# Biodegradation of polycyclic aromatic hydrocarbons in Basrah heavy crude oil by *Aspergillus terreus*

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## Abstract

One of the most harmful environmental contaminants is thought to be crude oil. Polycyclic aromatic hydrocarbons (PAHs) are thought to be among the most significant and hazardous petroleum molecules that are released into the environment. Currently, one of the most significant and ecologically beneficial methods for treating these toxins is bioremediation. Therefore, the purpose of this study was to find out how well the fungus *Aspergillus terreus* Thom, which was isolated from a soil that had been heavily contaminated by oil, could biodegrade the polycyclic aromatic hydrocarbons found in Basrah heavy crude oil. It was also determined how various factors affected this fungus capacity for biodegradation. Findings revealed that following a 15-day incubation period, *A. terreus*, degraded 43% of the crude oil, and this percentage was increased to 75% after 30 days of incubation. GC-MC analysis revealed that *A. terreus* had a good ability to degrade PAHs, and the residual total PAHs reached to 755 µg $mL^{-1}$ after 15 days and decreased to 491 µg $mL^{-1}$ after 30 days compared with the control with 1006 µg $mL^{-1}$ PAHs. Three compounds namely napthalene, acenaphthnen and fluorene**,** were completely degraded. The temperature 30 °C and pH 5.5 were the optimum conditions for degradation. The degradation percentage reached to 29%, and the total PAHs concentration was decreased to 397 µg $mL^{-1}$ after 7 days of incubation at the optimum conditions. Two aromatic compounds *viz.* napthalene and fluorene were completely degraded after 15 days and the concentration of PAHs was decreased to 235 µg $mL^{-1}$. Hence, *A. terreus* exhibited the potential for the degradation of PAHs.

**Keywords:** Biodegradation, Contaminated soil, Heavy crude oil, Polycyclic aromatic hydrocarbons.

## Introduction

Crude oil and its derivatives are one of the most important energy resources worldwide. The research emphasis now to utilize heavy oil reserves because of the reduction of light oil incomes and the escalation in energy request (Al-Sayegh *et al*., 2015). Crude oil is a complex combination of diverse compounds, which can be divided into four main groups one of the most important one is polycyclic aromatic compounds (PAHs) (Liu *et al*., 2014). Crude oil with heavy nature comprises an extraordinary proportion of PAHs that showed extra hardy to degradation than the light type (Darvishi *et al*., 2011). PAHs with organic nature having up to seven rings are toxic and environmentally stable and act as carcinogenic compounds in humans and other organisms (Abdullatif *et al*., 2016; Al-Dossary *et al*., 2020). These mixtures can possibly accrue in the environment to considerable levels. PAHs may harmfully disturb the physical and chemical properties of soil, including soil texture and water capacity, moreover they may inter to the food chain and drift to groundwater, producing different damages to existing organisms, particularly because of their mutagenic or carcinogenic nature (Govarthanan *et al*., 2017; Adenji *et al*., 2019).

PAH are removed from the environment by several methods, including biodegradation, which is a natural process that uses microbial consortium for degradation; they harness the ability of microorganisms to degrade pollutants and convert them to less lethal materials than their parental complexes (Al-Dossary *et al*., 2019; Al-Dhabaan *et al*., 2021). Fungi own certain advantages over bacteria because of their mycelial development and their confrontation to PAHs (Abdel-Shafy and Mansour, 2016; Al-Hawash *et al*., 2018). Fungal flora was readily isolated from soils polluted with PAHs and have an extraordinary capability to reduce their concentrations. The fungus *Aspergillus terreus* is commonly isolated fungus from the environment, and it has increased consideration as a possible agent for degradation of different PAHs (Lotfinasabasl *et al*., 2012; Al-Harbi *et al*., 2022). This study designed to determine the ability of *A. terreus*, which was isolated from soil polluted with oil in Basrah province, to degrade PAHs of heavy crude oil and assess the effectiveness of variable temperatures and pH on the process.

