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Isolation, Assessment and Identification of Potent Biosurfactant Producing Microorganisms from Oil Contaminated Sites

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Abstract: ***Oil spill damages marine flora and fauna to a great extent by causing physiological as well as biochemical damage. All the current measures being taken for oil spill recovery has their own drawbacks, and thus the last fate happens to be bioremediation. In our present study we have demonstrated that the organisms responsible for bioremediation are ubiquitous and how we can isolate them. The Bushnell and Hass medium is suitable for growing such hydrocarbonoclastic organisms as it provides salt composition similar to sea water as well as gives us choice to select carbon source. From various oil contaminated sites, we isolated 16 morphologically different organisms which were able to produce biosurfactant. One organism was screened as potent biosurfactant producer on basis of following screening test; drop collapse assay, hemolysis assay, oil displacement assay and emulsification assay. The potent isolate was further identified on the basis of morphological characteristics and biochemical test and it was identified as Halomonas sp. with help of Bergey's manual of systematic bacteriology-Vol.2.***

Keywords: Bioremediation, Biosurfactant, Emulsification Index, Oil displacement assay, Oil spill

1. Introduction

Although we have several resources of energy, petroleum resources have contributed greatly in field of energy. The petroleum industry also shows great effect in economic and financial growth of a country [1]. The rapid evolution of motorcars and other applications which use fuels made from crude oil for their internal combustion engine has increased demand for petroleum hydrocarbons [2]. In general, crude oils contain elements like nitrogen, sulphur, oxygen compounds as minor part and major contribution is of Hydrocarbons [3]. Class of hydrocarbons can include paraffins, naphthenes, aromatics and asphaltics [4]. Saudi Arabia, United States, Russia, Iran and Canada are top 5 countries leading in petroleum production [5]. The oil refineries are not distributed worldwide and thus, there is trade of crude oil or petroleum products between countries; most of this trade is done via sea. Oil spill is defined as unintentional release of oil or petroleum hydrocarbon in environment. Every year more than 3 million tons of oil gets spilled in sea [6]. Oil spill covers sea surface by oil thus endangering aquatic life by obstructing passage of light, exchange of oxygen and other essential nutrients. Oil can cause either physiological or biochemical injury to the marine life. Oil can get absorbed, inhaled or ingested and cause hindrance in carrying out daily functions by marine organisms. When specific chemical compound in oil interacts and causes damage to organism's cellular metabolism it is referred as biochemical injury [7]. Continuous exposure to petroleum hydrocarbons can cause damage to central nervous system in humans as well as animals. It can also result in respiratory malfunction, disturbances in endocrine system and eventually can lead to cancer of liver, lungs, kidney, skin or bladder [8].So, by looking over damage caused due to oil spill it is necessary to implement certain methods to overcome problem of oil spill. Currently there are four major strategies that are being implemented to overcome problem of oil spill and they are, use of chemicals like dispersants and emulsion breakers, use of booms, skimmers and adsorbents which are mechanical methods, in-situ burning and finally bioremediation [9]. Microorganisms degrade petroleum hydrocarbons by producing surface active compounds called Biosurfactant and Bioemulsifiers. The chemical agents that are used for treating oil spill are hazardous for environment but the biosurfactants and bioemulsifiers are environmentally friendly; more over they are biodegradable, less toxic, and are active at extreme pH, salinity and temperatures [10]. Biosurfactant are amphiphilic compounds that consist of both hydrophilic as well as hydrophobic domains. Hydrophobic domain contains long chain fatty acids whereas the hydrophilic domain can contain Phosphate group, amino acid, Carbohydrate or some other compound [11]. These biosurfactant molecules are capable of forming micelles due to their amphipathic nature. Micelles have interior part which is hydrophobic, and external part which is hydrophilic and thus exposed to water. These bilayer sheets are unstable and thus fold up forming micelle having hollow zone. During formation of hollow zone, oil moieties are engulfed in hollow space and thus can travel inside microorganism to satisfy its carbon source [12].

