ORIGINAL PAPER

Bioremediation of crude oil by indigenous species isolated from oil sludge contaminated soil. A case study: Karun Gas Oil Production Company, (IRAN)

Parviz Behdarvandan[1], Reza Jalilzadeh Yengejeh[1,✉], Sima Sabzalipour[2], Laleh Roomiani[3], Khoshnaz Payandeh[4]

1. Department of Environmental Engineering, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran
2. Department of Environment, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran
3. Department of Fisheries, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran
4. Department of Soil Science, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran

Date of submission: 16 Oct 2020, *Date of acceptance:* 02 Nov 2020

ABSTRACT

The present study aimed to investigate the biodegradability of the native species isolated from a site contaminated with crude oil (Karun Gas Oil Production Company, Iran). According to the findings, the species isolated from *bacillus* could grow at the concentrations of 1 and 3% of crude oil within the pH range of 3-5 and at various temperatures. *Bacillus sonorensis* had higher efficiency at the concentration of 1%, temperature of 30 °C, and near-neutral pH compared to the second species. Therefore, it could be used in biological remediation processes through the reduction of biosurfactant and surface tension to a certain extent (24.87 mN/m) compared to the control samples (59 mN/m). Furthermore, the chemical analysis showed that the bioremediation efficiency of chrysene, fluorene, naphthalene, dibenz (a, h) anthracene, and pyrene was 35.85, 39.56, 27.14, 28.45, and 27.5% within four days, respectively. With the reduction of the surface tension, aromatic compounds could be better decomposed compared to aliphatic compounds.

Keywords: Bioremediation, Oil-contaminated soil, Crude oil, Biosurfactant, Environment

Introduction

Oil and its derivatives are of utmost importance in terms of the environment and public health due to the presence of organic compounds that are resistant to biological decomposition, such as hydrocarbon and non-hydrocarbon compounds.[1-5] Crude oil contains carbon hydrocarbons such as paraffinic hydrocarbons (e.g., C_nH_2n+2), petroleum hydrocarbons (e.g., cycloparaffins [CnH_2N]), aromatic hydrocarbons (e.g., benzoids ([H_2n-6]), and various non-hydrocarbons; the last category includes heteroatomic organic compounds, which are subcategorized into sulfur compounds (e.g., disulfides, thiols/mercaptans, and thiophenes), oxygen compounds (e.g., phenols, furans, benzofurans, carboxylic acids, and esters), nitrogen compounds (e.g., aromatics, amines, carbazoles, and pyridines), and organometallic compounds (e.g., resins and asphaltenes).[1, 6]

The environmental pollution of water, soil, and air caused by oil and its derivatives is a monumental challenge across the globe, which occurs due to leakage from drilling and exploration operations (drilling rigs and wastes), crude oil transmission lines and its products, transport tankers, storage tanks, petroleum products sales outlets, transportation, and similar cases.[2, 7] Various physical and chemical methods are used for the

✉ *Reza Jalilzadeh Yengejeh*
r.jalilzadeh@iauahvaz.ac.ir

Citation: Behdarvandan P, Jalilzadeh Yengejeh R, Sabzalipour S, Roomiani L, Payandeh Kh. Bioremediation of crude oil by indigenous species isolated from oil sludge contaminated soil. A case study: Karun Gas Oil Production Company, (IRAN). J Adv Environ Health Res 2020; 8(4): 234-241

reduction and elimination of environmental pollution, including air sparging, chemical oxidation, soil vapor extraction, advanced oxidation processes, enhanced bioremediation, and solidification/stabilization. Some of the biological methods in this regard are enhanced bioremediation, biopiles, bioreactors, phytoremediation or a combination of these techniques.