## Materials and Methods

### Samples collection

From September 2021 to November 2021, ten samples of soil polluted with oil were taken through the top level nearly five to ten cm of various oil fields in the southern Iraqi area of Basrah. Samples of soil were gathered and kept at 4 °C until later usage in sterile plastic bags. (Latha and Kalaivani, 2012).

**Crude oil**

Crude oil (heavy type) was obtained from a southern oil company (Basrah, Iraq) and kept till use.

**Chemicals**

Different media and chemicals were achieved from the Indian company Hi-Media (India).

**Fungal isolation and identification**

Dilution technique which was defined formerly by Wicklow and Wittingham (1974) was used to isolate fungi from soil samples. Ninety milliliters of sterilized normal saline were used to dissolve10 g from each soil sample to get a dilution of $10^{-1}$ then it will shake well for 10 min. One millilitre of the diluted sample was placed on a Petri dish, and about fifteen millilitres of the sterile medium PDA were added separately. To prevent the growth of bacteria the antibiotic chloramphenicol 250 mg $L^{-1}$ was added to the growth medium. Before solidification the Petri dishes were moved to blend the components well, then incubated at 25 °C for one to two weeks. Fungal species were purified and identified to species level based on cultural and morphological features and maintained on PDA medium at 4 °C for future works.

**PAHs degradation by a single fungal isolate**

Before assessing the fungal isolate's capacity to break down heavy crude oil, it was first activated for seven days on PDA medium. The broth medium for biodegradation experiment (MSM) mineral salts medium had the next composition (g $L^{-1}$):NaCl, 10.0; KCl, 0.12; $K_2HPO_4$, 0.83; $MgSO_4.7H_2O$, 0.42; $Na_2HPO4$, 1.25 and $NaNO_3$, 0.42. The outer edge of the chosen colony was used to take 2 pieces by a cork borer 5 mm in diameter, then placed into conical flasks holding 100 millilitres of the MSM (pH 4.5). Each flask received one millilitre of crude oil (heavy type) as the only source for carbon and energy. The culture flasks were incubated in a rotating shaker incubator with  rpm of 110 at 25 °C for 15 and 30 days. Medium having heavy crude oil only attended as a control. Triplicate flasks were used for all experiments (Okerentugba and Ezeronye, 2003).

**Effect of some physical and chemical limitations on biodegradation**

Following a previously published protocol by Hamzah *et al*. (2012), the following parameters' effects on fungal biodegradation capacity were assessed:

**Incubation temperature**

Two flask sets having the fungal species, the formerly designated MSM with pH 4.5 and crude oil were constructed. The first set was maintained under 30 °C, the second one under 35 °C, in a rotating shaker incubator at 110 rpm for two periods 15 and 30 days. As a control, a uniform medium containing just heavy crude oil was used as a control. Triplicate flasks were used for all experiments

**pH**

To evaluate the outcome of diverse pH values on the process of biodegradation. Two rates of pH 5.5 and 6.5 were used in the culture media to study their effect on fungal growth and the operation of biodegradation. Conical flasks with crude oil and MSM medium only used as a control. Flasks in triple sets were used for each treatment and all flasks incubated in rotating incubator under 25 °C plus 110 rpm for one and two weeks.

**Effect of optimal conditions on PAHs biodegradation**

Conical flasks (250 mL) having 100 mL of MSM were kept in the optimum temperature and pH, which were definite from earlier trials, to estimate the result of bio stimulation on PAHs degradation by *A. terreus*. Each flask was inoculated with the fungal isolate. Conical flasks with crude oil and MSM medium only used as a control. Flasks in triple sets were used for each treatment and all flasks incubated in rotating incubator under 30 °C plus 110 rpm for one and two weeks.