So, the main focus of the study was to isolate such organisms from oil contaminated sites that can produce surface active compounds and use them for bioremediation of oil.

2. Material and Methods

1) Sample collection:
Three petrol contaminated soil samples were collected from HP and Indian oil petrol pump located in and around the Kalyan-Dombivli area. Water samples were collected from three dock sites that were contaminated with crude oil or its by-products: a) Ganesh ghat located in Kalyan (19.2470° N, 73.1183° E), b) Ganesh ghat located in Dombivli (19.2355° N, 73.0933° E), c) Indira dock located in Mumbai (18.9416° N, 72.8409° E). Two water samples were collected from each site; water samples were acquired from 5 cm depth in sterile 25 ml containers and transported in an icebox to the lab.

2) Enrichment and Isolation of biosurfactant producing microorganisms
Samples were enriched aerobically in 250 ml Erlenmeyer flask. The quantity of soil sample taken for enrichment was 1 gram and that of water sample was 10 ml. Samples were added in 100 ml of Bushnell Haas (BH) broth (Himedia M350) and as the sole carbon source phenol (1%, v/v) was used. The enrichment was carried out for one week on a rotary shaker (180rpm) at 30˚C. After incubation 1 ml of aliquots were inoculated in 100 ml of fresh BH broth containing crude oil (1%, v/v) as the only carbon source in 250 ml Erlenmeyer flask and were incubated again for one week at 30˚C at 180 rpm. Once enrichment was done, loop full from flask was used for isolation on BH agar medium containing 1% v/v crude oil as the only carbon source and it was observed for phenotypically different colonies. The procedure was repeated at least three times to isolate those organisms that exhibited pronounced growth on crude oil by transferring them to fresh agar plates to obtain pure cultures. The purity of isolates was verified through microscopic observation of Gram-stained cultures. The selected isolates were examined for the formation of biosurfactants using screening methods mentioned below.

The pure cultures were enriched in BH broth for 3 days in order to allow production of biosurfactant. Content was centrifuged from each flask for 15 minutes at 6000 rpm and 4˚C, after that the cell-free supernatant (CFS) was obtained by passing through 0.45µm pore size filter paper. The CFS that was obtained was further used for screening tests like emulsification index, hemolysis assay, drop collapse assay, and oil displacement assay.

3) Screening of Biosurfactant Producing Isolate

a) Hemolysis assay:
Hemolysis assay was determined by using freshly prepared blood agar plates consisting of 5% (v/v) blood. 50µl of enriched bacterial cultures were spot inoculated in the center of blood agar plates and were then kept for incubation overnight at 37°C. The hemolysis pattern around the colonies was analyzed in blood agar plates, indicating the production of biosurfactant. The blood agar screening technique is usually used as a primary test for microorganisms that may produce biosurfactants on hydrophilic media [12, 13].

b) Oil – displacement test:
The oil-displacement test was performed as described [14]. A Petri dish having a diameter of 15 cm was filled with 20 mL of deionized water. Then, on the top of the water 20µL of crude oil was released to form a layer above the surface of the water; it was then followed by the addition of 10µL cell-free supernatant of the enriched culture gently at the center of the plate. Also, positive control was evaluated with 0.1% Triton X-100 and negative control was evaluated with distilled water. After the 30s, the development of a clear zone was checked under visible light. If biosurfactant is present in the supernatant, then a clear zone is formed at the center of the oil layer.

c) Drop collapse assay:
Drops of CFS were put on the crude oil coated surface and the stability of the drop was observed. If the liquid contains surfactants, the drop may collapse or spread because of the decrease in interfacial surface tension between the liquid drop and the hydrophobic surface. Distilled water and 0.1% Triton X-100 were taken as positive control and negative control respectively [15].

d) Emulsification Index:
This test was performed to measure the emulsification caused by the biosurfactant that was produced by bacteria. Cell free supernatant and crude oil were taken in test tubes with a ratio of 1:1 (v/v). Test tubes were subjected to vortex for 5 min and kept in room temperature for 24 hours. The E24 index was calculated in percentage according to the following formula: [16].

$$E24\ index\ = \frac{H_e\ (mm)}{H_t\ (mm)}$$

Where, Ht is total height of liquid column and He is height of emulsified layer.

