Discover Water

Review

Biosurfactant, a green and effective solution for bioremediation of petroleum hydrocarbons in the aquatic environment

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Abstract
When pollutants like petroleum hydrocarbons as one of the major origins of aquatic pollution, enter the environment, they alter the biological and/or physicochemical characteristics of the aforementioned sites due to their potential of bioaccumulation, biomagnification, and resistance against biodegradation besides its toxicity and carcinogenicity in nature. Thus, the importance of degradation, deterioration and remediation of these pollutants from environments such as aquatic environments via a green method such as bioremediation is undeniable. Biosurfactants as secondary metabolites of microorganisms, enhance the bioremediation rate of petroleum hydrocarbons. Using oleophilic microorganisms with the capability of biosurfactant production which this paper calls "potential microorganisms" in a bioremediation system is promising. This article reviews the effective factors on bioremediation and the share of biosurfactants on the rate of bioremediation process, chemical surfactants and their limiting factors as biosurfactant's chemical counterpart, the rising market of biosurfactant and its promising future, various types of biosurfactants, and the requirements to develop an optimized biosurfactant-base bioremediation system. Furthermore, this paper based on former studies suggests a novel in-situ biosurfactant-based bioremediation system integrated with Biochar called "Potential Microorganisms Immobilized on Biochar system" (PMIBC system) as a cost-effective in-situ bioremediation system for decontamination of aquatic environments like groundwater, lakes, marshes, etc. from petroleum hydrocarbons and oil spills which requires further study.

Keywords Petroleum hydrocarbons · Aquatic pollution · Bioremediation · Biosurfactant · Microbial Enhanced Oil Recovery (MEOR) · Biochar

1 Introduction

Environmental pollution due to its vast and adverse effects on public health, mental health, ecosystems, and socioeconomic factors, requires prompt and proper measures to be restored and rehabilitated [1–3].

Investigates show the fact that approximately 2 million tons of oil enter aqueous environments per year based on sea-based activities [12]. After an oil spill occurrence, petroleum may remain in aquatic environments for a long time [13]. Petroleum hydrocarbon pollution may cause serious and harmful effects on the aforementioned ecosystems as well

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as human security because of its potential of bioaccumulation, biomagnification, and resistance against biodegradation besides its toxicity and carcinogenicity in nature [12, 14, 15]. Petroleum is a multiplex combination of aromatics, heterocyclic hydrocarbons, and natural gases. Besides crude oil which is one of the most prevailing global sources of energy contains a complex and variable mixture of hydrocarbons mostly including alkanes, saturates, aromatics, resins, asphaltenes, naphthenes, etc. [11, 16, 17].

Bioremediation is a valuable biotechnology method that simply can be defined as the process of decontamination and mitigation of pollutants from the projected contaminated environment via microbial activities. Since biosurfactant is an eco-friendly and effective compound that has the properties of surfactants generated by some microorganisms, it can enhance the breaking of petroleum hydrocarbons by increasing the bioavailability of mentioned contaminations for oleophilic microorganisms existing in the polluted aquatic environments and by this mean the bioremediation process enhances. Therefore, biosurfactant is a crucial factor in an optimized bioremediation process [20–22].

The objective of the present review is to evaluate the biosurfactant-producing microorganisms and their potentiality in bioremediation of petroleum hydrocarbon in aquatic environments.

2 The effects of petroleum hydrocarbons on marine environments

The marine environment is among the most significant aquatic environments as they are extremely valuable due to the existence of various useful and applicable ecosystems and natural resources. Marine pollution is alarming because when pollutants, especially oil spills or chemicals as the major cause of marine pollution enter to those environments, it can alter the biological and/or physicochemical characteristics of aquatic sites [4, 5]. Around 35 million petroleum barrels are conveyed by transoceanic travels annually and numerous ship accidents occur every year and most of the accidents lead to an extensive release of petroleum in marine environments. Usually, oil spill accidents in the marine sites are enormous and noxious [6]. Crude oil leakage and spillage may lead to disastrous damages to the aquatic environments. Fractions of oil can change population dynamics and derange structures and interactions of ecosystems related to contaminated sites and impact the population of most of the native organisms [12, 18]. As an example, several studies and experiments confirmed the diminishing of Persian Gulf biodiversity triggered by the petroleum contamination [19]. Increasing industrial activities for use of energy and raw materials and large-scale release of various contaminating factors during oil drilling, transportation, and accidents results in environmental pollution, for instance, the collision and explosion accident of Sanchi ship in 2018 resulted in a vast oil spill in water [7, 8]. Petroleum is the most profitable source of energy and simultaneously is among the most prevalent and hazardous environmental pollutant factors which can lead to the contamination of terrestrial and aquatic environments and ecosystems like groundwater, surface water, and soil due to its qualities like toxicity and recalcitrant nature [9–11].