Today, biological refining methods have attracted attention owing to their compatibility with the environment and the fact that water and soil microorganisms use petroleum compounds as a source of carbon and energy, causing their biological decomposition through the production of biosurfactants.[8-11] Several studies have been focused on the elimination or reduction of oil pollution in contaminated water and soil. In terms of the biological experiments and the kinetics study of the biological refining of the soils contaminated with crude oil using organic and mineral materials,[12] the investigation of the biodegradability of crude oil and its derivatives by Streptomyces. The findings in this regard have indicated that the species are able to remove phenanthrene (63%), anthracene (83%), and pyrene (93%) in addition to proper growth.[3] Another study in this regard investigated the consortium of the bacteria isolated from oil-contaminated soils (especially pseudomonas species), indicating their ability to remove n-alkanes and polycyclic aromatic hydrocarbons by 95% through bioremediation.[13] Other studies have also reported the use of the effective microorganism technology, which could proceed to the biological purification of contaminated oil sludge through the optimization of food ratios (e.g., carbon-to-nitrogen),[14] while other biological studies and the related documents have also proven accurate in this regard.[15-17] Various physical and chemical methods have yielded different results regarding the biological analysis of oil compounds and their derivatives. [18-21]

The present study aimed to investigate the oil-contaminated soils in a new oil region located in the south of Iran in terms of the biological ability of the isolated species at various concentrations (1 and 3% of crude oil) based on biosurfactant production and reduction of the surface tension.

Materials and Methods

After the sampling of Karun Gas Oil Production Company in Iran in the summer of 2019 as the study area, oil-contaminated soil and oil sludge were assessed to identify and isolate the native soil bacteria and determine the level of bacterial surface tension activity. In addition, chemical analysis was performed to determine their ability to biodegrade oil compounds.

Sampling

After the sampling of the soil and oil sludge, oil-contaminated soil and sludge were harvested from the depth of 15 centimeters of the soil, and the obtained samples were immediately transferred to the laboratory in an ice box in sterile conditions (Fig. 1).

Separation and identification of bacteria

At this stage, organic culture medium (nitrate agar) and mineral culture medium (mineral salt medium) were used. The media contained various compounds, including $NaNO_3$ (2.0 g/L), NaCl (0.8 g/L), KCl (0.8 g/L), $CaCl_2 \cdot 2H_2O$ (0.1 g/L), KH_2PO_4 (2.0 g/L), $Na_2HPO_4 \cdot 12H_2O$ (2.0 g/L), $MgSO_4$ (0.2 g/L), and $FeSO_4 \cdot 7H_2O$ (0.001 g/L). The pH of the media was 6.8, and they were completed with 2 mL of a trace element stock solution composed of $FeCl_3 \cdot 6H_2$-O (0.08 g/L), ZnSO-$4 \cdot H_2O$ (0.75 g/l), $COCl_2 \cdot 6H_2O$ (0.8 g/L), $CuSO_4 \cdot 5H_2O$ (0.75 g/L), $MgSO_4.H_2O$ (0.75 g/L), H_3BO_3 (0.15 g/L), and $Na_2MoO_4 \cdot 2H_2$-O (0.05 g/L).[22] At this stage, the soil suspension was prepared, cultured in the nutrient agar and nutrient broth culture media, and preserved in an incubator for 24 h. After observing the grown colonies on the culture media, the microbial consortium was re-cultured using a four-stage method and continued until the purification of the bacteria and observing single colonies.[1, 23, 24]

