Degradative pathways of polycyclic aromatic hydrocarbons (PAHs) by Phanerochaete chrysosporium under optimum conditions

M. A. M. Abo-State\textsuperscript{a}, M. E. Osman\textsuperscript{b}, O. H. Khattab\textsuperscript{b}, T. A. El-Kelani\textsuperscript{c} and Z. M. Abdel-Rahman\textsuperscript{b}

\textsuperscript{a}Radiation Microbiology Department, National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority (EAEA), Nasser City, Egypt; \textsuperscript{b}Botany and Microbiology Department, Faculty of Science, Helwan University, Cairo, Egypt

ABSTRACT
Polycyclic aromatic hydrocarbons (PAHs) constitute one group of priority environmental contaminants which must be disposed. Phanerochaete chrysosporium is one of the white rot fungi (WRF) used in the present study to investigate different conditions affecting its ability to utilize PAHs as a sole carbon and energy sources. \textit{P. chrysosporium} was grown under optimum conditions, and its degradation percentage and degradative pathways have been determined by HPLC and GC/MS. The results showed that the optimum condition for the growth of \textit{P. chrysosporium} was five discs inoculum size at 25°C for 7 days incubation period on 100 mg/L of each PAHs in BSM supplemented with 2000 \(\mu\)M MnSO\(_4\) with shaking. Four groups (I–IV) of optimum conditions were used to determine degradation percentage. The results cleared that the best PAHs in degradation was pyrene (Pyr.). \textit{P. chrysosporium} degraded (100%) Pyr. under the four groups. \textit{P. chrysosporium} degraded the six PAHs (Acen.; Anth.; Flu.; Naph.; Phen.; and Pyr.) efficiently. The intermediates resulted from degradation indicated that \textit{P. chrysosporium} first oxidized the middle ring or hetero ring followed by ring fission. \textit{P. chrysosporium} followed the phthalate route in its degradative pathway. The intermediates finally interred TCA cycle and give CO\(_2\) and H\(_2\)O or short chain aliphatic polymerized to give long chains.

1. Introduction
Polycyclic aromatic hydrocarbons (PAHs) are fused ring aromatic compounds of two or more fused rings. Science PAHs are carcinogenic, mutagenic, and teratogenic, and they represent a great environmental concern (Torres-Farrada et al., 2019; Abo-State & El-kelani, 2020).

The United States Environmental Protection Agency (USEPA) classified 16 PAHs as the most priority pollutants that must be disposed (White, 1986; Eggen & Majcherczyk, 1998). As the number of rings in PAHs increased, resistance to degradation and their risk increased (Garon et al., 2000; Bishnoi et al., 2005; Bishnoi et al., 2008; Agrawal et al., 2019; Lee et al., 2020). With increasing molecular weight, PAHs tend to have increased in hydrophobicity (low aqueous solubility) and accumulate in the environment (soil and/or water) (Haritash & Kaushik, 2009).

White rot fungi (WRF) can represent as a good candidate to remove such PAHs compounds efficiently and ecofriendly (Biotechnological approach) for both low molecular weight (LMW) and high molecular weight (HMW) – PAHs. And can transform these compounds to non-harmful, simpler nontoxic or even CO\(_2\) and H\(_2\)O, which means complete mineralization (Berekaa, 2013; Kuppusamy et al., 2017; Adnan et al., 2018; Agrawal et al., 2019; Lee et al., 2020).

WRF produce a battery of extracellular ligninolytic enzymes including lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase (Lac) which are involved in PAHs degradation process (Augustin & Muncnerova, 1994; Abo-State et al., 2011a, Abo-State et al., 2011b; Lee et al., 2020). These enzymes oxidize the bonds in PAH molecule via stimulating radical formation to destabilize the bonds. The treatment can be applied either by extracellular culture extract or the whole cell WRF (Paszczynski & Crawford, 2000; Baborovaa et al., 2006; Al-Hawash et al., 2018; Bishnoi et al., 2008).

\textit{Phanerochaete chrysosporium}, \textit{Pleurotus ostreatus}, \textit{P. sajor-caju}, \textit{Pleurotus pulmonarius}, \textit{Bjerkandera adusta}, \textit{Trametes versicolor}, \textit{Pycnoporus sanguineus} \textit{Pseudotrametes gibbosa}, and \textit{Peniophora incarnata} are the most WRF used for PAHs degradation of both LMW and HMW. These WRF utilize PAH – compounds as a sole carbon and energy source. (Li et al., 2010, 2014; Abo-State et al., 2018a; Abo-State et al., 2011a, 2011b; Wen et al., 2011; Hadibarata & Kristanti, 2015; Luis et al., 2015; Kosnar et al., 2019; Vasiliadou et al., 2019; Lee et al., 2020).

Optimization of PAHs degradation depends on efficient screening and selection of good candidates WRF and changing degradation conditions. Different microorganisms have optimum conditions for growth and also have different degrading efficiencies due to toxic compounds (Bao et al., 2013; El-Borai et al., 2016). For

CONTACT Z. M. Abdel-Rahman eosziab@gmail.com Botany and Microbiology Department, Faculty of Science, Helwan University, Cairo, Egypt © 2021 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

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2. Materials and methods

2.1. Microorganism

Phanerochaete chrysosporium ATCC 32629 is one of the white rot fungi (WRF). P. chrysosporium used in the present study was purchased from Microbiological Resources Center (MIRCEN), Faculty of Agriculture, Ain-Shams University, Cairo, Egypt.

