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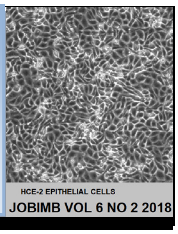

Bioremediation of Hydrocarbon: A Mini Review

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ABSTRACT

Several contaminants that are present in the environment are able to be removed through the physical and chemical approach. Environmental rehabilitation is a challenge especially in large water bodies like rivers due to the volume and fastmoving property of river water. The exorbitant cost, safety and efficiency of physicochemical methods are important issues especially to remediate dissolved compounds such as pesticides, drug, and heavy metals. Nowadays, environmental rehabilitation using biological approach or known as bioremediation gets great attention as the treatment processes are environmentally friendly, low cost and easy to handle. Moreover, the result is highly satisfactory and at the same time cause no harm to the environment. This mini-review highlights the application of bioremediation to clean contaminated environment. The advantages and disadvantages will also be discussed.

INTRODUCTION

All through the world, the issues of environmental contamination by harmful chemicals are huge and increase from a year to a year. The expected expenses of environmental rehabilitation that require accessible technologies run into many billions of dollars. Sometimes, no suitable tools are available at any expenditure. However, as an alternative, this issue can be solved through the process known as bioremediation. The word "remediate" means to cure or treat the problem. Thus, the word "bioremediate" means the use of biological organisms to treat environmental problems such as contaminated surface water (river) and groundwater. United States Environmental Protection Agency (EPA) defined bioremediation is a biological treatment such microorganisms to eliminate toxicant by converting to nontoxic form or less harmful substances [1–3].

Hence, the user of microorganisms to eliminate contaminants or pollutants such as oil is called “bioremediation” because biological agents (microbes) are used to remedy the situation. In a non-polluted environment, microorganisms constantly degrade the organic matters which utilise as carbon and nitrogen sources for their survival. However, it has their limitation which needs a favourable condition to enhance the remediation process in a shorter time, and the treated are fully recovered. The existence of xenobiotic such metal ion and pesticides may negatively effect to the biological function of the organism [4–6]. The area or treatment can be done artificially either in-situ or ex-situ. In-situ bioremediation uses the technology directly in the river without excavating and taking away any pollutants from the contaminated river. In ex-situ bioremediation, an amount of the polluted river water is taken out to a specialised treatment plan for remediation [7].

In-situ bioremediation is favourable as this process might save a lot of costs as well as effectively to eliminate contaminant. This process involves the stimulation of naturally occurring microbial populations via biostimulation or biosparging to enhance and increase the remediation rate on the contaminants of concern. In order to efficiently carry out this activity, the source of the pollutants must be identified and stop before further remedial action can be taken. The present of other toxicants can also present a difficult task as these co-contaminants may include nonbiodegradable pollutants including heavy metals that inhibit the remediation process. In the event that this pose a risk, other co-remediation strategy perhaps with the introduction of physicochemical methods may be the only way pollutant can be remediated efficiently [8–12].

Unfortunately, the induction of microbial populace experienced difficulty when there are developments of the subsurface by the massive amount of biomass that are produced or generated using microbial development on hydrocarbons, failure to supply oxygen to the subsurface, furthermore the incapability to transport nutrients to all locales of the subsurface area [13]. Ex-situ refers to the remediation process that is done only after the contaminated waste has been removed and transported to a treatment area. Commonly, ex-situ bioremediation involves composting which included the addition of essential chemicals or nutrients to stimulate and increase the microbial population associated with the increasing biological activities at the polluted site. Several parameters should take account to maximise the remediation process including microbial population, oxygen concentration, temperature, pH and substrate concentrations [14–18]. Numerous restricting elements happen to be proven to impact the biodegradation of petroleum hydrocarbons, a few of which have already been mentioned by numerous researchers [2,12,19–25].

The structure and built in biodegradability of the petroleum hydrocarbon contaminant is the most notably essential consideration in the event the appropriateness of a remediation strategy is to be evaluated. Amongst the physical elements, temperature takes on a crucial role in the biodegradation of hydrocarbons by specifically impacting the chemistry of the contaminants in addition to impacting the physiology and variety of the microorganisms make up. It is anticipated that the viscosity of the oil will be very high at low temperatures and the volatility of the low molecular weight hydrocarbons, which is toxic will be reduced, hence, stalling the start of biodegradation [20,26].

