Research Article

IJCRR
Section: Healthcare
Sci. Journal
Impact Factor
4.016

PROXY MODEL FOR OPTIMIZATION OF BIODEGRADATION OF PYRENE BY CORYNEBACTERIUM SP AND *PSEUDOMONAS PUTIDA*

Azeez Taofik Oladimeji[1], Owabor Chiedu Ngozi[2], Nwakaudu Madueke Stanley[3], Opebiyi Samson[4]

[1]Biomedical Technology Department, Federal University of Technology, P. M. B. 1526, Owerri, Imo State, Nigeria; [2]Chemical Engineering Department, University of Benin, Technology, P. M. B. 1526, Benin City, Edo State, Nigeria; [3-4]Chemical Engineering Department, Federal University of Technology, P. M. B. 1526, Owerri, Imo State, Nigeria.

ABSTRACT

A proxy model for optimization of operating conditions (pyrene concentration, biodegradation time and aeration) for biodegradation of pyrene by Corynebacterium sp and Pseudomonas putida was aimed to be investigated. The proxy model for biodegradation of pyrene with activity of Corynebacterium sp and Pseudomonas putida was developed from experimental data using response surface methodology (RSM) with central composite design (CCD) of the design of experiments software. Corynebacterium sp degraded 96.71 % of pyrene at optimal conditions of 68.16 mg/L of pyrene concentration, biodegradation time of 82.57 hours and aeration condition of 3.0125vvm, while Pseudomonas putida degraded 93.84 % of pyrene at optimal conditions of 69.90 mg/L of pyrene concentration, biodegradation time of 84 hours and aeration condition of 3.4995 vvm. The developed proxy model of biodegradation of hazardous pyrene disposal under the stated operating conditions is fit and acceptable for optimization.

Key Words: Pyrene, Biodegradation, Optimization, Response surface methodology, Proxy model

INTRODUCTION

Pyrene is a peri-condensed four-ring, hydrophobic compounds, high molecular weight (HMW) polycyclic aromatic hydrocarbon (PAH) and biochemical persistence within ecosystem as a result of dense clouds of p-electrons on both sides of the ring structures, making them resistant to nucleophilic attack (Johnsen et al, 2005; Obayori et al, 2009; Azeez, 2012). Pyrene contains domains of carcinogenesis and mutagenesis in its molecule and it has been classified as pollutant by the US-EPA as the 16 priority polycyclic aromatic hydrocarbons (Obayori et al, 2009; Valentin et al, 2007).

The structural symmetry and stability enable pyrene hard to be biologically attacked. It has been reported that microbes that can specifically degrade pyrene include *Mycobacterium* sp., *Rhodococcus* sp., *Saccharothrix* sp, *Gordona* sp., *Pseudomonas* sp., *Cycloclasticus* sp., *Corynebacterium sp, Sphingomonas yanoikuyae* and it could degrade or merely convert pyrene by co-metabolism (Hu et al, 2003; Hu et al, 2003; Rentz et al, 2005; Sanghvi, 2005; Liang et al, 2006; Mahanty et al, 2008; Kim et al, 2004; Azeez et al, 2013; Azeez, 2012; Lease et al, 2011). Versatility of this species makes it a probable inoculant in the remediation of pyrene contaminated sites (Van Hamme et al, 2003). *Corynebacterium variabilis* sp. Sh42 completely metabolized all representative compounds to CO_2 and H_2O (El-Gendy et al, 2006). Rentz et al (2005) reported that mineralization of $_{14}C_7$ benzo [a] pyrene by S. yanoikuyae JAR02 yielded 0.2 to 0.3% $_{14}CO_2$ when grown with plant root products which indicated that the enhancement of phytoremediation of high molecular weight PAH indicated that co-metabolism of plant/microbe interaction plays an important role in rhizoremediation. Valentin (2007) reported that about 80.6 % pyrene was degraded by Bjerkandera sp with no significant effect of soil microflora presence. The identification of key organisms that play a role in pollutant degradation processes is relevant to the develop-

Corresponding Author:
Azeez Taofik Oladimeji, Department of Biomedical Technology, School of Health Technology, Federal University of Technology, P. M. B. 1526, Owerri, Nigeria; E-mail: thaophic@yahoo.com