3 Bioremediation of petroleum hydrocarbons and the effects of biosurfactant

Remediation of petroleum hydrocarbons is a vital procedure for environments and/or organisms. Besides, some remediation strategies such as physical or chemical are not sufficient for decontamination of environments because of the unceasing entry of various pollutants throughout environments as a consequence of anthropogenic activities and also these methods mostly are costly and time-consuming. For this reason, bioremediation as a cost-effective, eco-friendly, non-toxic biotechnology method has been widely recommended [6, 23]. Bioremediation is the process of degradation, detoxification, mineralization, or transformation of a hazardous pollutant to a safe state relying on microorganisms' activity and their enzymes [24]. Aquatic bacteria have a wide range of bioremediation applications like biodegradation and removal of petroleum, diesel, heavy metals, other recalcitrant, etc. [25]. Bioremediation of petroleum hydrocarbons depends on microbial metabolic activities in presence of factors that optimize the process which in some cases just requires the addition of some factors like nutrients, surfactants, etc. to the polluted media [26, 27]. The efficacy of petroleum hydrocarbon bioremediation strategies may depend on different factors in aimed sites (Fig. 1) which researchers must consider all these factors before starting a bioremediation process [14, 28]. Sufficient concentration of nutrients, oxygen, and also suitable temperature along with pH between 6 and 9 in aimed sites optimize the rate of growth and hydrocarbon biodegradation. Studies determine that the maximum rate of hydrocarbon degradation or in other words hydrocarbon degradation in marine environments and freshwater environments respectively are 15–20 °C and 20–30 °C

[29, 30]. The addition of nitrogen and phosphorous in bioremediation sites can enhance the biodegradation rate of petroleum [31].

Biosurfactants are amphiphilic molecules that have surface tension reduction abilities that can be used in environmental, industrial, agricultural, therapeutic activities and can also enhance petroleum bioremediation [32–35]. As a secondary metabolite of microorganisms, biosurfactants can be processed by the cultivation of biosurfactant producing microorganisms in the stationary phase on many sorts of low-priced substrates like biochar, plant oils, carbohydrates, wastes and high-level production of biosurfactants can be controlled by regulation of environmental factors and growth circumstances [36, 37]. Cultivation of biosurfactant-producing microorganisms requires an appropriate media and some factors (Fig. 1). Important factors for biosurfactant production that should be taken into consideration include nitrogen source, carbon source, carbon to nitrogen ratio, minerals, vitamins, metabolic regulators, inhibitors, inducers, salinity, pH level, and water. For the best result of microbial growth on the media, researchers need data about the properties of the intended microorganism and its optimized media [38]. Based on Khan et al., an increase in nutrient availability and accessibility for microbial strains with the ability of hydrocarbon utilization and biosurfactant production, leads to an increase in biosurfactant production and eventually the rate of hydrocarbon bioremediation increases (Fig. 1) [39].

In-situ and ex-situ bioremediation techniques are respectively defined as bioremediation of pollutants at the contaminated site and out of the contaminated site. Usually, the ex-situ bioremediation techniques are more expensive rather in-situ techniques. Examples of ex-situ techniques are land farming, bioreactor, windrow, biopile and examples of in-situ bioremediation techniques include natural attenuation, phytoremediation, biosparging and, bioventing Additionally, biostimulation and bioaugmentation enhance the bioremediation process in both techniques. Nevertheless, researchers decide on ex-situ or in-situ bioremediation methods considering the location and type of pollutant [24, 40, 41].

Using indigenous bacteria which utilize hydrocarbons and produce biosurfactants, is a green and promising bioremediation method that ameliorates the efficiency of the bioremediation process by making petroleum hydrocarbons bioavailable and facilitating its degradation [42]. On exposure to petroleum, a major group of microorganisms that degrade hydrocarbons, also generate biosurfactants and bioemulsifires [43, 44]. Biosurfactants promote the surface area of hydrophobic water-insoluble substrates and following this phenomenon, hydrocarbon utilizing bacteria can utilize petroleum hydrocarbons more efficiently for its growth and logically, after all, rates of biodegradation, bioremediation, and biocontrol are enhanced [45]. The biodegradation of petroleum hydrocarbons requires a wide spectrum of enzymatic action, therefore the use of microbial consortium or biofilm to remediate these hydrocarbons could be appropriate [46, 47]. The bioremediation process cannot be effective if there are no microorganisms with the ability of hydrocarbon degradation available in the contaminated site [31]. Dozens of bacteria can utilize petroleum hydrocarbons in the aquatic environments which some of the bacterial species that have a satisfying ability to tolerate high concentrations of petroleum hydrocarbons and degrade them, have been listed in (Table 1). Microorganisms that generate biosurfactants mostly simplify the utilization of petroleum by two common functions: (1) boosting the complexation and solubilization of apolar substrates which finally leads to an increase in bioavailability of petroleum and (2) enhancing the affinity between oil–water interface and cell surface through metabolic activities which leads to stimulation of oil–water interface film's deformation [48].

4 Chemical surfactants and dispersants

Dispersants are chemical mixtures containing surfactants and solvents. They decrease the surface tension between oil and water interfaces leading to the oil-microdroplets formation which is more biodegradable in the water column rather than large spills. This process is called emulsification [36, 61]. In the above-mentioned process, dispersed oil may become more bioavailable for the marine organisms [62]. Dispersants are applied world-widely and commonly as an urgent response to the oil spills in aquatic ecosystems, which results in the formation of oil-microdroplets and ultimately make it more bioavailable for both hydrocarbons utilizing microorganisms and other marine organisms [63, 64]. The use of chemical dispersants in case of oil spill leakages and accidents in aquatic environments may result in the change of activity and community composition of microorganisms including hydrocarbon-degrading microorganisms [59]. Besides, dispersants in turn can contain amounts of toxic and hazardous compounds. For instance, Corexit EC9500A and Corexit EC9527A both include hazardous components such as: Organic sulfonic acid salt (10–30% w/w), propylene glycol (15% w/w). Also, Corexit EC9500A and Corexit EC9527A contain hydrotreated light petroleum (10–30% w/w) and 2-butoxyethanol (30–60% w/w) respectively [65]. Furthermore, information confirms that most of the industrial dispersants and chemical surfactants are petroleum based [42].