**PAHs separation and determination**

The culture medium of each flask was filtered following the end of every test by GFF filter paper and filtration device. The filtrate was collected and put to a separating funnel. The filtrate was extracted with one hundred millilitres of chloroform and constant shaker. The extraction procedure was recurrent for three intervals. The liquefied part was allowed to settle for a time, resulting in two layers the organic fraction which having the remaining oil and an aqueous fraction. The organic layer was moved to a pre-weighed flask and left to dry by air, then reweighed. The percentage of degradation was measured as follows Outdot (1984):

$$\text{Degradation (\%)} = \frac{\text{mg of crude oil control} - \text{mg of crude oil test}}{\text{mg of crude oil control}} \times 100$$

To separate the aromatic components, a silica gel column was used to pass the separated oil through it. Five millilitres from n-hexane were used to liquefy the samples after drying them by air. The liquefy fragments were then move through a column of silica gel. Thirty millilitres of benzene were used to split the PAHs component. Rotating evaporator was used to evaporate the fractions to dryness, then the dryness fractions were liquefied again in five millilitres of n-hexane and transferred to the gas chromatography instrument mass spectrometer GC-MS to detect PAHs (UNEP, 1989).

**Statistical analysis**

Minitab version 16 software was used to analyse the outcomes from the experiments using ANOVA. Significant differences among fungal processes were determined by calculating the relative least-significant difference values. A totally randomised design was implemented.

## Results and Discussion

**PAH biodegradation using *Aspergillus terreus***

Twenty fungal species belonging to nine genera were identified, eight of them belonged to the genus *Aspergillus*. The fungus *A. terreus*, which showed the higher ability in the preliminary test to degrade crude oil (Ahmed *et al*., 2023), was selected to determine its efficiency in biodegrading the aromatic compounds of heavy crude oil in liquid media and in the form of single isolate with two incubation periods of 15 and 30 days. As the fungus showed a good ability to degrade heavy crude oil, degradation-related changes were observed, including the growth of fungal hyphae in the liquid medium and in the form of mycelial attached to the crude oil. The biomass of the fungal hyphae increased over time. The tested fungus proved its ability to biodegrade heavy crude oil, in which the percentage of biodegradation reached 43% after 15 days of incubation and increased to 75% at the completion of the period of incubation. Statistical analysis revealed substantial differences ($P \leq 0.01$) in the proportion of PAHs degraded between 15 and 30 days.

Gas chromatography was used to analyse PAHs extracted from liquid growth cultures. The results showed that *A. terreus* had a good ability to biodegrade aromatic compounds. The total residual concentrations of aromatic compounds reached 756 µg $mL^{-1}$ after the end of the 15-day growth period and continued to decrease to reach 490 µg $mL^{-1}$ after the end of the 30-day growth period. Statistical analysis revealed substantial differences ($P \leq 0.01$) in the proportion of PAHs degraded between 15 and 30 days of incubation, compared to the control treatment 1006 µg $mL^{-1}$ (Table 1, Fig. 1).

Major differences ($P \leq 0.01$) in the percentage of PAHs degraded between the two incubation periods of 15 and 30 days and compared with control treatment were showed. *A. terreus* completely degraded three aromatic compounds, namely, naphthalene, acenaphthene, and fluorene, after 30 days of incubation.

Fungi possess a great resistance to hydrocarbons toxicity and they exhibit an active capability for hydrocarbons degradation because of their physiology. The excretion of multiple types of degradative enzymes in high amounts enable this fungus to have potential degradation capacity for PAHs. Moreover, *A. terreus* has an active and efficient enzyme system and can adapt and consume oil compounds, resulting in its high ability to biodegrade aromatic compounds (Mohsenzadeh *et al*., 2012; Barnes *et al*., 2018; Hassaine and Bordjiba, 2019). These results are constant with earlier studies that stated the degradation capability of *Aspergillus* for hydrocarbons (Al-Dossary *et al*., 2020; Al-Harbi *et al*., 2022).