2.2. Culturing of WRF

Phanerochaete chrysosporium ATCC 32629 was cultured on 2% (w/v) Malt Extract Agar (ME) (Oxoid, 1982) plates and slants. The slants were kept at 4°C until needed. Phanerochaete chrysosporium can be activated by subculturing on Potato dextrose agar (PDA) plates (Oxoid, 1982) or on ME plates and incubated at 28°C for 14 days.

2.3. PAHs used

Low and high molecular weight—polycyclic aromatic hydrocarbons (PAHs) utilized in this study were Fluoranthene (Flu.); Acenaphthene (Acen.); Phenanthrene (Phen.); Pyrene (Pyr.); and Anthracene (Anth.). All these PAH compounds were purchased from Sigma-Aldrich, USA, (all 99% purity). While Naphthalene (Naph.) (99% purity) was obtained from El-Gomhoria Company, Cairo, Egypt. Stock solution of PAHs were prepared by dissolving these PAH compounds in chloroform.

2.4. Culture medium for PAHs degradation

Basal Salt Medium (BSM) (Wen et al., 2011) was a liquid medium with modification (Abo-state et al., 2013a; Abo-State et al., 2013b, 2014 and 2018) was used for biodegradation of PAHs by P. chrysosporium. This medium was used for PAHs degradation by P. chrysosporium. BSM was amended by 100 mg/L of each PAH compound.

2.5. P. chrysosporium inoculum preparation

From the margin of actively growing (14 days) fungal culture (P. chrysosporium) on PDA agar plates, agar discs (6 mm diameter) were cut out. These discs were used to inoculate BSM for biodegradation of PAHs.

2.6. Optimizing biodegradation of PAHs by P. chrysosporium under certain conditions

In 500 ml conical flasks containing 150 ml BSM and sterilized by autoclaving, then amended by 100 mg L⁻¹ concentration of each one of the six examined PAHs, the biodegradation experiment was performed.

To find the most efficient biodegradation performance of Phanerochaete chrysosporium to six examined PAHs, the BSM were incubated according to four different parameters [Inoculum size (1, 3, 4, 5, 6 agar plugs or discs), MnSO₄ concentration (0, 600, 1000, 1500, 2000, 3000 µM/L), temperatures degree (20°C, 25°C, 30°C, 35°C, 40°C), shaking stagnant (150 rpm and static state)].

Three replicates were used for each PAH compound, for each parameter. After incubation period, all BSM were filtrate, extracted and analyzed to know the fungal growth, fungal activity and degradation rate of six PAHs tested.
2.7. **P. chrysosporium growth and protein determination**

The *P. chrysosporium* mycelia at the end of incubation period were removed from BSM cultures by filtration via Whatman filter paper and dried at 60°C for constant weight. Biomass (fungal mycelia) was weighed for the quantitative determination of *P. chrysosporium* growth (Dry weight).

The fungal filtrates of cultures were utilized for the determination of the efficient degradation rate of PAHs. The fungal activity was determined by measuring the extracellular protein secreted according to Lowry et al. (1951) periodically for each incubation period by using a Spectrophotometer (LW-V-200 RS UV/VIS, Germany) at National Center for Radiation Research and Technology (NCRTT), Egyptian Atomic Energy Authority (EAEA), Naser City, Cairo, Egypt.

2.8. **Determination of degradation percentage of PAHs by *P. chrysosporium* under optimum conditions**

From the previous experiment, four groups of optimum conditions were designed to determine its effect on PAHs degradation. The concentration used in all groups was 100 mg/L of each PAHs. The optimum condition Group I (5 disc inoculums size = 600 µM MnSO₄ + 7 days incubation + 25°C Temp. + shaking); Group II (5 discs size + 2000 µM MnSO₄ + 7 d. + 25°C + stagnant); Group III (5 discs + 600 µM MnSO₄+7 d. + 25°C + stagnant); and Group IV (5 discs + 2000 µM MnSO₄+7 d. + 25°C + shaking). At the end of this experiment, a quantitative determination of PAHs degradation has been done, using HPLC.

2.9. **High performance liquid chromatography (HPLC) analysis**

Quantitative analysis of residual PAHs in BSM was performed by Liquid/Liquid (1:1 v/v) extraction. *P. chrysosporium* (BSM): Chloroform (1: 1 v/v) was used. The chloroform layer (extracted samples) was dried by evaporation to a fixed volume, then analyzed by HPLC at Micro Analytical Center, Fac. Sci, Cairo University, Giza, Egypt. HPLC system (YL 9100), (South Korea) with pump No. YL 9110, occupied by UV/V detector No. 9120 and Column compartment No. 9131 with 150 mm reversed phase column (hypersil) ODS-C₁₈, 5 µm. Acetonitrile and deionized water (85:15 v/v) represented the mobile phase. Manual injection with flow rate (1 ml/min) at 40°C and 254 nm UV was used.