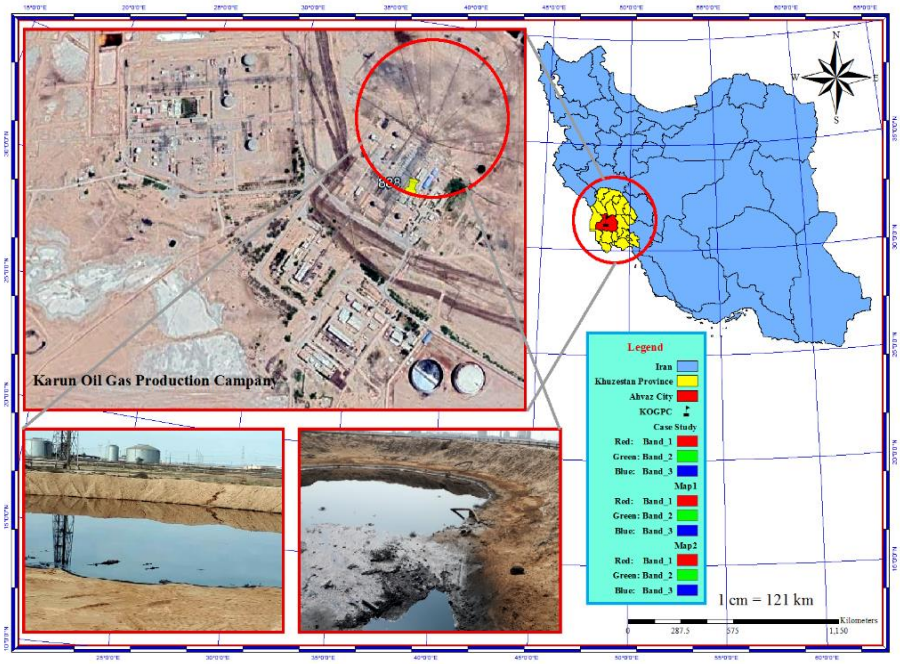

Fig. 1. Area study; Karun Gas Oil Production Company, (IRAN)

In the next step, biochemical tests were performed for the initial identification of the bacterial genus and species, including methyl red, catalase, starch hydrolysis, IMViC, arabinose fermentation, mannitol fermentation, and growth at various salt concentrations. In order to determine the species of the pure isolated bacteria, DNA extraction operations and sequencing were initially performed by polymerase chain reaction (PCR), and 16s rRNA was amplified using the forward primer 5-AGAGTTTGATCCTGGCTCAG -3 and reverse primer 5-TAAGGAGGTGATCCAGCC-3.[1, 22, 25-27]

Biosurfactant measurement

The surface tension was measured using a tensiometer and the ring method.[1, 11, 23, 24] For his purpose, 25 mL of the 48-hour culture of each sample was poured into the sample container of the device. Before measuring the surface tension, the sample temperature was raised to 25 °C, and the test was repeated thrice for each sample. For each measurement, the surface tension of the distilled water and non-bacterial culture medium was also measured as controls.

Chemical analysis

Initially, 100 mL of sterile mineral medium containing 1% yeast extract and varied concentrations of sterile crude oil (1 and 3%) was poured into a 250-milliliter Erlenmeyer. Following that, the reduction of the oil compounds (i.e., biological decomposition) by the bacteria was measured at 48 and 96 h using the high-performance liquid chromatography (HPLC) and gas chromatography (GC). Notably, the test samples were evaluated and compared with the control samples.[22]

Results and Discussion

The identified strains of *B. sonorensis* and *Bacillus* sp. were assessed aerobically after culturing in the mineral medium at various pH, temperatures, and incubation cycles. Finally, *B. sonorensis* was selected as the superior species, which was also a new strain in this contaminated area based on previous studies. According to the biochemical tests, PCR, and NCBI database, *bacillus* and the isolated species (especially *B. sonorensis*) were the bacteria decomposing hydrocarbon compounds and another *bacillus* species. This is similar (86%) to the strain found in salt

environments (*Bacillus sp.* 2BSG-MG-1; GenBank: AB533779.1), which has been identified as a new strain. In the present study, the biological ability of both bacteria was investigated. In several other studies, Bacilli such as *Bacillus sonorensis* and *Bacillus cereus* have also been identified as the decomposers of petroleum hydrocarbons and crude oil in oil-contaminated areas. Furthermore, various species of *bacillus* and pseudomonas have been isolated from oil-contaminated soils, and their biodegradability has been investigated and confirmed.[1, 22, 23, 27-30]

Measurement of bacterial growth at different temperatures and pH

Figs. 2 to 4 show the results of optical density (OD_{600}) as a microbial growth index after 48 h at the temperatures of 20, 30, and 40 °C and wavelength of 600 nm.

