ISSN: 2520-7687 (Print) and XXXX-XXXX (Online)
Journal of Agriculture, Food and Natural Resources
J. Agric. Food Nat. Resour., Jan-Apr 2017, 1(1): 65-70
Journal Homepage: http://www.afnrjournal.com/
http:/www.wollegajournals.com/

Original Research

Isolation of Diesel, Engine Oil and Crude Oil Degrading Fungi from Oil Contaminated Soil for their Bioremediation Potential

Manjunatha B.K* and Praveen Kumar S.V

Department of Biotechnology, The Oxford College of Engineering, Bengaluru-560068, Karnataka, India

Abstract

Polycyclic aromatic hydrocarbons (PAHs) are considered to be most significant environmental pollutants and are to be removed. PAHs exposure causes damage to lungs, liver, kidneys, intestines and other internal organs and many PAHs are reported as carcinogenic, teratogenic, immuno-toxic and genotoxic. In the present study, the biodegradation of used engine oil, diesel oil and crude oil (as model PAHs) using fungal isolates was carried out. Total of 20 indigenous fungal isolate has been successfully isolated from oil contaminated soil. All the pure fungal isolates were checked for their PAHs utilization capability. Among the isolates tested 2 fungi namely, OK-6 and CSN-01 isolates showed potent crude oil, diesel and engine oil degradation ability with 52.45 %, 63.50 % and 48 % and 58.21 %, 69.64 % and 43.6 % respectively. The preliminary screening tests were conducted in MSM agar plates for diesel and engine oil degradation studies, followed by DCPIP method. Further characterization of the isolates, biosurfactant and enzyme properties are studied to optimize and enhance the bioremediation process.

Article Information

Article History:
Received : 15-01-2017
Revised : 20-03-2017
Accepted : 16-04-2017

Keywords:
Biodegradation
PAHs
Fungi
Crude oil

***Corresponding Author:**

Manjunatha B.K

E-mail:
professorbkm@gmail.com

INTRODUCTION

In the modern developing world, contamination of environment on the earth is a major issue (Briggs 2016). Bioremediation is an ecologically acceptable technology that employs the use of microorganisms to efficiently degrade pollutants (Praveen *et al.,* 2016). Oil contamination has severe impacts in the plant and animal ecosystem including human health (Mandal *et al.,* 2007; EPA, undated). Crude oil exposure may cause damage to lungs, liver, kidneys, intestines and other internal organs. Polycyclic aromatic hydrocarbons (PAHs) may lead to cancer, Inhalation leads to headache, nausea, dizziness, respiratory irritation, BTEX (Benzene, Toluene, Eethyl benzene and Xylene) cause mutations, cancers, birth defects, nervous disorders, and liver disease, depression, irregular heartbeats etc. (Gomer *et al.,* 1980; Knafla *et al.,* 2006; Zhang *et al.,* 1992; Carpenter *et al.,* 1977; Lee *et al.,* 2006; Chen *et al.,* 2008 and Rice *et al.,* 2007). Oil contaminated soil loose its fertility and have impact on seed germination (Skulachev, 2006 and Gong *et al.,* 2001). The disposal of the oily waste in an improper manner may cause a serious environmental problem (Yuste *et al.,* 2000). The effects on human health will depend mainly on the length and route of exposure, the amount or concentration of PAHs one is exposed to, and of course the innate toxicity of the PAHs. A variety of other factors can also affect health impacts including subjective factors such as pre-existing health status and age. The ability of PAHs to induce short-term health effects in humans is not clear. Occupational exposures to high levels of pollutant mixtures containing PAHs have resulted in symptoms such as eye irritation, nausea, vomiting, diarrhea and confusion. However, it is not known which components of the mixture were responsible for these effects and other compounds commonly found with PAHs may be the cause of these symptoms. Mixtures of PAHs are also known to cause skin irritation and inflammation. anthracene, benzo(a)pyrene and naphthalene are direct skin irritants while anthracene and benzo(a)pyrene are reported to be skin sensitizers, i.e. cause an allergic skin response in animals and humans (IPCS, 1998). Health effects from chronic or long term exposure to PAHs may include decreased immune function, cataracts, kidney and liver damage (e.g. jaundice), and breathing problems, asthma like symptoms, and lung function abnormalities, and repeated contact with skin may induce redness and skin inflammation. Naphthalene, a specific PAH, can cause the breakdown of red blood cells if inhaled or ingested in large amounts. If exposed to PAHs, the harmful effects that may occur largely depend on the way people are exposed. Although un-metabolized PAHs can have toxic effects, a major concern is the ability of the reactive metabolites, such as epoxides and dihydrodiols, of some PAHs to bind to cellular proteins and DNA. The resulting biochemical disruptions and cell damage lead to mutations, developmental malformations, tumors, and cancer. Evidence indicates that mixtures of PAHs are carcinogenic to humans. The evidence comes primarily from occupational studies of workers exposed to mixtures containing PAHs and these long-term studies have shown an increased risk of predominantly skin and lung, but as well as bladder and gastrointestinal cancers.