Table 1 bacteria with the ability of petroleum hydrocarbons utilization and toleration in aquatic environments

#	Bacterial species	Petroleum hydrocarbon compound	References
1	*Aeromonas*		[50]
2	*Acinetobacter* spp.	Aliphatics, Monoaromatics, C5-C16 alkanes, C10-C30 alkanes	[6, 49, 50]
3	*Achromobacter*	Polyaromatics	[6, 49]
4	*Alcaligenes* spp.		[6, 51]
5	*Alcanivorax* spp.	Aliphatics	[49, 52]
6	*Alkanindiges*		[6]
7	*Alteromonas*		[50, 53]
8	*Arthobacter*		[54]
9	*Bacillus* spp.	Aliphatics, Monoaromatics, Polyaromatics	[6, 49]
10	*Brevibacterium*	Aliphatics	[6, 49]
11	*Burkholderia*	C5-C16 alkanes	[6, 49]
12	*Corynebacterium* spp.		[6]
13	*Cycloclasticus*	Polyaromatics	[49, 55]
14	*Dietzia*		[50, 55]
15	*Enterobacter*		[50]
16	*flavobacterium* spp.		[6, 50]
17	*Geobacillus*		[56]
18	*Halomonas*	Monoaromatics,	[49, 56]
19	*Kocuria* sp.		[50, 57, 58]
20	*Marinobacter* sp.	Aliphatics	[49, 59]
21	*Micrococcus roseus*	Aliphatics,	[49, 59]
22	*Mycobacterium*	Fatty acids, Cycloalkanes, Alkyl benzenes	[6, 49]
23	*Nocardia*		[60]
24	*Oleiphilus*		[56]
25	*Oleispira*		[55, 56]
26	*Pseudomonas aeruginosa*	Aliphatics, Monoaromatics, Resins, C5-C16 alkanes	[6, 49]
27	*Pseudomonas fluorescens*	Aliphatics, Monoaromatics, Resins, C5-C16 alkanes	[6, 49]
28	*P. putida*	-	[25]
29	*Rhodococcus*	Aliphatics, Monoaromatics, Alkyl benzenes, Fatty acids	[6, 49]
30	*Sphingomonas*	Monoaromatics	[6, 49]
31	*Staphylococcus*		[6]
32	*Streptomyces* sp.		[25]
33	*Thallassolituus*	Aliphatics	[49, 55]
34	*Vibrio*	Polyaromatics	[6, 49]
35	*Xanthomonas* sp.		[55]

Deepwater Horizon (DWH) occurred on April 20th, 2010, followed by an explosion on DWH drilling rig and led to oil and gas blowout [66, 67]. Statistics show that 74% of saturated hydrocarbons, 16% of aromatic hydrocarbons, and 10% of polar hydrocarbons formed the DWH oil spill [68]. Corexit EC9500A is an oil dispersant which was largely employed in the Gulf of Mexico after the aforementioned catastrophe both below water and at the surface. Studies after the DWH accident reveal that both Corexit 9500 and 9527 were genotoxic and cytotoxic for marine mammals. Meanwhile, Corexit 9500 was less genotoxic but more cytotoxic than Corexit 9527 [69]. Aquatic studies show this fact that this oil dispersant is low toxic for many crustaceans, corals, and fishes but is more toxic to planktons, daphnia, and many species in the early stages of life [67]. After using 7 million liters of dispersants in DWH to stimulate crude oil degradation by microbial processes, oxidation rate measurements of alkanes and aromatic hydrocarbons revealed the fact that neither suppression nor stimulation of oil biodegradation process in presence of dispersants will change. Although further studies demonstrate that Corexit EC9500A affects some characteristics of *Marinobacter* sp. TT1 including metabolisms of hydrocarbon, formation of biofilm, and chemotactic motility, it also induces mechanisms of solvent tolerance in the mentioned bacterium [59, 66].

After the DWH accident, some dispersants listed on the U.S. Environmental Protection Agency's (EPA) National Contingency Plan (NCP) were employed in the Gulf of Mexico [70]. Eight of these dispersants include Corexit 9500, Dispersit SPC 1000, Jd-2000, Nokomis 3-AA, Nokomis 3-F4, Saf-Ron Gold, Sea Brat #4, and ZI-400. Over the past few years, acute toxicity tests were conducted on the mentioned dispersants and revealed their relative toxicity [71]. Nevertheless, various studies demonstrate that application of chemical surfactants can impede the process of biodegradation of some petroleum hydrocarbons for the particular reason that petroleum degrading microorganisms may utilize those applied surfactants rather than petroleum hydrocarbons as growing substrate [72]. The use of chemical dispersants, besides being relatively inefficient, can also result in the accumulation of its toxic compounds in the aquatic and any other environment [18].

Several studies demonstrate that there are some green and non-toxic chemical surfactants that can be used in a petroleum bioremediation process like choline laurate or choline alkylsulfates. Further studies, though, indicate that mixtures of a green surfactant and a biosurfactant can improve the rate of petroleum bioremediation more efficiently. Shah and his colleagues [73] suggested a new strategy in the remediation of crude oil. A binary mixture as a green dispersant including a glycolipid-type biosurfactant produced by *Starmerella bombicola* named lactonic sophorolipid, and a liquid and ionic surfactant named choline laurate were reported. At a 40:60 (w/w) ratio of choline laurate and lactonic sophorolipid with a dispersant to oil ratio of 1:25 (v/v), the efficiency of dispersion achieved 83%. This binary mixture is also classified as non-toxic agent [73, 74].