Received: 17.12.2014 **Revised:** 26.01.2015 **Accepted:** 02.03.2015

ment of optimal in situ biodegradation strategies (Viggiani et al, 2004; Abed et al, 2002). The discovery of aligned bacteria for remediation with hazardous environmental pollutants has been a driven agent of a large research community and generated biochemical, genetic and physiological knowledge about the degradation capacities of microorganisms and their applications in bioremediation (Van Hamme et al, 2003; Dua et al, 2002). Biodegradation, a bioremediation technique employed for cleaning up contaminants of petroleum hydrocarbons because it is simple to maintain, leads to the destruction of contaminants, applicable over large areas and cost effective (Vila et al, 2001). Different genera of bacteria are known for their potential oil degradation which contains different degradative enzymes involved in the metabolism of hydrocarbons (Palmroth et al, 2005; Pazoa et al, 2004). Researchers have developed models for kinetics, transport and diffusivity of biodegradation of pyrene with activity of *Corynebacterium sp* and *Pseudomonas putida* without considered the optimal conditions of the variables in the reactors and the medium (Owabor et al, 2010; Azeez et al, 2014). The stimulation of removal of contaminants from soil could not only be brought about through several mechanisms of plant/soil interaction but by increase in soil microbial activity, increase in microbial association with the root and toxic compounds, and changes in the physical and chemical properties of the contaminated soil (Singh and Jain, 2003). Wang et al (2009) reported that pyrene degradation efficiency increases by the presence of Iron oxides as a photocatalyst. It has also been demonstrated that the addition of nitrogen and phosphorus as essential nutrients obtained from mineral salt medium (MSM) composition with continuously mixed slurry or field-wet soil incubations not only increased the rate of mineralization but increased the extent of mineralization of pyrene and decreases the lag period before significant pyrene mineralization could occur (Jones et al, 2008). Aeration condition, nitrogen and light in the presence of TiO_2 and iron oxide enhanced degradation or loss of PAHs concentrations with variable degradation time (Świetlik et al, 2002; Wang et al, 2009) but the optimal conditions for biodegradation of pyrene have not been studied. The aim of this research was to develop and examine optimal model for biodegradation of pyrene by the activity of *Corynebacterium sp* and *Pseudomonas putida* with aeration condition, biodegradation time and pyrene concentration which contributes significantly to in - situ biodegradation.

MATERIALS AND METHOD

Pyrene, dichloromethane and hexane (Analytical grade Chemicals) were purchased from Patanne Chemicals, a renowned laboratory chemicals and equipment dealer in Benin City.

Preparation of Mineral Salt Medium and Isolation of Microbes

The microorganisms *Corynebacterium sp* and *Pseudomonas putida* for the experiment were isolated from the subsurface soil of about 0-15cm depth obtained from an uncultivated land in the Nigerian Institute for oil palm research (NIFOR), Benin City in Nigeria. The subsurface soil used for isolation of microbes has been described by (Azeez et al, 2010). The method described by Azeez et al (2012) was employed in which the soil was sieved using 2mm mesh screen for uniform particle size and stored in sterilized polyethylene bag at room temperature covered with aluminium foil for further use. Mineral salt medium (MSM) was used to avoid drastic fluctuation of pH which may be detrimental to the viability of the microbes in the batch medium and it was carbon free before pyrene was added after autoclaved at 121_0C for 15 minutes. The MSM was prepared with Analytical grade chemicals composition: KH_2PO_4 (9.0g/l), K_2HPO_4 (1.5g/l), NH_4Cl (1.5g/l), $CaCl_2$ (20mg/l), and $MgSO_4$ (0.2g/l) at a standardized pH of 7.2 using 0.1N NaOH. The MSM was sterilized in an autoclaved at 121_0C for 15 minutes and then stored in a secured corner in the laboratory until the experiment was set up. 0.5 g of soil samples were added into 100 ml MSM. The medium containing the soil sample and 0.1w/v % pyrene was incubated at $28\pm2_0$C on a rotary incubator shaker at 150 revolutions per minute for 24 h. The pure culture of colonies of *Corynebacterium sp* and *Pseudomonas putida* were maintained on nutrient agar plates for 72 hours at $28\pm2_0$C temperature for production of the microbes' enmasse mainly for reduction of the lag phase and suitability of the inoculums in pyrene contaminated environment before the biodegradation.

Biodegradation Analysis of Pyrene

The quantity of pyrene as presented in the Table 1 was dissolved in 10% dichloromethane solution and make up to 1 liter by water. The solvent was volatized from pyrene solution under fume-hood. 250 ml of each of the pyrene solution measured into bioreactor vessel and 10 ml of inoculums was transferred from each agar plate of *Corynebacterium sp* and *Pseudomonas putida* into pyrene contaminated water and incubated at $28\pm2_0$C on a rotary incubator shaker at 150 revolutions per minute and supernatant was withdrawn for analysis at designed biodegradation time (3.546, 24, 54, 84 and 104.45 hour), centrifuged, decanted and cells of *Corynebacterium sp* and *Pseudomonas putida* settled down at the bottom of the centrifuged tube were scooped and dried in an oven at 60_0C for 8 hours.

The method described by (Azeez, 2012) was employed using UV visible spectrophotometer to measure absorbance of the pyrene in aliquot. The absorbance of the pyrene was recorded at a wave-

length of 267 nm in the UV region after isolation of the microbes from centrifuge of 10 ml aliquots at 10,000 revolutions per minute for 20 minutes and allowed to settle for 30 minutes to obtain a clear supernatant. Pyrene was extracted from 5 ml of the clear supernatant using 5ml of hexane for 10 minutes in a separating funnel. The top solution in a separating funnel was a solution of pyrene in hexane and poured into the corvettes of the spectrophotometer and absorbance readings at a wavelengths of 267 nm was recorded. The procedure was repeated in designed biodegradation time immediately after inoculation with *Corynebacterium sp and Pseudomonas putida* for a period of 104.45 hours of incubation and solutions of pyrene in the hexane were prepared to give a concentration of 0.3mg/ml. The absorbance of the solutions was read at the appropriate wavelengths 267nm for the pyrene solution. The standard model obtained by Azeez (2012) was used for the conversion of pyrene absorbance to mg/L. The percentage of biodegradation of pyrene was evaluated as follows:

$$\%\ Degraded\ (Y_i) = \frac{C_0 - C_i}{C_0}\ x\ 100\% \qquad (1)$$

Where γ_i is the concentration of pyrene utilized or degraded, C_0 and C_i is the initial concentration of anthracene and concentration of pyrene at any time after inoculums respectively measured in mg/L.