4) Identification of potent isolate
The strains that showed potential activity were selected and then were identified on basis of their morphological and standard biochemical tests. The biochemical tests carried out were Gram staining, VogesProskauer Test, Gelatin hydrolysis, Phenylalanine deaminase, Oxidase, Catalase, Starch hydrolysis, Indole Test, Methyl Red Test, Urease, Salt tolerance test, Triple sugar iron (TSI), Coagulase, Citrate Test, H2S production, Lysine decarboxylase, Nitrate reduction and production of acid from carbohydrates. Results obtained from morphological and standard biochemical test were used to find the close correspondence with known bacterial genus in accordance to Bergey`s Manual of Systematic Bacteriology [17].

3. Results

1) Isolation of biosurfactant producing microorganism
12 different samples were collected from selected sampling sites. Samples were enriched and total 16 morphologically different isolates were obtained on BH agar medium plate. Each of them was further kept for production of biosurfactants in BH broth as well as was stored on BH agar slants at 4°C till further use.

2) Hemolytic activity

Each of the isolate was checked for hemolysis activity. This test is based on fact that biosurfactant causes breakdown of RBCs leading to clear zone around colonies. Isolates DOK1, DOK4, DUG2, HPK1, HPK2, IOP2, ASW2 and ASW3 showed beta hemolysis. Whereas, isolates DOK2, DOK3, DUG1, DUG3, HPK3, HPK4, IOP1 and ASW1 showed gamma hemolysis that is no hemolysis.

3) Oil displacement assay

Oil displacement assay was implemented as described by K.V. Deepika et al. The results were scored by comparing test sample's cell free broth results with positive and negative control. Isolate DOK1 and ASW2 showed highest degree of oil displacement amongst other isolates. Isolate DOK3 didn't showed displacement of oil. The results of oil displacement assay are tabulated in Table 1.

4) Drop collapse assay

Drop collapse assay was performed as described by A. K. Pradhan et al. This assay is based on fact that biosurfactantcauses reduction in interfacial tension between oil and water, causing drop to collapse. The diameter of drop was measured with aid of magnifying glass. Cell-free broth of isolates DOK1, HPK2, IOP2, ASW2 and ASW3 showed drop diameter of 3 mm, whereas, rest others showed diameter of 2 mm. Negative control that was used, showed drop diameter of 1 mm and positive control showed drop diameter of 3 mm.

5) Emulsification index

The emulsification assay was performed as mentioned by K.M. Barakat et al. Isolate IOP2 showed highest emulsifying activity which is 42.3% which is higher than positive control which was triton X-100. Isolate DOK1 showed emulsifying activity similar to that of positive control which is 38.46%. The emulsification capacity of each cell-free broth containing biosurfactants was obtained as E24 % and is as presented in Table 1.

6) Identification of potent isolate

The biochemical tests of four potential isolates i.e., DOK1, HPK2, IOP2 and ASW3 were carried out. The results of test lead us to know the closest genus of our isolates according to Bergey's Manual of Systematic Bacteriology-Vol.2. The morphological characteristics and biochemical test results of potent isolate IOP2 showed similarity with standard biochemicals of Halomonas sp. such as Indole test, Methyl red test, citrate utilization test,VoguesProskauer test, catalase, gelatinase, etc. Hence, using Bergey's Manual of systematic Bacteriology, we can say that IOP2 belongs to Halomonas sp. The results of biochemical test for each potent isolate are shown in Table 2.