5 The rising market of biosurfactant and its challenges

Biosurfactants do not process any secondary pollutants but a lot of chemical surfactants do [75]. Employment of these agents for remediating oil-contaminated sites is beneficial due to its characteristics, like biodegradability, specificity, activation at very low concentrations, high surface activity, the capability to reduce interfacial tension, environmentally safe, low toxicity, and efficacy in vast ranges of temperatures and pH [9, 76–80].

Based on former studies, the use of biosurfactants in a short period of time enhances the removal rate of petroleum hydrocarbons (achieving almost 80% removal rate within 1 week of biosurfactant cure) [81]. Compared to the chemical dispersants, biosurfactants appear more effective in bioremediation applications on oil-contaminated aquatic environments [82].

Regardless of the aforementioned advantages of using biosurfactants, these biological agents are not commercially common yet as their final production cost is approximately 12 times higher than chemical surfactants [83]. 70–75% of all common and synthetic surfactants used in industrial countries are based on petroleum, therefore developing economical processes and approaches to find a cost-effective biosurfactant is a critical key to expanding the petroleum hydrocarbons bioremediation methods [42]. Some strategies have been developed to overcome the problem of large-scale biosurfactant production [84]. Some of the challenges along the commercialization process of biosurfactants include downstream processing, the requirements of pretreatment, large-scale production, and the availability of raw materials as feedstock. Various wastes can be applied as feedstock for biosurfactant-producing microorganisms to lower the production price of biosurfactants, like agro-industrial waste, glycerol, and oily waste, fruit and vegetable waste, dairy industrial waste, municipal waste, and industrial waste [86].

The global demand for biosurfactants is growing each year and these biological agents form a major share of the surfactant market. Estimations indicate that revenue generation of the biosurfactant market is expected to reach 2.6 billion US dollars by 2023 from 1.8 billion US dollars in 2016 which indicates the global market tendency for the replacement of chemical surfactants with biosurfactants [85].

6 Types and classifications of biosurfactants

Biosurfactants are amphipathic molecules with both hydrophilic and hydrophobic moieties. Hydrophobic moieties can include a fatty acid carbon chain with 10 to 18 atoms of carbon or a peptide, aprotein with a high portion of lipophilic side chains. Hydrophilic moieties can include carbohydrate, phosphate, carboxylate, or ester groups, an amino acid or similar compounds [87, 88].

Generally, biosurfactants include different types (Fig. 2) and Different types of biosurfactants also contain various subdivisions (Table 2) [89–92]. These biological surfactants can be extracted from certain kinds of plants, animals, bacteria, filamentous fungi by fermentation, and yeast which can synthesize biosurfactants. Among all microorganisms, bacteria are the major biosurfactant producing group [79, 93, 94].

Numerous numbers of biosurfactants are produced by aerobic microorganisms in aqueous media using a carbon source [87]. Biosurfactants are divided into high molecular weight (HMW) and low molecular weight (LMW). HMW biosurfactants are known for their emulsifying properties and LMW biosurfactants are suitable for decreasing surface and interfacial tensions between oil and water. Altogether, biosurfactants with higher molecular mass are considered more effective as emulsification agents [102]. The significant classes of LMW surfactants usually are glycolipids (as rhamnolipids), lipopeptides (as surfactin), and phospholipids, whereas HMW surfactants are polymeric [87, 103].

7 Requirements to develop a novel and optimized biosurfactant-base bioremediation system

A biosurfactant-based bioremediation system is an accelerated or engineered bioremediation technique in which the main focus is to increase microbial biosurfactant production by providing a suitable environment for the microbes [24, 109]. Typically, microorganisms with biotechnological applications are chosen for industrial approaches [104]. Biosurfactants considerably apply in various industrial applications, like in medicine, food, cosmetics, agriculture, petroleum. [105]. One of the major benefits of remediation of contaminated sites by the use of microbes is its high efficiency and low cost in a sustainable fashion [106]. due to the fact that a vast variety of microorganisms have the potential for biosurfactant production and they are ubiquitous in aqueous sites, The idea of using these biological agents in order to improve the bioremediation processes of petroleum in contaminated aquatic sites has become more promising. [79, 107]. Studies and results declare the fact that a lot of biosurfactants are bio-compatible and have an acceptable application to bioremediation of petroleum contaminated aquatic environments. Biosurfactants produced by microorganisms remarkably can reduce the surface tension of petroleum hydrocarbons and enhance the bioremediation rate of petroleum hydrocarbons [108].