Experimental design and Statistical Optimization

A 2_3 full factorial Central Composite Design (CCD) with response surface methodology of Design – Expert software version 6.0.8 (2002 East Hennepin ave., Suite 480 Minneapolis, MN 55413, stat Ease, Inc.) was used. Eight hundred milliliters of the autoclaved MSM and 0.4 liters of pyrene solution with variable concentration and inoculum of 200 mL were introduced aseptically to make up 0.5 liters of the working volume. Three factors were considered to perform response surface methodology (RSM) with CCD at variable concentration of pyrene (X_1), fermentation time (X_2) and aeration (X_3). The bioreactor was operated for variable concentration (9.66, 25, 47.5, 70, 85.34 g/L), aeration (2.159, 2.5, 3.0, 3.5 and 3.841 vvm) and biodegradation time (3.55, 24, 54, 84 and 104.45 hours) at a temperature of $28\pm2_0$C as presented in the Table 1. Aliquot was withdrawn for analysis based on designed factors from bioreactor as presented in the Table 2 for experimental variables. The range of these values was considered since it characterized the optimum range for the microbes and the expected range in which the process could be operated.

The experiments were performed in triplicates and the average of pyrene degraded by *Corynebacterium sp* (*Y1*) and *Pseudomonas putida* (*Y2*) obtained were taken as the response function (*Yi*) of the factors. The Second degree polynomials equation (2) which contains factors with interaction terms were used to calculate the predicted response:

$$Y_i = \beta_0 + \sum_{i=1}^{n}\beta_i X_i + \sum_{i=1}^{n}\beta_{ii}X_i^2 + \sum_{i=1}^{n}\sum_{j=i+1}\beta_{ij}X_iX_j + \varepsilon \qquad (2)$$

Where Y_i is the response of anthracene degraded by *Corynebacterium sp* and *Pseudomonas putida* as dependent variables; *n* is the number of independent variables (factors), *Xi* (*i* =1, 2, 3…) and *Xj* $j_{=1,2,3\ldots)\ are}$ the concentration of pyrene degraded, degradation time and aeration respectively; ε is the random error; $\beta_{0\ is\ offset}$ term, and βi, βij and βii are the coefficients of linear, interaction and quadratic term respectively.

The model was developed based on experimental data using response surface methodology with statistical optimization using analysis of variance (ANOVA). The quadratic models were represented as 3D with contour plots and response surface curves were generated for variables.

RESULTS AND DISCUSSION

The results of the experimental data shows that the degradation of pyrene by the activity of *Corynebacterium sp* is more fit than that of *Pseudomonas putida* due to high correlation coefficient (R_2) of *Corynebacterium sp* 0.96108 (> 0.9) compared with *Pseudomonas putida* 0.61095 (< 0.9) as shown in the Figure 1 and 2 respectively. This indicates 96.11 % for *Corynebacterium sp and 61.10 for Pseudomonas putida ar*e variability in the response that could be explained by the quadratic and linear model respectively.

The Model F-value of 27.44 with 0.01% error and 8.38 with 0.014 error for *Corynebacterium sp* and *Pseudomonas putida respectively* as presented in the Table 3. The error values for the activity of *Corynebacterium sp* and *Pseudomonas putida* indicated that the model terms are significant. The determination of the significant parameters was performed through a hypothesis test (*p – value)* with a 5 % level of significance. Parameters with p – value higher than 0.05 is significance while parameter with p – value less than 0.05 is significant. The response surface model obtained for *Corynebacterium sp was* for quadratic while for *Pseudomonas putida was* linear and presented as equation (3) and (4) respectively:

$$Y_1 = -109.322 - 0.0012438X_1 + 1.7390X_2 + 65.921X_3 - 0.0045045X_1^2 - 0.011624X_2^2 - 10.578X_3^2 + 0.011069X_1X_2 + 0.008111X_1X_3 - 0.01625X_2X_3 \qquad (3)$$

$$Y_2 = 32.98853 + 0.08201X_1 + 0.594053X_2 + 1.492211X_3 \qquad (4)$$

Though, the adjusted correlation coefficient (adj. R_2 = 0.92606) for the activity of *Corynebacterium sp on pyrene* was also satisfactory for confirming the significance of the response surface model but fairly significant in the case of

activity of *Pseudomonas putida on pyrene due to low correlation coefficient* (adj. R_2 = 0.538) *(see Table 3).*

Furthermore, an adj. R_2 close to the R_2 values insures a satisfactory adjustment of the quadratic models to the experimental data by the activity of the two microbes used for this research as presented in the Table 3. Therefore, the regression models explained the removal efficiency well.