All the experiments and measurements were done in duplicates, and arithmetic averages were used throughout the data analysis and calculations. Efficient degradation percentages were analyzed and calculated for 100 mg L⁻¹ concentrations of each PAHs examined for each parameter.

2.10. **Determination of biodegradation intermediates by GC/MS analysis**

The optimum conditions of group IV were used to determine the degradative pathways of the six PAHs (Ace., Anth., Flu., Naph., Phen., and Pyr.) according to Abo-State et al. (2018b).

Abo-State et al. (2018a) illustrated the qualitative and quantitative determination of various compounds performed using Gas Chromatography/Mass Spectrometry (GC/MS) in The Regional Center for Food and Feed (R.C.F.F.), Giza, Egypt. The analysis was carried out using a GC (Agilent Technologies 7890A) interfaced with a mass-selective detector (MSD, Agilent 7000 Triple, Quad) equipped with Agilent HP5ms (5%- phenyl methyl polysiloxane) capillary column (30 m x 0.25 mm i. d. and 0.25 µm film thickness) Santa Clara, California, USA. The carrier gas was helium with the linear velocity of 1 ml/min. The injector and detector temperatures were 200°C and 250°C, respectively. The volume injected 1 µl of the sample. The MS operating parameters were as follows: ionization potential 70 eV, interface temperature 250°C, and acquisition mass range 50–600 (Oberoi et al., 2015). The identification of components was based on a comparison of their mass spectra and retention time with those of the authentic compounds and by computer matching with NIST and WILEY library as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in the literature.

3. **Results and discussion**

3.1. **Growth and protein secretion by *P. chrysosporium* under different conditions**

Biodegradation of PAH compounds (100 mg/L) after different incubation periods using different inoculum sizes, MnSO₄ concentrations, incubation temperatures under shaking or stagnant states have been determined.

The growth of *P. chrysosporium* on PAHs was indicated in Figures 1–6. The results revealed that maximum growth of *P. chrysosporium* was recorded at 5 and 6 discs of inoculum after 7 days of incubation period except Naph. need more time (14 d.). Also, protein secretion followed the same trend of growth (i.e. five out of the six PAHs secreted maximum protein with 5 discs after 7 days incubation except Naph. need more time (14 days)).
Growth of *P. chrysosporium* on BSM supplemented by different concentrations of MnSO₄ as inducer for MnP enzyme were shown in Figures 7–12. Results cleared that the highest concentrations of MnSO₄ (2000, 3000 µM) gave the highest growth but it may need more incubation period (14 d.). *P. chrysosporium* secreted maximum protein at concentration (1500–3000 µM) after 14 days incubation.
Maximum growth of *P. chrysosporium* has been recorded at 25°C incubation period for all PAHs tested in the present study after 7 days incubation (Figures 13–18). However, the maximum protein secretion for Flu., Naph., and Phen. was recorded at 40°C as shown in Figures 13–18.

Figure 19 shows the maximum growth has been recorded for Phen. with shaking after 7 d. incubation. Generally, all PAHs supporting the growth of *P. chrysosporium* under shaking conditions than the stagnant state. Also, the maximum protein secretion is shown in Figure 20.
3.2. Degradation rate under optimum conditions

Table 1 shows that pyrene was the best compound degraded by *P. chrysosporium* (100%) under four groups (I–IV) followed by Ace. (I, III, IV), and Phen. (III, IV). However, the worst PAHs in degradation was Anth. (III, IV) by degradation percentage 66.99%. The previous results were confirmed by the results of other investigators as follows:

Initial biodegradation indicated 75.2% and 54.3% phenanthrene and pyrene degraded by *C. sakazakii MMO45* (KT933253) within 24 h. After CCD optimization,
100% degradation was achieved for each of phenanthrene and pyrene, resulting in the formation of intermediate metabolites (Umar et al., 2017).

3.3. Degradation intermediates as determined by GC/MS

*P. chrysosporium* grown in BSM amended by 100 mg/L of each PAHs under optimum condition degraded these compounds by oxidation followed by ring fission.

First step for degradation of Acenaphthene (Ace.) was oxidation to form 1- Acenaphethol with more oxidation gave Acenaphthyl – dihydro -, then with ring fission (hetero ring) became β-(1-Naphthyl) acrylic acid which converted to Bis – (2-methoxy ethyl) phthalate (i.e. *P. chrysosporium*) followed the phthalate route in degradation as indicated in Figure 21.

Anthracene (Anth.) degradation by *P. chrysosporium* was first oxidized to 9, 10 Anthracenedione with further oxidation followed by ring fission gave 1,2-
benzene dicarboxylic acid, bis – (1- methyl ethyl) ester, which inter TCA cycle to gave finally CO₂ and H₂O as shown in Figure 22.

Fluoranthene (Flu.) was degraded by P. chrysosporium via oxidation and hetero ring fission to give benzene methanol, 3- phenoxy with more oxidation it gave Di - n- octyl phthalate (i.e. followed the phthalic pathway) as cleared in Figure 23.

