*Biological Forum – An International Journal* **15(5): 93-98(2023)**

**ISSN No. (Print): 0975-1130**
**ISSN No. (Online): 2249-3239**

# Influence of Supplementary Carbon and Nitrogen Sources on Pyrene Degradation by *Aspergillus lacticoffeatus* Isolated from Crude Oil-contaminated Soil

***Veeramani Veerasamy, Gowri Manogari Boovaragamoorthy and Thamaraiselvi Kaliannan****
*Department of Environmental Biotechnology,*
*School of Environmental Sciences, Bharathidasan University, Tiruchirappalli (Tamil Nadu), India.*

*(Corresponding author: Thamaraiselvi Kaliannan*)*
*(Received: 27 February 2023; Revised: 15 April 2023; Accepted: 20 April 2023; Published: 20 May 2023)*
*(Published by Research Trend)*

**ABSTRACT: Polycyclic aromatic hydrocarbon is the most prevalent environmental pollutant. In this study, pyrene degrading fungi, *Aspergillus lacticoffeatus,* was isolated from crude oil-contaminated soil from Oil and Natural Gas Corporation (ONGC) Karaikal. *A. lacticoffeatus* is optimized for pyrene degradation in the medium amended with different nitrogen and carbon sources. Among the carbon sources, it was found that pyrene degradation was significant with sucrose compared to glucose, fructose, maltose, and starch. The ammonium chloride nitrogen source provided better degradation than ammonium sulfate, ammonium nitrate, calcium nitrate, and sodium nitrate. Maximum pyrene removal was obtained in the combination of sucrose and ammonium chloride (84.91%), and physical parameters like pH (6.00) and temperature (35°C) provided a better degradation in a period of 30 days. Overall, the study identified glucose and sucrose as suitable carbon sources and ammonium chloride as a suitable nitrogen source for pyrene degradation.**

**Keywords:** Pyrene degradation, Polycyclic aromatic hydrocarbon, Carbon sources, Nitrogen sources, *Aspergillus lacticoffeatus.*

## INTRODUCTION

The soil, a source of infinitive life, is an essential determinant of agricultural productivity and sustainability. Since soil is a sink for pollutants, soil pollution is a hot issue in research circles worldwide. Crude oil, a naturally occurring petroleum product, comprises polycyclic aromatic hydrocarbons (PAHs) and is considered a global environmental problem(Imam *et al.,* 2019; Tomás *et al.*, 2019). Crude oil contamination changes the composition of soil flora and fauna and the soil structure (Andriani and Tachibana 2016; Argumedo-Delira *et al*., 2012; Zafra *et al.*, 2014). Alterations in soil's biological and physicochemical properties can be attributed to PAHs' tenacious and hydrophobic nature. These changes include variations in the microbial population, soil enzyme activities, water-holding capacity, and soil organic matter (Ghosal *et al.,* 2016; Huang *et al.*, 2019). They can attach to DNA and transform normal cells into malignant ones (Ghosal *et al*., 2016; Mallick *et al.*, 2011). Also, they interfere with enzymes of hormone metabolism in thyroid glands, causing toxicity to the reproductive and immune systems (Oostingh *et al.*, 2015; Kaur *et al.*, 2021). PAHs affect human beings via the cytochrome P450-mediated mixed-function oxidase system. Because it is fat soluble, PAHs are easily absorbed in the digestive tract of mammals (Qiu *et al.*, 2015).

Pyrene has been listed as a priority pollutant by USEPA among the 16 PAHs (Ghosal *et al.*, 2016; Oostingh *et al.*, 2015). Pyrene, composed of four fused aromatic rings, is the most abundant compound in polluted PAHs environments. Many microorganisms, like bacteria, yeast, fungi, and algae, could metabolize PAHs and were isolated from petroleum-contaminated sites (Essabri *et al.*, 2019; Mallick *et al.*, 2011). The PAHs have a bay region and a K region where microbial attacks occur. Recentre search has indicated that indigenous Ascomycetes dominate polluted environments, and its occurrence controls the accumulation of toxic compounds in soil. The high diversity of this fungal species has demonstrated an influential role in removing a broad range of xenobiotics due to their oxidation capacity (Latimer and Zheng 2003). In addition, Ascomycetes use intracellular enzymes (the cytochrome P450 pathway) to degrade xenobiotics, unlike the white rot fungus, which uses extracellular enzymes (Agrawal and Shahi 2017; Bezalel *et al*., 1996).