Benzo(a)pyrene is the most common PAH to cause cancer in animals and this compound is notable for being the first chemical carcinogen to be discovered. Based on the available evidence, both the International Agency for Research on Cancer (IARC, 1987) and US EPA (1994) classified a number of PAHs as carcinogenic to animals and some PAHs-rich mixtures as carcinogenic to humans. The EPA has classified seven PAH compounds as probable human carcinogens: benz (a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k) fluoranthene, chrysene, dibenz(ah)anthracene, and indeno(1,2,3-cd)pyrene. Teratogenicity-Embryo toxic effects of PAHs have been described in experimental animals exposed to PAH such as benzo (a) anthracene, benzo (a) pyrene, and naphthalene.

Bacteria and fungi are known to be the principle agents of biodegradation of hydrocarbons. Fungi have a higher tolerance to the toxicity of hydrocarbons due to their physiology and adaption to such variations to the environment and have the mechanism for elimination of spilled oil form the environment. Hence fungi are found to better degraders of petroleum than traditionally bioremediation techniques with bacteria (Ojo, 2005). Fungi have also demonstrated ability to degrade and mineralize phenols, halogenated phenolic compounds, petroleum hydrocarbons, polycyclic aromatic hydrocarbons and polychlorinated biphenyls (Singh, 2006). The advantages isolated with fungi bioremediation are primarily in the versatility of the technology and its cost efficiency compared to other remediation technologies such as incineration, thermal desorption and extraction.

The use of fungi is expected to be relatively economical as they grown on a number of inexpensive agricultural or forest wastes such as corncobs and saw dust. More so, their utilization is a gentle non-aggressive approach hence the aim of this study is to evaluate the hydrocarbon degradation potentials fungi associated with these oil-contaminated soils. Hence in the present study, the potent isolated fungi from oil contaminated soils were checked for their potential use in bioremediation process.

MATERIALS AND METHODS

Soil Sampling and Pretreatment

Soil samples were collected from different localities of Bengaluru of Karnataka State covering oil spilled areas like garages, automobiles and workshops. Each collection was made from 10-15 cm depth of the soil. Collected soil samples were transported to the laboratory in air tight pouches and stored in cold condition. Further processing was carried out by reported method (Al- Nasrawi, 2012) and used for isolation studies.

Enumeration and Isolation of Indigenous Fungi from Soil Samples

After careful sorting of stones and debris using 2 mm sieve, serially diluted soil samples were plated onto PDA medium supplemented with 0.1 % tetracycline, and total heterotrophic fungi were enumerated by dilution plate count method. Distinct colonies were isolated through repeated passages (Dhar *et al.,* 2014).

Identification of Petroleum Hydrocarbon Degrading Fungi

The indigenous fungi exhibiting degradation of petroleum hydrocarbon were grown on PDA and SDA agar medium to examine morphology, viz., size, mycelia and sporulation, and culture, viz., color, texture, substrate color and colonial appearances. The observed characteristics of the isolates were recorded and compared with the established identification key given in the Manual of Soil Fungi (Dhar *et al.,* 2014).

Screening of Engine Oil and Diesel Degrading Fungi

For preliminary screening of engine oil and diesel degrading fungi, minimal salt agar medium was used. The composition of MSM was NaCl (10.0g), MgSO4.7H2O (0.42g), KCl (0.29g), KH2PO4 (0.83g), Na2HPO4 (1.25g), NaNO3 (0.42g), agar (20g), distilled water (1L) and pH 7.2. The MSM plates were inoculated with spore suspension of each isolate, incubated at 26°C, and the appearance of substantial -growth was monitored daily up to 7-10 days.