Table 1. Degradation rate of PAHs by Phanerochaete chrysosporium under four groups of defined conditions.

| Polycyclic aromatic hydrocarbons compounds – PAHs (100 mg L⁻¹) | Degradation percentage % |
|---------------------------------------------------------------|--------------------------|
|                                                              | Group (I) | Group (II) | Group (III) | Group (IV) |
| Acenaphthene                                                  | 100%      | 89.03%     | 100%        | 100%       |
| Anthracene                                                   | 94.35%    | 92.69%     | 66.99%      | 66.99%     |
| Fluoranthenne                                                | 95.77%    | 94.18%     | 98.94%      | 98.94%     |
| Naphthalene                                                  | 82.33%    | 80.97%     | 79.79%      | 79.79%     |
| Phenanthrene                                                 | 98.50%    | 99.25%     | 100%        | 100%       |
| Pyrene                                                       | 100%      | 100%       | 100%        | 100%       |
However, Naphthalene (Naph.) first gives 1,2 – Naphthalene dihydro-, followed by oxidation and ring fission to give benzoic acid, which finally converted to short aliphatic chains. It may be via polymerization converted to long chain of aliphatic compound as indicated in Figure 24.

Phenanthrene (Phen.) degradation showed that Phen. may be degraded first from the middle ring to give Cis – Stibene or from peripheral ring to give Naph. via oxidation and ring fission as shown in Figure 25. Pyrene (Pyr.) was first oxidized to give 3, 4-dihydro-phenanterene followed by ring fission to convert to phenanthrene as shown in Figure 26.

In spite of a large number of research being conducted on PAHs degradation by different bacteria and their pathways nearly screened by Nzila (2019) and Abo-State and El-kelani (2020), little has been known about PAHs pathways by fungi. White rot fungi (WRF) take great attention in biodegradation of PAHs, because of their ability to secrete a battery of liginolytic enzymes. It was clear that P. chrysosporium undergo oxidation followed by ring fission in the proposed six pathways for the degradation of different PAHs used in the present study. Also, from the previous proposed pathways, it was obvious that P. chrysosporium followed the phthalic pathway then inter TCA cycle, and convert finally to CO₂ and H₂O (Abo-State & El-kelani, 2020; Lyu et al., 2014; Peng et al., 2008; Sawulski et al., 2014).
The previous findings of the present study were confirmed by other investigators as shown in Figure 26. Biodegradation pathways encompass the breakdown of PAHs, being ring fission by intracellular oxidation and hydroxylation, which represent the typical initial steps (Abo-State et al., 2018a). The microorganisms cleave the benzene ring in different ways. Ortho- or meta-cleavage path leading to the formation of central intermediates which further converted to tricarboxylic acid (TCA) cycle intermediate (Abbasian et al., 2015). It was also observed the formation of long linear chains of aliphatic polymers (Tetradecane 2, 6, 10 trimethyl-; Octacosane; Hexacosane) and branched chains of polymer (Triacontane, 11, 20- didecyl-). These chains may be used by the microorganisms to build up their cell wall; it was observed the same phenomena by other investigators. Octanoic acid may inter tricarboxylic acid cycle (TCC) or with reduction and polymerization converted to nanodecane or heneicosane. These aliphatic intermediates degraded to CO₂ and H₂O or may serve in the formation of the cell wall of the microorganisms as Bacillus spp. and Rhodococcus sp. (Fritsche & Hofrichter, 2008; Saleh et al., 2013).
Initial oxidative attach followed by ring cleavage of the benzene ring is the key step in degradation of PAHs. The oxidation results in the formation of a diol with further cleavage forming dicarboxylic acids (Hendrickx et al., 2006; Abo-State et al., 2014). The first step is oxidation catalyzed by monooxygenase and dioxygenase (Kanaly & Harayama, 2000; Zhang et al., 2011).

Degradation of Naphthalene starts through Naphthalene dioxygenase, which converts Naph. to Cis-dihydrodiol, which transformed to 1, 2 dihydroxynaphthalene followed by metabolites of ring fission leading the formation of phthalic or salicylic pathway. Both the two pathways enter the Krebs cycle (TCA) (Haritash & Kaushik, 2009; Se et al., 2009).

Z. Li et al. (2018) found that 2-methyl phenol was the metabolite that resulted from Phen. degradation and 2, 3-dihydroxy-3-phenyl propanoic acid, citramalic acid and B[α]anthracene, 7, 12- dione were the degradation metabolites of B[α]anth. by WRF (Pycnoporus sanguineus 14).