**Optimisation of PAHs degradation using *A. terreus***

The influence of the two parameters (pH and temperature) on the biodegradation process was investigated for 15- and 30-days using *A. terreus*. Fungal growing can be disturbed by different limits and temperature is one of the most important limits (Delille *et al*., 2004). A variety of temperatures, 25–35 °C were used to cultivate *A. terreus* and 30 °C was the best temperature for degradation and growth. At 30 °C, the original concentration of PAHs 1006 µg $mL^{-1}$ was reduced significantly to 709 µg $mL^{-1}$ after 15 days and continued to decrease to 474 µg $mL^{-1}$ after 30 days of incubation (Table 2). The degradation percentage reached 63% at 30 °C after 15 days and increased to 75% after 30 days. The temperature 35 °C negatively affected the degradation. The percentage of heavy crude oil degraded was 13% after 15 days and 38% after 30 days. The amount of total residual PAHs was 911 µg $mL^{-1}$ after 15 days and decreased to 568µg $mL^{-1}$ after 30 days. The present result is in line with previous experiments (Al-Hawash *et al*., 2018; Al-Dossary *et al*., 2020), which found that optimum temperature is 30 °C for fungal growth through oil degradation. Increased fungal growth may improve energy efficacy in the fungal structure by promoting interaction between the energy source and cell surface. Yet, at temperatures more than 30 °C, the mechanism perhaps deceleration possibly because of the distortion of cell layer existing as positions for substratum linking (Al-Asheh and Duvnjak, 2018; DSouza *et al*., 2021).

Fungi favour acidic conditions for growth, the optimal pH in the present research was 5.5, at which the percentage degradation for heavy crude oil reached 43% after 15 days and continued to increase to 75% after 30 days of incubation. The total PAH concentration decreased considerably to 601µg $mL^{-1}$after 15 days and decreased to 213 µg $mL^{-1}$ after 30 days (Table 3). pH 6.5 had negative effects on the degradation, that is, the percentage of degrading heavy crude oil reached 25% after 15 days and increased to 63% after 30 days. The total PAH concentrations were 779 µg $mL^{-1}$ after 15 days of incubation and decreased to 366 µg $mL^{-1}$after 30 days. The increasing of the hydroxyl ions in the medium may possibly inhibits the adsorption of PAHs to cation-binding sites (Jalali *et al*., 2002).

Comparable outcomes were stated in previous studies (Hamzah *et al*., 2012; Al-Dossary *et al*., 2020), which demonstrated that microbial growth

and degradation directly affected by the conditions in the culture medium. The PAHs concentrations significantly differed ($P \leq 0.01$) between 15 and 30 days of incubation under different temperatures and pH levels.

**Biodegradation of PAHs under bio-stimulation conditions**

Growing and enzymatic action of the microorganisms stimulate by the modification of environmental situations. Effects of the optimal conditions, namely, 30 °C and pH 5.5, on PAHs biodegradation by *A. terreus* were estimated. At the seven days of the experiment, the percentage of biodegradation was 29%, and the overall concentration of PAHs decreased to 397µg $mL^{-1}$. A high biodegradation efficacy was reached with bio stimulation (Fig. 5), where the concentration of the total PAHs decreased to 397 µg $mL^{-1}$ within 7 days. This value is lower than the concentration of the residual PAHs by *A. terreus* in 15 days without bio stimulation. This finding can be attributed to that exposing the fungus to the best temperature and pH give rise to greater enzymatic action and fungal growing, along with more mycelial growth coming in contact with the heavy crude oil, thereby improving breakdown of PAHs (Zeneli *et al*., 2019; Al-Dossary *et al*., 2020). Several previous studies congruent with the current results (Kuappi *et al*., 2011; Wu *et al*., 2016). After 15 days of incubation, there was an increase in degradation efficiency. The PAHs concentration decreased to 236 µg $mL^{-1}$ (Table 4), and the degradation percentage was 63%. The degradation percentage significantly differed ($P \leq 0.01$) among the fungus in this trial and that in the earlier trials without bio stimulation. Numerous researches stated that biodegradation was maximised when the best circumstances for growing were attained (Govarthanan *et al*., 2017; Al-Dossary *et al*., 2020).