4. Discussion

Biosurfactants are widely utilized in field of hydrocarbon bioremediation as they enhance the growth of organisms on hydrophobic surface and helping with the poor availability of nutrients to microorganisms by increasing the uptake of hydrophobic substrates [18]. Biosurfactants are eco-friendly, least toxic, biodegradable and have high specificity and thus they can be good alternative to chemical surfactants [19]. The aim of this study was the examination of potent microorganisms from oil contaminated sites and the analysis of their biosurfactant-producing ability. Many researchers have reported the presence of biosurfactant-producing microorganisms in environments polluted with hydrocarbons [20, 21, 22]. Oil displacement, emulsification of oil in water, reduction in viscosity of crude oil and surface activity are the properties of a biosurfactant which make it important for oil spills bioremediation. Therefore, isolation of biosurfactant producing microorganisms to increase the process of bioremediation is an essential area of research.

In the present study, total 12 samples were enriched to isolate biosurfactant producing microorganisms. From 12 samples, total 16 morphologically different isolates were obtained on 1% oil containing Bushnell and Hass agar medium. The bacteria that were isolated were scrutinized for potential biosurfactant producers by using four screening method as suggested earlier [23]. The first test that is hemolytic activity appears to be a favorable screening method that can be used primarily for exploring bacteria that produce biosurfactants [24]. In the present study, 50% of the isolates were positive for hemolysis assay by exhibiting beta hemolysis around the colonies. However, only hemolysis can't be the criteria to rule out other isolates as biosurfactant non-producers. Thus, all organisms were exposed to other screening tests.

The secondary screening to further investigate biosurfactant production consists of three tests: drop collapse assay, oil displacement assay, and emulsification index. The oil

Table 1: Screening of biosurfactant producing microorganism

Sr. no.	*Sample*	*Hemolysis*	*Oil displacement assay*	*Drop collapse assay (mm)*	*Emulsification Index (%)*	*Potency ranking*
1	DOK1	β	++++	3	38.46	2nd
2	DOK2	γ	++	2	29.57	
3	DOK3	γ	-	2	22.25	
4	DOK4	β	+	2	24.33	
5	DUG1	γ	+	2	28.9	
6	DUG2	β	+	2	27.55	
7	DUG3	γ	++	2	21.6	
8	HPK1	β	+	2	23.86	
9	HPK2	β	+++	3	33.33	4th
10	HPK3	γ	++	2	25.63	
11	HPK4	γ	+	2	22.53	
12	IOP1	γ	+	2	26.75	
13	IOP2	β	+++	3	42.3	1st

14	ASW1	γ	+	2	24.95	
15	ASW2	β	++++	3	35.71	3rd
16	ASW3	β	+++	3	30.35	
17	NE. CTR	NA	-	1	0	
18	PO. CTR	NA	++++	3	38.46	

Key: NE. CTR = negative control; PO. CTR = positive control; β = beta haemolysis; γ = gamma haemolysis; NA = not applicable; ++++ = highest degree of oil displacement; - = no oil displacement.

Table 2: Biochemical Identification Test

Biochemical tests	*Isolate*			
	IOP2	*DOK1*	*ASW2*	*HPK2*
Gram Staining	Negative rods	Negative rods	Negative rods	Positive rods
Indole	+	-	-	-
Methyl red	-	-	-	+
VogesProskauer	-	-	-	+
Citrate utilisation	+	+	+	+
Gelatine Hydrolysis	+	+	-	-
Phenylalanine deaminase	+	-	+	+
Oxidase	+	+	-	-
Catalase	+	+	+	+
Starch hydrolysis	-	-	-	+
Urease	+	-	-	+
7% Salt tolerance	+	+	+	+
Triple sugar iron	Red slant, butt yellow, no gas	Red slant, Red butt, no gas	Red slant, yellow butt, no gas	Red slant, yellow butt, no gas
Coagulase	+	-	-	-
H_2S production	-	-	-	-
Lysine decarboxylase	+	-	+	-
Nitrate reduction	+	+	-	+
Glucose	+	+	+	+
Maltose	+	-	+	+
Fructose	-	+	-	+
Galactose	-	-	+	+
Sucrose	+	-	-	+
Lactose	-	-	+	+
Mannitol	+	+	-	+
Xylose	+	+	+	-
Genus and species	*Halomonas sp.*	*Pseudomonas sp.*	*Acinetobacter sp.*	*Bacillus sp.*

