Biodegradation of Some Polycyclic Aromatic Hydrocarbons by a Bacterial Consortium Isolated from the Red Sea of Jeddah

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are considered organic pollutants, which are stable, highly toxic, and carcinogenic. Therefore, it was necessary to find an environmentally friendly way to degrade these compounds and remove them from polluted environments. Water samples polluted with petroleum hydrocarbons were collected from the coast of Jeddah on the Red Sea - Saudi Arabia. The results of the current study showed the ability of bacterial consortium that was enriched from the coast of Jeddah, Saudi Arabia to degrade petroleum hydrocarbons wastewater, which proved its ability to degrade PAHs in saline conditions. The bacterial union degraded BENZ compound by more than 95% at the concentration of 100 and 250 mg/L, while the degradation of this compound at the concentration of 1000 mg/L was recorded about 83% while the ANT degradation rate was recorded at more than 90% at different concentrations. In addition, this study revealed the ability of bacterial consortium to treat petroleum hydrocarbons wastewater in bioreactor (CSTR) with 92.7% of COD removed under saline conditions. Hence, this study recommends the investment of bacterial consortium in the treatment of petroleum hydrocarbons wastewater in marine environments and to remove pollutants from them.

Keywords: Polycyclic aromatic hydrocarbons, Biodegradation, Red Sea, Benzene, Anthracene.

Introduction

Due to the importance of energy used in many human needs and the significant increase in development and technology, more energy is required for the increase in human activities. This increase led to the need for more exploration, distribution, and extraction of oil as well as manufacturing, refining, and transportation operations in order to provide an energy source for the world (Welsby et al., 2021). The increase in human activities, led to more pollution problems in the aquatic or terrestrial environments, including the pollution by petroleum hydrocarbons, which were caused by many oil spills, such as transportation, extraction, prospecting, and distribution accidents, and thus these spills affect the environment and the living organisms that live in them (Juhasz and Naidu, 2000; Ramirez et al., 2017; Davoodi et al., 2020).

Polycyclic aromatic hydrocarbons (PAHs) are considered as organic pollutants, which are stable, highly toxic, and carcinogenic. These compounds are considered toxic that cause many mutations and are carcinogenic (Landvik et al., 2007; Rengarajan et al., 2015; Ewa and Danuta, 2017; Stading et al., 2021; Tazdaït and Salah-Tazdaït, 2021). These pollutants are mainly produced by in-complete combustion of organic matter (Baali and Yahyaoui, 2019; José et al., 2019; Ali et al., 2020; Zungum and Imam, 2021).

Polycyclic aromatic hydrocarbons are distributed in different ecosystems of the world, where they are ubiquitous in sediments, soil, air, and water (Gereslassie et al., 2018; Wu et al., 2019; Ofori et al., 2021; Zhang et al., 2021). They can also be found in drinking water (Aygun and Bagcevan, 2019; Ambade et al., 2021), food (Al-Thaiban et al., 2018; Kafouris et al., 2020), feed (Fernandez-Gonzalez et al., 2012; Eyring et al., 2021), and others. Because of their harmful effects on humans, the environment, and living organisms, this attracted many researchers to conduct more studies and find solutions to get rid of these pollutants(Meador, 2010; Rubio-Clemente et al., 2014).There are several measures that can be taken when oil spills occur such as photo-oxidation, volatilization, use of chemical dispersants, and leaching, which can be effective in reducing the level of environmental toxicity of PAHs (Santos et al., 2011; Lewis and Prince, 2018; Wang et al., 2021).

Therefore, removing these compounds from the environment is a major problem that drowns researchers; thus, it was necessary to find a safe and environmentally friendly way to degrade these compounds and remove them from polluted environments. Microbes play an important role in the degradation and elimination of PAHs (Hassanshahian and Boroujeni, 2016). Recently, the use of the biological treatment method has been preferred as an alternative to physical or chemical treatment due to its low cost and environmentally friendly properties (Fakhru'l-Razi et al., 2009; Lyon and Vogel, 2013; Rubio-Clemente et al., 2014; Abegunde et al., 2020).

Many microorganisms such as bacteria, fungi and algae play their ability to degrade, destroy, and bioremediate PAHs. The economic aspect is a source of concern for many bioremediation processes, and therefore bioremediation has recently proven its efficiency in mitigating pollution problems in different sites in environments (Thavamani et al., 2012; Ndimele et al., 2018; Gaur et al., 2021). Bacteria are one of the most important groups involved in the microbial degradation of organic pollutants in polluted sites(Kuppusamy et al., 2016; Gaur et al., 2018; Zoppini et al., 2020; Espinosa-Ortiz et al., 2021).Therefore, bioremediation by bacterial consortium plays an important role in removing PAHs from contaminated sites.

In this current study, the aim of the study was to determine the efficiency of a bacterial consortium that was enriched from the coast of Jeddah - Red Sea - Saudi Arabia in the degradation of the studied PAHs compounds and to determine the rates of degradation.

Materials and methods

Sample collection

Water samples polluted with petroleum hydrocarbons were collected from the coast of Jeddah on the Red Sea - Saudi Arabia in sterile glass bottles (100 ml), stored under 4°C until transferred to the laboratory for further experiments.

Chemicals

Benzene (BENZ) and Anthracene (ANT)and other chemicals such as: $MgSO_4.7H_2O$, K_2HPO_4, $FeCl_3.6H_2O$, $Na_2HPO_4.7H_2O$, KH_2PO_4, $CaCl_2$, NH_4Cl, and NaCl were obtained from Sisco Research Laboratory (Delhi, India), Na_2SO_4 and ethyl acetate was purchased from Fisher Scientific, UK.

Culture medium

In a (250 ml) flask the contaminated water samples were enriched by using 20 ml of water contaminated with petroleum hydrocarbons with 80 ml of the modified mineral salts medium (MMSM),which consists of $Na_2HPO_4.7H_2O$ (668mg), $MgSO_4.7H_2O$ (22.5 mg), KH_2PO_4 (170 mg), K_2HPO_4 (435 mg), NH_4Cl (85 mg), $FeCl_3.6H_2O$ (0.25 mg), $CaCl_2$ (27.5 mg) per litter (Liu et al., 1995), and this medium was modified by adding 40 g of NaCl, then all compounds were dissolved in 1000 ml of distilled water, and sterilized in an autoclave for use in bacteria enrichment and biodegradation experiments. BENZ or ANT were added to MMSM as the sole carbon source for the growth of bacteria that degrade them.