The carbon sources used for microbial respiration or fermentation is also critical for the growth of organisms (Adnan *et al.*, 2017; Souza *et al.*, 2017). Carbon and nitrogen sources are essential for their role in amino acid, protein, nucleic acids, enzymes, ADP, and ATP synthesis in fungi (Diano et al., 2006; Nehls *et al.*, 2001). Moreover, studies have yet to be done on the carbohydrate nutrition of Ascomycetes species. *Aspergillus lacticoffeatus, an Ascomycetes species,* was

investigated for pyrene degradation using different carbon (glucose, fructose, sucrose, maltose, and starch) and nitrogen sources (ammonium chloride, ammonium sulfate, calcium nitrate, ammonium nitrate, and sodium nitrate) based on previous studies. A carbon source is required to help metabolize PAHs, while a macronutrient such as nitrogen is needed for producing biomass (Dutta and Das 2017). *Aspergillus lacticoffeatus* is a novel fungal species with excellent pyrene degradation potential. In the present study , we assess the role of different combinations of carbon and nitrogen sources on the growth of *Aspergillus lacticoffeatus* and enha nce its ability to degrade pyrene.

## MATERIALS AND METHODS

**Sample collection.** The crude oil-contaminated soil samples were collected at seven different places from Oil and National Gas Corporation (ONGC) Karaikal, Tamil Nadu, India(10.8251918,79.7903966) in-depth 5cm. Samples were collected in sterile containers, carefully kept in an ice box, transferred, and maintained at 4°C in the laboratory.

**Isolation of pyrene-degrading fungi.** According to Accensi *et al*. (1999), pyrene-degrading fungi were isolated. About 1g of air-dried, sieved (2 mM) soil sample was mixed with 10 ml of double distilled water and agitated for three hours at 120rpm. The soil mixture was then serially diluted and poured on PDA media enriched with different concentrations of pyrene (10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75mg$L^{-1}$) and incubated at 27°C for 7 to 10 days.

**Extraction of fungal genomic DNA.** Fungal genomic DNA was extracted according to Accensi *et al*. (1999). After 5 days of incubation, the mycelium was separated and washed thrice with double distilled water and air dried. 1g of mycelium was crushed with 10mL of fungal extraction buffer (Tris-HCl (pH- 8.0)- 50 mM, EDTA –50mM, 3% SDS, and 1% β-mercaptoethanol and keptfor 45 min in 60°C water bath. The genomic DNA was obtained using phenol:chloroform: isoamyl alcohol (25:24:1). The resulting pellet was air-dried and dissolved in TE Buffer (pH8.0) and stored at 4°C for further analysis.

**PCR Amplification and phylogenetic analysis.** The 18S rRNA was amplified and sequenced for molecular identification according to the manufacturer's instructions. Extracted DNA was amplified with universal primers ITS1 (3' TCCGTAGGTGAACCTGCGG5') and ITS4 (3' TCCTCCGCTTATTGATATATATGC 5').The PCR reaction was carried out in an automated thermal cycler (Bio-Rad T100TM) with the following cyclic conditions: initial denaturation at 94°C for 5min followed by 35 cycles of denaturation at 94°C for 1min, annealing 55°C for 30sec, extension at 72°C for 1min and final extension at 72°C for 10min. Amplified PCR products were detected using agarose gel electrophoresis, and images were captured under the Gel documentation system (Bio-Rad Gel Doc$^{TM}$ XR+).The amplified nucleotide sequence was analyzed in BLAST search to determine the closest related line. Sequence alignment was generated with CLUSTAL W Program. Construction of a phylogenetic tree was done using phylotree software.

**Role of different carbon sources on *Aspergillus lacticoffeatus* growth.** *Aspergillus lacticoffeatus* was grown with different carbon sources growth in plate and flask culture methods. *Aspergillus lacticoffeatus* maintained on PDA media slant at 4°C was recovered by subculturing in PDA media at 27°C for 7 days. Experiments were conducted in different conical flasks containing different carbon sources in MSM. MSM was prepared according to HAMAD et al. (2014). Carbon sources were glucose, fructose, sucrose, maltose, and starch (30g$L^{-1}$).A loop of fungal colonies was inoculated into the conical flasks and kept in an orbital shaker at 27°C (120rpm) for 30days.The fungal biomasses in the conical flasks were carefully filtered using Whatman No.1 filter paper. The wet weight of the fungal biomass was measured. Also, experiments were carried out in the plates which contained different carbon sources (30g$L^{-1}$) in MSM. A loop of *Aspergillus lacticoffeatus* was kept in these plates and incubated at 27°C for 7 days. The hyphal growth diameter (mm) was noted every 24h.