The availability of a big volume of data (big data) on microorganisms' behavior under various environmental stress levels, genetics, catabolic potentials, functional pathways, enzymes, metabolites like biosurfactants and their characteristics, promise researchers a promising future of developing an optimized biosurfactant-based bioremediation system for bioremediation of pollutants like petroleum in the aquatic environments. Considering the fact that in-situ generation of biosurfactants is almost the most sustainable approach through bioremediation, the next generation biosurfactant-base systems must be combined with sensors and genetically engineered microorganisms. based on available data of the biosurfactants producing microorganisms on the bioremediation process, researchers and engineers can develop a biosurfactant-base bioremediation system that constantly detects the number of pollutants, pH, temperature, salinity, availability of nutrients, and other effective factors on bioremediation via sensors for in-situ detection. Followed the detection, the system should provide the suitable condition for proper biosurfactant-producing microorganisms to enhance the bioremediation rate of petroleum and oil spills in aquatic environments as a sustainable clean-up technique using big data and sensors [23, 110–118]. The development of such an optimized biosurfactant-based bioremediation system requires at least two key factors besides having sufficient knowledge about the projected environment. First, is knowing about biosurfactant-producing microorganisms and choosing the most suitable microorganisms for the system and the second one is genome sequencing and genetic engineering of biosurfactant producers to optimization of the bioremediation system.

Table 2 subdivisions of biosurfactants

Biosurfactant classification:	Subdivisions:	References:
Glycolipids	Rhamnolipids, di-rhamnolipids, sophorolipids, trehalolipids, Mannosylribitol lipid, Mannosylarabitol lipid, Mannosylmannitol lipid, Cellobiolipid, Xylolipid	[95–98]
Lipopeptides	Surfactin, Lichenysin, Polymyxin, Psudofactin II, Fengycin, Iturin, Syringafactin, Amphisin, Kurstakin, Pumilacidin, Viscosin, Serrawettin, Subtilisin, Arthrofactin, Ornithine, Bacillomycin D, Fusaricidins,	[16, 97–99]
Polymeric surfactants	Liposan, Alasan, Emulsan, Protein PA, Mannoprotein, Biodispersan, rufisan	[90, 100, 101]
Phospholipids/Fatty acids/Neutral lipids	Phosphatidylethanolamine, Corynomycolic acid, Spiculisporic acid, Flavolipid,	[98, 100]

7.1 Biosurfactant-producing microorganisms

A bioremediation method including in-situ or ex-situ applications rely on microbial activity, therefore a major requirement to develop a biosurfactant-based bioremediation system is to thoroughly know about biosurfactant producing microorganisms' qualities and characteristics [24].

A study suggests the use of biosurfactant-producing bacterium, from *Enterobacter hormaechei* species, to combat accidental marine oil spills.The produced lipoprotein biosurfactant is an anionic HMW biosurfactant (48 KDa) and has unique emulsification and surface activities. *E. hormaechei,* also, has the ability to degrade 85% of petroleum hydrocarbons of crude oil in 10 days of incubation [119].

A laboratory experiment was performed in 2018 to test the bioremediation rate of contaminated seawater with petroleum products (motor oil) using biosurfactant produced by *Pseudomonas aeruginosa* UCP 0992 that cultivated in industrial wastes. After 30 days, the experiment recorded more than 90% rate of oil degradation [76]. *Pseudomonas aeruginosa* mostly produces rhamnolipids, surface-active compounds which belong to the class of glycolipid biosurfactants [120].

In another study, *Bacillus cereus* strain BCS0, a biosurfactant producer isolated from seawater and cultivated with different carbon and nitrogen sources, augmented to a motor oil-contaminated sample. Within 27 days, the degradation rates enhanced up to 96%. Besides, the biosurfactant remained stable in a vast range of temperatures (5–120 °C), pHs (2–10), and salinity (2–10%), which demonstrated the potential of mentioned microorganism and its biosurfactant in the bioremediation process of aquatic environments [121]. The biosurfactants produced by some species of the genus *Bacillus* have also anticancer activity and non-pathogenic characteristics, therefore this genus has attracted a lot of attention in the biosurfactant industry [122, 123].

Acinetobacter baumannii OCB1, isolated from an aquatic site, grown in seawater contaminated with petroleum crude oil, and supplemented with glucose (1.0 g/L), demonstrated 69.69% C8-C14 hydrocarbons degradation. Plus, the addition of 0.05 g/L of yeast extract enhanced the degradation of C8-C14 hydrocarbons. Additionally, the lipopeptide biosurfactant produced by *A. baumannii* OCB1 remained completely stable in a wide spectrum of pHs (2–12) and NaCl concentrations (2–12%), meaning that *it* produces a halotolerant biosurfactant [43]. Lipopeptide biosurfactants can take action as the replacement of chemical surfactants if only the final cost of production of these biological agents become more reasonable by developing new methods and strategies [99].

A study suggested the use of a biosurfactant/phenol system to improve Polycyclic Aromatic Hydrocarbons (PAHs) bioavailability. Phenol in turn reduced the biosurfactant's critical micelle concentration (CMC) and enlarged the dissolution of PAHs in biosurfactant solutions. After adding biosurfactant, the bioavailability of PAHs in sludge improved from 27.7% to 43.1% and after the addition of phenol, bioavailability reached 49.2%. Phenol also improved the bioremediation of PAHs in biosurfactant solutions [124]. While phenol enhances the rate of bioremediation, the use of biosurfactant/phenol system is not recommended in marine ecosystems due to the hazardous properties of phenol and phenolic compounds. Based on the discussed data, biosurfactants can also be used for the bioremediation of aromatic compounds such as phenol or phenolic derivatives [125].