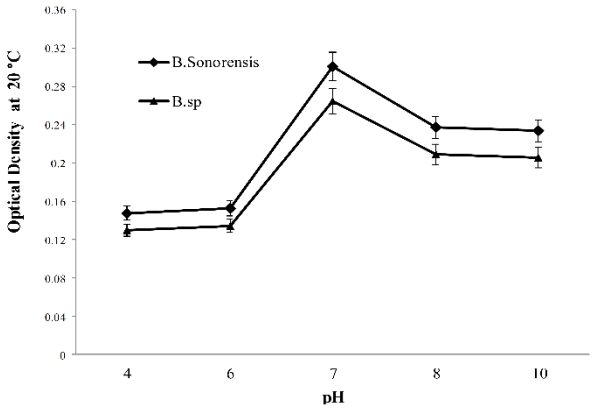

Fig. 2. Growth (optical density) of *B. Sonorensis* and *B.* sp. at 20 °C in different pHs (48 h)

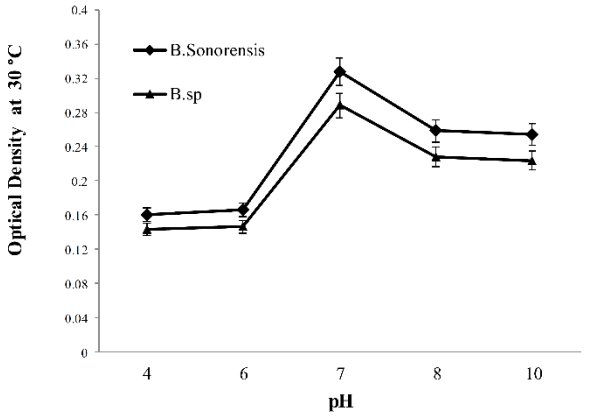

Fig. 3. Growth (optical density) of *B. Sonorensis* and *B.* sp. at 30 °C in different pHs (48 h)

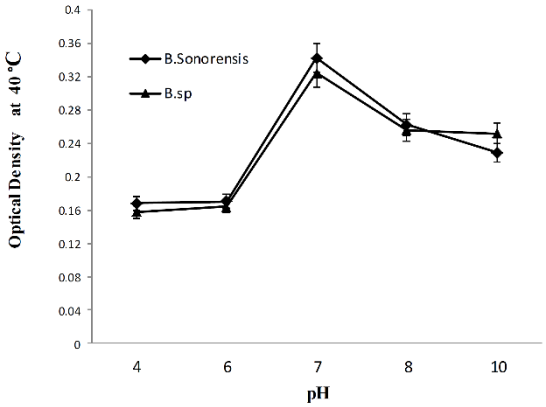

Fig. 4. Growth (optical density) of *B. Sonorensis* and *B.* sp. at 40 °C in different pHs (48 h)

As can be seen, the optimal pH at the temperatures of 20, 30, and 40 °C was within the neutral range (~7), and the microbial growth rate increased at higher temperatures. The optical density of *B. sonorensis* at the temperatures of 20, 30, and 40 °C was within the range of 0.14 - 0.3, 0.16 - 0.32, and 0.16 - 0.32, respectively. The optical density of the other *Bacillus* (second species) was estimated to be 0.12 - 0.2, 0.28 - 0.14, and 0.32 - 0.15 after 48 h with 1% crude oil, indicating the high microbial growth by *B. sonorensis*.

Research regarding the correlation of microbial growth and temperature has shown that bacterial growth and its rate are affected by temperature. The results of a study indicated that the bacterial growth of *Bacillus cereus* increased by increasing temperature from 28 to 37 °C in an MTBE-infected environment, and the highest removal efficiency was observed in the neutral range. Another study regarding the Bacilli decomposing 1% crude oil at the temperature of 30-55 °C showed the reduction of bacterial growth, and the optimal pH was observed within the neutral range (~6.8).[22]