Though, the "Pred R_2" for both microbes are not as close to the "Adj R_2" as presented in the Table 3 and it does not justify that the proxy model cannot be used for design since the adequate precision 19.777 (> 4) and 10.139 (>4) for the response surface model of *Corynebacterium sp* and *Pseudomonas putida respectively.*

Based on linear regression analysis presented in the Table 4, parameters or model terms with a level of significance higher than p value (p > 0.05) were dismissed (Azeez et al, 2013; De Lima et al, 2010; Nwabanne and Ngwu, 2013). The significant model terms for pyrene degradation by the activity of *Corynebacterium sp* include the intercept (β_0), biodegradation time (X_1) quadratic of the biodegradation time (X_1^2) and interaction of the pyrene concentration and biodegradation time (X_1X_2) while for *pseudomonas putida* were intercept (β_0)and biodegradation time (X_1). The equation (3) and (4) reduced to equation (5) and (6) which represents proxy model for biodegradation of pyrene by the activity of *Corynebacterium sp* and *Pseudomonas putida* respectively.

$$Y_1 = -109.322 + 1.7390X_2 - 0.011624X_2^2 + 0.011069X_1X_2 \quad (5)$$

$$Y_2 = 32.98853 + 0.594053X_2 \quad (6)$$

The obvious prominence in the response surfaces indicated that the optimal conditions were located exactly inside the design boundary indicating the stationary point and it was a single point of maximum response. The model gave a maximum solution of 96.711 % pyrene degraded by *Corynebacterium sp* with pyrene concentration of 68.16 mg/L, biodegradation time of 82.57hours and aeration condition of 3.0125vvm, while the highest pyrene degraded by *Pseudomonas putida* obtained was 93.843% under conditions of 69.90mg/L pyrene concentration, biodegradation time of 84 hours and aeration 3.4995 as shown in the Figure 3 and 4.

The biodegradation of pyrene with activity of *Corynebacterium sp* (96.71 %) and *Pseudomonas putida* (93.84) using response surface methodology with central composite design gave the best result compared with 89.1 % and 79.4 % pyrene degraded by *Mycobacterium sp reported by* Farshid (2013), 80.6 % pyrene degraded by *Bjerkandera sp* with no significant effect of the presence of soil microflora as reported by Valentin (2007), and 70 % of pyrene degraded by *Corynebacterium variabilis sp. Sh42* to carbon (VI) oxide and water reported by El-Gendy et al (2010) as well as report of Shokrollahzadeh et al (2012) in which 78 % of pyrene was degraded by *Sphingopyxis sp*. This may be attributed to enrich mineral salt medium composition and kinetics.

To validate the agreements of the results achieved from the model and experiments, two additional experiments were conducted by using pyrene concentration, biodegradation time and aeration condition in the optimum region. As shown in Table 5, the degraded pyrene by the activity of *Corynebacterium sp* and *Pseudomonas putida* obtained from the additional experiments are very close to those estimated using the model, implying that the response surface methodology approach was appropriate for optimizing the conditions of the biodegradation process of pyrene.

CONCLUSION

The central composite design and response surface methodology successfully enabled in obtained proxy model for the determination of optimal operating conditions for biodegradation of pyrene with the activity *Corynebacterium sp* and *Pseudomonas putida in a* short period of time with the least number of experiments. The proxy model validity of the model was proven by repeating the experiment at optimal conditions. The response surface methodology demonstrated the best optimal conditions of pyrene degraded by *Corynebacterium sp* with 96.71 % of 68.16 mg/L of pyrene concentration, biodegradation time of 82.57 hours and aeration condition of 3.0125vvm, while the pyrene degraded by *Pseudomonas putida* was 93.84 % at optimal conditions of 69.90 mg/L pyrene concentration, biodegradation time of 84 hours and aeration of 3.4995vvm. The validated results of an experiment were found to be in good agreement with the optimal solution predicted by the model.

Source of funding

The source of funding of this work is financed by the authors.

ACKNOWLEDGEMENT

Authors acknowledge the immense help received from the scholars whose articles are cited and included in references of this manuscript. The authors are also grateful to authors / editors / publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

REFERENCES

1. Abed R. M. M., Safi N.M.D., Koster J., de Beer D., El-Aahha Y., Rullkoter J. and Garcia-Piche F. (2002). Microbial diversity of a heavily polluted microbial mat and its community changes following degradation of petroleum compounds. *J. Appl. Environ.*