**Combined effect of glucose and sucrose on different nitrogen sources in pyrene degradation.** The experiments were conducted using glucose and sucrose (since these two carbon sources showed good growth of *Aspergillus lacticoffeatus*). Different nitrogen sources(3g$L^{-1}$), namely - ammonium sulfate, ammonium chloride, ammonium nitrate, calcium nitrate, and sodium nitrate, were added in a separate conical flask containing MSM. Also, glucose and sucrose were amended individually to these flasks to determine the combined effects of carbon and nitrogen sources. A loop of *Aspergillus lacticoffeatus* was added to the flasks and kept in the shaker(120rpm) at 27°Cfor 30days. After incubation, the contents of the conical flasks were filtered using Whatmann No. 1 filter paper. The residual pyrene was extracted by adding an equal volume of hexane and vortexed for 2min. The aqueous phase was separated by centrifuging for 10mins, and the pyrene concentration was measured spectrophotometrically (Shimadzu UV-1700) at 254 nm.Pyrene quantification was done according to Das and Mukherjee (2007). The wet weight of the fungal biomass was also measured. The experiments were carried out in duplicates.

## RESULTS AND DISCUSSION

**Isolation and identification of pyrene-degrading fungi.** The isolated strain effectively grew on PDA media by utilizing pyrene (60mg$L^{-1}$), and it was identified as *Aspergillus* species using lactophenol cotton blue staining. Further, using 18S rRNA nucleotide sequencing, the organism was identified as *Aspergillus lacticoffeatus*, and it was submitted to Gen Bank in the accession number KTSMBNL-84 (Fig. 1).

**Pyrene tolerance capacity of *Aspergillus lacticoffeatus.*** The pyrene tolerance capacity for *Aspergillus lacticoffeatus* was studied at different concentrations (10 to 75mg$L^{-1}$) at an incubation period

of seven days. At 10mgL$^{-1}$ of pyrene, the number of fungal colonies was high, and the growth was observed on the 5th day itself. With the increase in pyrene concentration, the number of fungal colonies decreased. Beyond 60mgL$^{-1}$, fungal growth was not observed for seven days of incubation. This indicates that *Aspergillus lacticoffeatus* could tolerate 60mgL$^{-1}$ of pyrene (Fig. 2). Similar results, where a high concentration of pyrene inhibited the growth of fungi have been reported by (Souza *et al.*, 2017; Zafra *et al.*, 2014). Pyrene tolerance capacity of *Aspergillus lacticoffeatus* is influenced by many factors such as solubility, adsorption, etc. (Argumedo-Delira *et al*., 2012; Latimer and Zheng 2003).

**Role of different carbon sources on *Aspergillus lacticoffeatus* growth.** Usually, fungi need suitable carbon sources, nitrogen sources, pH, temperature, etc. Among these, carbon sources are significant for the development and metabolism of fungi. Right carbon usually enhances the growth of fungi (Reddy and Mohan, 2012). The role of carbon sources on *Aspergillus lacticoffeatus* growth was done in a plate and batch culture methods. To find a suitable carbon source for the development of *Aspergillus, lacticoffeatus* was grown on media augmented with different carbon sources like glucose, fructose, sucrose, maltose, and starch. The length and density of fungi cultured with and without carbon source were recorded. The plate experiments showed that the thickness of the fungal mycelia was higher in the presence of sucrose, followed by glucose. In the case of fructose, maltose, and starch, the density was insignificant (Fig. 3). Results showed that the growth diameter for *Aspergillus lacticoffeatus* was 0.1mM for 24 h of incubation for all the carbon sources tested. At 48 h, the growth diameter was 0.3mM for glucose, 0.2mm for fructose, maltose, and sucrose, and 0.1mM for starch. The growth diameter increased with an increase in time for all the carbon sources (Table. 1). It was noted that in the plate augmented with glucose, there was a rapid growth in the diameter of mycelium, but density was less compared to the plate supplemented with sucrose in 3 to 4 days. Maximum density was seen at 168 h for glucose and sucrose compared to fructose, maltose, and starch. Carbon sources have been found to modify fungal hyphal morphology, thickness, and rate of metabolite release. Reports show that without a carbon source, fungi could not grow due to nutritive stress (Costa and Nahas 2012).

The wet weight of the fungal mycelia in the presence of different carbon sources for observed after 30 days. The damp weight was 11.8g, 11.4g, 12.5g, 11.3g, and 9.6g in the presence of glucose, fructose, sucrose, maltose, and starch, respectively. Withouta carbon source, *i.e.*, only MSM, the wet weight was 2.5g. Sucrose showed higher biomass growth than flasks containing other carbon sources (Costa and Nahas 2012). Based on these results, glucose and sucrose were used in further experiments.