In another research regarding the growth of a microbial population and its association with salinity, various temperatures (10, 30, and 50 °C), and pH of 7.3, it was observed that microbial activity intensified in the early stages with increased nutrients and temperature. In the mentioned study, the microbial activity and products associated with

aerobic reactions (e.g., carbon dioxide) were also measured up to 50 °C in the biological refining process of soil.[31]

Measurement of surface tension

The studied strains were placed in the liquid mineral medium containing 1% yeast extract and 1 and 3% crude oil at different temperatures, and the surface tension and reduction rate were evaluated in two species after 48 h using a shaker at 180 rpm, as well as different temperatures and pH. At this stage, the capacity and emulsification ability of the two bacterial species were initially measured, and the values were estimated at 81.16% and 75.35% for *B. sonorensis* and other *bacillus* species, respectively, indicating the ability of both species in emulating the test environment and producing biosurfactant, while *B. sonorensis* was more capable than the second species of bacillus in this regard. This capability has been investigated in other studies, such as those conducted by Rizi *et al.*[22] and Jalilzadeh *et al*., the results of which indicated that the biological refining ability of microorganisms could increase with high biosurfactant production.[1]

According to the information in Table 1, varied levels of surface tension and its reduction could be observed in the two studied species depending on environmental conditions such as temperature, pH, and percentage of crude oil. Based on the preliminary experiments on the two bacterial species and considering the ability of both bacteria to grow and multiply within a neutral pH range, it was expected that the greatest reduction in surface tension would be achieved at this stage, and the hypothesis was confirmed by the measured data in both cases. In the case of 1% crude oil, the rate for *B. sonorensis* at the pH of 7 was 38.5, 34.22, and 33.5%, and the reduction in the surface tension was equal to 20.5, 24.78, and 25.5% at the temperatures of 20, 30, and 40 °C, respectively. According to the measured data with 3% crude oil, the reduction was estimated to be 19.7, 23.9, and 24.3 mN/m at the temperatures of 20, 30, and 40 °C, respectively. In addition, the highest reduction of the surface tension occurred with 1% crude oil at the temperature of 30 °C.

Table 1. The amount of *B. Sonorensis* and *B.* sp. surface tensions (temperature: 20, 30, 40 °C; C: 1, 3%; time: 48 h)

Bacteria			Surface tension (mN/m)						Emulsification capacity in pH=7
			Crude oil % (at 20 °C)		Crude oil % (at 30 °C)		Crude oil % (at 40 °C)		
	pH	Blank	1	3	1	3	1	3	
B. Sonorensis	4	59-60	44.5	44.9	40.13	41.5	36.5	37.8	81.16%
	6	59-60	39.4	40.5	35.76	36.78	34.1	35.3	
	7	59-60	38.5	39.3	34.22	35.91	33.5	34.7	
	8	59-60	38.7	39.6	35.55	36.81	33.6	34.9	
	10	59-60	38.6	39.3	36.12	37.71	34.9	35.8	
B. sp.	4	59-60	49.8	51.15	45.6	45.87	45.65	45.98	75.35%
	6	59-60	48.8	49.58	44.95	45.35	43.15	43.95	
	7	59-60	48.15	49.113	44.87	45.01	42.35	42.87	
	8	59-60	48.3	49.266	44.97	45.13	42.49	43.36	
	10	59-60	49.4	50.388	45.35	45.93	43.95	44.35	

On the same note, the findings of the current research regarding the second bacillus species at 1% crude oil concentration at the pH of 7.7 were 48.15, 44.87, and 42.35%, and the surface tension reduction was estimated at 11.85, 15.33, and 17.67% at the temperatures of 20, 30, and 40 °C, respectively. According to the measured data at 3% crude oil, the reduction rate was 10.88, 14.99, and 17.13 mN/m at the temperatures of 20, 30, and 40 °C, respectively, and the most significant surface tension reduction was observed with 1% crude

oil at the temperature of 30 °C.