Experimental Setup

The biodegradation experiments for BENZ and ANT were carried out in a duplicate manner, where the design of the experiments was based on the use of control flasks (Con1 and Con2) and test flasks (Te1 and Te2). The control flasks (Con1 and Con2) contain MMSM with the PAHs to be biodegrade as the sole carbon source, according to the concentration specified in the study, BENZ (100, 250 and 1000 mg/L) or ANT (50, 100 and 500) mg/,while the test flasks (Te1 and Te2), contained MMSM, bacterial consortium (BC) and PAHs with the specific concentration as mentioned previously.

BENZ and ANT extraction

All flasks for biodegradation experiments were enriched in 100 ml in a 250 ml capacity flasks. These flasks were incubated in a vibrating shaker at 150 rpm for the duration of the experiment. Then 10 ml of control and test flasks were taken and placed in test tubes of 20 ml capacity, 10 ml of ethyl acetate was added in 3 phases (5 ml, 3 ml and 2 ml)respectively, in order to extract the remaining PAHs from degradation processes. The test tubes were mixed for 2 minutes, and then an aqueous phase layer containing residues of PAHs was retained. This process was repeated 3 times in order to ensure the complete extraction of residual compounds from the samples that had been degraded. Small amounts of Sodium sulphate anhydrous (NA2SO4) were added, and the remaining extracts were filtered by syringe filter 0.2 µm and placed in a glass vial for analysis by GC-MS and HPLC (Sánchez-Avila et al., 2011).

Gas chromatography-mass spectrometry (GC-MS) Analysis

The GC-MS device was utilized to identify the metabolites created after the biodegradation of PAHs. A 30 m × 0.25 mm × 0.25 µm fuse-silica capillary column was used for PAHs analysis, and the temperature program (T) was used and adjusted to follows: on 100 °C hold for 1 min to 160 °C at 15 °C/min, 265 °C to 300°Cat 5 °C/min, hold for 5 min.Helium gas was utilized as a transporter with an influx rate of 1 mL/min and was controlled by electronic pressure control, and the injector temp was suspended in (splitless) mode at 270°C for the duration of the injection.The results were calibrated with the internal GC-MS library for PAHs.

High-Performance Liquid Chromatography (HPLC) Analysis

The aromatic hydrocarbons remaining in the flasks of the biodegradation experiments were analyzed by HPLC (Agilent, USA). This device was fitted with a C18 column (150 mm x 4.6 mm), Acetonitrile was used at 1 mm flow rate and aromatic compounds were detected at 252 nm, the injection volume was 20 µL. Biodegradation rates were measured by comparison with the peak area and the results were compared with aromatic hydrocarbon standards.

Phylogenetic analysis

The bacterial communities of the study samples were analyzed using bacterial 16S rDNA, Greengene (default): V201305. The taxonomic composition distribution in samples of Genus-level was analyzed. The tags number of the Genus taxonomic rank in the sample was summarized in a histogram, and the histogram was drawn with the software R (v3.1.1).

Treatment of PAHs in bioreactor

Petroleum hydrocarbon wastewater samples were collected from the coast of Jeddah, Saudi Arabia for treatment and a bioreactor study, continuous stirred tank reactor (CSTR) was conducted at the lab scale and the reactor was temperature controlled (30 ºC). The total capacity of the bioreactor was estimated at 10 liters and the working sample volume was about 7 liters (6.5 L of wastewater petroleum hydrocarbons + 0.5 L of bacterial consortium).The bioreactor was operated at the organic loading rates (OLR) 0.128 kg/m^3d, with hydraulic retention time (HRT) for 10 days as mentioned by (Jamal and Pugazhendi, 2018).The bioreactor was studied within 36 days. Chemical oxygen demand COD, mixed liquor suspended solids (MLSS), and mixed liquor volatile suspended solids (MLVSS) were analyzed using standard methods of analysis (Federation and Association, 2005).

Results and Discussion

Bacterial consortium was enriched in modified MMSM medium at 40 g/L of NaCl concentration from water samples from the coast of Jeddah, Saudi Arabia. Alternatively, ANT or BENZ were used as the sole carbon source in this study. The results of the preliminary biodegradation study of ANT and BENZ revealed complete degradation of these two compounds at a concentration of 50 and 100 mg/l for 10 days, respectively, thus confirming the ability of bacterial consortium to degrade PAHs.

Degradation of PAHs at diverse concentrations

Degradation of BENZ

The biodegradation of BENZ was studied at different concentrations (100, 250 and 1000 mg/L) by bacteria consortium under saline conditions. The degradation rate of the BENZ compound was about 38.4 and 66.7% at a concentration of 100 mg/L within 4 and 6 days, respectively, while the rate of degradation was about 81.3% in 8 days, and then the compound was completely degraded within 10 days of the experiment (Figure 1).

When the concentration of the BENZ compound was increased to 250 mg/L, the degradation rates were recorded at this concentration in different values, which reached 24 and 46.56% within 4 and 6 days, respectively, while the degradation rates reached about 62.48 and 86.76% within 8 and 10 days, respectively.