Bioremediation application

Bioremediation is a well-known concept to eliminate contaminant in the environment — the first commercial application in the 1970s which the experiment was conducted during the oil spill at Sun Oil pipeline near Ambler, Pennsylvania [27]. However, only at the end of the 1980s, bioremediation has become widely known as a technology for clean-up of shorelines contaminated with spoiled oil in the United States. Bioremediation gets great attention since the incident occurred in 1989 where The Exxon Valdez oil spill in Prince William Sound, Alaska [28]. During the 1990s, most of the researcher accentuation changed to more noteworthy dependence on natural microorganisms and procedures to improve their implementation and effect [27].

Bioremediation was developed due to the combination of skills and expertise to understand the mechanism involves during the remediation process, contamination behaviour and level by the geology of the site, contaminant management and technology application associated with the civil and process engineering industry. The needs for rehabilitation of contaminated land and river were drastically increasing for a year to a year, and this is the fact to the several developing countries to confront this problem. Other than physical and chemical approach, bioremediation was accepted as an alternative option for the treatment of polluted sites as their capability to completely remove or detoxify the contaminant [29,30,30–35].

Bioremediation for river rehabilitation

Bioremediation is the processes by which we used and stimulate the microbial activities to clean up contaminated environments such as the oil spills in the river or ocean, the toxic chemicals or another medium. There are many bioremediation processes that have been proposed to clean up the toxic chemicals, and the most successful of bioremediation is cleaning up spills of crude oil. Before going deeper into the processes of how microbes clean up the oil spills, we need to know the composition of the oil itself and why microorganisms can degrade the oils easily. Theoretically, petroleum which is the crude oil is rich with organic matter. It is formed with hydrocarbon which is the compound that is made up of carbon and hydrogen and also with the small addition of certain substances. Petroleum is the fossil fuel which is formed when the plants or animals die and remain buried under the sand or mud for thousands or even more years. When petroleum is pumped out to Earth’s surface, some of it might get in contact with the air or the water. This leakage of hydrocarbons in soil and water is known as pollutants and must be clean up as soon as possible. The bioremediation of river from oil and other pollutants have been carried out with varying success [2,12,19–25] dur to the constraints mentioned before.

Petroleum-based products are the major source of energy for industry and daily life. Leaks and accidental spills occur regularly during the exploration, production, refining, transport, and storage of petroleum and petroleum products. The amount of natural crude oil seepage was estimated to be 600,000 metric tons per year with a range of uncertainty of 200,000 metric tons per year [1]. Release of hydrocarbons into the environment whether accidentally or due to human activities is a main cause of water and soil pollution [2]. Soil contamination with hydrocarbons causes extensive damage of local system since accumulation of pollutants in animals and plant tissue may cause death or mutations [3]. The technology commonly used for the soil remediation includes mechanical, burying, evaporation, dispersion, and washing. However, these technologies are expensive and can lead to incomplete decomposition of contaminants [9,24,36–45].

The operation of bioremediation, understood to be the application of microbes to purify or eliminate contaminants due to their diversified metabolic features is definitely a changing opportunity for the elimination and degradation of numerous environmental contaminants such as the products of the petroleum sector. Additionally, bioremediation technologies are believed to be noninvasive and relatively cost-effective. Biodegradation by natural populations of microorganisms represents one of the greatest systems through which petroleum along with other hydrocarbon contaminants can be taken off from the surroundings [6] and is less expensive than some other removal systems.

From here onwards, microbial activities play an important role to clean up the spills. Physical methods are often employed as the first stage to remove the bulk of the oil spills, and the action of microbes can remove residual oil from the river or the ocean. Sometimes, microbe takes several periods of times to break down the hydrocarbon depending on the concentration and the compound of the spills. Microbial oil degradation can occur by aerobic respiration or anaerobic respiration depending on the presence of the oxygen. If there is oxygen molecule, then it will be aerobic respiration while in the absent of oxygen, anaerobic respiration is being used, but potentailly more toxic by-products are often produce under latter conditions. Numerous microbial hydrocarbon degradation occurs by aerobic respiration by which the oil-degrading microorganisms consume the oxygen molecule and utilise oil hydrocarbon for the carbon sources. For the anaerobic metabolism, microbes have other pathways to degrade the hydrocarbon for energy and sometimes slower compared to the aerobic respiration [6].