Crude Oil Degradation Studies for the Fungal Isolates using DCPIP Method

The method of Kumar *et al.,* 2016 was adopted for the biodegradation studies. Fungal spore suspension was prepared and inoculated into FSM broth incorporated with sterile 2 % crude oil and redox indicator 2 % DCPIP. The uninoculated flask serves as control. The flasks were incubated at 30° C with constant shaking at a 180 rpm/min for 10 days. The aliquots in the flasks were monitored daily for color change (from deep blue to colorless).

Gravimetric Analysis of Residual Hydrocarbons

Residual oil was extracted by adding hexane to equal volume of broth culture and shaking thoroughly as described by Obayori *et al*., 2009 after removing the aqueous phase with separating funnel. The residual oil concentration of the after treatment and before treatment using the fungal isolates and consortium was determined, % of Total Petroleum Hydrocarbon (TPH) by gravimetrically and results were expressed as percentages of respective controls.

UV-Spectrophotometer Analysis of Residual Crude Oil

The optical density (OD) of the residual crude oil was measured/scanned in between 200-800 nm by Visible-UV spectrophotometer (Thermo evalution-501). The extent of degradation was noticed and recorded by observing the change in the absorption maximum and decrease/ increase in the UV spectra compare to the control (Kumar and Manjunath, 2015).

RESULTS AND DISCUSSION

Isolation of PAHs Degrading Indigenous Fungi and Degradation Studies

Total of 20 fungal strains were isolated from the oil contaminated soil samples collected from local garages and service centers in Bengaluru city. The fungal isolates viz., OK-1, OK-2, OK-3(1), OK-4, OK-5, OK-8, CSN-1, CSN-5, CSN-8, CSN-12, CSN-17, OK-16, BN-3, BN-3a, BN-4, BN-8, BN-9 and BN-10 (Fig-2). The degradation potential of the organisms were observed and the degradation rate was graded as strong (++++), good (+++), moderate (++), weak (+) and no (-) degrading potential as following the method of Ekundayo and Obire, 1987 (supplementary table-S1) were successfully isolated from the oil contaminated soil samples as depicted in table-01. All the isolates were checked for their efficiency to degrade the diesel and engine oil degradation ability on

MSM agar medium supplemented with 2 % engine oil/ diesel as sole carbon source. Among the isolates tested 2 fungi namely, OK-6 and CSN-1 showed maximum degradation potential (Fig-01).

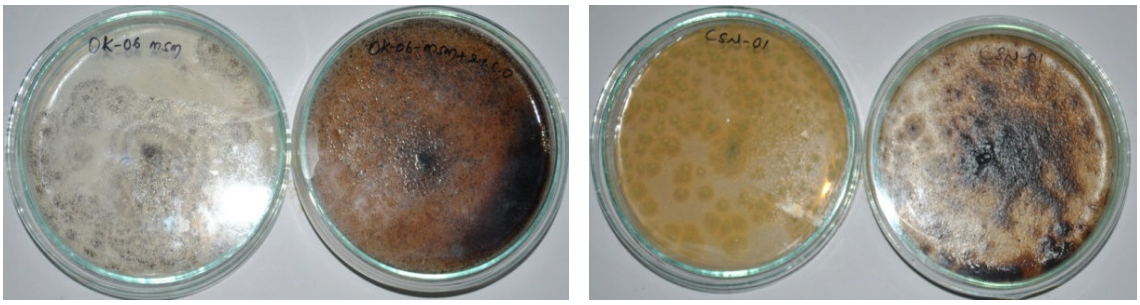

Figure 1: Degradation of diesel and engine oil on MSM agar plates supplemented with 2% of engine oil and diesel as sole carbon source by the fungal isolate OK-06 and CSN-01.

Table 1: Consolidated table shows, the diesel and engine oil degrading potentials of isolated indigenous fungi from soil.