According to the literature, biosurfactant production reduces the surface tension of the environment, thereby providing the proper conditions for the increased degradation of bacteria. In a study on *Bacillus cereus* for the biological refining of 1% crude oil, the bacterium could diminish the surface tension reduction from 37 to 31 mN/m at the temperature of 37 °C and to 32.5 mN/m at 20 °C.[1] In another study, the biodegradation of hydrocarbon groups (c16: c13) was evaluated, and different results were obtained regarding the ability of *Bacillus subtilis* to reduce surface tension, including the ability of the bacterium to reduce the surface tension in a sample containing n-Hexadecane from 30.1 to 7.5 mN/m, from 42.3 to 9.9 mN/m in crude oil medium, and from 36.4 m^3 to 3.8 mN/m.[30] Similar results in another study showed that two bacilli species could decrease surface tension from 60 to 31 and 38 mN/m, while also biologically decomposing crude oil and similar compounds.[23]

In another study on the species derived from crude oil-contaminated sites in Aramco (Saudi Arabia), the ability of bacillus and pseudomonas species was investigated, and one species (strain 2-IV; *Pseudomonas stutzeri*) was considered optimal as it was able to achieve high emulsification strength and cause the significant surface tension reduction of approximately 30.5 mN/m in the tested samples.[26]

Chemical analysis of samples

Based on the findings regarding *B. sonorensis* at 1% concentration and after the re-cultivation of the selected species, the biological degradation rate of crude oil was measured at 48 and 96 h. The analysis of the tested samples is presented in Table 2 in the GC and HPLC analysis sections, which indicate the ability of crude oil to decompose into simpler derivatives. The obtained results confirmed the efficiency of the biological decomposition of various compounds, such as chrysene (35.85%), fluorene (39.56%), naphthalene (27.14%), dibenz(a, h)anthracene (28.45%), and pyrene (27.5%). Furthermore, other compounds and petroleum derivatives were biodegraded at varied rates within four days, and aromatic compounds were better decomposed compared to aliphatic compounds.

Table 2. Chemical analysis of samples by GC and HPLC in %1 concentration

Type of analysis	Sample	Blank sample (mg/L)	48 h	96 h
GC analysis	n-C18	4.4	4.25	3.98
	n-C10	1.67	1.55	1.41
	n-C11	5.7	5.2	4.89
	C13	9.2	8.95	8.61
	C16	7.9	7.77	7.47
	C7	3	2.75	2.48
HPLC analysis	Pyrene	12	8.9	7.87
	Chrysene	4.1	2.87	2.63
	Fluorene	16	10.8	9.67
	Phenanthrene	246	229	215
	Dibenz(a,h) anthracene	1.23	0.92	0.88
	Benzo(ghi) perylene	6.55	6.5	6.46
	Naphthalene	210	187	1.53
	Acenaphthene	2.8	2.45	2.06

In some studies, chemical analysis has been performed to determine the decomposition rate of hydrocarbon or non-hydrocarbon compounds *in-vitro*, the results of which have confirmed the biological decomposition of crude oil and its derivatives, as well as the ability of the bacillus group to biologically decompose aromatic and aliphatic compounds,[1, 2, 22, 23, 32] such as resins, asphaltenes,[33] and *pseudomonas*.[2, 7, 32]

Conclusion

In this study, we investigated the ability of native bacteria isolated from oil-contaminated soil and sludge. According to the findings, both bacteria could produce biosurfactant at the two concentrations of 1 and 3%, three temperatures of 20, 30, and 40 °C, and different pH. The highest efficiency was observed at the temperature of 30 °C under neutral conditions for both bacteria, especially *B. sonorensis*. A wide range of bacteria

(especially the bacillus group) are able to biologically decompose crude oil and its derivatives. Although these methods are time-consuming on an industrial scale, they could be used in combination with other methods to clear the sites that are contaminated with oil and its derivatives.

Acknowledgements

This article was extracted from a PhD dissertation conducted by Mr. Parviz Behdarvandan at the Department of Environmental Engineering of Islamic Azad University, Ahvaz Branch, Iran.