Microorganisms such as bacteria and yeast, although some of the can assimilate hydrocarbon, considerable energy is needed to overcome cell wall and membrane damage due to the solvent effect of hydrocarbons [46]. The stripping of cellular membrane through the solvent effects of hydrocarbon causes leaking of cellular compartment that ultimately lead to cellular deaths. A number of classes of organic compounds are harmful for microorganisms as they quite simply build up in and break up cell membranes. In these instances, the dose-dependent toxicity of hydrocarbon fits in accordance to the logarithm of its partition coefficient in between water (logP) and octanol. in general, compounds having a logP value in between 1 and 5 are poisonous for bacterial cells [11]. Consequently, poisonous outcomes of hydrocarbons on bacteria could cause difficulties in teh process of bioremediation especially in region where the sites are heavily polluted. The poisonous proeprties of the majority of hydrocarbons is because, of the nonspecific effects on the fluidity of membrane structure as these hydrocarbons tends to accumulate in the hydrophobic phospolipid bilayer [8].

The majority of substances having a greater hydrophobicity than logP of 4 have low water solubility, and examples include PAHs, biphenysls and alkanes. Consequently, the low bioavailability exerts a less toxic effect. Microorganisms can overcome this effect through the production of biosurfactants that can protect the bacteria from the toxic effect of hydrocarbons. Other protective strategy includes the formation of biofilms and exopolysaccharides that can alleviate the toxic effects of hydrocarbons. The ability to break down the hydrocarbon is present in a variety of bacteria and fungi [47–60]. The mechanism on how the microbes degrade the oil spills can be understood by the analogy of the automobile. The microbes utilised the hydrocarbon as the fuel and finally release carbon dioxide (CO_2) and water as the products. This situation occurred when the microbe metabolised the aromatic hydrocarbon through the activity of the enzymes and consumption of oxygen in the river. This is due to the ability of microbial genetic to generate and synthesis various oil-degrading enzymes [16,17].

Same with other living things or enzymes, they need to be at the optimum temperature to maximize the activity [18–20]. Bioremediation occurs best when it is near the surface of the river where the sunlight reached easil, and hence warm-water bacteria can thrive. The deeper the waters, the colder it becomes. The cold condition sometimes can inhibit the microbial growth, and thus, they cannot perform the degradation of the oil spills. Sometimes, the process of oil degradation can occur at low temperature, but the speed for the microbes to degrade oil will be very low. For the aerobic environment, as long as there is oxygen, the oil will get chewed up. For the anaerobic environment, it can be developed locally by the river itself because of the ready supply of the oil and also the microbes that are eager to devour it [21,22]. It is possible to add fertilizers such as nitrogen to stimulate the growth of such bacteria. In human view, the microbe is helping us to degrade the oil spills in the river and eventually clean the contaminated environment. While for microbial view, consuming the oil spills to provide energy and materials that are needed for them to live and growth development [23].

Before bioremediation can be implemented, it is necessary to determine the present state of water quality of that river in question by taking in samples to determine certain parameters that can reveal the overall health condition of that river [24]. These parameters can be used to better inform the design of bioremediation strategy. The parameters are; 1) Total Suspended Solids (TSS) represents the total insoluble substances or solids in the river, 2) Total Dissolved Solids (TDS) represents the total concentration of dissolved minerals, such as salts in the river, 3) Turbidity represents the amount of suspended organic substances in the river, such as clay, that causes the water to appear muddy, and 4) Biochemical Oxygen Demand or BOD, represents the amount of oxygen in the river water required for aquatic aerobic bacteria metabolism in the river.

The metabolism of those aerobes will aid in breaking down of biological waste and organic matter, which will then reduce contamination level of the river naturally. Chemical Oxygen Demand, according to the American Society of Testing and Materials, is defined as the amount of oxygen that is equivalent to the level of organic matter present in the sample taken from the river, that are susceptible to oxidation by potassium dichromate. This is an important parameter to determine the concentration of the contaminated river water [6]. River bioremediation is carried out by using natural or lab-grown microbes that will be placed in a controllable environment. The process of bioremediation in the river can divide into two types, natural and artificial bioremediation. Some species of aquatic plants can also be exploited as a tool for remediation. Aquatic plants have naturally fixed the degree of filter and purification, especially in water pollution.

Aquatic plants such as water hyacinth and grain leaf pondweed, are chosen due to their strong ability to absorb pollutants as well as able to live extremely in water with polluted conditions [58,61–71]. Removal or fixation of toxic substance from the river water is carried out by the process of adsorption, absorption, accumulation, and degradation by the aquatic plants that will then enable them to purify water [4,25]. Certain aquatic animals can also be used for remediating the river water, especially in alleviating the pollution problems caused by eutrophication of algae such as phytoplankton. Filter-feeding fish such as silver and common carp can be used to control eutrophication in the water by adjusting the composition and density of the fishes in their habitat [4]. Microorganism dosing uses certain compound microorganisms that can decompose, transform and absorb contaminants in the water, which in turn aid to clean the river water. Good arrangement for the overall structure of the overall process is important to determine the effectiveness in microorganism dosing.