Abo-State et al. (2017) found that the degradation of Pyrene proposed pathway by Bacillus altitudinis MAM-8 revealed the formation of the following metabolites: [(hexadeulenio) phynel] naphthalene; Trians-4, 4 dimethoxy beta methylchakone; phthalic acid monocyclohexyl ester; phthalic acid monobutyl...
ester; dimethoxy benzyl-ide neacetne and phthalic anhydride. The previous data indicated that *B. altitudinis* MAM-8 followed the phthalic path in Pyrene degradation. Abo-State et al. (2018a) reported that Pyrene degradation by *Pseudomonas panipatensis* MAM-39 with accession No. MF 150314 b isolated contaminated soil of Suez Canal, Egypt produced 14 intermediates, including, 3-methyl 2-butoenoic acid; 3-methyl pent-1; 3-methyl- but-2 enyl ester; 4-diene-3-ol; 3- hexanone; benzene; 2- pentyl ester and (3, 3- dimethyl 4- pentyl-). The results of GC/MS analysis revealed that *Bordetella avium* MAM-P22 degrade naphthalene to give rise to six intermediates. These intermediates were Butyl-2, 4 dimethyl; 2- nitro- 4- pentanoate; 1, 2- Benzene dicarboxylic acid; 1- Nonen- 3- ol; anacoseno and Eicosane (Abo-State et al., 2018).

Hadibarata and Yuniarto (2020) detected 1- hydroxyl –2 naphthoic acid; salicylic acid; diphenic acid; catechol; benzoic acid; antraquinone and phthalic acid as metabolites of anthracene, phenanthrene and pyrene. These finding confirmed the results of the present study.

4. Conclusion

*Phanerochaete chrysosporium* was able to utilize each one of PAHs (Acen., Anth., Flu., Naph., Phen., and Pyr.) as a sole carbon and energy source and reached to the maximum growth and extracellular protein secretion under four certain conditions [5 agar plugs inoculum size (6 mm diameter), 2000 µM/L MnSO₄ concentration, 25°C temperature degree with shaking stagnant).

*P. chrysosporium* degraded the six PAHs (Acen.; Anth.; Flu.; Naph.; Phen.; and Pyr.) efficiently with optimum conditions.

Pyrene was the best compound degraded by *P. chrysosporium* (100%) under four groups (I–IV) of optimum conditions followed by Ace. (I, III, IV) and Phen. (III, IV).

*P. chrysosporium* followed the phthalate route in its degradative pathway for PAHs, and finally, its intermediates interred TCA cycle and gave CO₂ and H₂O or short chain aliphatic polymerized to give long chains.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

**References**

Abbasian, F., Lockington, R., Mallavarapu, M., & Naidu, R. (2015). A comprehensive review of aliphatic hydrocarbon degradation by bacteria. *Applied Biochemistry Biotechnology, 176*, 670–699. https://doi.org/10.1007/s12010-015-1603-5

Abo-State, M. A., El-Gendy, N. S., El-Temtamy, S. A., Mahdy, H. M., & Nassar, H. N. (2014). Modification of Basal Salts Medium for Enhancing Dibenzo thiophene Biodesulfurization by *Brevibacillus Invocatus* C19 and Rhodococcus erythropolis IGTS8. *World Applied Sciences Journal, 30(2)*, 133–140. DOI:10.5829/idosi.wjas.2013.30.02.14023

Abo-State, M. A., Saleh, Y., Aziz, N., & Partila, A. M. (2013a). Isolation of polycyclic aromatic hydrocarbon degrading bacterial strains isolated from indigenous microbial communities of petroleum contaminated soils. *World Applied Sciences Journal, 23(4)*, 554–564. DOI: 10.5829/idosi.wjas.2013.23.04.13079

Abo-State, M. A., Saleh, Y., & Partila, A. M. (2013b). Identification of polycyclic aromatic hydrocarbon degrading bacterial strain and its ability to degrade pyrene. *World Applied Sciences Journal, 23(14)*, 515–525. DOI: 10.5829/idosi.wjas.2013.23.04.13078

Abo-State, M. A. M., Abdallah, N., & Nader, B. (2017, March). Biodegradation of high molecular weight polycyclic aromatic hydrocarbons (HMW-PAHs) mixture by bacteria isolated from soil polluted with petroleum oil. In *1 st international conference on biological and environmental science and Application (ICBEA)* 23–25 March, Luxor, Egypt. *Journal of Ecology of Health and Environment, 6(2)*, 63–76. https://doi.org/10.18576/jeh/060202

Abo-State, M. A. M., & El-kelani, T. A. (2020). Bacterial Biodegradation pathways of low and high molecular weight polycyclic aromatic hydrocarbons (PAHs). *Archives of Petroleum & Environmental Biotechnology, 5(01)*, 158. DOI:10.290011/2574-7614.100038

Abo-State, M. A. M., Khatob, O., Abo- El Nasr, A. A., & Mahmoud, B. (2011b). Factors affecting laccase production by *Pleurotus ostreatus* and *Pleurotus sajor-caju*. *World Applied Sciences Journal, 14(11)*, 1607–1619. IDOSI publications. https://www.researchgate.net/publication/285207001_Factors_affecting_laccase_production_by_Pleurotus_ostreatus_and_Pleurotus_sajor-caju

Abo-State, M. A. M., Othman, M., Khatob, O, & Abd-Elfattah, E. A. (2011a). Enhancement production of MnP enzyme produced by *Pleurotus sajor-caju* exposed to Gamma Radiation. *World Applied Science Journal, 14(10)*, 1457–1468.