No.	Isolate Code	Rate of Degradation
1	OK-1	++
2	OK-2	-
3	OK-3(1)	+
4	OK-6	++++
5	OK-5	+
6	OK-8	-
7	CSN-1	++++
8	CSN-5	+
9	CSN-8	++
10	CSN-12	+
11	CSN-17	+++
12	OK-16	-
13	BN-3	-
14	BN-3a	++
15	BN-4	+
16	BN-8	++
17	BN-9	++
18	BN-10	-

Note: -: no growth, +: poor growth; ++: moderate growth, +++: good growth, ++++: excellent growth.

The potent strain OK-6 and CSN-1 were selected based on their diesel and used engine oil degradation. Further these strains were identified by their morphological and cultural characteristics. From the preliminary identification results the isolate OK-06 was found be it belongs to *Aspergillus spp.* and the isolate CSN was found to be *Fusarium Sp.* as showed in table-2.

Table 2: Shows cultural and morphological characteristics of the fungal isolates OK-6 and CSN-1 on PDA agar medium.

Isolate Code	Cultural Characteristics	Morphological Characteristics
OK-6 (*Aspergillus sp.*)	Black colored spores/colony with yellow color substrate was observed on PDA media with rapid growth.	Long conidiophores, smooth, unbranched light with initial brown in color with terminated round vesicles, conidial head with dark brown to block color spores.
CSN-1 (*Fusarium sp.*)	Shows rapid growth on PDA medium with wooly white colony and mycelia is branched and septate.	Thread like conidiophores with both micro and macro conidia was observed, septate.