Advantages and disadvantages of bioremediation

Bioremediation is very useful to completely or partially detoxify a wide variety of toxic contaminants. There are high possibilities for microbes to degrade the contaminant at a certain level. Until at a period, the contaminant is degraded, and at the same time, the biological activities will decline associated with the decreasing population of microbes. Normally, the secondary product from the bioremediation is harmless products such as carbon dioxide, water, and cell biomass. The future liability that caused from the bioremediation process and disposal of contaminated material could eliminate [26]. There are several key advantages to using bioremediation which is based on current technological method.

Bioremediation method can be the most cost-effective option it does not need sophisticated and costly technology to operate including the transportation of toxic materials. The main purposes of bioremediation eliminate or reduces the chances of the contaminants to scatter further in any medium. Since it manipulates the biological processes to remove the contaminants; bioremediation may cause side effects towards the surrounding environment caused by secondary metabolite produced. However, the techniques commonly can be expected to have minimal negative impacts on the environment, since bioremediation is a natural process. The process appears to have only minor and short-lived adverse effects when used correctly. Otherwise, the residues can be removed by evaporation alone; the bioremediation process capable of eliminating a number of the toxic components of petroleum from a spill site more rapidly [42,72–77].

However, there are some disadvantages that could happen which encountered with bioremediation as well especially environmental factor such as pH, temperature, salinity and also the presence of multiple contaminants as well as compete with other types of microorganisms, which are very hard to predict the consequences. Moreover, the existence of non-organic pollutants such heavy metal is possible to cause inhibition to the rate of bioremediation but still manages to work best when it is used to degrade organic compounds. Another disadvantage is the limitation of information about the interaction between microorganism and toxicant as well as the biological processes and its reactions which is required to continuously calculate all of the effects of using bioremediation on a site.

Besides, it is possible the organic contaminants might be not degraded or disintegrates fully if the process is not controlled well. This will cause the toxicant easily to spread than the initial contamination. This process is sensitive to the toxicant concentration and environmental stressors on the ground. It is advisable to use field monitoring to trace the rate of biodegradation of the nonorganic and organic contaminants. Several researchers confirm that the range of contaminant concentration that can be treated with effectively is limited to substance or compounds that are biodegradable. Likewise, the number of organisms must be increased in order to successfully reduce contamination levels. For this, their growth conditions must be determined and maintained at the contaminated sites.

Controlling the development states of microbes forms may demonstrate difficulty, especially as conditions may change so drastically among the mediums. Thus, to maintain the optimal conditions might be extensive, especially in the long-term. Although in an ideal environment, an organism may prefer to consume or metabolize other more readily available nutrients within a polluted area, or the toxic material may be isolated to the degrading organism. Moreover, the medium may consist of compounds or organism that inhibits or slowing down the growth of the degrading population.

CONCLUSION

As a conclusion, bacteria are favorable from an environmental, medical, and economic standpoint as they possess a multiple potentials usage especially bioremediation purpose. Bioremediation has been introduced to stimulate the naturally occurring metabolic activities of microorganisms for degrading, transforming, as well as accumulating harmful pollutants or compounds such as hydrocarbons, chemical substances, and heavy metals. Nowadays, bioremediation is the only technology that is possible to clean polluted environments, such as in river rehabilitation. However, bioremediation is still a non-advanced technology that has not been implemented in non-developed countries yet. Bioremediation may be simple in concept, but it is difficult in practice. Nevertheless, the role of bioremediation in river rehabilitation is continuously increasing. Further studies and research on bioremediation will contribute to the improvement of ecological restoration.