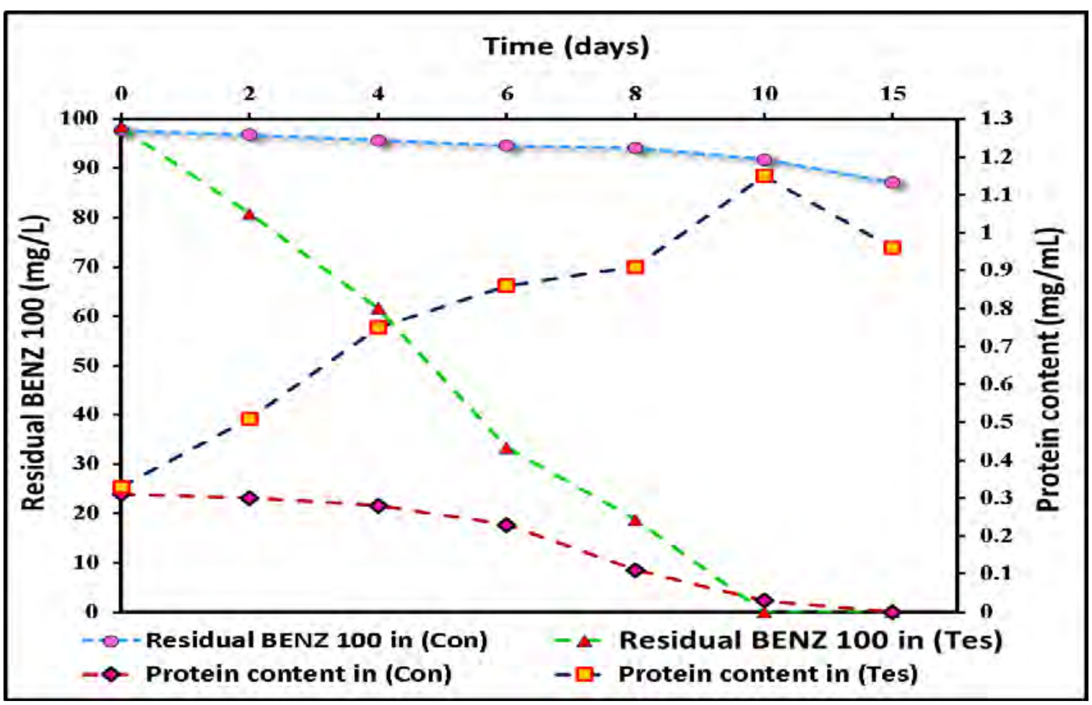

Figure 1. BENZ degradation at 100 mg/L by bacterial consortium

The highest degradation rate for this compound was recorded, reaching 95.08% in 15 days (Figure 2). Godambe and Fulekar (2016) in a study they conducted on the degradation of benzene at different concentrations, where it recorded a degradation rate of 99% at a concentration of 200 mg/L within 10 days, while in this study the rate of degradation was 95.08% at a concentration of 250 mg/L, compared with the time to deterioration (15 days) due to increased focus from the Godambe and Fulekar study.

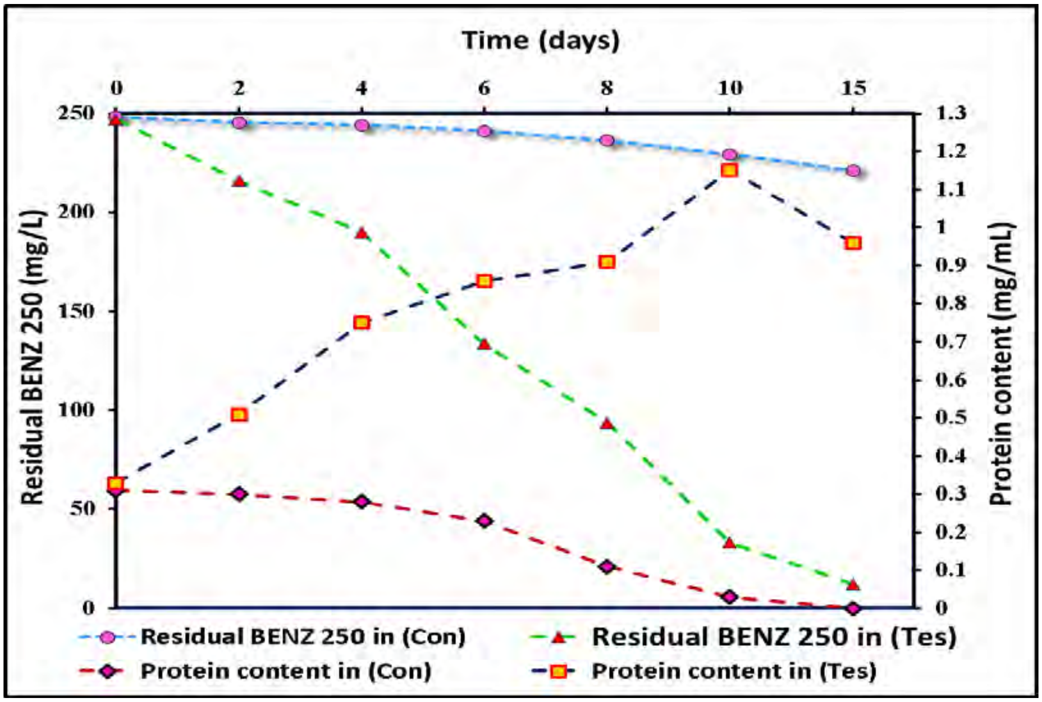

Figure 2. BENZ degradation at 250 mg/L by bacterial consortium

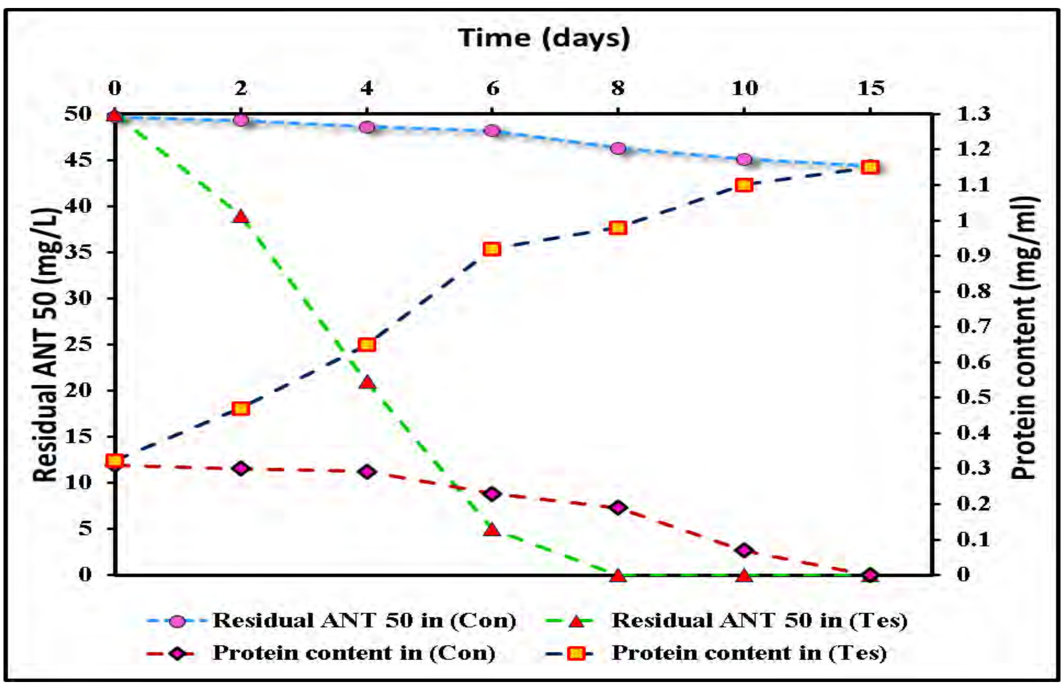

Figure 3. BENZ degradation at 1000 mg/L by bacterial consortium

At a concentration of 1000 mg/L of BENZ, more than 50% of the compound was degraded in 6 days. In addition, the deterioration rate increased, as it recorded degradation rates of about 69% and 76.5% within 8 and 10 days, respectively. In 15 days of the experiment, the highest degradation rate of this compound was recorded, reaching 82.94%, as the increase in the concentration of this compound led to a slow degradation of the compound due to its severe toxicity, which affects the efficiency of the bacterial consortium, which in turn reduced the rates of degradation compared to low concentrations (Figure 3).