Author contribution

Parviz Behdarvandan: conceptualization, methodology, investigation, formal analysis, writing, and original draft; Reza Jalilzadeh Yengejeh: methodology, writing-review & editing, and investigation; Sima Sabzalipour: methodology, writing - review & editing, and project administration; Laleh Roomiani: Methodology, writing-review & editing, and project administration; Khoshnaz Payandeh: methodology, investigation, formal analysis, writing, and project administration.

Conflicts of interest

None declared.

References

1. Jalilzadeh Yengejeh R, Pourjafarian V, Afrous A, Gholami A, Maktabi P, Sharifi R. Studying *Bacillus cereus*'s ability to biodegrade crude oil in hot areas. Pet Sci Technol 2017; 35(3): 287-91.
2. Xu X, Liu W, Tian S, Wang W, Qi Q, Jiang P, *et al*. Petroleum hydrocarbon-degrading bacteria for the remediation of oil pollution under aerobic conditions: A perspective analysis. Front Microbio 2018; 9: 2885.
3. Baoune H, Aparicio JD, Acuña A, El Hadj-khelil AO, Sanchez L, Polti MA, *et al*. Effectiveness of the *Zea mays-Streptomyces* association for the phytoremediation of petroleum hydrocarbons impacted soils. Ecotoxicol Environ Saf 2019; 184: 109591.
4. Qiao K, Tian W, Bai J, Wang L, Zhao J, Du Z, *et al*. Application of magnetic adsorbents based on iron oxide nanoparticles for oil spill remediation: A review. J Taiwan Inst Chem Eng 2019; 97: 227-36.
5. Patel N, Khan MD Z A, Shahane S, Rai D, Chauhan D, Kant C, *et al*. Emerging pollutants in aquatic environment: Source, effect, and challenges in biomonitoring and bioremediation-A review. Pollution 2020; 6(1): 99-113.
6. Wauquier JP. Petroleum Refining: Crude oil, petroleum products, process flowsheets. Editions Technip; Vol. 1. Editions Technip, 1995.
7. Abbaspour M, Javid AH, Jalilzadeh Yengjeh R, Hassani AH, Mostafavi PG. The biodegradation of methyl tert-butyl ether (MTBE) by indigenous *Bacillus cereus* strain *RJ1* isolated from soil. Pet Sci Tech 2013; 31(18): 1835-41.
8. Environmental Protection Agency. Office of Solid Waste, Emergency Response. Office of Superfund Remediation, Technology Innovation. Technologies for Treating MTBE and Other Fuel Oxygenates. US Environmental Protection Agency, Office of Solid Waste and Emergency Response, Office of Superfund Remediation and Technology Innovation; 2004.
9. Akhavan Sepahi A, Dejban Golpasha P, Emami M, Nakhoda AM. Isolation and characterization of crude oil degrading *Bacillus* spp. Iran J Environ Health Sci Eng 2008; 5(3): 149-54.
10. Speight JG. Reaction Mechanisms in Environmental Engineering: Analysis and Prediction. 2018; Butterworth-Heinemann.
11. Jimoh AA, Lin J. Bioremediation of contaminated diesel and motor oil through the optimization of biosurfactant produced by *Paenibacillus* sp. D9 on waste canola oil. Bioremediat J 2020; 24(1): 21-40.
12. Mbah GC, Obahiagbon KO. Kinetics of bioremediation of crude oil contaminated soil using organic and inorganic particulates. Pet Sci Tech 2018; 36(1): 9-15.
13. Xu GL, Liu H, Li MJ, Li ZM, Peng ZH, Zuo LM, *et al*. In situ bioremediation of crude oil contaminated site: A case study in Jianghan oil field, China. Pet Sci Tech 2016; 34(1): 63-70.
14. Ahmad J. Bioremediation of petroleum sludge using effective microorganism (EM) technology. Pet Sci Tech 2017; 35(14): 1515-22.