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REFERENCES

1. Espeche ME, MacCormack WP, Fraile ER. Factors affecting growth of an n-hexadecane degrader Acinetobacter species isolated from a highly polluted urban river. Int Biodeterior Biodegrad. 1994;33(2):187–96.
2. Claassens S, Van Rensburg L, Riedel KJ, Bezuidenhout JJ, Van Rensburg PJJ. Evaluation of the efficiency of various commercial products for the bioremediation of hydrocarbon contaminated soil. Environmentalist. 2006;26(1):51–62.
3. Dahalan FA, Yunus I, Johari WLW, Shukor MY, Halmi MIE, Shamaan NA, et al. Growth kinetics of a diesel-degrading bacterial strain from petroleum-contaminated soil. J Environ Biol. 2014;35(2):399–406.
4. Aidil MS, Sabullah MK, Halmi MIE, Sulaiman R, Shukor MS, Shukor MY, et al. Assay for heavy metals using an inhibitive assay based on the acetylcholinesterase from *Pangasius hypophthalmus* (Sauvage, 1878). Fresenius Environ Bull. 2013;22(12):3572–6.
5. Sabullah MK, Ahmad SA, Shukor MY, Gansau AJ, Syed MA, Sulaiman MR, et al. Heavy metal biomarker: Fish behavior, cellular alteration, enzymatic reaction and proteomics approaches. Int Food Res J. 2015;22(2):435–54.
6. Sabullah MK, Sulaiman MR, Shukor MS, Yusof MT, Johari WLW, Shukor MY, et al. Heavy metals biomonitoring via inhibitive assay of acetylcholinesterase from *Periophthalmodon schlosseri*. Rendiconti Lincei. 2015;26(2):151–8.
7. Abdel MA, Mueller R. Degradation of long chain alkanes by a newly isolated *Pseudomonas frederiksbergensis* at low temperature. Bioremediation Biodivers Bioavailab. 2009;3:55–60.
8. Chayabutra C, Ju L-K. Degradation of n-hexadecane and its metabolites by Pseudomonas aeruginosa under microaerobic and anaerobic denitrifying conditions. Appl Environ Microbiol. 2000;66(2):493–8.
9. Ruberto LAM, Vazquez S, Lobalbo A, Mac Cormack WP. Psychrotolerant hydrocarbon-degrading *Rhodococcus* strains isolated from polluted Antarctic soils. Antarct Sci. 2005;17(1):47–56.
10. Shukor MY, Dahalan FA, Jusoh AZ, Muse R, Shamaan NA, Syed MA. Characterization of a diesel-degrading strain isolated from a hydrocarbon-contaminated site. J Environ Biol. 2009;30(1):145–50.
11. Asok AK, Jisha MS. Biodegradation of the anionic surfactant linear alkylbenzene sulfonate (LAS) by autochthonous pseudomonas sp. Water Air Soil Pollut. 2012;223(8):5039–48.
12. Das R, Tiwary BN. Isolation of a novel strain of *Planomicrobium chinense* from diesel contaminated soil of tropical environment. J Basic Microbiol. 2013;53(9):723–32.
13. Islahuddin NKS, Halmi MIE, Manogaran M, Shukor MY. Isolation and culture medium optimisation using one-factor-at-time and Response Surface Methodology on the biodegradation of the azo-dye amaranth. Bioremediation Sci Technol Res. 2017;5(2):25–31.
14. Jeyasingh J, Philip L. Bioremediation of chromium contaminated soil: Optimization of operating parameters under laboratory conditions. J Hazard Mater. 2005;118(1–3):113–20.