Degradation of ANT

The biodegradation of ANT was studied by bacterial consortium at different concentrations such as (50, 100 and 500) mg/l .At a concentration of 50 mg/L of ANT, about 90% of the compound was degraded by the bacterial consortium in 6 days of the experiment, while the complete degradation rate of this compound was recorded at this concentration within 8 days (Fig 4). Somtrakoon et al. (2008) recorded a degradation rate of more than 90% at the initial concentration of 50 mg/L of anthracene compound in 6 days and this agrees with the results in this study. Arulazhagan et al., (2017) in a study they conducted on the degradation of PAHs by acidophilic *Stenotrophomonas maltophilia* strain AJH1 recorded a degradation rate of about 93% of anthracene compound at a concentration of 50 mg/L in 4 days.

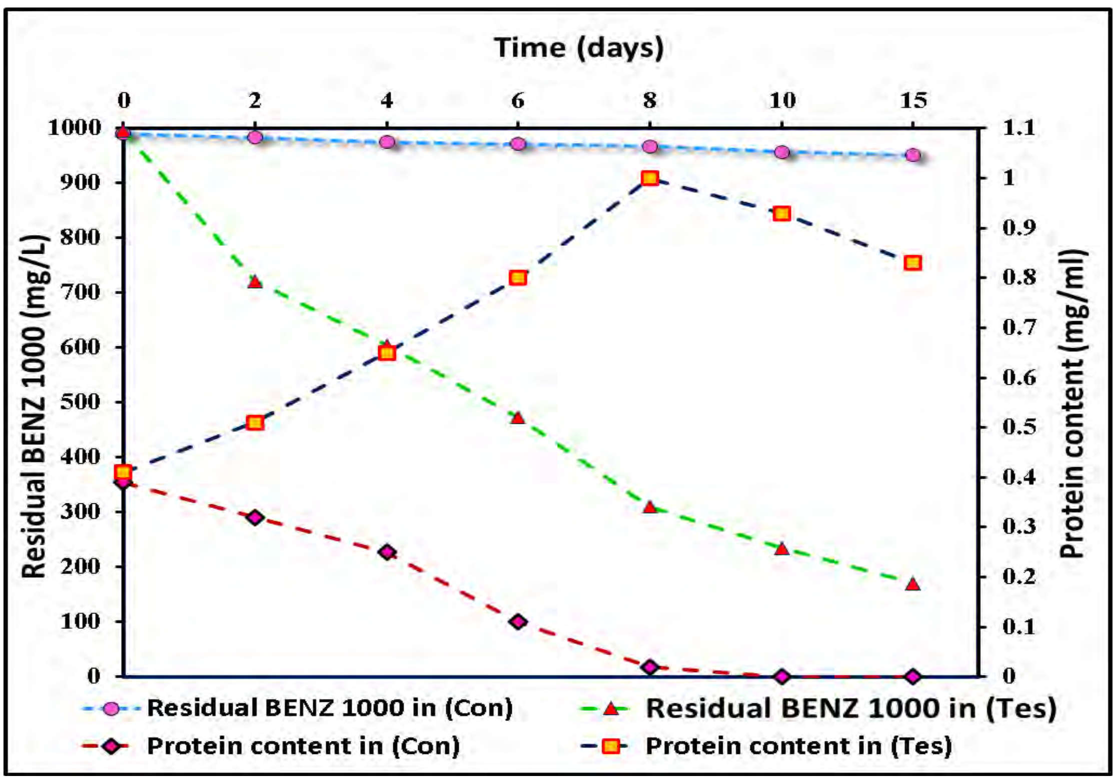

Figure 4. ANT degradation at 50 mg/L by bacterial consortium

When the concentration was increased to 100 mg/L, degradation rates for this compound were recorded, reaching 36.9 and 50.4% in 4 and 6 days, respectively. In 8 and 10 days of the experiment, the deterioration rates were about 68.3 and 76.5%, respectively. The highest degradation rate of this compound was recorded at this concentration, about 97%, within 15 days of the experiment by bacterial consortium (Fig 5). These results are in agreement with the results of a study by

Hesham et al. (2014) recorded the degradation of anthracene about 98% by *Sphingomonas koreensis* Strain ASU-06 at a concentrationof 100 mg/l within 15 days.