Key: + = positive; - = negative

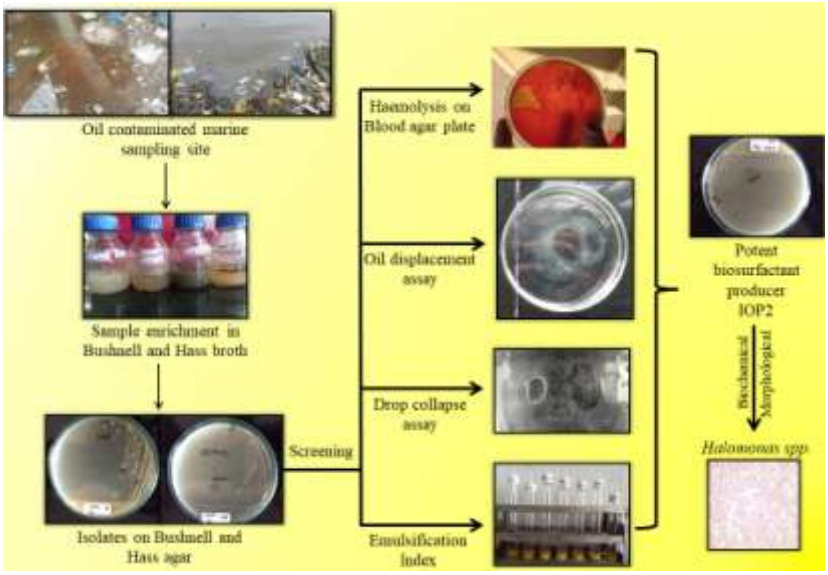

Figure 1: Graphical representation of work presented in this paper

Figure 2: Oil contaminated sampling site

displacemsent assay is a very reliable and rapid test as it needs only a small amount of sample and doesn't require any specific equipment [25]. It was observed that50% of isolates were turned out to be efficient by oil displacement assay.

The drop-collapse assay established earlier [26] confide on the property of destabilizing the liquid droplets which is due to the activity of surfactants. The drop collapse assay is easy to perform as well as very sensitive. It also has several advantages like requirement of very less amount of sample, not requiring specialized equipment, and being rapid & simple to perform [27], only 31% isolates were positive for this test. The emulsification index is one of the standard measures that can be used to determine the potential of biosurfactants, and only 5 isolates were found with emulsification index more than 30%. The four isolates that had higher results for drop collapse assay also showed higher emulsification index value, while IOP2 amongst these was excellent. Thus, a positive correspondence can be obtained linking the above-mentioned properties. Those isolates that are able to produce biosurfactants in culture media substantially can be a potential candidate for bioremediation of xenobiotic compounds as they have the ability to decrease the surface tension effectively, which leads to more solubilisation and absorption thus leading to biodegradation [28, 29]. For determining the identity of all the four isolates they were subjected to various biochemical tests, and the results were then compared with standard biochemical tests mentioned in Bergey's manual of determinative bacteriology-Vol.2. The isolate IOP2 was found out to be Halomonas sp., DOK1 was found out to be Pseudomonas sp., ASW2 was found out to be Acinetobacter sp., and HPK2 was found out to be Bacillus sp.

The isolation of several biosurfactant-producing bacteria can demonstrate the microorganism's natural ability to survive in hydrocarbon-contaminated environments by synthesizing biosurfactants [30].

5. Conclusion

In conclusion, sixteen different bacterial colonies were isolated from oil contaminated soil and water samples. Among them, four potent isolates were selected based on results of tests like Oil displacement assay, emulsification index, hemolytic activity test and drop collapse assay. Of which three isolates were gram negative rods while one was gram positive rods. Based on microscopic analysis and biochemical tests the four isolates DOK1, HPK2, ASW2 and ASW3 were identified as Halomonas sp., Pseudomonas sp., Acinetobacter sp. and Bacillus sp. respectively using Bergey's Manual of Systematic Bacteriology. The ability of these isolates to produce biosurfactants with significant results suggests its potential application in oil spills bioremediation.