## Conclusion

High biodegradation prospective was revealed by *Aspergillus terreus* under laboratory situations. It decreased the concentration of the total PAHs of heavy crude oil to 236 µg $mL^{-1}$ at the end of day 15 of incubation. Bio stimulation below the greatest temperature and pH stimulated the degradation process. Moreover, the time of incubation also affected biodegradation, with about 63% of heavy crude oil being biodegraded at the end of day 15 of the experiment. This experiment revealed that *A. terreus* is a good biodegrader organism which might be applied in the remediation of PAHs in the environment.

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## Contribution of authors

MAA wrote the main text of the manuscript, ZAA analyzed the data and formatted tables while FMA carried out analysis of PAHs.

## Conflict of interest

Authors declare that there is no conflict of interest.

**Table 1:** Residual concentrations of PAHs for heavy crude oil in the liquid medium culture of *Aspergillus terreus.*

| PAHs compounds | Control (µg $mL^{-1}$) | PAHs compounds | Concentration (µg $mL^{-1}$) After 15 days | PAHs compounds | Concentration (µg $mL^{-1}$) After 30 days |
|---|---|---|---|---|---|
| Naphthalene | 16 | Naphthalene | 0 | Naphthalene | **0** |
| 2-Methylnaphtha | 279 | 2-Methylnaphtha | 97 | 2-Methylnaphtha | **85** |
| 1-Methylnaphtha | 38 | 1-Methylnaphtha | 24 | 1-Methylnaphtha | **21** |
| Acenaphthyene | 49 | Acenaphthyene | 56 | Acenaphthyene | **41** |
| Acenaphthnen | 51 | Acenaphthnen | 43 | Acenaphthnen | **0** |
| Fluorene | 8 | Fluorene | 7 | Fluorene | **0** |
| Phenanthrene | 126 | Phenanthrene | 123 | Phenanthrene | **114** |
| Anthracene | 107 | Anthracene | 112 | Anthracene | **75** |
| Fluoranthene | 39 | Fluoranthene | 39 | Fluoranthene | **27** |
| Pyrene | 152 | Pyrene | 102 | Pyrene | **42** |
| Chrysene | 19 | Chrysene | 21 | Chrysene | **7** |
| Benzo(a)anthracene | 10 | Benzo(a)anthracene | 14 | Benzo(a)anthracene | **14** |
| Benzo(b) fluoranthene | 37 | Benzo(b) fluoranthene | 45 | Benzo(b) fluoranthene | **27** |
| Benzo(k) fluoranthene | 9 | Benzo(k) fluoranthene | 13 | Benzo(k) fluoranthene | **5** |
| Benzo(a) pyrene | 50 | Benzo(a) pyrene | 45 | Benzo(a) pyrene | **26** |
| Indeno(1,2,3-cd)pyrene | 16 | Indeno(1,2,3-cd)pyrene | 15 | Indeno(1,2,3-cd)pyrene | **6** |
| Benzo(g,h,i)perylene | 0 | Benzo(g,h,i)perylene | 0 | Benzo(g,h,i)perylene | **0** |
| **Total** | **1006** | **Total** | **756** | **Total** | **490** |