**Influence of glucose on different nitrogen sources in pyrene degradation.** The removal of pyrene in the presence of glucose increased for the nitrogen sources studied. In the absence of glucose, there was insignificant removal of pyrene. The combination of glucose with different nitrogen sources was 22.09%, 31.65%, 31.95%, 69.62%, and 79.68% for calcium nitrate, ammonium sulfate, ammonium nitrate, sodium nitrate, and ammonium chloride, respectively (Fig. 4a). The results show that the combination of glucose and ammonium chloride was adequate for the removal of pyrene. Glucose has been identified as a suitable carbon source for the degradation of 4-tert-butylphenol and decane by CHY-2 strain (Govarthanan *et al.*, 2017). Ron and Rosenberg (2014) reported that glucose amended to the medium increased the rate of n-eicosane degradation (Ron and Rosenberg 2014). Reports show that fungal PAH degradation occurs only in the presence of an additional carbon source (Ravelet *et al.*, 2000; Sack *et al.*, 1997). Studies using *Cunninghamellae legends* illustrated that they could not utilize PAHs as the sole carbon and energy source (Cerniglia *et al.*, 1989; Cerniglia, 1997). White rot fungi, the deuteromycete *Penicillium* species, could co-metabolize pyrene (Boonchan *et al.*, 2000; Ravelet *et al.*, 2000).Similarly, the pyreneoxidation was increased by *P. janthinellum* by optimizing the co-substrate (glucose, nitrate) concentration and bioconversion time (Saraswathy and Hallberg 2002). Eibes *et al*. (2005) reported the role of extracellular enzymes, namely, manganese peroxidase and lignin peroxidize, in PAH biodegradation (Eibes *et al.*, 2005). Reports also show that *A. fumigatus* produced the above two enzymes, effectively degrading anthracene (Lahkar and Deka 2017; Ye *et al.*, 2011). Metabolites formed from the anthracene are phthalic anhydride, anthrone, and anthraquinone (Cajthaml *et al.*, 2002), and (Wu *et al.*, 2009) identified similar metabolites with *L.lacteus* and *F.solani* on anthracene. Studies show that benzo[a]pyrene was effectively degraded in the medium supplemented with glucose (Hadibarata and Kristanti 2012; Mao *et al.*, 2016).

**Influence of sucrose on different nitrogen sources in pyrene degradation.** The removal of pyrene for the combination of sucrose and different nitrogen sources is 20.51%, 22.38%, 43.09%, 60.74%, and 84.91% for ammonium sulfate, calcium nitrate, ammonium nitrate, sodium nitrate, and ammonium chloride, respectively (Fig. 4b). Results show that *Aspergillus lacticoffeatus* effectively removed pyrene in the presence of sucrose and ammonium chloride. Usually, PAH degradation occurs through mineralization or co-metabolism (Cerniglia, 1993). Studies indicate that microbes can use and degrade LMW-PAHs as sole carbon and energy sources for growth. In the case of HMW-PAHs, microbes cannot utilize them as LMW-PAHs (Nam *et al.*, 2001). Mostly fungal degradation of HMW-PAHs occurs with alternate carbons sources (Hwang *et al.*, 2007; Tekere *et al.*, 2005) and very few HMW-PAHs as sole carbon and energy sources (Wu *et al.*, 2009). Co-substrates induce the secretion of catabolic enzymes, which enhances the degradation of HMW-PAHs (Acevedo *et al*., 2011).

All these studies and present results show that fungi evolved in the natural selection process can be

effectively used to remediate pyrene-contaminated sites. Fungi are best chosen because of their wide range of PAHs hydroxylation. Thus, the isolated fungal species can be further investigated for developing wastewater treatment methods for pyrene degradation.

**Table 1: Fungal growth diameter.**

| | Glucose | Fructose | Sucrose | Maltose | Starch |
|---|---|---|---|---|---|
| 1 Day | 0.1mm | 0.1mm | 0.1mm | 0.1mm | 0.1mm |
| 2 Day | 0.3mm | 0.2mm | 0.2mm | 0.2mm | 0.1mm |
| 3 Day | 0.8mm | 0.7mm | 0.5mm | 0.6mm | 0.3mm |
| 4 Day | 1.5mm | 1.2mm | 0.9mm | 1.2mm | 1.2mm |
| 5 Day | 2.8mm | 2.0mm | 1.7mm | 1.9mm | 2.1mm |
| 6 Day | 3.8mm | 3.2mm | 3.0mm | 3.0mm | 3.4mm |
| 7 Day | 5.2mm | 4.8mm | 5.1mm | 4.5mm | 4.2mm |