6. Future Prospect

Further study on optimum pH, optimum temperature and utilization of Agricultural and industrial wastes as a substrate for the large-scale production is recommended. As well as FTIR or HPLC analysis of biosurfactant can give us molecular insight of biosurfactant. The potent organisms isolated from the current study can be used for replacing traditional chemical method by the natural biosurfactants.

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References

[1] Ite, A. E., Harry, T. A., Obadimu, C. O., Asuaiko, E. R., &Inim, I. J. (2018). Petroleum hydrocarbons contamination of surface water and groundwater in the Niger Delta region of Nigeria. Journal of Environment Pollution and Human Health, 6(2), 51-61.

[2] Eneh, O. C. (2011). A review on petroleum: source, uses, processing, products, and the environment. Journal of Applied Sciences, 11(12), 2084-2091.

[3] Jukić, A. (2013). Petroleum refining and petrochemical processes. Natural Gas Composition, Classification, Processing.

[4] Chen, C. C., &Que, H. (2018). U.S. Patent No. 9,934,367.

[5] Capuano, L., Nalley, S., Leckey, T., LaRose, A. &Corriere, M. (1995, July 1). Independent Statistics & Analysis U.S. Energy Information Administration (EIA). Retrieved from https://www.eia.gov/international/data/world.

[6] Prasad, G., &Anuprakash, M. V. V. S. (2016). Pollution due to oil spills in marine environment and control measures. IOSR J Environ SciToxicol Food Technol, 10, 1-8.

[7] Buskey, E. J., White, H. K., &Esbaugh, A. J. (2016). Impact of oil spills on marine life in the Gulf of Mexico: effects on plankton, nekton, and deep-sea benthos. Oceanography, 29(3), 174-181.