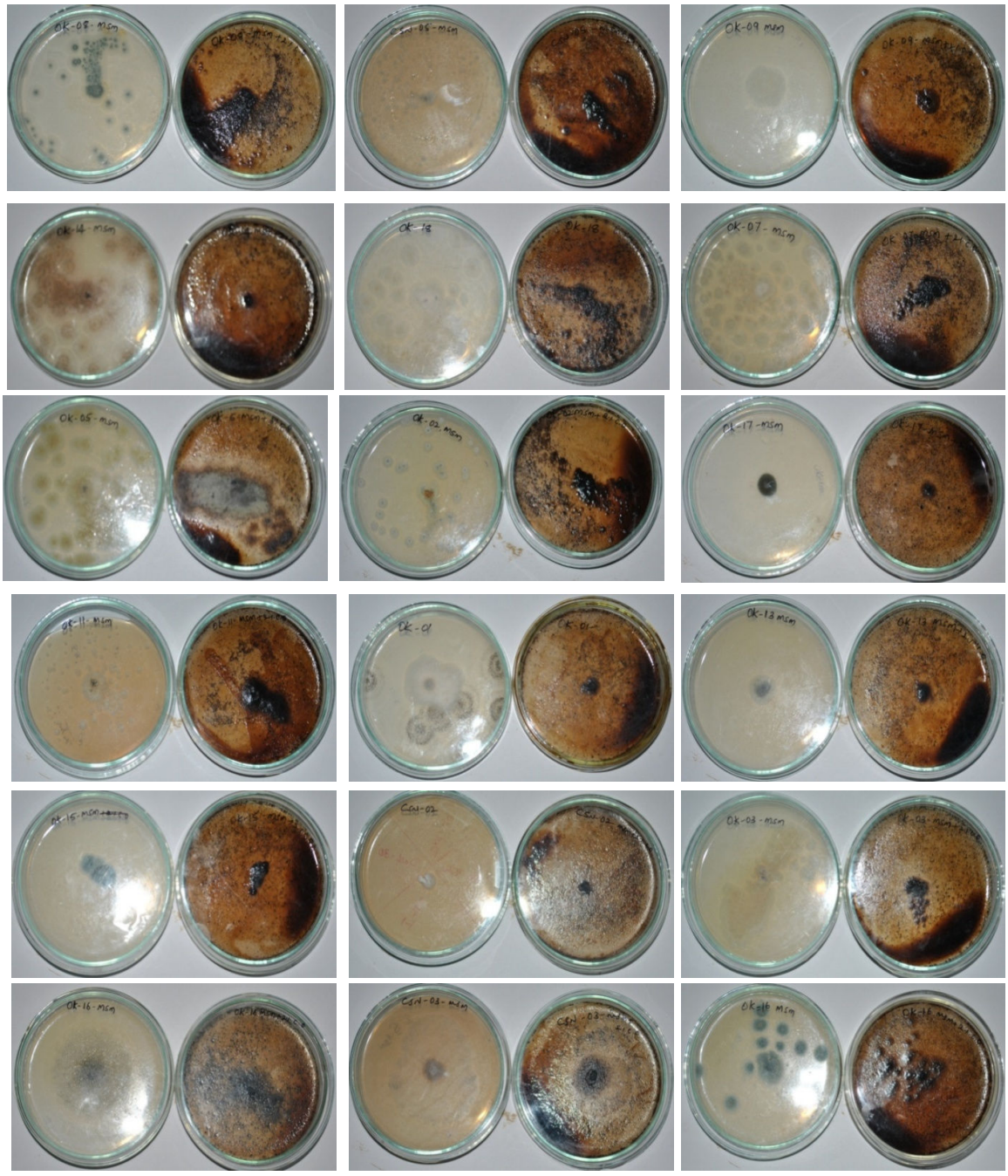

Figure 2: Engine oil and diesel utilisation studies on MSM agar using isolated fungal strains

Crude Oil Degradation Studies using DCPIP Indicator

The isolates OK-06 and CSN-01 were selected based upon their diesel and engine oil utilization capability. The reduction in the dye color from deep blue to colorless was observed in both the fungal isolates (Figure 02). This could be visually inferred, as those isolates that are utilized crude oil were able to produce a turbid mixture with sand like color and consistency, while the rest of flasks retain considerable amount of crude oil which forms immiscible disc like layer the degradation potency of the isolate was assessed continuously by measuring the % TPH and by UV spectrophotometer.

Figure 3: Shows the degradation of (2%) crude oil, which was indicated by the reduction in the color of dye DCPIP**.** In both the isolate OK-6 and CSN-1 shows potential degradation of crude oil at 15 day of incubation.

Estimation of Residual Oil after the Degradation by %TPH and UV-spectrophotometer Analysis

The amount of residual oil left after the degradation studies were estimated by gravimetric analysis. The highest degradation of engine oil and diesel was observed in the isolate CSN-1 with 58.21% and 69.64% respectively. Where in case of isolate OK-6 the engine oil and diesel degradation was found to be 52.45 % and 63.50 % respectively. It was noticed that, the highest crude oil degradation from the isolate OK-6 (48 %) followed by CSN-1 (43.6 %) respectively at 15th day of incubation (Figure 04). The % TPH results were correlates with UV-spectra, where the change in the λ_{max} from 528 nm (control crude oil) to 288 nm in OK-6 and 253 nm in CSN-1 which indicated by the shifting of peaks, many major hydrocarbon components present in the crude oil are disappears after the treatment with fungal isolate (Figure 05).

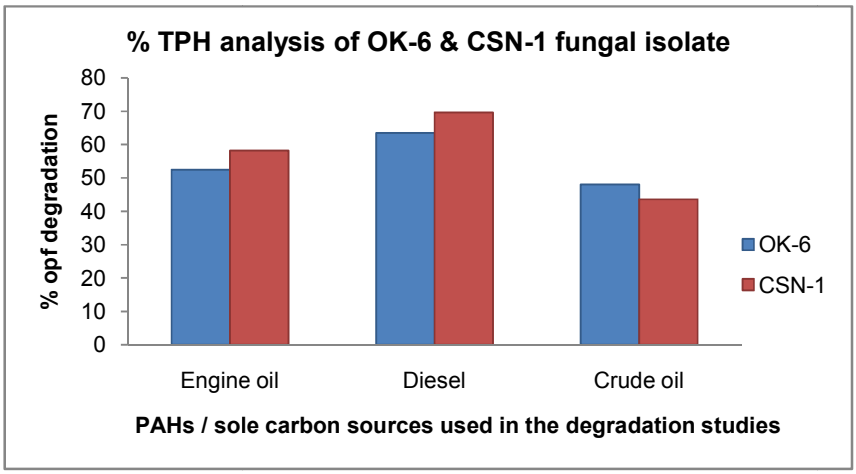

Figure 4: Shows the % TPH degradation potential of engine oil, diesel and crude oil by the fungal isolate CSN-1 and OK-6.