Hydrocarbons' degradation capability of *Staphylococcus pasteuri* CO100 as a halotolerant microorganism under high salinity, was studied. Results of the study demonstrate that *Staphylococcus pasteuri* CO100 degraded 72% of aliphatic hydrocarbons existing in crude oil, they can also grow on PAHs like pyrene, fluoranthene, and phenanthrene. The lipopeptide biosurfactant produced by *Staphylococcus pasteuri* CO100 can enhance oil degradation more efficiently than some synthetic surfactants. The biosurfactant-CO100 remained stable in a vast range of temperatures (4–121 °C), pHs (24.3–12), and salinities (0–300 g/L NaCl) [126].

Standing on various researches and studies on microbial strains, some of the potential and promising microbial strains which produce promising biosurfactants that can enhance the bioremediation rate of petroleum hydrocarbons in aquatic environments have listed (Table 3).

Based on the diversity of chemical components of petroleum, the use of bacterial consortiums, which produce biosurfactants in order to biostimulate petroleum pollutants bioremediation rates, is recommended. A consortium of microorganisms has a greater possibility of covering more spectrum of enzymatic actions rather than a single bacterium [100, 106]. In an experiment, 5 marine bacteria candidates, where sampled from sediments of four different places to examine their potential of PAHs removal, The bacterial consortium consist of *Ochrobactrum*, *Streptococcus*, *Pseudomonas* sp., *Pseudomonas aeruginosa* and *Achromobacter xylosoxidans* demonstrated more than 90% removal of PAHs [144].

Chen et al. suggested the combination of two hydrocarbon-degrading microorganisms including *Dietzia* sp. CN-3 and *Acinetobacter* sp. HC8–3S as a consortium in which both microorganisms were isolated from petroleum-contaminated

Table 3 Strains that produce promising biosurfactants and utilize hydrocarbons as carbon source (potential microorganisms) to enhance bioremediation rates in different aquatic environments

#	Bacterial strain	Type of produced biosurfactant	Type of pollutant—Biodegradation efficiency	Lab scale/ field scale	Stability of produced BS under various conditions	Ref
1	*Achromobacter kerstersii* LMG3441	Glycolipid	Crude oil—53%	Field scale	-	[127]
2	*Acinetobacter baumannii* OCB1	Lipopeptide	C8-C14 hydrocarbons—69.69%	Field scale	pH (2–12), NaCl (2–12%)	[43]
3	*Bacillus cereus* BCS0	Lipopeptide (surfactin)	Motor oil— ~ 96%	Labe scale	pH (2–10), temperature (5–120 °C), NaCl (2–10%)	[121]
4	*Bacillus cereus* DRDU1	Lipopeptide (surfactin)	Crude oil—86%	Lab scale	-	[128]
5	*Bacillus methylotrophicus* SSNPLPB5	Lipopeptide (surfactin and iturin)	C8-C19 hydrocarbons— ~ 92.1%, C20—C33 hydrocarbons—42.4%	Field scale	-	[16]
6	*Bacillus* stratosphericus FLU5	Lipopeptide (surfactin)		Lab scale	pH (2.1–12), temperature (4–121 °C), NaCl (0–120 g/L)	[129]
7	*Bacillus subtilis* ATCC 21,332	Lipopeptide (surfactin)		Lab scale	-	[130]
8	*Bacillus subtilis* CN2	Lipopeptide	Motor oil— ~ 82%	Lab scale	pH (5–12), temperature (25–125 °C), NaCl (5–20% W/v)	[131]
9	*Bacillus subtilis* RSL-2	Lipopeptide (surfactin)	Crude oil—72%	Lab scale	-	[132]
10	*Bacillus velezensis* KLP2016	Lipopeptide (surfactin)	Engine oil—52.3% to 65.7%	Field scale	-	[133]
11	*Brevibacterium casei*-4AB	Lipopeptide	Aromatic amine 4-Aminobiphenyl—80%	Lab scale	-	[134]
12	*Marinobacter hydrocarbonoclasticus* SdK644	Glycolipid		Field scale	-	[135]
13	*paracoccus* sp. MJ9	Glycolipid (rhamnolipid)	Diesel oil—81%	Lab scale	-	[136]
14	*Pseudomonas aeruginosa* NAPH6	Glycolipid	Naphthalene and aliphatic hydrocarbons— ~ 100%	Lab scale	pH (2.2–12), temperature (4–121 °C), NaCl (0–300 g/L)	[137]
15	*Pseudomonas aeruginosa* PAO1	Glycolipid (rhamnolipid)	-	Lab scale	-	[130]
16	*Pseudomonas aeruginosa* S5	Glycolipid	LMW and HMW PAHs -	Field scale	pH (3.5–9.5), NaCl (0–15%)	[138]
17	*Pseudomonas aeruginosa* UCP 0992	Glycolipid	Motor oil—90%	Field scale	–	[76]
18	*Pseudomonas sihuiensis* SNPLPB7	Glycolipid (di-rhamnolipids)	C8-C19 hydrocarbons— ~ 92.1%, C20—C33 hydrocarbons—42.4%	Field scale	–	[16]
19	*Pseudoxanthomonas* sp. PNK-04	Glycolipid (rhamnolipid)		Lab scale	-	[139]
20	*Rhizopus arrhizus* UCP 1607	Glycoprotein	Diesel oil -	Lab scale	pH (2–12), temperature (0–100 °C), NaCl (2–10%)	[92]
21	*Rhodococcus erythropolis* HX-2	-	Petroleum and PAHs -	Lab scale	pH (3–10), temperature (20–100 °C), NaCl (5–2- g/L)	[140]
22	*Serratia marcescens* ZCF25	Lipopeptide	PAHs and alkane – 64% and 65.57% respectively	Lab scale	pH (2–12), temperature (50–100 °C), NaCl (10–100 g/L)	[141]
23	*Staphylococcus pasteuri* CO100	Lipopeptide	Aliphatic hydrocarbons – 72%	Lab scale	pH (4.3–12), temperature (4–121 °C), NaCl (0–300 g/L)	[126]