[8] Gkorezis, P., Daghio, M., Franzetti, A., Van Hamme, J. D., Sillen, W., &Vangronsveld, J. (2016). The interaction between plants and bacteria in the remediation of petroleum hydrocarbons: an environmental perspective. Frontiers in microbiology, 7, 1836.
[9] Ivshina, I. B., Kuyukina, M. S., Krivoruchko, A. V., Elkin, A. A., Makarov, S. O., Cunningham, C. J., ... &Philp, J. C. (2015). Oil spill problems and sustainable response strategies through new technologies. Environmental Science: Processes & Impacts, 17(7), 1201-1219.
[10] Pacwa-Płociniczak, M., Płaza, G. A., Piotrowska-Seget, Z., &Cameotra, S. S. (2011). Environmental applications of biosurfactants: recent advances. International journal of molecular sciences, 12(1), 633-654.
[11] Joshi, P. A., Shekhawat, D. B. (2016). Biosurfactants: Screening, Production, Applications. Delhi: Techno World Press.
[12] Youssef, N. H., Duncan, K. E., Nagle, D. P., Savage, K. N., Knapp, R. M., &McInerney, M. J. (2004). Comparison of methods to detect biosurfactant production by diverse microorganisms. Journal of microbiological methods, 56(3), 339-347.
[13] Mouafi, F. E., Elsoud, M. M. A., &Moharam, M. E. (2016). Optimization of biosurfactant production by Bacillus brevis using response surface methodology. Biotechnology Reports, 9, 31-37.
[14] Deepika, K. V., Kalam, S., Sridhar, P. R., Podile, A. R., &Bramhachari, P. V. (2016). Optimization of rhamnolipidbiosurfactant production by mangrove sediment bacterium Pseudomonas aeruginosa KVD-HR42 using response surface methodology. Biocatalysis and agricultural biotechnology, 5, 38-47.
[15] Pradhan, A. K., Pradhan, N., Mall, G., Panda, H. T., Sukla, L. B., Panda, P. K., & Mishra, B. K. (2013). Application of lipopeptidebiosurfactant isolated from a halophile: Bacillus tequilensis CH for inhibition of biofilm. Applied biochemistry and biotechnology, 171(6), 1362-1375.
[16] Barakat, K. M., Hassan, S. W., &Darwesh, O. M. (2017). Biosurfactant production by haloalkaliphilic Bacillus strains isolated from Red Sea, Egypt. The Egyptian Journal of Aquatic Research, 43(3), 205-211.
[17] Singh, V., Haque, S., Singh, H., Verma, J., Vibha, K., Singh, R., ...&Tripathi, C. K. M. (2016). Isolation, screening, and identification of novel isolates of actinomycetes from India for antimicrobial applications. Frontiers in microbiology, 7, 1921.
[18] Chandran, P., & Das, N. (2010). Biosurfactant production and diesel oil degradation by yeast species Trichosporonasahii isolated from petroleum hydrocarbon contaminated soil. Int J EngSciTechnol, 2(12), 6942-6953.
[19] Banat, I. M., Makkar, R. S., &Cameotra, S. S. (2000). Potential commercial applications of microbial surfactants. Applied microbiology and biotechnology, 53(5), 495-508.
[20] Bodour, A. A., Drees, K. P., & Maier, R. M. (2003). Distribution of biosurfactant-producing bacteria in undisturbed and contaminated arid southwestern soils. Applied and environmental microbiology, 69(6), 3280-3287.
[21] Yateem, A., Balba, M. T., Al-Shayji, Y., & Al-Awadhi, N. (2002). Isolation and characterization of biosurfactant-producing bacteria from oil-contaminated soil. Soil and Sediment Contamination, 11(1), 41-55.
[22] Das, K., & Mukherjee, A. K. (2005). Characterization of biochemical properties and biological activities of biosurfactants produced by Pseudomonas aeruginosamucoid and non-mucoid strains isolated from hydrocarbon-contaminated soil samples. Applied microbiology and biotechnology, 69(2), 192-199.
[23] Walter, V., Syldatk, C., &Hausmann, R. (2010). Screening concepts for the isolation of biosurfactant producing microorganisms. In Biosurfactants (pp. 1-13). Springer, New York, NY.
[24] Carter, G. R. (1967). Diagnostic procedures in veterinary bacteriology and mycology. Diagnostic procedures in veterinary bacteriology and mycology.
[25] Płaza, G. A., Zjawiony, I., & Banat, I. M. (2006). Use of different methods for detection of thermophilicbiosurfactant-producing bacteria from hydrocarbon-contaminated and bioremediated soils. Journal of Petroleum Science and Engineering, 50(1), 71-77.
[26] Jain, D. K., Collins-Thompson, D. L., Lee, H., &Trevors, J. T. (1991). A drop-collapsing test for screening surfactant-producing microorganisms. Journal of Microbiological Methods, 13(4), 271-279.
[27] Shoeb, E., Ahmed, N., Akhter, J., Badar, U., Siddiqui, K., ANSARI, F., ...& BAIG, R. (2015). Screening and characterization of biosurfactant-producing bacteria isolated from the Arabian Sea coast of Karachi. Turkish Journal of Biology, 39(2), 210-216.
[28] Kuyukina, M. S., Ivshina, I. B., Philp, J. C., Christofi, N., Dunbar, S. A., &Ritchkova, M. I. (2001). Recovery of Rhodococcusbiosurfactants using methyl tertiary-butyl ether extraction. Journal of Microbiological Methods, 46(2), 149-156.
[29] Fiebig, R., Schulze, D., Chung, J. C., & Lee, S. T. (1997). Biodegradation of polychlorinated biphenyls (PCBs) in the presence of a bioemulsifier produced on sunflower oil. Biodegradation, 8(2), 67-75.
[30] Margesin, R., &Schinner, F. (2001). Bioremediation (natural attenuation and biostimulation) of diesel-oil-contaminated soil in an alpine glacier skiing area. Applied and environmental microbiology, 67(7), 3127-3133.

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