15. Tsang YF, Ok YS, Sarmah AK, Cao B, Wong MH. Special issue: Bioremediation of contaminated soil and water: GeoTrop 2017. Biodegradation 2018; 29: 311-12.
16. Kumari B, Kriti, Singh G, Sinam G, Singh D. Microbial Remediation of Crude Oil-Contaminated Sites. Environmental Concerns and Sustainable Development: Springer, 2020, p; 333-51.
17. Dai X, Lv J, Yan G, Chen C, Guo S, Fu P. Bioremediation of intertidal zones polluted by heavy oil spilling using immobilized laccase-bacteria consortium. Bioresour Technol 2020; 309: 123305.
18. Broje V, Keller AA. Effect of operational parameters on the recovery rate of an oleophilic drum skimmer. J Hazard Mater 2007; 148(1-2): 136-43.
19. Castro A, Iglesias G, Carballo R, Formoso F. Floating boom performance under waves and currents. J Hazard Mate 2010; 174(1-3): 226-35.
20. Al-Majed AA, Adebayo AR, Hossain ME. A sustainable approach to controlling oil spills. J Environ Manage 2012; 113: 213-27.
21. Pinto J, Heredia-Guerrero JA, Athanassiou A, Fragouli D. Reusable nanocomposite-coated polyurethane foams for the remediation of oil spills. Int J Environ Sci Technol 2017; 14: 2055-66.
22. Rizi MS, Sepahi AA, Tabatabaee MS. Crude oil biodegradation by a soil indigenous *Bacillus* sp. isolated from Lavan Island. Bioremediat J 2012; 16(4): 218-24.
23. Deka H, Lahkar J. Biodegradation of Benzo(a)anthracene employing *Paenibacillus* sp. HD1PAH: A novel strain isolated from crude oil contaminated soil. Polycycl Aromat Compd 2017; 37(2-3): 161-9.
24. Banat IM. Characterization of biosurfactants and their use in pollution removal–State of the Art. Acta Biotechnol 1995; 15(3): 251-67.
25. Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 1991; 173(2): 697-703.
26. Anwar Y, El-Hanafy AA, Sabir JSM, Al-Garni SMS, Al-Ghamdi K, Almehdar H, *et al*. Characterization of mesophilic bacteria degrading crude oil from different sites of Aramco, Saudi Arabia. Polycycl Aromat Compd 2020; 40(1): 135-43.
27. Viesser JA, Sugai-Guerios MH, Malucelli LC, Pincerati MR, Karp SG, Maranho LT. Petroleum-tolerant Rhizospheric Bacteria: Isolation, characterization and Bioremediation potential. Sci Rep 2020; 10: 2060.
28. Al Azad S, Farjana M, Mazumder B, Abdullah-Al-Mamun M, Haque AI. Molecular identification of a Bacillus cereus strain from Murrah buffalo milk showed in vitro bioremediation properties on selective heavy metals. J Adv Vet Anim Res 2020; 7(1): 62-8.
29. Pourbabaei AA, Khoshhal Nakhjiri E, Torabi E, Farahbakhsh M. Dissipation of butachlor by a new strain of *Pseudomonas* sp. isolated from paddy soils. Pollution 2020; 6(3): 627-35.
30. Bharti V, Gupta B, Kaur J. Novel bacterial strains *Pseudomonas* sp. and *Bacillus* sp. isolated from petroleum oil contaminated soils for degradation of flourene and phenanthrene. Pollution 2019; 5(3): 657-69.
31. Abed RM, Al-Kharusi S, Al-Hinai M. Effect of biostimulation, temperature and salinity on respiration activities and bacterial community composition in an oil polluted desert soil. Int Biodeterior Biodegradation 2015; 98: 43-52.
32. Jalilzadeh Yengejeh R, Sekhavatjou MS, Maktabi P, Arbab SN, Khadivi S, Pourjafarian V. The biodegradation of crude oil by Bacillus subtilis isolated from contaminated soil in hot weather areas. Int J Environ Res 2014; 8(2): 509-14.
33. Tavassoli T, Mousavi SM, Shojaosadati SA, Salehizadeh H. Asphaltene biodegradation using microorganisms isolated from oil samples. Fuel 2012; 93: 142-8.