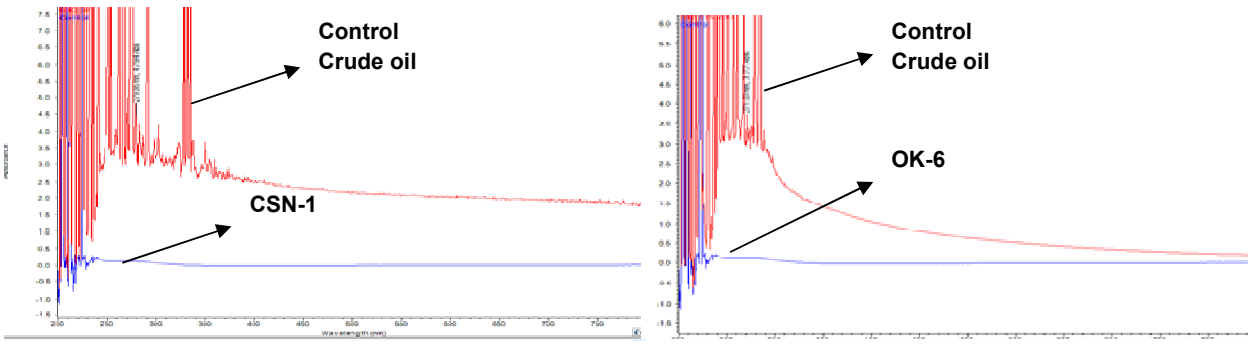

Figure 5: The UV spectra of CSN-1 and OK-6 at 15th day after the degradation treatment. From the UV-spectra, shifting of peaks and disappearance of many peaks in control indicates the degradation potential of the fungal isolates.

During the bioremediation experimentation, it was observed that, the increase in bio mass of fungal isolates with correspondence to the degradation. The % TPH results, UV indicates the remediation process after 15 days of incubation period.

DISCUSSION

Fungi are known to be efficient degraders of a variety of compounds, both simple and complex. In this study we were able to isolate a number of potential oil degrading fungal strains. The isolates CSN-1 and OK-6 were able to degrade diesel, engine oil and crude oil effectively the microbes that are able to utilize crude oil as the carbon source eventually degrade oil and thus reduce the oil into turbid sand like mixture. The potential of these fungi to use diesel, engine oil and crude oil as sole carbon source confirms with the reports of several other research works carried out in the same direction towards bioremediation PAHs wherein it has been reported that indigenous microorganisms can play an effective role as biological agents for cleanup of oil spills. Similarly the UV spectral studies of the crude oil were recorded and compared with control. The change in λ_{max} value for the control and for the sample indicates the loss of conjugation and breakdown in the molecular structure of the oil and it confirms the degradation of crude oil by fungal isolate. Another explanation to the appearance of these peaks is that, as the long chain hydrocarbons break, the C,C linkage at which the breaking occur results in the shift of the atom's energy state and hence the peak symbolizes the highest absorption for that atom.

CONCLUSIONS

The Present study revealed that, fungal isolates collected from crude oil contaminated soil sample holds promise for the development and discovery of new bioremediation approach for the effective PAHs treatment. In order to enhance the bioremediation, further the properties of biosurfactant and enzymes and mechanism of degradation is necessary.

Conflict of Interest

None Declared

Acknowledgement

We thank Naval Research Board- Material Panel-DRDO (DNRD/05/4003/NRB/291) Govt. of India for financial support for this work. We are thankful to Management, Principal and Head Department of Biotechnology, The Oxford College of Engineering Bengaluru for their constant support and facility.

REFERENCES

Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profile for Polycyclic Aromatic Hydrocarbons (PAHs) Aug 1995. Accessed 12.09.2010.

Al-Nasrawi, H. (2012). Biodegradation of Crude oil by fungi isolated from Gulf of Mexico. *Journal of Bioremediation and Biodegradation* 1(4): 21-23.

Carpenter, C.P., Geary, D.L.J., Myers, R.C., Nachreiner, D.J., Sullivan, L.J., King, J.M. (1977). Petroleum hydrocarbon toxicity studies, XIV. Animal and human response to vapors of "High Aromatic Solvent". *Toxicology and Applied Pharmacology* 41(2), 235-249.