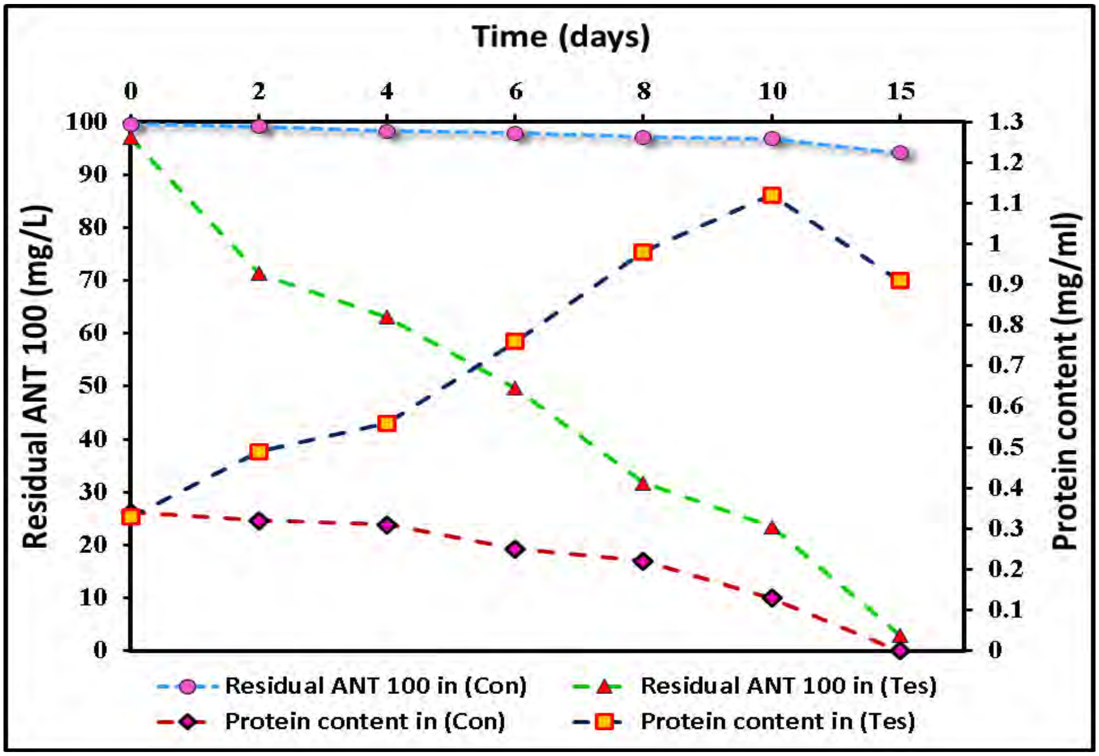

Figure 5. ANT degradation at 100 mg/L by bacterial consortium

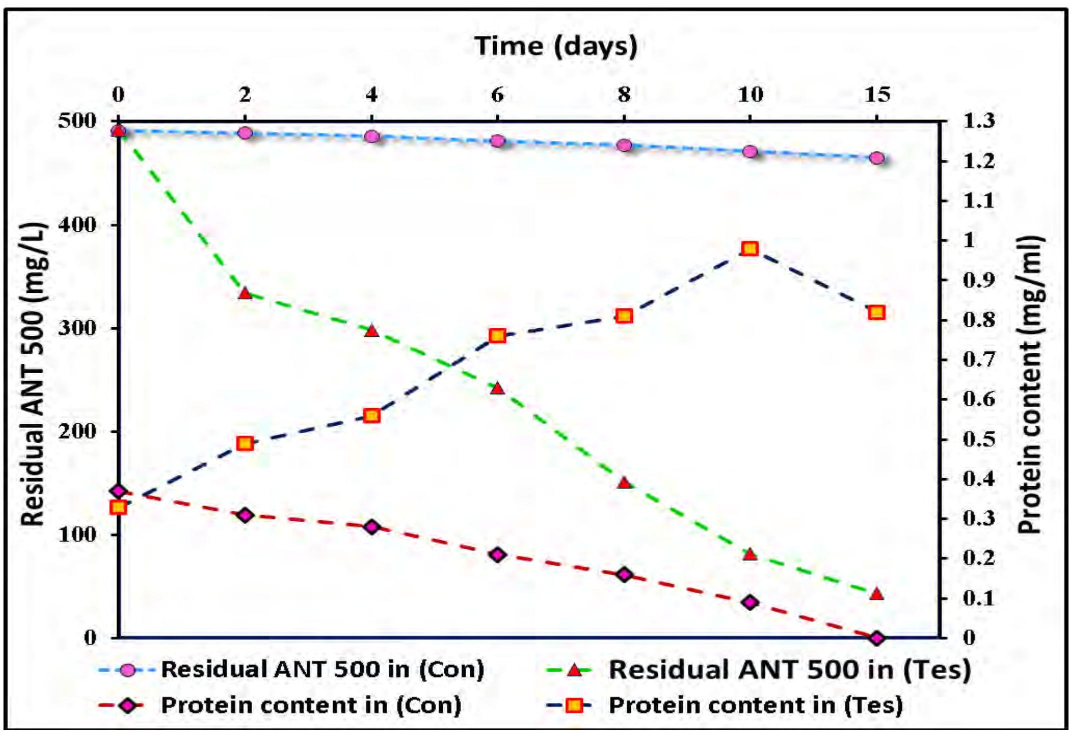

Figure 6. ANT degradation at 500 mg/L by bacterial consortium

At 500 mg/L concentration of ANT, the degradation of this compound was studied by bacterial consortium, where the degradation rates of this compound were recorded about 40.4 and 51.6% within 4 and 6 days, respectively. More degradation was recorded with the passage of the experimental days, as the percentages reached 69.72 and 83.7% of degradation in 8 and 10 days, respectively.

The highest degradation rate of 500 mg/L of ANT was recorded in this study, which was about 91.4% in 15 days of the experiment (Fig 6). It was noted that when the concentration of the compound was increased, the degradation rates decreased due to the high toxicity of this concentration and therefore it takes a longer time to remove these pollutants from the environment. Therefore, the complete degradation of this compound was not recorded at this concentration, perhaps due to the fact that it needs a longer time for its complete degradation. There are several studies that have documented the degradation of anthracene and benzene at different concentrations by bacterial consortium (Arulazhagan and Vasudevan, 2011; Nikolopoulou et al., 2013; Bae et al., 2018; Birolli et al., 2018; Jauhari et al., 2020; Eziuzor et al., 2021; Toth et al., 2021).

Treatment of PAHs in bioreactor study

In this study, wastewater contaminated with petroleum hydrocarbons collected from the coast of Jeddah, Saudi Arabia was used. Bacterial consortium probably degrades the hydrocarbon constituents of these contaminants in addition to reducing COD in CSTR (Fig 7). The reactor (CSTR) was operated under optimal conditions of organic loading rates (OLR) at 0.106 kg/m^{3}d with hydraulic retention time (HRT) for 10 days under saline condition. About 92.7% of COD in the bioreactor was removed in 36 days by the bacterial consortium under saline conditions (Fig 8). The MLSS and MLVSS concentration levels were preserved at 3.8 to 3.1 g/l, and 3.2 to 2.3 g/l, respectively.

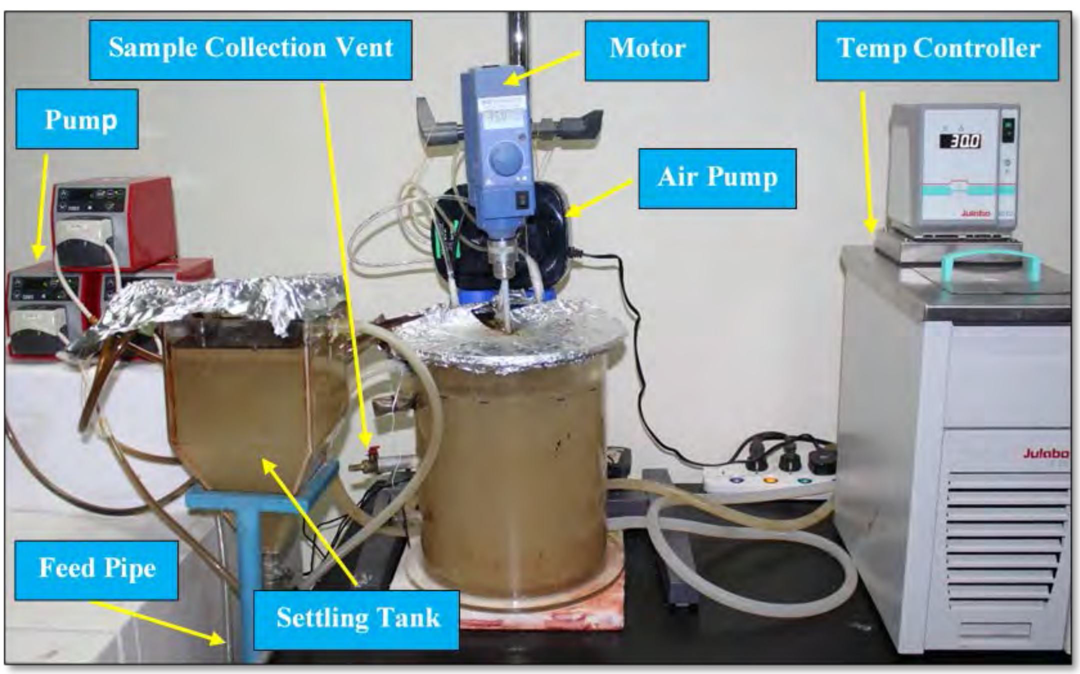

Figure 7. Treatment of petroleum hydrocarbon wastewater by the bacterial consortium in CSTR

