (C6H6O2), salicylic acid (C7H6O3) and phthalic anhydride (C8H6O3) (Fig. 7D) at the retention time of 10.588, 12.446 and 12.769 min, respectively, during degradation. Seo et al. reports the metabolites formed during degradation of fluorene.

**Growth kinetics for biodegradation of naphthalene and fluorene**

The growth kinetics of *P. pseudoalcaligenes* NRSS3 on naphthalene and fluorene could be adequately described by Monod kinetics. The model has used to find out the kinetic parameters for free cell at each concentration of 20-200 mg/L for naphthalene and fluorene in separate bioreactor using *P. pseudoalcaligenes*. The value of specific growth rate (μmax) and half saturation rate constant (Ks) were found to be 0.3057 per day and 112.87 mg/L, respectively for naphthalene (Fig. 8; Table 1). In the case of fluorene, the value of μmax and Ks were found to be 0.2921 per day and 114.75 mg/L, respectively (Fig. 8; Table 1). The low value of Ks and high value of μmax are desirable during biodegradation and it is better to use ratio (μmax/ Ks) as useful quantity index to show the biodegradation potential of microbes for particular substrate. In the present study, the values of μmax/ Ks were found to be 0.0027 and 0.0025 (L/mg.day) for naphthalene and fluorene, respectively. These ratios indicate that *P. pseudoalcaligenes* NRSS3 has slightly more potential for naphthalene than fluorene during biodegradation. Guha et al. applied, Monod model for multi substrate and obtained μmax (0.23 per hour) and Ks (23.75 mg/L) for naphthalene.

The concentration of naphthalene and fluorene needs to be high enough to insure better growth. However, too high concentration of naphthalene and fluorene addition can lead to residual naphthalene and fluorene levels. *P. pseudoalcaligenes* NRSS3 is highly sensitive to the naphthalene and fluorene concentration and at high concentration there is no further

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Fig. 6 — SEM analysis of Sterculia alata (A) without immobilized, (B) immobilized in the presence of naphthalene, (C) immobilized in the presence of fluorine
Fig. 7 — GC-MS of intermediate metabolites identified from naphthalene and fluorene degradation by *P. pseudoalcaligenes* NRSS3 (A) naphthalene control; (B) naphthalene metabolites; (C) fluorene control; and (D) fluorene metabolites

Fig. 8 — Monod model fitted with observed and experimental data for naphthalene and fluorene

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<th>Compound</th>
<th>$\mu_{\text{max}}$ (per day)</th>
<th>$K_s$ (mg/L)</th>
<th>$\mu_{\text{max}}/K_s$ (L/mg day)</th>
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<td>Fluorene</td>
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growth. This may be the possibility of inhibition at higher concentration of naphthalene and fluorene. Kinetic study degradation of PAHs compound was also studied by previous researchers.\(^{42,43}\)

**Conclusion**

Performance of combined system containing UV-Fenton and PBBR was evaluated under varying process condition for degradation of naphthalene and fluorene up to the concentration of 400 mg/L each. The
Sterculia alata immobilized with P. pseudoalcaligenes (NRSS3) sp. was used as packing media in the bioreactor. The maximum removal efficiency of the combined system was found to be 96 and 94.5% for naphthalene and fluorene, respectively. The compounds such as catechol, 1-naphthol, salicylic acid and phthalic anhydride were found as metabolites in the biodegradation of naphthalene and fluorene thus confirm the successful biodegradation. The biodegradation kinetics of naphthalene and fluorene were studied using Monod model and found to be \( \mu_{\text{max}}: 0.3057 \text{ per day; } K_s: 112.87 \text{ mg/L for naphthalene and } \mu_{\text{max}}: 0.2921 \text{ per day; } K_s: 114.75 \text{ mg/L for fluorene.} \) The combined system was found more effective than the individual systems, particularly at high concentration of the effluent.

References