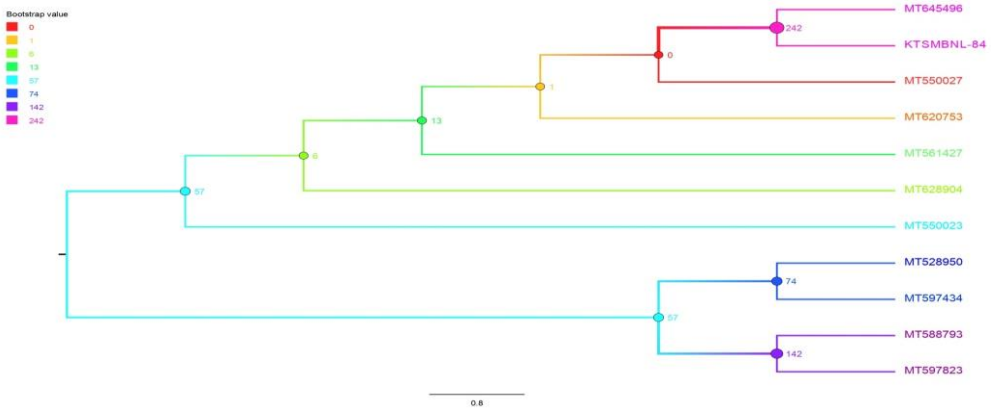



**Fig. 1** Phylogenetic relationship showing ascomycetes fungal communities isolated from crude oil contaminated soil based on 18S rRNA sequence.

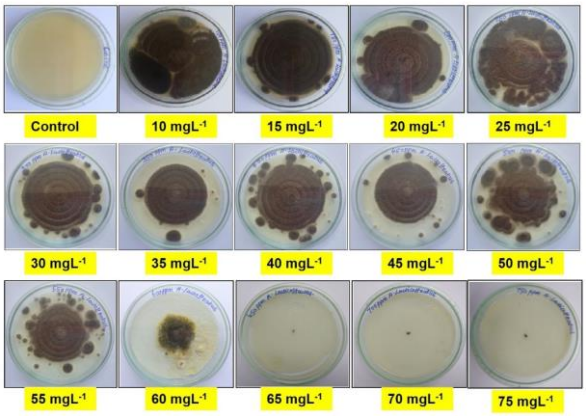



**Fig. 2.** Tolerance of *Aspergillus lacticoffeatus* on pyrene.

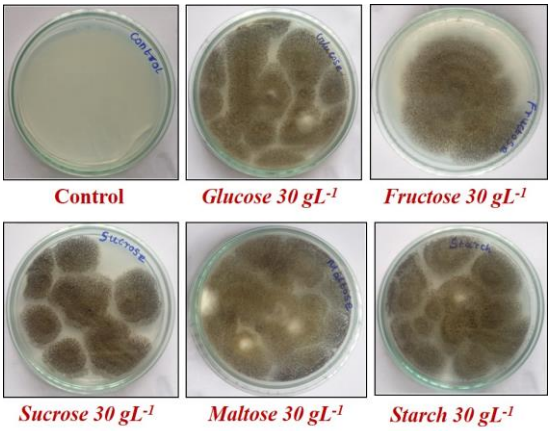
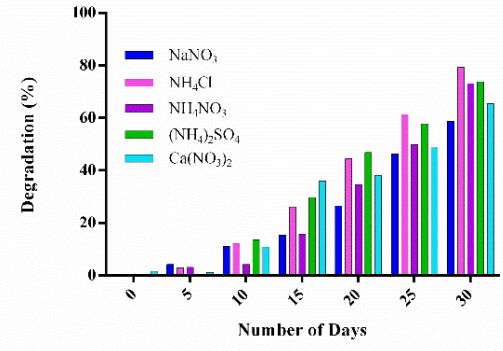
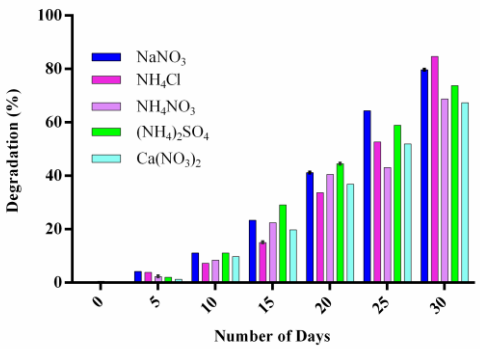