Abo-State, M. A. M., Riad, B. Y., & Abdel Aziz, M. F. (2018b). Biodegradation of naphthalene by *Bordetella avium* isolated from Petroleum refinery wastewater in Egypt and its pathway. *Journal of Radiation Research and Applied Sciences, 11(2018)*, 1–9. https://doi.org/10.1016/j.jrras.2017.10.001

Adnan, B. A.-H., Alkooranee, J. T., Xiaoyu, Z., & Fuying, M. (2018). Fungal degradation of polycyclic aromatic hydrocarbons. *International Journal of Pure & Applied Bioscience, 6(2)*, 8–24. http://dx.doi.org/10.18782/2320-7051.6302

Agrawal, P. K., Shrivastava, R., & Verma, J. (2019). Bioremediation approaches for degradation and detoxification of polycyclic aromatic hydrocarbons (Springer Nature Singapore). Emerging and Eco-Friendly Approaches for Waste Management.Pte Ltd. https://doi.org/10.1007/978-981-10-8669-4_6

Al-Hawash, A. B., Jawad any, T., Alkooranee, X. Z., & Fuying, M. (2018). Fungal degradation of polycyclic aromatic hydrocarbons. *International Journal of Pure & Applied Bioscience, 6(2)*, 8–24. https://doi.org/10.18782/2320-7051.6302

Augustin, J., & Muncnerova, D. (1994). Degradation pathways of aromatic hydrocarbons fungi and bacteria. *Biologia, 49* (6), 289–299.

Baborovoa, P., Moderb, M., Baldriana, P., Cajthamllovaa, K., & Cajtham, T. (2006). Purification of a new manganese peroxidase of the white-rot fungus *Ir pex lucteus*, and...
degradation of polycyclic aromatic hydrocarbons by the enzyme. Research in Microbiology, 157(3), 248–253. https://doi.org/10.1016/j.resmic.2005.09.001

Bao, M., Chen, Q., Gong, Y., Li, Y., Wang, H., & Jiang, G. (2013). Removal efficiency of heavy oil by free and immobilized microorganisms on laboratory scale. The Canadian Journal of Chemical Engineering, 91(1), 1–8. https://doi.org/10.1002/cjce.20688

Berekaa, M. M. (2013). Towards efficient crude oil degradation by Pseudomonas sp. strain-O2: Application of plackett-Burman design for evaluation of cultivation conditions. African Journal of Microbiology Research, 7(39), 4722–4729. https://doi.org/10.5897/AJMR2012.2280

Bishnoi, K., Kumar, R., & Bishnoi, R. N. (2008). Biodegradation of polycyclic hydrocarbons by white rot fungi Phanerochaete chrysosporium in sterile and unsterile soil. Journal of Scientific and Industrial Research, 67(04), 538–542. http://hdl.handle.net/123456789/1798

Bishnoi, N. R., Mehta, U., & Sain, U. (2005). Quantification of polycyclic aromatic hydrocarbons in tea and coffee samples of Mumbaicity (India) by high performance liquid chromatography. Environmental Monitoring and Assessment, 107, 399–406. https://doi.org/10.1007/s10661-005-3547-7

Chang, A. J., Fan, J., & Wen, X. (2012). Screening of fungi capable of highly selective degradation of lignin in rice straw. International Biodeterioration & Biodegradation, 72, 26–30. https://doi.org/10.1016/j.ibiod.2012.04.013

Eggen, T., & Majcherczyk, A. (1998). Removal of polycyclic aromatic hydrocarbons (PAH) in contaminated soil by white rot fungus Pleurotus ostreatus. International Biodeterioration & Biodegradation, 41(2), 111–117. https://doi.org/10.1016/S0964-8305(98)00002-X

El-Borai, A. M., Eltayeb, K. M., Mostafa, A. R., & El-Assar, S. A. (2016). Optimization and statistical evaluation of medium components affecting crude oil biodegradation by some locally isolated bacteria Egypt. Journal of Botany, 56(3), 753–767. DOI: 10.21608/EJBO.2016.2733

Eriksson, M., Ka, J. O., & Mohn, W. W. (2001). Effects of low temperature and freeze-thaw cycles on hydrocarbon biodegradation in Arctic Tundra soil. Applied and Environmental Microbiology, 67(11), 5107–5112. https://doi.org/10.1128/AEM.67.11.5107-5112.2001

Fritsche, W., & Hofrichter, M. (2008). Aerobic degradation by microorganisms in biotechnology: Environmental process II vol 11B. In J. Klein (Ed.), Soil decontamination (pp. 146–164). Elsevier Science.