15. Singh KD, Sharma S, Dwivedi A, Pandey P, Thakur RL, Kumar V. Microbial decolorization and bioremediation of melanoidin containing molasses spent wash. J Environ Biol. 2007;28(3):675–7.
16. Lima D, Viana P, André S, Chelinho S, Costa C, Ribeiro R, et al. Evaluating a bioremediation tool for atrazine contaminated soils in open soil microcosms: The effectiveness of bioaugmentation and biostimulation approaches. Chemosphere. 2009;74(2):187–92.
17. Du L-N, Li G, Xu F-C, Pan X, Wen L-N, Wang Y. Rapid decolorization of methyl orange by a novel *Aeromonas* sp. strain DH-6. Water Sci Technol. 2014;69(10):2004–13.
18. Sopian NA. Isolation, characterization and growth optimization of a chromate-reducing bacterium. Bioremediation Sci Technol Res. 2015;2(2):18–24.
19. Atlas RM, Cerniglia CE. Bioremediation of petroleum pollutants. BioScience. 1995;45(5):332–8.
20. Margesin R, Schinner F. Biodegradation and bioremediation of hydrocarbons in extreme environments. Appl Microbiol Biotechnol. 2001;56(5–6):650–63.
21. Hong JH, Kim J., Choi OK, Cho K-S., Ryu HW. Characterization of a diesel-degrading bacterium, *Pseudomonas aeruginosa* IU5, isolated from oil-contaminated soil in Korea. World J Microbiol Biotechnol. 2005;21(3):381–4.
22. Kaszycki P, Czechowska K, Petryszak P, Międzobrodzki J, Pawlik B, Kołoczek H. Methylotrophic extremophilic yeast *Trichosporon* sp.: A soil-derived isolate with potential applications in environmental biotechnology. Acta Biochim Pol. 2006;53(3):463–73.
23. Lee M. b, Kim MK. c, Singleton I., Goodfellow M., Lee S-T. d. Enhanced biodegradation of diesel oil by a newly identified Rhodococcus baikonurensis EN3 in the presence of mycolic acid. J Appl Microbiol. 2006;100(2):325–33.
24. Mohammed D., Ramsubhag A., Beckles DM. An assessment of the biodegradation of petroleum hydrocarbons in contaminated soil using non-indigenous, commercial microbes. Water Air Soil Pollut. 2007;182(1–4):349–56.
25. Yudono B, Said M, Hakstege P, Suryadi FX. Kinetics of indigenous isolated bacteria Bacillus mycoides used for ex-situ bioremediation of petroleum contaminated soil in PT Pertamina Sungai Lilin South Sumatera. J Sustain Dev. 2009;2(3):64–71.
26. Affandi IE, Suratman NH, Abdullah S, Ahmad WA, Zakaria ZA. Degradation of oil and grease from high-strength industrial effluents using locally isolated aerobic biosurfactant-producing bacteria. Int Biodeterior Biodegrad. 2014;95(PA):33–40.
27. Brown RA, Hinchee RE, Norris RD, Wilson JT. Bioremediation of petroleum hydrocarbons: A flexible, variable speed technology. Remediat J. 1996;6(3):95–109.
28. Etkin DS. Historical review of oil spills from all sources. 1999 Int Oil Spill Conf. 1999;1097–102.
29. Hazen TC. Test plan for in situ bioremediation demonstration of the Savannah River Integrated Demonstration Project DOE/OTD TTP No.: SR 0566-01. Revision 3. Westinghouse Savannah River Co., Aiken, SC (United States); 1991.
30. Hazen TC, Looney BB, Fliermans CB, Eddy-Dilek CA, Lombard KH, Enzien MV, et al. Summary of in-situ bioremediation demonstration (methane biostimulation) via horizontal wells at the Savannah River site integrated demonstration project. Battelle Press, Columbus, OH (United States); 1994.
31. Travis BJ, Rosenberg ND. Modeling in situ bioremediation of TCE at Savannah River: Effects of product toxicity and microbial interactions on TCE degradation. Environ Sci Technol. 1997;31(11):3093–3102.
32. Venosa AD, Lee K, Suidan MT, Garcia-Blanco S, Cobanli S, Moteleb M, et al. Bioremediation and biorestoration of a crube oil-contaminated freshwater wetland on the St. Lawrence River. Bioremdiation J. 2002;6(3):261–281.
33. Jackson VA, Paulse AN, Bester AA, Neethling JH, Khan S, Khan W. Bioremediation of metal contamination in the Plankenburg River, Western Cape, South Africa. Int Biodeterior Biodegrad. 2009;63(5):559–568.