**Fig. 3.** Growth of *Aspergillus lacticoffeatus* in the presence of different carbon sources.




(a) Combined effect of glucose with different nitrogen sources (79.68%) for pyrene degradation by *Aspergillus lacticoffeatus*




(b) Combined effect of sucrose with different nitrogen sources (84.91%) for pyrene degradation by *Aspergillus lacticoffeatus.*

**Fig. 4.**

## CONCLUSIONS

This study dealt with isolating a novel strain of *Aspergillus lacticoffeatus* isolated from a crude oil-contaminated site for effective pyrene degradation. Results showed significant variance in the growth and pyrene removal pattern for *Aspergillus lacticoffeatus* for different carbon and nitrogen sources. High mycelial biomass and pyrene degradation in glucose and sucrose-containing medium was seen in the presence of ammonium chloride as a nitrogen source. Among glucose and sucrose, sucrose was seen to be more effective for removing pyrene with ammonium chloride by *Aspergillus lacticoffeatus.* Hence this fungal strain showed promising properties for the effective degradation of pyrene.

## FUTURE SCOPE

This study is designed to enhance the degradation of pyrene. We can conclude that *Aspergillus lacticoffeatus* effectively degraded pyrene, which can be used for cleaning hydrocarbon contamination.

**Acknowledgment:** We thank UGC- SAP and DST-FIST for providing infrastructure in the Department of Environmental Biotechnology.

**Conflict of Interest.** None.

## REFERENCES

Accensi, F., Cano, J., Figuera, L., Abarca, M. L. and Cabanes, F. J. (1999). New PCR method to differentiate species in the *Aspergillus niger* aggregate. *FEMS microbiology letters*, *180*(2), 191-196.

Acevedo, F., Pizzul, L., del Pilar Castillo, M., Cuevas, R. and Diez, M. C. (2011). Degradation of polycyclic aromatic hydrocarbons by the Chilean white-rot fungus *Anthracophyllum dis* color. *Journal of hazardous materials*, *185*(1), 212-219.

Adnan, M., Zheng, W., Islam, W., Arif, M., Abubakar, Y. S., Wang, Z. and Lu, G. (2017). Carbon catabolite repression in filamentous fungi. *International Journal of Molecular Sciences*, *19*(1), 48.

Agrawal, N. and Shahi, S. K. (2017). Degradation of polycyclic aromatic hydrocarbon (pyrene) using novel fungal strain Coriolopsisbyrsina strain APC5. *International Biodeterioration & Biodegradation*, *122*, 69-81.

Andriani, A. and Tachibana, S. (2016). Lignocellulosic materials as solid support agents for Bjerkanderaadusta SM46 to enhance polycyclic aromatic hydrocarbon degradation on sea sand and seawater media. *Biocatalysis and Agricultural Biotechnology*, *8*, 310-320.

Argumedo-Delira, R., Alarcón, A., Ferrera-Cerrato, R., Almaraz, J. J. and Peña-Cabriales, J. J. (2012). Tolerance and growth of 11 Trichoderma strains to crude oil, naphthalene, phenanthrene and benzo [a] pyrene. *Journal of environmental management*, *95*, S291-S299.

Bezalel, L., Hadar, Y., Fu, P. P., Freeman, J. P. and Cerniglia, C. E. (1996). Initial oxidation products in the metabolism of pyrene, anthracene, fluorene, and dibenzothiophene by the white rot fungus Pleurotusostreatus. *Applied and Environmental Microbiology*, *62*(7), 2554-2559.

Boonchan, S., Britz, M. L. and Stanley, G. A. (2000). Degradation and mineralization of high-molecular-weight polycyclic aromatic hydrocarbons by defined fungal-bacterial cocultures. *Applied and environmental microbiology*, *66*(3), 1007-1019.

Cajthaml, T., Möder, M., Kačer, P., Šašek, V. and Popp, P. (2002). Study of fungal degradation products of polycyclic aromatic hydrocarbons using gas chromatography with ion trap mass spectrometry detection. *Journal of Chromatography A*, *974*(1-2), 213-222.

Cerniglia, C. E. (1993). Biodegradation of polycyclic aromatic hydrocarbons. *Current opinion in biotechnology*, *4*(3), 331-338.

Cerniglia, C. E. (1997). Fungal metabolism of polycyclic aromatic hydrocarbons: past, present and future applications in bioremediation. *Journal of Industrial Microbiology and Biotechnology*, *19*(5-6), 324-333.

Cerniglia, C. E., Campbell, W. L., Freeman, J. P. and Evans, F. E. (1989). Identification of a novel metabolite in phenanthrene metabolism by the fungus *Cunninghamella* elegans. *Applied and environmental microbiology*, *55*(9), 2275-2279.