Chen, C.S., Hseu, Y.C., Liang, S.H., Kuo Jar-Yi., Chen, S. C. (2008). Assessment of genotoxicity of methyl-tert-butyl ether, benzene, toluene, ethylbenzene, and xylene to human lymphocytes using comet assay. *Journal of Hazardous Materials* 153(1-2): 351-356.

Dhar, K., Dutta, S., Anwar, M.N. (2014). Biodegradation of Petroleum Hydrocarbon by indigenous Fungi isolated from Ship breaking yards of Bangladesh. *International Research Journal of Biological Sciences* 3(9): 22-30.

Gomer, C.J., Smith, D.M. (1980). Acute skin phototoxicity in hairless mice following exposure to crude shale oil or natural petroleum oil. *Toxicology* 18(1): 75-85.

Gong, P., Wilke, B.M., Strozzi, E., Fleischmann, S. (2001). Evaluation and refinement of a continuous seed germination and early seedling growth test for the use in the eco-toxicological assessment of soils. *Chemosphere* 44(3): 491-500.

International Programme On Chemical Safety (INCHEM) Polycyclic aromatic hydrocarbons, selected non-heterocyclic (EHC 202, 1998) Accessed 12.11.2010.

Knafla, A., Phillipps, K.A., Brecher, R.W., Petrovic, S., Richardson, M. (2006). Development of a dermal cancer slope factor for benzo[a]pyrene. *Regulatory Toxicology and Pharmacology* 45(2): 159-168.

Kumar, P.S.V., Manjunatha, B.K., Pavani Bhat., Veena, R., Pawate, S.S. and Yogashree, M. (2016). Bioremediation of poly aromatic hydrocarbons and crude oil by fungal consortium from West Coast of Karnataka. *International Journal of Current Microbiology and Applied Science* 5(10): 386-396.

Kumar, P.S.V., Manjunatha, B.K. (2015). Studies on hydrocarbon degradation by the bacterial isolate *Stenotrophomonas rhizophila* (PM-1) from the oil spilled regions of Western Ghats of Karnataka. *Science Technology and Arts Research Journal* 4(3): 139-144.

Lee Ada, S., Michael R. Bye., Robert B. Mellins (2006). Lung Injury from Hydrocarbon Aspiration and Smoke Inhalation, Kendig's Disorders of the Respiratory Tract in Children, (Seventh Edition), 653-660.

Obayori, O.S., Ilori, M.O., Adebusoye, S.A., Oyetibo, G.O., Omotayo, A.E., Amund, O.O. (2009). Degradation of hydrocarbons and biosurfactant production by Pseudomonas sp. strain LP1. *World Journal of Microbiology and Biotechnology* 25: 1615-1623.

Ojo, O.A. (2005). Petroleum-hydrocarbons utilization by nature bacterial population from a waste water canal south west Nigeria. *African Journal of Biotechnology*. 5: 333-337.

Praveen, K.S.V., Manjunatha, B.K., Mishra, G. and Mehkri, M A. (2016). Optimization of crude oil and PAHs degradation by *Stenotrophomonas rhizophila* KX082814 strain through response surface methodology using Box-Behnken design. *Biotechnology Research International* 2016, Article ID 4769542, 13 pages.

Public Health SA Polycyclic Public Health Fact Sheet. Accessed 13.11.2010.

Rice, S.D., Short, J.W., Carls, M.G., Moles, A., Spies, R. B. (2007). The Exxon Valdez Oil Spill, Long-term Ecological Change in the Northern Gulf of Alaska, 419-520.

Singh, H. (2006). Mycoremediation: fungal remediation. New York: Wiley Interscience 592.

Skulachev, V. (2006). Rotary Catalysis of ATP Synthase *Biochimica et Biophysica Acta (BBA) – Bioenergetics* 1757(1): 1-551.

Yuste, L., Corbella, M.E., Turiegano, M.J., Karlson, U., Puyet, A., Rojo, F. (2000). Characterization of bacterial strains able to grow on high molecular mass residues from crude oil processing. *FEMS Microbiology Ecology* 32: 69-75

Zhang Litai., Kristin Sannes., Alan J. Shusterman., Corwin Hansch (1992). The structure-activity relationship of skin carcinogenicity of aromatic hydrocarbons and heterocycles. *Chemico-Biological Interactions* 81(1-2): 149-180.