Table 3 (continued)

#	Bacterial strain	Type of produced biosurfactant	Type of pollutant—Biodegradation efficiency	Lab scale/ field scale	Stability of produced BS under various conditions	Ref
24	*Stenotrophomonas* sp. S1VKR-26	Glycolipid (rhamnolipid)	Naphthalene, phenanthrene, fluoranthene, TPHs, pyrene and phenolic compounds – 93%, 86%, 92%, 72,33%, 98,3% and 93,06% respectively	Lab scale	-	[142]
25	*Streptomyces* sp. DPUA1566	Lipoprotein (bioelan)		Lab scale	pH (6–12)	[143]

marine sediments of Bohai Bay. The biosurfactant-producing bacterial consortium is recommended for the biodegradation of crude oil since this halotolerant and oil-degrading bacterial consortium reached 95.8% degradation capability of crude oil in 10 days. The mentioned bacterial consortium effectively degraded crude oil in a vast spectrum of salinities (0–120 g L^{-1}) and pHs (4–10) [145].

For the very first time in 2020 in Isfahan, Iran, *Achromobacter kerstersii* LMG3441 was identified as a glycolipid biosurfactant producer and hydrocarbon consumer. Results showed an extensive crude oil degradation capability (53%) of this strain. Besides, yeast strain *Rhodotorula muciloginosa* SKF2 produces sophorolipid biosurfactants and has the ability of crude oil degradation. This novel study suggests the use of these two mentioned strains as a microbial consortium due to their high potential for biosurfactant production and crude oil biodegradation [127].

7.2 Genome sequencing and genetic engineering of biosurfactant producers

Increasing progressions in genomic data and genome sequencing of microorganisms with the capability of hydrocarbon degradation and biosurfactant production, provide researchers opportunities to develop promising methods and approaches for the bioremediation of petroleum in the aquatic environments [146]. The use of microbial enzymes involved in the process of bioremediation such as oxidoreductase, lyases, peroxidases, hydrolase, dehalogenases, etc. is a secure, effective, and cost-effective bioremediation method. The new and promising genetic engineering methods such as clustered regularly interspaced short palindromic repeat—CRISPR associated proteins (CRISPR-Cas) technology give scientists a suitable approach to increase the production of enzymes and biosurfactants by transferring the coded genes of enzymes and biosurfactants into another microbial host with desired characteristics or knocking-out some impediment genes [98, 147–150]. Due to the significant advances in molecular biology and available data about DNA sequences of biosurfactant-producing microorganisms, it is achievable to overproduce recombinant strains with more efficient biosurfactant production and hydrocarbon utilization rather than wild-type strains which can be used in a bioremediation system and increase the rate of the petroleum hydrocarbon bioremediation [123]. Table 4 represent genomic data of some hydrocarbon utilizing and biosurfactant producing bacterial strains which have been completely sequenced in recent years.

To show the potential of genetic engineering of biosurfactant-producing strains for the development of a cost-effective bioremediation system, Wu et al., established a systematical genetic engineering approach in which 53 genes of *Bacillus subtilis* 168 were modified for the biosynthesis of surfactin biosurfactant. Five major steps have been taken in their systematical experiment to the goal of a rise in the surfactin titer. First, they resorted to the biosynthetic activity of the biosurfactant by the combination of the whole *sfp* gene into *Bacillus subtilis* 168. In the second step, they tried to decrease competition by deletion of 3.8% of the whole genome of the intended strain which was responsible for biofilm formation and pathways of polyketide synthase. The third step of the experiment includes potential self-resistance-associated protein overexpression which results in tolerance amelioration of the cell to the surfactin biosurfactant. In the fourth step, by the branched-chain fatty acid biosynthesis pathway engineering, they increased the precursor branched-chain fatty acids supply. In the last step, they improved the *srfA* transcription which resulted in the diversion of Acetyl-CoA from the process of cell growth to the biosynthesis of surfactin as a green microbial biosurfactant. What encodes surfactin biosynthesis in the *Bacillus subtilis* 168 is the *srfA* operon. In this experimentation, biosurfactant titer reached a maximum value of 12.8 g/l which indicated the great potential of genetic engineering methods and the principal role of genome sequencing for developing an optimized biosurfactant-base bioremediation system [151].