34. Kuhn TK, Hamonts K, Dijk JA, Kalka H, Stichler W, Springael D, et al. Assessment of the intrinsic bioremediation capacity of an eutrophic river sediment polluted by discharging chlorinated aliphatic hydrocarbons: a compound-specific isotope approach. Environ Sci Technol. 2009;43(14):5263–5269.
35. Hale SE, Meynet P, Davenport RJ, Jones DM, Werner D. Changes in polycyclic aromatic hydrocarbon availability in River Tyne sediment following bioremediation treatments or activated carbon amendment. Water Res. 2010;44(15):4529–4536.
36. Fialová A, Boschke E, Bley T. Rapid monitoring of the biodegradation of phenol-like compounds by the yeast Candida maltosa using BOD measurements. Int Biodeterior Biodegrad. 2004;54(1):69–76.
37. Nagamani A., Lowry M. Phenol biodegradation by Rhodococcus coprophilus isolated from semi arid soil samples of Pali, Rajasthan. Int J Appl Environ Sci. 2009;4(3):295–302.
38. Morgante V, López-López A, Flores C, González M, González B, Vásquez M, et al. Bioaugmentation with Pseudomonas sp. strain MHP41 promotes simazine attenuation and bacterial community changes in agricultural soils. FEMS Microbiol Ecol. 2010;71(1):114–26.
39. Tu C, Teng Y, Luo Y, Li X, Sun X, Li Z, et al. Potential for biodegradation of polychlorinated biphenyls (PCBs) by Sinorhizobium meliloti. J Hazard Mater. 2011;186(2–3):1438–44.
40. Wasi S, Tabrez S, Ahmad M. Suitability of immobilized Pseudomonas fluorescens SM1 strain for remediation of phenols, heavy metals, and pesticides from water. Water Air Soil Pollut. 2011;220(1–4):89–99.
41. Zakaria ZA, Ahmad WA, Zakaria Z, Razali F, Karim NA, Sum MM, et al. Bacterial reduction of Cr(VI) at technical scale - The Malaysian experience. Appl Biochem Biotechnol. 2012;167(6):1641–52.
42. Bahar MM, Megharaj M, Naidu R. Arsenic bioremediation potential of a new arsenite-oxidizing bacterium Stenotrophomonas sp. MM-7 isolated from soil. Biodegradation. 2012 Nov 1;23(6):803–12.
43. Guo Q, Wan R, Xie S. Simazine degradation in bioaugmented soil: Urea impact and response of ammonia-oxidizing bacteria and other soil bacterial communities. Environ Sci Pollut Res. 2014;21(1):337–43.
44. Fuller SJ, Burke IT, McMillan DGG, Ding W, Stewart DI. Population changes in a community of alkaliphilic iron-reducing bacteria due to changes in the electron acceptor: Implications for bioremediation at alkaline Cr(VI)-contaminated sites. Water Air Soil Pollut [Internet]. 2015;226(6). Available from: http://www.scopus.com/inward/record.url?eid=2-s2.0-84930959090&partnerID=40&md5=4b913f2dc527f69f8f1d756d28e9901e
45. Purwanti IF, Abdullah SRS, Hamzah A, Idris M, Basri H, Mukhlisin M, et al. Biodegradation of diesel by bacteria isolated from *Scirpus mucronatus* rhizosphere in diesel-contaminated sand. Adv Sci Lett. 2015;21(2):140–3.
46. Ku Ahamad KE, Halmi MIE, Shukor MY, Wasoh MH, Abdul Rachman AR, Sabullah MK, et al. Characterization of a diesel-degrading strain isolated from a local hydrocarbon-contaminated site. J Environ Bioremediation Toxicol. 2013;1(1):1–8.
47. Atlas RM, Raymond RL. Stimulated petroleum biodegradation. Crit Rev Microbiol. 1977;5(4):371–86.
48. Lal B, Khanna S. Degradation of crude oil by Acinetobacter calcoaceticus and Alcaligenes odorans. J Appl Bacteriol. 1996;81(4):355–62.
49. Sutton SD, Pfaller SL, Shann JR, Warshawsky D, Kinkle BK, Vestal JR. Aerobic biodegradation of 4-methylquinoline by a soil bacterium. Appl Environ Microbiol. 1996;62(8):2910–4.
50. Alvarez HM. Relationship between ß-oxidation pathway and the hydrocarbon-degrading profile in actinomycetes bacteria. Int Biodeterior Biodegrad. 2003;52(1):35–42.
51. Guo W, He M-C, Yang Z-F. A review of studies on the degradation of petroleum hydrocarbon in soils and sediments by microorganism. Bull Mineral Petrol Geochem. 2007;26(3):276–83.
52. Kwapisz E, Wszelaka J, Marchut O, Bielecki S. The effect of nitrate and ammonium ions on kinetics of diesel oil degradation by Gordonia alkanivorans S7. Int Biodeterior Biodegrad. 2008;61(3):214–22.
53. Hadibarata T, Tachibana S. Characterization of phenanthrene degradation by strain Polyporus sp. S133. J Environ Sci. 2010;22(1):142–9.