Garon, D., Krivobok, S., & Seigle-Murandi, F. (2000). Fungal degradation of fluoride. Chemosphere, 40(02), 91–97. https://doi.org/10.1016/S0045-6535(99)00250-7

Hadibarata, T., & Chuang, T. (2014). Optimization of pyrene degradation by white-rot fungus Pleurotus pulmonarius F043 and characterization of its metabolites. Bioprocess and Biosystems Engineering, 37(8), 1679–1684. https://doi.org/10.1007/s00449-014-1140-6

Hadibarata, T., & Kristanti, R. A. (2015). Biotransformation studies on fluoranil, a four-ring polycyclic aromatic hydrocarbon, by White-Rot Fungus Armillaria sp. F022Tonym. Agriculture and Agricultural Science Procedia, 3 (1), 45–50. https://doi.org/10.1016/j.aaspro.2015.01.011

Haritash, A., & Kaushik, C. (2009). Biodegradation aspects of poly-cyclic aromatic hydrocarbons (PAHs): A review. Journal of Hazardous Materials, 169(1-3), 1–15. https://doi.org/10.1016/j.jhazmat.2009.03.137

Hendrickx, B., Junca, H., Vosahlova, I., Lindner, A., Ruegg, I., Bucheli-Witschel, M., Faber, F., Egli, T., Mau, M., Schlömann, M., Brennerova, M., Brenner, V., Pieper, D. H., Top, E. M., Dejonghe, W., Bastiaens, L., & Springael, D. (2006). Alternative primer sets for PCR detection of genotypes involved in bacterial aerobic BTEX degrading isolates and in subsurface soils of BTEX-contaminated industrial site. Journal of Microbiological Methods, 64(2), 250–256. https://doi.org/10.1016/j.mimet.2005.04.018

Hofrichter, M., Scheibner, K., Schneegaß, I., & Fritsche, W. (1998). Enzymatic combustion of aromatic and aliphatic compounds by manganese peroxidase from Nematoloma frowardii. Applied and Environmental Microbiology, 64(2), 399–404. https://doi.org/10.1128/AEM.64.2.399-404.1998

Kadri, T., Rouissi, T., Brar, S. K., Cledon, M., Sarma, S., & Verma, M. (2017). Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by fungal enzymes: A review industrial crops and products. Journal of Environmental Sciences, 51 (3), 52–74. https://doi.org/10.1016/j.jes.2016.08.023

Kanaly, R. A., & Harayama, S. (2000). Biodegradation of high-molecular-weight polycyclic aromatic hydrocarbons by bacteria. Journal of Bacteriology, 182(14), 2059–2067. https://doi.org/10.1128/JB.182.8.2059-2067.2000

Kosnar, Z., Castkova, T., Wiesnerova, L., Praus, L., Jablonsky, I., Koudela, M., & Tlustoš, P. (2019). Comparing the removal of polycyclic aromatic hydrocarbons in soil after different bioremediation approaches in relation to the extracellular enzyme activities. Journal of Environmental Sciences (China), 76, 249–258. https://doi.org/10.1016/j.jes.2018.05.007

Kuppusamy, S., Thavamani, P., Venkateswarlu, K., Lee, Y. B., Naidu, R., & Megharaj, M. (2017). Remediation approaches for polycyclic aromatic hydrocarbons (PAHs) contaminated soils: Technological constraints, emerging trends and future directions. Chemosphere, 168, 944–968. https://doi.org/10.1016/j.chemosphere.2016.10.115

Lee, A. H., Lee, H., Heo, Y. M., Lim, Y. W., Kim, C., Kim, G., Chang, W., & Kim, J. (2020). A proposed stepwise screening framework for the selection of polycyclic aromatic hydrocarbon (PAH)-degrading white rot fungi Bioprocess and Biosystems Engineering. Springer-Verlag GmbH Germany. https://doi.org/10.1007/s00449-019-02272-w

Leys, M. N., Bastiaens, L., Verstraete, W., & Springael, D. (2004). Influence of the carbon/nitrogen/phosphorus ratio on polycyclic aromatic hydrocarbon degradation by Mycobacterium and Sphingomonas in soil. Applied Microbiology and Biotechnology, 66(6), 726–736. https://doi.org/10.1007/s00253-004-1766-4

Li, X., Lin, X., Yi, R., Wu, Y., Chu, H., Zeng, J., & Yang, T. (2010). Optimization of Laccase-mediated Benzo[a]pyrene oxidation and bioremedial application in aged polycyclic aromatic hydrocarbons-contaminated soil. Journal of Health Science, 56(6), 534–540. https://doi.org/10.1248/jhs.56.534

Li, X., Wang, Y., Wu, S., Qiu, L., Gu, L., Li, J., Zhang, B., & Zhong, W. (2014). Peculiarities of metabolism of anthracene and pyrene by laccase-producing fungus Pycnoporus sanguineus H. International Union of Biochemistry and Molecular Biology, 61(4), 549–554.
Li, Z., Pan, Y., Hu, S., Sheng, Y., Cheng, Y., Wang, Y., Youjing, A., Wu, K., Zhang, S., & Yang, S. (2018). Diversity of phenanthrene and Benz[a]anthracene metabolic pathways in white rot fungus Pycnoporus sanguineus. Journal of Science and Total Environment, 134(3), 25–30.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with folin phenol reagent. Journal of Biological Chemistry, 193(2), 265–275. https://doi.org/10.1016/0021-9258(51)92451-6

Luis, F. B., Gabriel, M., & Raquel, S. (2015). Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by laccase from Trametes versicolor covalently immobilized on amino-functionalized SBA-15. Chemosphere, 136(6), 273–280. https://doi.org/10.1016/j.chemosphere.2015.05.071