Microbiol., 68: 1674 - 1683.
2. Azeez, T. O. (2012). Biodegradation of Pyrene Using Corynebacteria sp and Pseudomonas Putida in Contaminated Water. *International Journal of Biotechnology Research.* 5 (1): 31-38.
3. Azeez, T. O., Arinkoola, A.O., Salam, K.K and Nwakaudu, M.S. (2014). Effective Diffusivity and Kinetics Model for Biodegradation of PAHs in a Saturated Porous Matrix. *Journal of Emerging Treads in Engineering and Applied Sciences (JETEAS),* 5 (1): *62 - 69.*
4. Azeez, T. O., Onukwuli O. D., Araromi, D. O., Arinkoola, A. O., Salam, K. K., Iwuji, S. C., Ejeta, K. O., Dawodu, B. F., Ayinde, K. A, Nwacha, R. and Azeez, F. O. (2013). Optimization of Bioremediation of Cheese Whey with the Activity of *K. Pneumonia* Using Response Surface Methodology. *International Journal of Science and Emerging Technology with Latest Trends (IJSETT),* 11(1): 10 - 21.
5. *De Lima C. J. B., Coelho L. F. and Contiero J.* (2010). The Use of Response Surface Methodology in Optimization of Lactic Acid Production: Focus on Medium Supplementation, Temperature and pH Control, *Food Technol. Biotechnol. 48* (2): 175 –181.
6. Dua M., Singh A., Sethunathan N. and Johri A. K. (2002). Biotechnology and bioremediation: successes and limitations. *J. Appl. Microbiol. Biotechnol.*, 59: 143-152.
7. El-Gendy N. S., Moustafa Y. M., Habib S. A. and Ali S. (2010). Evaluation of *Corynebacterium variabilis* Sh42 as a degrader for different poly aromatic compounds. *Journal of American Science,* 6(11): 343 - 356.
8. Hu Y., Ren F., Zhou P., Xiam. and Liu S. (2003). Degradation of pyrene and characterization of *Saccharothrix* sp. PYX-6 from the oligotrophic Tianchi Lake in Xinjiang Uygur Autonomous Region, China. *Chinese Science Bulletin,* 48 (20): 2210 - 2215. doi: 10.1360/03wc0281.
9. Johnsen A. R., Wick L. Y. and Harms H (2005) Principles of microbial PAH-degradation in soil. *Environ Poll.,* 133: 71 – 84
10. Jones M. D, Singleton D. R., Carstensen D. P. et al (2008). Effect of incubation conditions on the enrichment of pyrene-degrading bacteria identified by stable-isotope probing in an aged, PAH contaminated soil. *Microb. Ecol.,* 56: 341 – 349.
11. Kim S-J., Jones R. C., Cha C-J., Kweon O., Edmondson R. D., Cerniglia C. E. (2004). Identification of proteins induced by polycyclic aromatic hydrocarbon in *Mycobacterium vanbaalenii* PYR-1 using two-dimensional polyacrylamide gel electrophoresis and *de novo* sequencing methods. *Proteomics,* 4: 3899 - 3908.
12. Lease C. W. M., Bentham R. H., Gaskin S. E. and Juhasz A. L. (2011). Isolation and Identification of Pyrene Mineralizing Mycobacterium spp. from Contaminated and Uncontaminated Sources. Applied and Environmental Soil Science, 2011: 1 – 11. doi:10.1155/2011/409643
13. Liang Y., Gardner D. R., Miller C. D., Chen D., Anderson A. J., Weimer B. C. and Sims R. C. (2006). Study of Biochemical Pathways and Enzymes Involved in Pyrene Degradation by *Mycobacterium* sp. Strain KMS^. *Applied and Environmental Microbiology*, 72 (12): 7821 - 7828. doi:10.1128/AEM.01274-06.
14. Mahanty B., Pakshirajan K. and Venkata Dasu V. (2008). Biodegradation of pyrene by Mycobacterium frederiksbergense in a two-phase partitioning bioreactor system. *Bioresource Technology,* 99: 2694 – 2698. doi:10.1016/j.biortech.2007.05.042
15. Nwabanne, J.T. and Ekwu, F. C. (2013). Experimental Design Methodology Applied to Bleaching of Palm Oil Using Local Clay. *International Journal of Applied Science and Technolog.,* 3(4): 69 - 77.
16. Obayori O. S., Adebusoye S. A., Ilori M. O. Oyetibo G. O. Omotayo A. E. and Amund O. O. (2009). Effects of Corn Steep Liquor on Growth Rate and Pyrene Degradation by Pseudomonas strains. *Curr Microbiol,* 60: 407 – 411. doi: 10.1007/s00284-009-9557-x
17. Owabor C. N., Agarry S. E. and Azeez T. O. (2010). Development of a Transport Model for the Microbial Degradation of Polycyclic Aromatic Hydrocarbons in a Saturated Porous Medium. *Journal of the Nigerian Association of Mathematical Physics (J of NAMP)*, 16: 317 - 324.
18. Palmroth, R.T., U. Munster, J. Pichtel and A. Puhakka, (2005). Metabolic responses of microbiota diesel fuel addition in vegetated soil. *J. Biodegradation*, 16: 91 - 101.
19. Pazos, F., D. Guizas, A. Valencia and V. De Lorenzo (2004). Meta Router: bioinformatics for bioremediation. *J. Nucleic Acids Res.,* 33: 53 - 65. doi:10.1016/j.jhazmat.2009.11.055
20. Rentz J. A, Alvarez P. J.J. and Schnoor J. L. (2005). Benzo[a] pyrene co-metabolism in the presence of plant root extracts and exudates: Implications for phytoremediation. Environmental Pollution 136: 477 - 484. doi:10.1016/j.envpol.2004.12.034.
21. Rentz J. A, Alvarez P. J.J. and Schnoor J. L. (2008). Benzo[a] pyrene degradation by Sphingomonas yanoikuyae JAR02. Environmental Pollution 151 (2008) 669 – 677. doi:10.1016/j.envpol.2007.02.018
22. Sanghvi S. (2005). Bioremediation of polycyclic aromatic hydrocarbon contamination using *Mycobacterium vanbaalenii* MMG 445. *eJournal,* 1: 1 - 7.
23. Shokrollahzadeh S., Golmohammad F. and Shokouhi H. (2012). Study of *Sphingopyxis* Isolates in Degradation of Polycyclic Aromatic Hydrocarbons. *Chemical Engineering Transactions. 27: 55-60. doi: 10.3303/Cet1227010*
24. Singh O. V. and Jain R. K. (2003). Phytoremediation of toxic aromatic pollutants from soil. *Appl Microbiol Biotechnol.,* 63:128 – 135. doi: 10.1007/s00253-003-1425-1
25. Świetlik R., Kowalczyk D. and Dojlido J. (2002). Influence of Selected Physicochemical Factors on the Degradation of PAHs in Water. *Polish Journal of Environmental Studies, 11(2): 165 - 169.*
26. Valentı´n L., Lu-Chau T. A., Lo´pez C., Feijoo G., Moreira M.T. and Lema J.M. (2007). Biodegradation of dibenzothiophene, fluoranthene, pyrene and chrysene in a soil slurry reactor by the white-rot fungus *Bjerkandera sp*. BOS55. *Process Biochemistry,* 42: 641 – 648. doi:10.1016/j.procbio.2006.11.011
27. Van Hamme, J. D., A. Singh and O.P. Ward, (2003). Recent advances in petroleum microbiology. *J. Microbiol. Molec. Biol. Rev.*, 67: 503 - 549.
28. Viggiani, A., Siani L., Notomista E., Birolo L., Pucci P. and Di Donato A. (2004). The role of the conserved residues His-246, His-199 and Tyr-255 in the catalysis of catechol 2, 3-dioxygenase from *Pseudomonas stutzeri* OX1. *J. Biol. Chem.*, 279: 48630 - 48639.
29. Vila, J., Lopez Z., Sabate J., Minguillon C., Solanas A. M. and Crifoll M. (2001). Identification of a novel metabolite in the degradation of pyrene by *Mycobacterium sp.* Strain AP1: action of isolates on two and three-ring polycyclic aromatic hydrocarbons. *J. Appl. Environ. Microbiol.,* 67: 5497-5505. doi: 10.1128/AEM.67.12.5497-5505.2001
30. Wang Y., Liu C.S., Li F.B., Liu C.P., and Liang J. B. (2009). Photodegradation of polycyclic aromatic hydrocarbon pyrene by iron oxide in solid phase. Journal of Hazardous Materials 162 (2009) 716–723. doi:10.1016/j.jhazmat.2008.05.086