**Table 2:** Effect of temperature at 30 °C on biodegradation of PAHs by *Aspergillus terreus.*

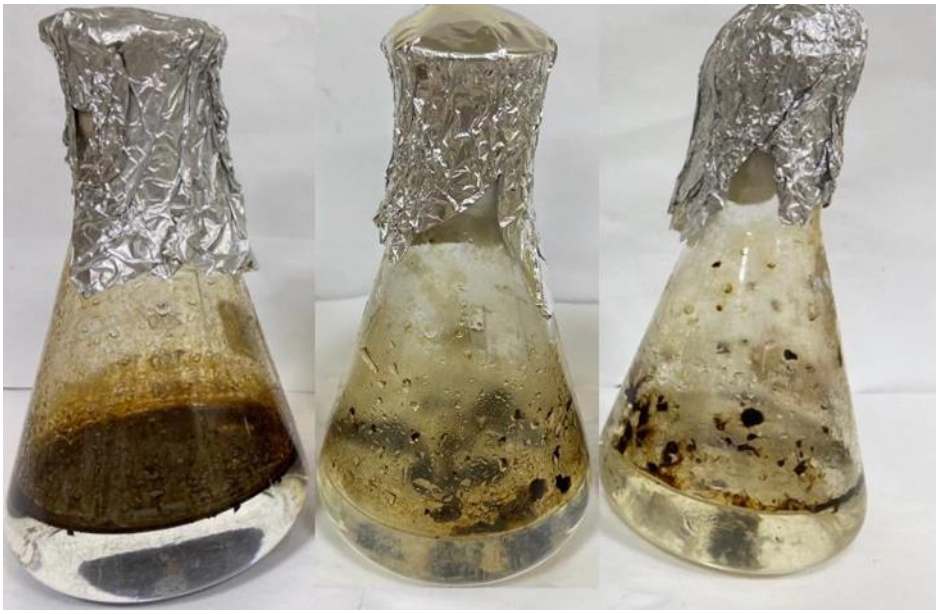
| PAHs compounds | Control (µg mL$^{-1}$) | PAHs compounds | Concentration (µg mL$^{-1}$) After 15 days | PAHs compounds | Concentration (µg mL$^{-1}$) After 30 days |
|---|---|---|---|---|---|
| Naphthalene | 16 | Naphthalene | 0 | Naphthalene | 0 |
| 2-Methylnaphtha | 279 | 2-Methylnaphtha | 96 | 2-Methylnaphtha | 83 |
| 1-Methylnaphtha | 38 | 1-Methylnaphtha | 25 | 1-Methylnaphtha | 21 |
| Acenaphthyene | 49 | Acenaphthyene | 48 | Acenaphthyene | 44 |
| Acenaphthnen | 51 | Acenaphthnen | 49 | Acenaphthnen | 0 |
| Fluorene | 8 | Fluorene | 0 | Fluorene | 0 |
| Phenanthrene | 126 | Phenanthrene | 121 | Phenanthrene | 90 |
| Anthracene | 107 | Anthracene | 103 | Anthracene | 81 |
| Fluoranthene | 39 | Fluoranthene | 38 | Fluoranthene | 23 |
| Pyrene | 152 | Pyrene | 101 | Pyrene | 43 |
| Chrysene | 19 | Chrysene | 20 | Chrysene | 18 |
| Benzo(a)anthracene | 10 | Benzo(a)anthracene | 10 | Benzo(a)anthracene | 7 |
| Benzo(b) fluoranthene | 37 | Benzo(b) fluoranthene | 35 | Benzo(b) fluoranthene | 25 |
| Benzo(k) fluoranthene | 9 | Benzo(k) fluoranthene | 4 | Benzo(k) fluoranthene | 2 |
| Benzo(a) pyrene | 50 | Benzo(a) pyrene | 43 | Benzo(a) pyrene | 37 |
| Indeno(1,2,3-cd)pyrene | 16 | Indeno(1,2,3-cd)pyrene | 16 | Indeno(1,2,3-cd)pyrene | 0 |
| Benzo(g,h,i)perylene | 0 | Benzo(g,h,i)perylene | 0 | Benzo(g,h,i)perylene | 0 |
| **Total** | **1006** | **Total** | **709** | **Total** | **474** |