Costa, B. D. O. and Nahas, E. (2012). Growth and enzymatic responses of phytopathogenic fungi to glucose in culture media and soil. *Brazilian Journal of Microbiology*, *43*, 332-340.

Das, K. and Mukherjee, A. K. (2007). Differential utilization of pyrene as the sole source of carbon by Bacillus subtilis and Pseudomonas aeruginosa strains: role of biosurfactants in enhancing bioavailability. *Journal of applied microbiology*, *102*(1), 195-203.

Diano, A., Bekker Jensen, S., Dynesen, J. and Nielsen, J. (2006). Polyol synthesis in *Aspergillus niger:* influence of oxygen availability, carbon and nitrogen sources on the metabolism. *Biotechnology and bioengineering*, *94*(5), 899-908.

Dutta, D. and Das, M. D. (2017). Effect of carbon and nitrogen sources on mycelial biomass and biosynthesis of antifungal protein from *Aspergillus giganteus* MTCC 8408 in submerged fermentation. *Journal of Scientific and Industrial Research*, *76*(06), 369-375.

Eibes, G., Lú-Chau, T., Feijoo, G., Moreira, M. T. and Lema, J. M. (2005). Complete degradation of anthracene by manganese peroxidase in organic solvent mixtures. *Enzyme and Microbial Technology*, *37*(4), 365-372.

Essabri, A. M., Aydinlik, N. P. and Williams, N. E. (2019). Bioaugmentation and biostimulation of total petroleum

hydrocarbon degradation in a petroleum-contaminated soil with fungi isolated from olive oil effluent. *Water, Air, & Soil Pollution*, *230*, 1-16.

Ghosal, D., Ghosh, S., Dutta, T. K. and Ahn, Y. (2016). Current state of knowledge in microbial degradation of polycyclic aromatic hydrocarbons (PAHs): a review. *Frontiers in microbiology*, 1369.

Govarthanan, M., Fuzisawa, S., Hosogai, T. and Chang, Y. C. (2017). Biodegradation of aliphatic and aromatic hydrocarbons using the filamentous fungus Penicillium sp. CHY-2 and characterization of its manganese peroxidase activity. *RSC advances*, *7*(34), 20716-20723.

Hadibarata, T. and Kristanti, R. A. (2012). Fate and cometabolic degradation of benzo [a] pyrene by white-rot fungus Armillaria sp. F022. *Bioresource Technology*, *107*, 314-318.

Hamad, H. (2014). The effect of some sugars on the growth of *Aspergillus niger*. *KSÜ DoğaBilimleriDergisi*, *17*(4), 7-11.

Huang, Y., Wang, Y., Feng, H., Wang, J., Yang, X. and Wang, Z. (2019). Genome-guided identification and characterization of bacteria for simultaneous degradation of polycyclic aromatic hydrocarbons and resistance to hexavalent chromium. *International Biodeterioration & Biodegradation*, *138*, 78-86.

Hwang, H. M., Hu, X. and Zhao, X. (2007). Enhanced bioremediation of polycyclic aromatic hydrocarbons by environmentally friendly techniques. *Journal of Environmental Science and Health Part C*, *25*(4), 313-352.

Imam, A., Suman, S. K., Ghosh, D. and Kanaujia, P. K. (2019). Analytical approaches used in monitoring the bioremediation of hydrocarbons in petroleum-contaminated soil and sludge. *TrAC Trends in Analytical Chemistry*, *118*, 50-64.

Kaur, J., Dhanjal, D. S. and Singh, R. (2021). Microbiological Perspectives of Hydrocarbon Degradation: A Review. *Biological Forum – An International Journal*, *13*(1), 440-445.

Lahkar, J. and Deka, H. (2017). Isolation of polycyclic aromatic hydrocarbons (PAHs) degrading fungal candidate from oil-contaminated soil and degradation potentiality study on anthracene. *Polycyclic Aromatic Compounds*, *37*(2-3), 141-147.

Latimer, J. S. and Zheng, J. (2003). The sources, transport, and fate of PAHs in the marine environment. *PAHs: an ecotoxicological perspective*, 7-33.

Mallick, S., Chakraborty, J. and Dutta, T. K. (2011). Role of oxygenases in guiding diverse metabolic pathways in the bacterial degradation of low-molecular-weight polycyclic aromatic hydrocarbons: a review. *Critical reviews in microbiology*, *37*(1), 64-90.

Mao, J. and Guan, W. (2016). Fungal degradation of polycyclic aromatic hydrocarbons (PAHs) by *Scopulariopsis brevicaulis* and its application in bioremediation of PAH-contaminated soil. *Acta Agriculturae Scandinavica, Section B—Soil & Plant Science*, *66*(5), 399-405.