Table1: Levels of Different Factors for Biodegradation

	Ranges and Levels				
Process Variables	-1.682	-1	0	1	+1.682
Concentration of Anthracene (X_1) (g/L)	9.660	25	47.5	70	85.34
biodegradation time (X_2) (hr)	3.546	24	54	84	104.45
Aeration(X_3)(vvm)	2.159	2.5	3.0	3.5	3.841

Table 2: CCD Matrix of Coded and Experimental Variables for Biodegradation of Pyrene

Run	Coded value			Actual Value		
	Concentration of Pyrene (X_1) (mg/L)	Degradation time (X_2) (hr)	aeration (X_3) (vvm)	Concentration of Pyrene (X_1) (mg/L)	Degradation time (X_2) (hr)	aeration (X_3) (vvm)
1	-1	-1	-1	25	24	2.5
2	1	-1	1	70	24	3.5
3	0	0	0	47.5	54	3
4	0	1.682	0	47.5	104.45	3
5	0	0	0	47.5	54	3
6	1	1	1	70	84	3.5
7	-1.682	0	0	9.660	54	3
8	0	0	1.682	47.5	54	3.841
9	-1	1	-1	25	84	2.5
10	0	0	-1.682	47.5	54	2.159
11	1	1	-1	70	84	2.5
12	0	0	0	47.5	54	3
13	0	0	0	47.5	54	3
14	-1	1	1	25	84	3.5
15	0	0	0	47.5	54	3
16	0	-1.682	0	47.5	3.546	3
17	1	-1	-1	70	24	2.5
18	1.682	0	0	85.340	54	3
19	-1	-1	1	25	24	3.5
20	0	0	0	47.5	54	3

Table 3: Analysis of Variance (ANOVA) of response surface model for biodegradation of Pyrene

Source	sum of square	Degree of freedom	Mean Square	F-value	Prob>F	R_2	Adj R_2	Pred R_2	Adeq Precision
Biodegradation of Pyrene by Corynebacterium sp									
Model	13692	9	1521.4	27.439	< 0.0001	0.961085	0.92606	0.70549	19.777
Residual	554.45	10	55.445						
Lack of Fit	554.45	5	110.89						
Pure error	0	5	0						
Total	14246.8	19							
Biodegradation of Pyrene by Pseudomonas putida									
Model	4391.6	3	1463.9	8.3753	0.0014	0.61095	0.5380	0.2940	10.139
Residual	2796.6	16	174.8						
Lack of Fit	2796.6	11	254.23						
Pure error	0	5	0						
Total	7188.2	19							