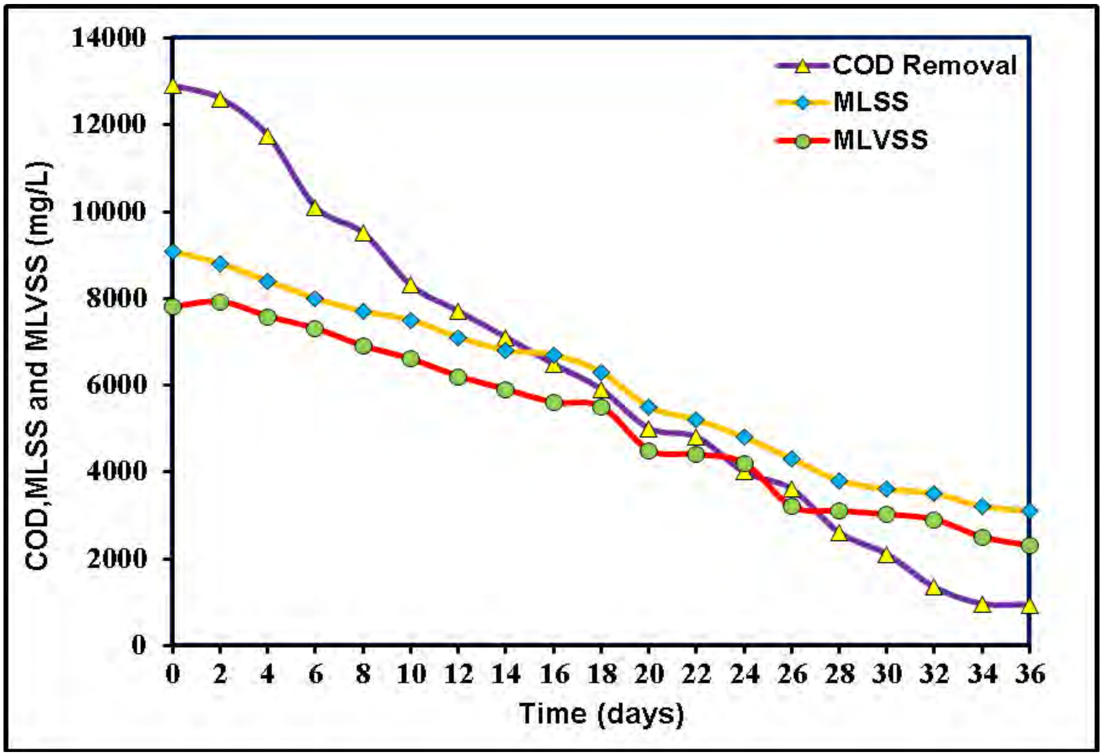

Figure 8. Analysis of COD, (MLSS) and (MLVSS) during the treatment process of petroleum wastewater by bacterial consortium in CSTR

Phylogenetic analysis

The bacterial communities of the study samples were analyzed using bacterial 16S rDNA, Greengene (default): V201305. The taxonomic assignment to major bacterial taxa revealed four main bacterial phyla dominating the sampled sites: Proteobacteria, Actinobacteria, Bacteriodetes and Firmicutes. The ratio of each species in certain sample is directly displayed (Fig. 9)

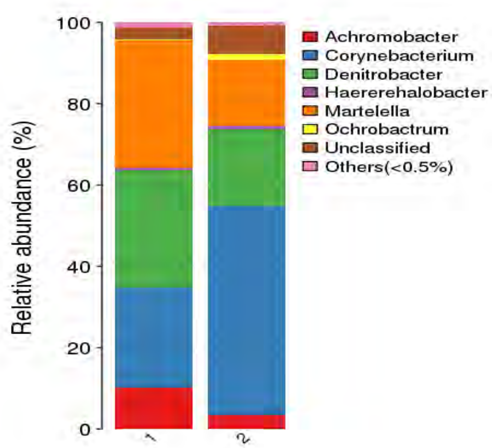

Figure 9. The taxonomic composition distribution in samples of Genus-level

Conclusion

The results of the current study showed the ability of bacterial consortium that was enriched from the coast of Jeddah, Saudi Arabia to degrade petroleum hydrocarbons wastewater, which proved its ability to degrade PAHs in saline conditions. The bacterial union degraded the BENZ compound by more than 95% at the concentration of 100 and 250 mg/L, while the degradation of this compound at the concentration of 1000 mg/L was recorded about 83%.While the ANT degradation rate was recorded at more than 90% at different concentrations.In addition, this study revealed the ability of bacterial consortium to treat petroleum hydrocarbons wastewater in bioreactor (CSTR) with 92.7% of COD removed under saline conditions.Hence, this study recommends the investment of bacterial consortium in the treatment of petroleum hydrocarbons wastewater in marine environments and to remove pollutants from them.

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References

Abegunde, S. M., Idowu, K. S., & Sulaimon, A. O. (2020). Plant-mediated iron nanoparticles and their applications as adsorbents for water treatment–a review. Journal of Chemical Reviews, 2(2), 103-113.

Al-Thaiban, H., Al-Tamimi, N., & Helaleh, M. (2018). Development of QuEChERS method for the determination of polycyclic aromatic hydrocarbons in smoked meat products using GC-MS from Qatar. Journal of analytical methods in chemistry, 2018.

Ali, M. U., Siyi, L., Yousaf, B., Abbas, Q., Hameed, R., Zheng, C., . . . Wong, M. H. (2020). Emission sources and full spectrum of health impacts of black carbon associated polycyclic aromatic hydrocarbons (PAHs) in urban environment: a review. Critical Reviews in Environmental Science and Technology, 1-40.

Ambade, B., Sethi, S. S., Kumar, A., Sankar, T. K., & Kurwadkar, S. (2021). Health risk assessment, composition, and distribution of polycyclic aromatic hydrocarbons (PAHs) in drinking water of southern Jharkhand, East India. Archives of Environmental Contamination and Toxicology, 80(1), 120-133.