54. Salam LB, Obayori OS, Akashoro OS, Okogie GO. Biodegradation of bonny light crude oil by bacteria isolated from contaminated soil. Int J Agric Biol. 2011;13(2):245–50.
55. Díaz J, Ricoy C, Moreno C, Ricoy V, Pérez V, Valbuena O. Alkane incorporation by hydrocarbon degradating bacteria mediated by a 70kda protein attached to membranes during the fuel diesel biodegradation [Incorporación de alcanos por bacterias degradadoras de hidrocarburos mediada por una proteína de 70 KDa unida a membranas durante la biodegradación de combustible diesel]. Interciencia. 2013;38(6):437–42.
56. Hadibarata T, Kristanti RA. Biodegradation and metabolite transformation of pyrene by basidiomycetes fungal isolate *Armillaria* sp. F022. Bioprocess Biosyst Eng. 2013;36(4):461–8.
57. Affandi IE, Suratman NH, Abdullah S, Ahmad WA, Zakaria ZA. Degradation of oil and grease from high-strength industrial effluents using locally isolated aerobic biosurfactant-producing bacteria. Int Biodeterior Biodegrad. 2014;95(PA):33–40.
58. Almansoory AF, Idris M, Abdullah SRS, Anuar N. Plant-microbe interaction of *Serratia marcescens* and *Scirpus mucronatus* on phytoremediation of gasoline contaminated soil. Int J ChemTech Res. 2014;6(1):556–64.
59. Almansoory AF, Idris M, Abdullah SRS, Anuar N. Screening for potential biosurfactant producing bacteria from hydrocarbon-degrading isolates. Adv Environ Biol. 2014;8(3 SPEC. ISSUE):639–47.
60. Prakash A, Bisht S, Singh J, Teotia P, Kela R, Kumar V. Biodegradation potential of petroleum hydrocarbons by bacteria and mixed bacterial consortium isolated from contaminated sites. Turk J Eng Environ Sci. 2014;38(1):41–50.
61. Neunhäuserer C, Berreck M, Insam H. Remediation of soils contaminated with molybdenum using soil amendments and phytoremediation. Water Air Soil Pollut. 2001;128(1–2):85–96.
62. Huang X-D, El-Alawi Y, Penrose DM, Glick BR, Greenberg BM. Responses of three grass species to creosote during phytoremediation. Environ Pollut. 2004;130(3):453–63.
63. Silva Gonzaga MI, Gonzaga Santos JA, Ma LQ. Arsenic phytoextraction and hyperaccumulation by fern species. Sci Agric. 2006;63(1):90–101.
64. Haferburg G, Kothe E. Metallomics: Lessons for metalliferous soil remediation. Appl Microbiol Biotechnol. 2010;87(4):1271–80.
65. Aggangan NS, Aggangan BJS. Selection of ectomycorrhizal fungi and tree species for rehabilitation of Cu mine tailings in the Philippines. J Environ Sci Manag. 2012;15(1):59–71.
66. Yang Q, Tu S, Wang G, Liao X, Yan X. Effectiveness of applying arsenate reducing bacteria to enhance arsenic removal from polluted soils by Pteris vittata L. Int J Phytoremediation. 2012;14(1):89–99.
67. Gutiérrez-Ginés MJ, Hernández AJ, Pérez-Leblic MI, Pastor J, Vangronsveld J. Phytoremediation of soils co-contaminated by organic compounds and heavy metals: Bioassays with Lupinus luteus L. and associated endophytic bacteria. J Environ Manage. 2014;143:197–207.
68. Selamat SN, Abdullah SRS, Idris M. Phytoremediation of lead (Pb) and Arsenic (As) by *Melastoma malabathricum* L. from Contaminated Soil in Separate Exposure. Int J Phytoremediation. 2014;16(7–8):694–703.
69. Al-Baldawi IAW, Abdullah SRS, Suja F, Anuar N, Idris M. Phytoremediation of contaminated ground water using *Typha angustifolia*. Water Pract Technol. 2015;10(3):616–24.
70. Al-Baldawi IAW, Abdullah SRS, Suja F, Anuar N, Idris M. The ratio of plant numbers to the total mass of contaminant as one factor in scaling-up phytoremediation process. J Teknol. 2015;74(3):111–4.
71. Nuraini Y, Arfarita N, Siswanto B. Isolation and characteristic of nitrogen-fixing bacteria and phosphate-solubilizing bacteria from soil high in mercury in tailings and compost areas of artisanal gold mine. Agrivita. 2015;37(1):1–7.
72. Delille D, Bassères A, Dessommes A. Effectiveness of bioremediation for oil-polluted Antarctic seawater. Polar Biol. 1998;19(4):237–41.
73. Liang R-X, Wu X-L, Wang X-N, Dai Q-Y, Wang Y-Y. Aerobic biodegradation of diethyl phthalate by Acinetobacter sp. JDC-16 isolated from river sludge. J Cent South Univ Technol Engl Ed. 2010;17(5):959–66.
74. Tripathi A, Upadhyay RC, Singh S. Mineralization of mono-nitrophenols by Bjerkandera adusta and Lentinus squarrosulus and their extracellular ligninolytic enzymes. J Basic Microbiol. 2011;51(6):635–49.
75. Che Zulzikrami Azner A, Naimah I, Faimah MR, Salsuwanda S. Removal of Cu(II) from industrial effluents by citric acid modified rice straw. Bioremediation Sci Technol Res. 2014;2(1):23–38.
76. Ahmad SA. Biodegradation of Chicken Feather Wastes in Submerged Fermentation Containing High Concentrations of Heavy Metals by Bacillus sp. khayat. J Environ Bioremediation Toxicol E-ISSN 2289-5884. 2015;2(2):38–41.
77. Ibrahim S, Shukor MY, Syed MA, Wan Johari WL, Ahmad SA. Characterisation and growth kinetics studies of caffeine-degrading bacterium *Leifsonia* sp. strain SIU. Ann Microbiol. 2016;66(1):289–98.