Table 4: Model Coefficient Estimated by Linear Regression

Model	Pyrene degraded by Corynebacterium sp			Pyrene degraded by Pseudomonas putida		
Term	Model Coefficient	F - Value	Prob > F	Model Coefficient	F - Value	Prob > F
Intercept	-109.322	27.43931	< 0.0001	32.98853	8.375289	0.0014166
X_1	-0.00124	4.638344	0.056711	0.08201	0.266039	0.61306
X_2	1.739025	204.573	< 0.0001	0.594053	24.81633	0.00013582
X_3	65.92078	0.236443	0.63727	1.492211	0.043496	0.83743
X_1^2	-0.0045	1.351623	0.27199			
X_2^2	-0.01162	28.44703	0.00033127			
X_3^2	-10.5782	1.81778	0.20732			
X_1X_2	0.011069	8.054036	0.017610			
X_1X_3	0.008111	0.001201	0.97303			
X_2X_3	-0.01625	0.008573	0.92806			

Table 5: Validation of experiments at optimum conditions

	Pyrene concentration (mg/L)	Biodegradation time (hr)	aeration (vvm)	Pyrene degraded (%)
Pyrene degraded by Corynebacterium sp				
Experimental value	68.16	82.57	3.0125	94.6
Predicted value	68.16	82.57	3.0125	96.71
Error (%)				2.23
Pyrene degraded by Pseudomonas putida				
Experimental value	69.9	84	3.4995	92.05
Predicted value	69.9	84	3.4995	93.84
Error (%)				1.94

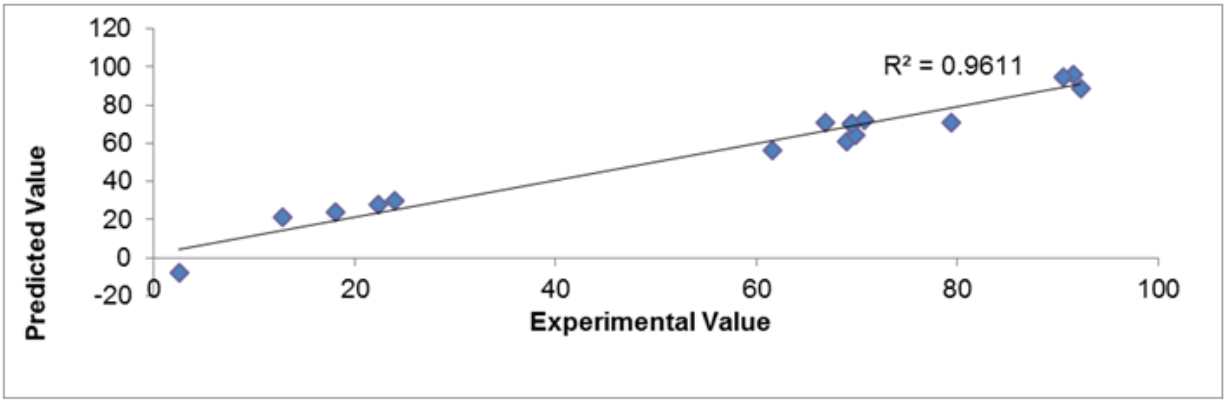

Figure 1: Predicted Value against experimental Value for Pyrene degraded by Corynebacterium sp

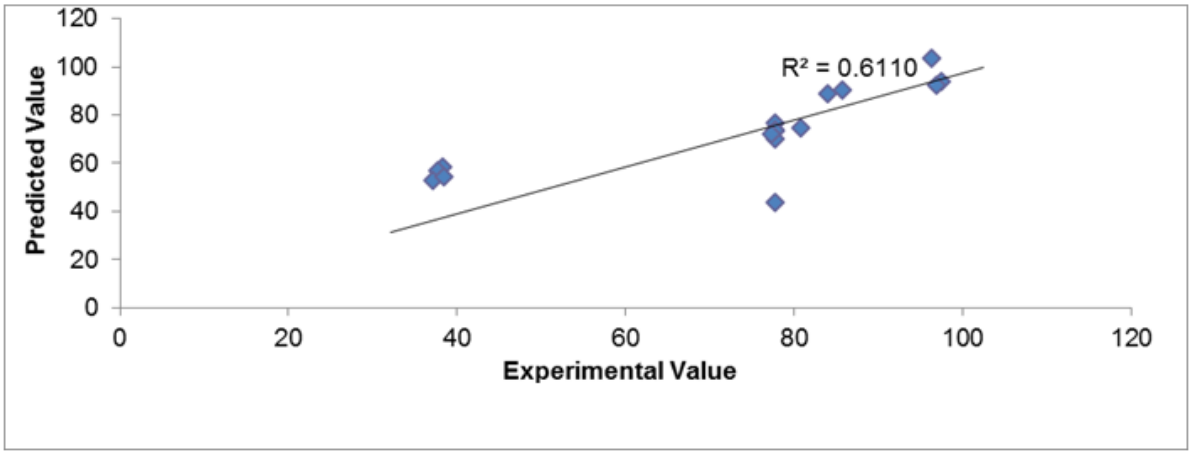

Figure 2: Predicted Value against experimental Value for Pyrene degraded by Pseudomonas putida