Arulazhagan, Al-Shekri, K., Huda, Q., Godon, J. J., Basahi, J. M., & Jeyakumar, D. (2017). Biodegradation of polycyclic aromatic hydrocarbons by an acidophilic Stenotrophomonas maltophilia strain AJH1 isolated from a mineral mining site in Saudi Arabia. Extremophiles, 21(1), 163-174.

Arulazhagan, & Vasudevan, N. (2011). Biodegradation of polycyclic aromatic hydrocarbons by a halotolerant bacterial strain Ochrobactrum sp. VA1. Marine Pollution Bulletin, 62(2), 388-394.

Aygun, S. F., & Bagcevan, B. (2019). Determination of polycyclic aromatic hydrocarbons (PAHs) in drinking water of Samsun and it's surrounding areas, Turkey. Journal of Environmental Health Science and Engineering, 17(2), 1205-1212.

Baali, A., & Yahyaoui, A. (2019). Polycyclic aromatic hydrocarbons (PAHs) and their influence to some aquatic species. In Biochemical Toxicology-Heavy Metals and Nanomaterials: IntechOpen.

Bae, S. S., Jung, J., Chung, D., & Baek, K. (2018). Marinobacterium aestuarii sp. nov., a benzene-degrading marine bacterium isolated from estuary sediment. International Journal of Systematic and Evolutionary Microbiology, 68(2), 651-656.

Birolli, W. G., Santos, D. d. A., Alvarenga, N., Garcia, A. C., Romão, L. P., & Porto, A. L. (2018). Biodegradation of anthracene and several PAHs by the marine-derived fungus Cladosporium sp. CBMAI 1237. Marine Pollution Bulletin, 129(2), 525-533.

Davoodi, S. M., Miri, S., Taheran, M., Brar, S. K., Galvez-Cloutier, R., & Martel, R. (2020). Bioremediation of unconventional oil contaminated ecosystems under natural and assisted conditions: a review. Environmental science & technology, 54(4), 2054-2067.

Espinosa-Ortiz, E. J., Rene, E. R., & Gerlach, R. (2021). Potential use of fungal-bacterial co-cultures for the removal of organic pollutants. Critical reviews in biotechnology, 1-23.

Ewa, B., & Danuta, M.-Š. (2017). Polycyclic aromatic hydrocarbons and PAH-related DNA adducts. Journal of applied genetics, 58(3), 321-330.

Eyring, P., Tienstra, M., Mol, H., Herrmann, S. S., Rasmussen, P. H., Frandsen, H. L., & Poulsen, M. E. (2021). Development of a new generic extraction method for the analysis of pesticides, mycotoxins, and polycyclic aromatic hydrocarbons in representative animal feed and food samples. Food Chemistry, 356, 129653.

Eziuzor, S. C., Schmidt, M., & Vogt, C. (2021). Anaerobic benzene mineralization by natural microbial communities from Niger Delta. Biodegradation, 32(1), 37-52.

Fakhru'l-Razi, A., Pendashteh, A., Abdullah, L. C., Biak, D. R. A., Madaeni, S. S., & Abidin, Z. Z. (2009). Review of technologies for oil and gas produced water treatment. Journal of hazardous materials, 170(2-3), 530-551.

Federation, W. E., & Association, A. (2005). Standard methods for the examination of water and wastewater. American Public Health Association (APHA): Washington, DC, USA.

Fernandez-Gonzalez, R., Yebra-Pimentel, I., Martinez-Carballo, E., & Simal-Gandara, J. (2012). Feed ingredients mainly contributing to polycyclic aromatic hydrocarbon and polychlorinated biphenyl residues. Polycyclic Aromatic Compounds, 32(2), 280-295.

Gaur, N., Narasimhulu, K., & PydiSetty, Y. (2018). Recent advances in the bio-remediation of persistent organic pollutants and its effect on environment. Journal of cleaner production, 198, 1602-1631.

Gaur, V. K., Gupta, S., & Pandey, A. (2021). Evolution in mitigation approaches for petroleum oil-polluted environment: recent advances and future directions. Environmental Science and Pollution Research, 1-17.

Gereslassie, T., Workineh, A., Liu, X., Yan, X., & Wang, J. (2018). Occurrence and ecological and human health risk assessment of polycyclic aromatic hydrocarbons in soils from Wuhan, central China. International journal of environmental research and public health, 15(12), 2751.

Godambe, T., & Fulekar, M. (2016). Cow dung Bacteria offer an Effective Bioremediation for Hydrocarbon-Benzene. Int J Biotech Trends Techn, 6(3), 13-20.

Hassanshahian, M., & Boroujeni, N. A. (2016). Enrichment and identification of naphthalene-degrading bacteria from the Persian Gulf. Marine Pollution Bulletin, 107(1), 59-65.

Hesham, A. E.-L., Mawad, A. M., Mostafa, Y. M., & Shoreit, A. (2014). Biodegradation ability and catabolic genes of petroleum-degrading Sphingomonas koreensis strain ASU-06 isolated from Egyptian oily soil. BioMed Research International, 2014.

Jamal, M. T., & Pugazhendi, A. (2018). Degradation of petroleum hydrocarbons and treatment of refinery wastewater under saline condition by a halophilic bacterial consortium enriched from marine environment (Red Sea), Jeddah, Saudi Arabia. 3 Biotech, 8(6), 1-10.

Jauhari, N., Mishra, S., Kumari, B., Singh, S., Chauhan, P. S., & Upreti, D. (2020). Bacteria induced degradation of anthracene mediated by catabolic enzymes. Polycyclic Aromatic Compounds, 40(2), 313-325.

José, M., Sánchez-Martín, Á. M., Campos, P., & Miller, A. Z. (2019). Effect of pyrolysis conditions on the total contents of polycyclic aromatic hydrocarbons in biochars produced from organic residues: Assessment of their hazard potential. Science of The Total Environment, 667, 578-585.

Juhasz, A. L., & Naidu, R. (2000). Bioremediation of high molecular weight polycyclic aromatic hydrocarbons: a review of the microbial degradation of benzo [a] pyrene. International Biodeterioration & Biodegradation, 45(1-2), 57-88.

Kafouris, D., Koukkidou, A., Christou, E., Hadjigeorgiou, M., & Yiannopoulos, S. (2020). Determination of polycyclic aromatic hydrocarbons in traditionally smoked meat products and charcoal grilled meat in Cyprus. Meat Science, 164, 108088.

Kuppusamy, S., Thavamani, P., Megharaj, M., & Naidu, R. (2016). Bioaugmentation with novel microbial formula vs. natural attenuation of a long-term mixed contaminated soil—treatability studies in solid-and slurry-phase microcosms. Water, Air, & Soil Pollution, 227(1), 1-15.