Nam, K., Rodriguez, W. and Kukor, J. J. (2001). Enhanced degradation of polycyclic aromatic hydrocarbons by biodegradation combined with a modified Fenton reaction. *Chemosphere*, *45*(1), 11-20.

Nehls, U., Mikolajewski, S., Magel, E. and Hampp, R. (2001). Carbohydrate metabolism in ectomycorrhizas: gene expression, monosaccharide transport and metabolic control. *New Phytologist*, *150*(3), 533-541.

Oostingh, G. J., Smith, K. E., Tischler, U., Radauer-Preiml, I. and Mayer, P. (2015). Differential immunomodulatory responses to nine polycyclic aromatic hydrocarbons applied by passive dosing. *Toxicology in Vitro*, *29*(2), 345-351.

Qiu, M., Sun, K., Jin, J., Han, L., Sun, H., Zhao, Y. and Xing, B. (2015). Metal/metalloid elements and polycyclic aromatic hydrocarbon in various biochars: The effect of feedstock, temperature, minerals, and properties. *Environmental Pollution*, *206*, 298-305.

Ravelet, C., Krivobok, S., Sage, L., and Steiman, R. (2000). Biodegradation of pyrene by sediment fungi. *Chemosphere*, *40*(5), 557-563.

Reddy, M. V. and Mohan, S. V. (2012). Effect of substrate load and nutrients concentration on the polyhydroxyalkanoates (PHA) production using mixed consortia through wastewater treatment. *Bioresource technology*, *114*, 573-582.

Ron, E. Z. and Rosenberg, E. (2014). Enhanced bioremediation of oil spills in the sea. *Current Opinion in biotechnology*, *27*, 191-194.

Sack, U., Heinze, T. M., Deck, J., Cerniglia, C. E., Martens, R., Zadrazil, F. and Fritsche, W. (1997). Comparison of phenanthrene and pyrene degradation by different wood-decaying fungi. *Applied and Environmental Microbiology*, *63*(10), 3919-3925.

Saraswathy, A. and Hallberg, R. (2002). Degradation of pyrene by indigenous fungi from a former gasworks site. *FEMS Microbiology Letters*, *210*(2), 227-232.

Souza, H. M. D. L., Barreto, L. R., Mota, A. J. D., Oliveira, L. A. D., Barroso, H. D. S. and Zanotto, S. P. (2017). Tolerance to polycyclic aromatic hydrocarbons (PAHs) by filamentous fungi isolated from contaminated sediment in the Amazon region. *Volume 39, Número 4*, pp, 481-488.

Tekere, M., Read, J. S. and Mattiasson, B. (2005). Polycyclic aromatic hydrocarbon biodegradation in extracellular fluids and static batch cultures of selected sub-tropical white rot fungi. *Journal of biotechnology*, *115*(4), 367-377.

Tomás, M. S. J., Carrasco, M. G., Lobo, C. B., Alessandrello, M. J., Sánchez, L. and Ferrero, M. A. (2019). PAH removal by simultaneous and sequential inoculation of Pseudomonas monteilii P26 and Gordonia sp. H19 in the presence of biostimulants. *International Biodeterioration & Biodegradation*, *144*, 104752.

Wu, Y. R., He, T. T., Lun, J. S., Maskaoui, K., Huang, T. W. and Hu, Z. (2009). Removal of benzo [a] pyrene by a fungus *Aspergillus* sp. BAP14. *World Journal of Microbiology and Biotechnology*, *25*, 1395-1401.

Ye, J. S., Yin, H., Qiang, J., Peng, H., Qin, H. M., Zhang, N. and He, B. Y. (2011). Biodegradation of anthracene by Aspergillus fumigatus. *Journal of hazardous materials*, *185*(1), 174-181.

Zafra, G., Absalón, Á. E., Cuevas, M. D. C. and Cortés-Espinosa, D. V. (2014). Isolation and selection of a highly tolerant microbial consortium with potential for PAH biodegradation from heavy crude oil-contaminated soils. *Water, Air, & Soil Pollution*, *225*, 1-18.

**How to cite this article:** Veeramani Veerasamy, Gowri Manogari Boovaragamoorthy and Thamaraiselvi Kaliannan (2023). Influence of Supplementary Carbon and Nitrogen Sources on Pyrene Degradation by *Aspergillus lacticoffeatus* Isolated from Crude Oil-contaminated Soil. *Biological Forum – An International Journal*, *15*(5): 93-98.