Lyu, Y., Zheng, W., Zheng, T., & Tian, Y. (2014). Biodegradation of polycyclic aromatic hydrocarbons by Novosphingobium pentaromativorans US6-1. PLOS ONE, 9(7), 1–8. https://doi.org/10.1371/journal.pone.0101438

Margesin, R., & Schinner, F. (2001). Biodegradation and bioremediation of hydrocarbons in extreme environments. Applied Microbiology and Biotechnology, 56(6), 650–663. https://doi.org/10.1007/s002530001007

Nziala, A. (2019). Current status of the degradation of aliphatic and aromatic petroleum hydrocarbons by thermophilic microbes and future perspectives. International Journal of Environmental Research and Public Health, 15, 2782. https://doi.org/10.3390/ijerph15122782

Oberoi, A. S., Philip, L., & Bhallamudi, S. M. (2015). Biodegradation of various aromatic compounds by enriched bacterial cultures: Part A–monocyclic and polycyclic aromatic hydrocarbons. Applied Biochemistry and Biotechnology, 176(1), 1870–1888. https://doi.org/10.1007/s12010-015-1684-1

Oxoid. (1982). The oxoid manual of culture media, ingredients and other laboratory services.

Paszczyński, A., & Crawford, R. L. (2000). Recent advances in the use of fungi in environmental remediation and biotechnology. Soil Biology & Biochemistry, 10(2), 379–422.

Peng, R. H., Xiong, A. S., Xue, Y., Fu, X. Y., & Gao, F. (2008). Microbial biodegradation of polycyclic aromatic hydrocarbons. Microbiol.Rev, 32(11), 927–955.

Saleh, Y. E., Abo-State, M. A. M., & Khalil, O. (2013). Aerobic degradation of 3-chlorobenzoic acid by bacterial strains isolated from petroleum polluted soils. World Applied Sciences Journal, 27(3), 1328–1340. 10.5829/idosi.wasj.2013.21.9.2932

Sawulski, P., Clipson, N., & Doyle, E. (2014). Effects of polycyclic aromatic hydrocarbons on microbial community structure and PAH ring hydroxylatingdioxygenase gene abundance in soil. Biodegradation, 25(1), 835–847. https://doi.org/10.1007/s10532-014-9703-4

Se, J. S., Keum, Y. S., & Li, Q. X. (2009). Bacterial degradation of aromatic compounds. International Journal of Environmental Research and Public Health, 6(7), 278–309. https://doi.org/10.3390/ijerph6010278

Torres-Farrada, G., Manzano-León, A. M., Rineau, F., Leal, M. R., Thijs, S., Jambon, I., Put, J., Czech, J., Rivera, G. G., Carlee, R., & Vangronsveld, J. (2019). Biodegradation of polycyclic aromatic hydrocarbons by native Ganoderma sp. strains: Identification of metabolites and proposed degradation pathways. Applied Microbiology and Biotechnology, 103(11), 7203–7215. https://doi.org/10.1007/s00253-019-09968-9

Umar, Z. D., Abd, A. N. A., Zulikifli, S. Z., & Mustafa, M. (2017). Rapid biodegradation of polycyclic aromatic hydrocarbons (PAHs) using effective Cronobacter sakazakii MM045 (KT933253). MethodsX, 4(1), 104–117. https://doi.org/10.1016/j.mex.2017.02.003

Vassiliadou, I. A., Molina, R., Pariente, M. I., Christoforidis, I., Martinez, F., & Melero, J. A. (2019). Understanding the role of mediators in the efficiency of advanced oxidation processes using white-rot fungi. Water, 359(2), 1427–1435.

Wang, C., Sun, H., Li, J., Li, Y., & Zhang, Q. (2009). Enzyme activities during degradation of polycyclic aromatic hydrocarbons by white rot fungus Phanerochaete chrysosporium in soils. Journal of Chemosphere, 77(3), 733–738. https://doi.org/10.1016/j.chemosphere.2009.08.028

Wen, J., Gao, D., Zhang, B., & Liang, H. (2011). Co-metabolic degradation of pyrene by indigenous white-rot fungus Pseudotrametes gibbsa from the northeast China. International Biodeterioration & Biodegradation, 65, 600–604. https://doi.org/10.1016/j.ibiod.2011.03.003

White, K. L. (1986). An overview of immune toxicology and carcinogenic/polycyclic aromatic hydrocarbons. Environmental Carcinoma Review, 4(2)4163–4202. https://doi.org/10.1080/10590508609373342

Yang, S., Hai, F. I., Nghiem, L. D., Price, W. E., Roddick, F., Moreira, M. T., & Magram, S. F. (2013). Understanding the factors controlling the removal of trace organic contaminants by white-rot fungi and their lignin modifying enzymes: A critical review. Bioresource Technology, 141(2), 97–108. https://doi.org/10.1016/j.biortech.2013.01.173

Zhang, Z., Hou, Z., Yang, C., Ma, C., & Tao, F. (2011). Degradation of n-alkanes and polycyclic aromatic hydrocarbons in petroleum by a newly isolated Pseudomonas aeruginosa. DQB. Bioresource Technology, 102, 4111–4116. https://doi.org/10.1016/j.biortech.2010.12.064