Landvik, N. E., Gorria, M., Arlt, V. M., Asare, N., Solhaug, A., Lagadic-Gossmann, D., & Holme, J. A. (2007). Effects of nitrated-polycyclic aromatic hydrocarbons and diesel exhaust particle extracts on cell signalling related to apoptosis: possible implications for their mutagenic and carcinogenic effects. Toxicology, 231(2-3), 159-174.

Lewis, A., & Prince, R. C. (2018). Integrating dispersants in oil spill response in Arctic and other icy environments. Environmental science & technology, 52(11), 6098-6112.

Liu, Z., Jacobson, A. M., & Luthy, R. G. (1995). Biodegradation of naphthalene in aqueous nonionic surfactant systems. Applied and environmental microbiology, 61(1), 145-151.

Lyon, D. Y., & Vogel, T. M. (2013). Bioaugmentation for groundwater remediation: an overview. Bioaugmentation for groundwater remediation, 1-37.

Manzetti, S. (2013). Polycyclic aromatic hydrocarbons in the environment: environmental fate and transformation. Polycyclic Aromatic Compounds, 33(4), 311-330.

Meador, J. (2010). Polycyclic aromatic hydrocarbons. In Ecotoxicology (pp. 314-323): Academic Press.

Ndimele, P. E., Saba, A. O., Ojo, D. O., Ndimele, C. C., Anetekhai, M. A., & Erondu, E. S. (2018). Remediation of crude oil spillage. In The political ecology of oil and gas activities in the Nigerian aquatic ecosystem (pp. 369-384): Elsevier.

Nicholson, C. A., & Fathepure, B. Z. (2004). Biodegradation of benzene by halophilic and halotolerant bacteria under aerobic conditions. Applied and environmental microbiology, 70(2), 1222-1225.

Nikolopoulou, M., Pasadakis, N., & Kalogerakis, N. (2013). Evaluation of autochthonous bioaugmentation and biostimulation during microcosm-simulated oil spills. Marine Pollution Bulletin, 72(1), 165-173.

Ofori, S. A., Cobbina, S. J., Imoro, A. Z., Doke, D. A., & Gaiser, T. (2021). Polycyclic Aromatic Hydrocarbon (PAH) Pollution and its Associated Human Health Risks in the Niger Delta Region of Nigeria: A Systematic Review. Environmental Processes, 1-28.

Parales, R. (2010). Hydrocarbon degradation by betaproteobacteria. In Handbook of hydrocarbon and lipid microbiology.

Ramirez, M. I., Arevalo, A. P., Sotomayor, S., & Bailon-Moscoso, N. (2017). Contamination by oil crude extraction–Refinement and their effects on human health. Environmental Pollution, 231, 415-425.

Rengarajan, T., Rajendran, P., Nandakumar, N., Lokeshkumar, B., Rajendran, P., & Nishigaki, I. (2015). Exposure to polycyclic aromatic hydrocarbons with special focus on cancer. Asian Pacific journal of tropical biomedicine, 5(3), 182-189.

Rubio-Clemente, A., Torres-Palma, R. A., & Peñuela, G. A. (2014). Removal of polycyclic aromatic hydrocarbons in aqueous environment by chemical treatments: a review. Science of The Total Environment, 478, 201-225.

Sánchez-Avila, J., Fernandez-Sanjuan, M., Vicente, J., & Lacorte, S. (2011). Development of a multi-residue method for the determination of organic micropollutants in water, sediment and mussels using gas chromatography–tandem mass spectrometry. Journal of Chromatography A, 1218(38), 6799-6811.

Santos, H. F., Carmo, F. L., Paes, J. E., Rosado, A. S., & Peixoto, R. S. (2011). Bioremediation of mangroves impacted by petroleum. Water, Air, & Soil Pollution, 216(1), 329-350.

Somtrakoon, K., Suanjit, S., Pokethitiyook, P., Kruatrachue, M., Lee, H., & Upatham, S. (2008). Enhanced biodegradation of anthracene in acidic soil by inoculated Burkholderia sp. VUN10013. Current microbiology, 57(2), 102-106.

Stading, R., Gastelum, G., Chu, C., Jiang, W., & Moorthy, B. (2021). Molecular mechanisms of pulmonary carcinogenesis by polycyclic aromatic hydrocarbons (PAHs): Implications for human lung cancer. Paper presented at the Seminars in Cancer Biology.

Tazdaït, D., & Salah-Tazdaït, R. (2021). Polycyclic Aromatic Hydrocarbons: Toxicity and Bioremediation Approaches. In Biotechnology for Sustainable Environment (pp. 289-316): Springer.

Thavamani, P., Megharaj, M., & Naidu, R. (2012). Bioremediation of high molecular weight polyaromatic hydrocarbons co-contaminated with metals in liquid and soil slurries by metal tolerant PAHs degrading bacterial consortium. Biodegradation, 23(6), 823-835.

Toth, C. R., Luo, F., Bawa, N., Webb, J., Guo, S., Dworatzek, S., & Edwards, E. A. (2021). Anaerobic Benzene Biodegradation Linked to the Growth of Highly Specific Bacterial Clades. Environmental science & technology.

Wang, S., Wang, D., Yu, Z., Dong, X., Liu, S., Cui, H., & Sun, B. (2021). Advances in research on petroleum biodegradability in soil. Environmental Science: Processes & Impacts, 23(1), 9-27.

Welsby, D., Price, J., Pye, S., & Ekins, P. (2021). Unextractable fossil fuels in a 1.5° C world. Nature, 597(7875), 230-234.

Wu, H., Sun, B., & Li, J. (2019). Polycyclic aromatic hydrocarbons in sediments/soils of the rapidly urbanized lower reaches of the River Chaohu, China. International journal of environmental research and public health, 16(13), 2302.

Zhang, R., Han, M., Yu, K., Kang, Y., Wang, Y., Huang, X., . . . Yang, Y. (2021). Distribution, fate and sources of polycyclic aromatic hydrocarbons (PAHs) in atmosphere and surface water of multiple coral reef regions from the South China Sea: A case study in spring-summer. Journal of hazardous materials, 412, 125214.

Zoppini, A., Bongiorni, L., Ademollo, N., Patrolecco, L., Cibic, T., Franzo, A., . . . Amalfitano, S. (2020). Bacterial diversity and microbial functional responses to organic matter composition and persistent organic pollutants in deltaic lagoon sediments. Estuarine, Coastal and Shelf Science, 233, 106508.

Zungum, I. U., & Imam, T. S. (2021). Ecotoxicity and Associated Threat of Polycyclic Aromatic Hydrocarbons (PAHs) to Biodiversity: A Review.