**Table 3:** Effect of potential of hydrogen ions at the pH 5.5 on biodegradation of PAHs by *Aspergillus terreus.*

| PAHs compounds | Control (µg mL$^{-1}$) | PAHs compounds | Concentration (µg mL$^{-1}$) After 15 days | PAHs compounds | Concentration (µg mL$^{-1}$) After 30 days |
|---|---|---|---|---|---|
| Naphthalene | 16 | Naphthalene | 13 | Naphthalene | 0 |
| 2-Methylnaphtha | 279 | 2-Methylnaphtha | 261 | 2-Methylnaphtha | 82 |
| 1-Methylnaphtha | 38 | 1-Methylnaphtha | 38 | 1-Methylnaphtha | 11 |
| Acenaphthyene | 49 | Acenaphthyene | 36 | Acenaphthyene | 16 |
| Acenaphthnen | 51 | Acenaphthnen | 37 | Acenaphthnen | 13 |
| Fluorene | 8 | Fluorene | 0 | Fluorene | 0 |
| Phenanthrene | 126 | Phenanthrene | 40 | Phenanthrene | 20 |
| Anthracene | 107 | Anthracene | 35 | Anthracene | 27 |
| Fluoranthene | 39 | Fluoranthene | 17 | Fluoranthene | 8 |
| Pyrene | 152 | Pyrene | 24 | Pyrene | 13 |
| Chrysene | 19 | Chrysene | 18 | Chrysene | 7 |
| Benzo(a)anthracene | 10 | Benzo(a)anthracene | 7 | Benzo(a)anthracene | 4 |
| Benzo(b) fluoranthene | 37 | Benzo(b) fluoranthene | 20 | Benzo(b) fluoranthene | 0 |
| Benzo(k) fluoranthene | 9 | Benzo(k) fluoranthene | 9 | Benzo(k) fluoranthene | 7 |
| Benzo(a) pyrene | 50 | Benzo(a) pyrene | 33 | Benzo(a) pyrene | 5 |
| Indeno(1,2,3-cd)pyrene | 16 | Indeno(1,2,3-cd)pyrene | 13 | Indeno(1,2,3-cd)pyrene | 0 |
| Benzo(g,h,i)perylene | 0 | Benzo(g,h,i)perylene | 0 | Benzo(g,h,i)perylene | 0 |
| **Total** | **1006** | **Total** | **601** | **Total** | **213** |

**Table 4:** Effect of biostimulation on biodegradation of PAHs by *Aspergillus terreus.*

| PAHs compounds | Control (µg mL$^{-1}$) | PAHs compounds | Concentration (µg mL$^{-1}$) After 15 days | PAHs compounds | Concentration (µg mL$^{-1}$) After 30 days |
|---|---|---|---|---|---|
| Naphthalene | 16 | Naphthalene | 1 | Naphthalene | **0** |
| 2-Methylnaphtha | 279 | 2-Methylnaphtha | 113 | 2-Methylnaphtha | **60** |
| 1-Methylnaphtha | 38 | 1-Methylnaphtha | 16 | 1-Methylnaphtha | **9** |
| Acenaphthyene | 49 | Acenaphthyene | 21 | Acenaphthyene | **14** |
| Acenaphthnen | 51 | Acenaphthnen | 17 | Acenaphthnen | **10** |
| Fluorene | 8 | Fluorene | 0 | Fluorene | **0** |
| Phenanthrene | 126 | Phenanthrene | 51 | Phenanthrene | **11** |
| Anthracene | 107 | Anthracene | 28 | Anthracene | **21** |
| Fluoranthene | 39 | Fluoranthene | 12 | Fluoranthene | **10** |
| Pyrene | 152 | Pyrene | 13 | Pyrene | **10** |
| Chrysene | 19 | Chrysene | 9 | Chrysene | **5** |
| Benzo(a)anthracene | 10 | Benzo(a)anthracene | 8 | Benzo(a)anthracene | **5** |
| Benzo(b) fluoranthene | 37 | Benzo(b) fluoranthene | 40 | Benzo(b) fluoranthene | **26** |
| Benzo(k) fluoranthene | 9 | Benzo(k) fluoranthene | 2 | Benzo(k) fluoranthene | **2** |
| Benzo(a) pyrene | 50 | Benzo(a) pyrene | 51 | Benzo(a) pyrene | **39** |
| Indeno(1,2,3-cd)pyrene | 16 | Indeno(1,2,3-cd)pyrene | 15 | Indeno(1,2,3-cd)pyrene | **14** |
| Benzo(g,h,i)perylene | 0 | Benzo(g,h,i)perylene | 0 | Benzo(g,h,i)perylene | **0** |
| **Total** | **1006** | **Total** | **397** | **Total** | **236** |



**Control** **15 days** **30 days**

**Fig. 1:** The ability of *Aspergillus terreus* to degrade heavy crude oil in liquid medium after 15 and 30 days of incubation compared with the control.

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