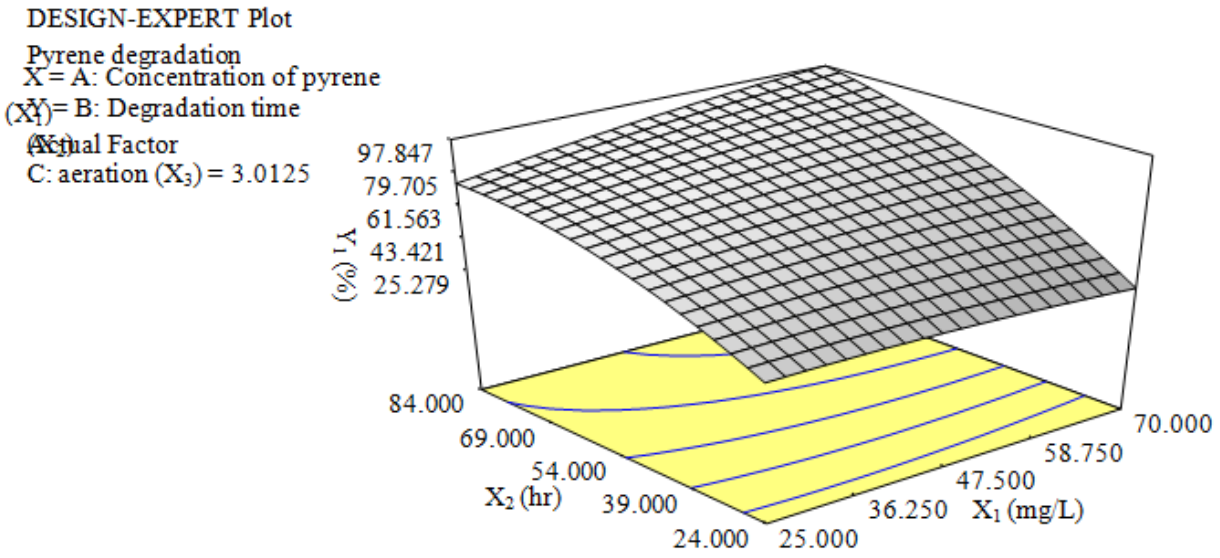

Figure 3: 3-D Plot Response Surface of biodegradation of Pyrene by Activity of *Corynebacterium sp*

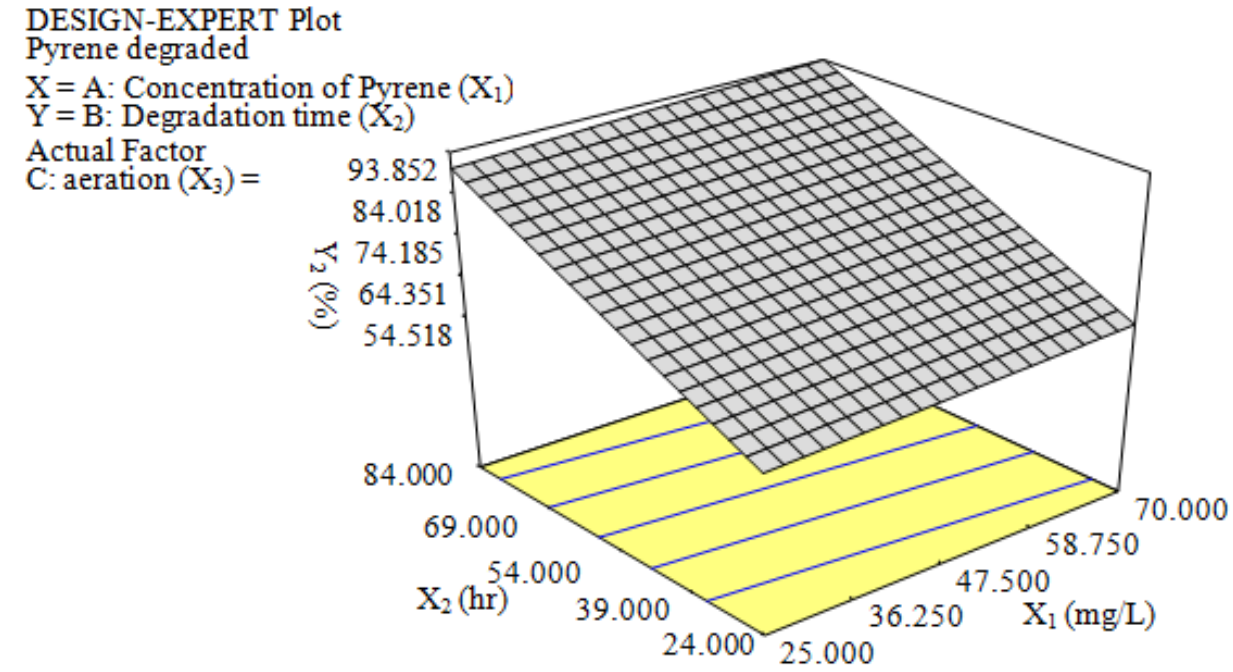

Figure 4: 3-D Plot Response Surface of biodegradation of Pyrene by Activity of *Pseudomonas putida*