8 Prospective of a biosurfactant-based bioremediation system integrated with Biochar

Biochar (BC) is a stable solid, porous, carbonaceous material obtained from biomass via hydrothermal and thermochemical processes like pyrolysis or gasification [160–162]. BC can preserve the organoleptic properties of water and moreover can remove biological, chemical, and physical pollutant factors in the aquatic systems [164,165). Owing to the carbonaceous and porous structure of BC, this material can remove various pollutants including organics and inorganics via the process of biosorption. Additionally, BC by providing solid support as a sustainable source of nutrients for the growth of hydrocarbon degrader microorganisms can be used as an aquatic oil spill recovery method besides fortifying the hydrocarbon biodegradation process [164, 165]. Microorganisms that utilize petroleum hydrocarbons and, in some cases, produce biosurfactants, can also be immobilized on BC [166, 167]. In a study, crude oil sorption capacity of BC derived from peat (BP) and its oil removal efficiency examined and the results after contact of BP and crude oil in

Table 4 Genomic data of some hydrocarbon degrading and biosurfactant producing bacterial strains, which can be applied to produce recombinant strains, and can be useful in the process of bioremediation of petroleum hydrocarbons

#	Strain name/ references	Feature	Value/count	NCBI Gene Bank accession no
1	*Achromobacter* sp. HZ01 / [146]	Genome size (bp)	5,532,918	**LWKV00000000**
		G + C content (%)	68.1	
		Total genes	5,162	
		Gene length (bp)	5,108,407	
		Gene average length (bp)	990	
		Pseudogenes	31	
		rRNAs	4	
		tRNAs	54	
2	*Rhodococcus erythropolis* B7g / [152]	Genome size (bp)	7,175,690	**LQWU00000000**
		G + C content (%)	62.4	
		Protein coding genes	7,153	
		Gene average length (bp)	901	
		Coding percentage (%)	89.8	
		Pseudogenes	-	
		rRNAs	16	
		tRNAs	53	
3	*Bacillus* sp. AKBS9 / [153]	Genome size (bp)	1,330,614,215	**POYG00000000.1**
		Total genes	5,253	
		Gene average length (bp)	83,613	
4	*Acinetobacter* sp. AKBS16 / [153]	Genome size (bp)	1,175,940,239	**POYH00000000.1**
		Total genes	3,656	
		Gene average length (bp)	91,744	
5	*Bacillus subtilis* UMX-103 / [154]	Genome size (bp)	4,234,627	-
		Total genes	4,399	
		Protein coding genes	4,301	
		RNA genes	98	
		Genes involve in biosurfactant production	25	
6	*Bacillus aquimaris* SAMM MCC 3014 / [155]	Genome size (bp)	4,414,932	**MINN00000000.1**
		G + C content (%)	44.8	
		Total genes	4,370	
		Protein coding genes	4,247	
		RNA genes	123	
		rRNAs	32	
		tRNAs	86	
		ncRNAs	5	
		Pseudogenes	153	
7	*Acinetobacter indicus* UBT1 / [156]	Genome size (Mb)	2.97	**JABFOI000000000**
		G + C content (%)	45.9	
		Total genes	2,863	
		Total CDS	2,789	
		RNA genes	74	
		Complete rRNA	1, 1 (5S, 16S)	
		rRNAs	1, 1 (5S, 16S)	
		tRNAs	68	
		ncRNAs	4	
		Pseudogenes	68	
		CRISPR arrays	1	

Table 4 (continued)

#	Strain name/ references	Feature	Value/count	NCBI Gene Bank accession no
8	*Bacillus sp.* AM 13 / [157]	Genome size (bp)	3,734,657	**LKCP00000000.1**
		G + C content (%)	41.6	
		Total genes	3,791	
		Total CDS	3,481	
		Complete rRNAs	9, 4, 5 (5S, 16S, 23S)	
		Partial rRNAs	3, 3 (16S, 23S)	
		rRNAs	9, 7, 8 (5S, 16S, 23S)	
		tRNAs	84	
		ncRNAs	0	
		Pseudogenes	202	
9	*Planococcus maritimus* SAMP / [158]	Genome size (bp)	3,216,408	**MINM00000000**
		G + C content (%)	47.2	
		Total genes	3,234	
		Total CDS	3,141	
		RNA genes	93	
		rRNAs	8, 8, 10 (5S, 16S, 23S)	
		Complete rRNAs	8, 1 (5S, 23S)	
		Partial rRNAs	8, 9 (16S, 23S)	
		tRNAs	63	
		ncRNAs	4	
		Pseudogenes	30	
10	*Halomonas desertis* G11 / [159]	Genome size (bp)	3,963,288	**LYXG00000000**
		G + C content (%)	57.82	
		Protein coding genes	3,639	
		RNA genes	76	
		rRNAs	8	
		tRNAs	58	
		DNA scaffolds	44	
11	*Bacillus cereus* NWUAB01 / [104]	Genome size (bp)	5,989,415	**QNGD00000000.3**
		G + C content (%)	35.01	
		Total genes	6,306	
		Total CDS	6,191	
		RNA genes	115	
		rRNAs	11, 4, 8 (5S, 16S, 23S)	
		Complete rRNAs	7 (5S)	
		Partial rRNAs	4, 4, 8 (5S, 16S, 23S)	
		tRNAs	87	
		ncRNAs	5	
		Pseudogenes	280	

70 min at 45 °C respectively demonstrate 32.5 g of crude oil per 1 g of adsorbent material and 91.2% which means BP can be used for cleaning up oil spills in aquatic environments [168]. In an experiment, rhizospheric microorganisms in BC-amended soil were isolated to screening biosurfactant production. *Psuedomonas* and *Bacillus* spp. were the major isolates. Biosurfactant derived from *Psuedomonas putida* in turn showed the potential of hydrocarbon degradation over 10 days [161]. Wei et al. investigated the merged application of BC, rhamnolipid biosurfactant, and nitrogen on remediation of petroleum hydrocarbons in a microcosm study where wetland soils were artificially contaminated with crude oil. In the study, BC + rhamnolipid biosurfactant, BC + nitrogen, and BC + rhamnolipid biosurfactant + nitrogen respectively

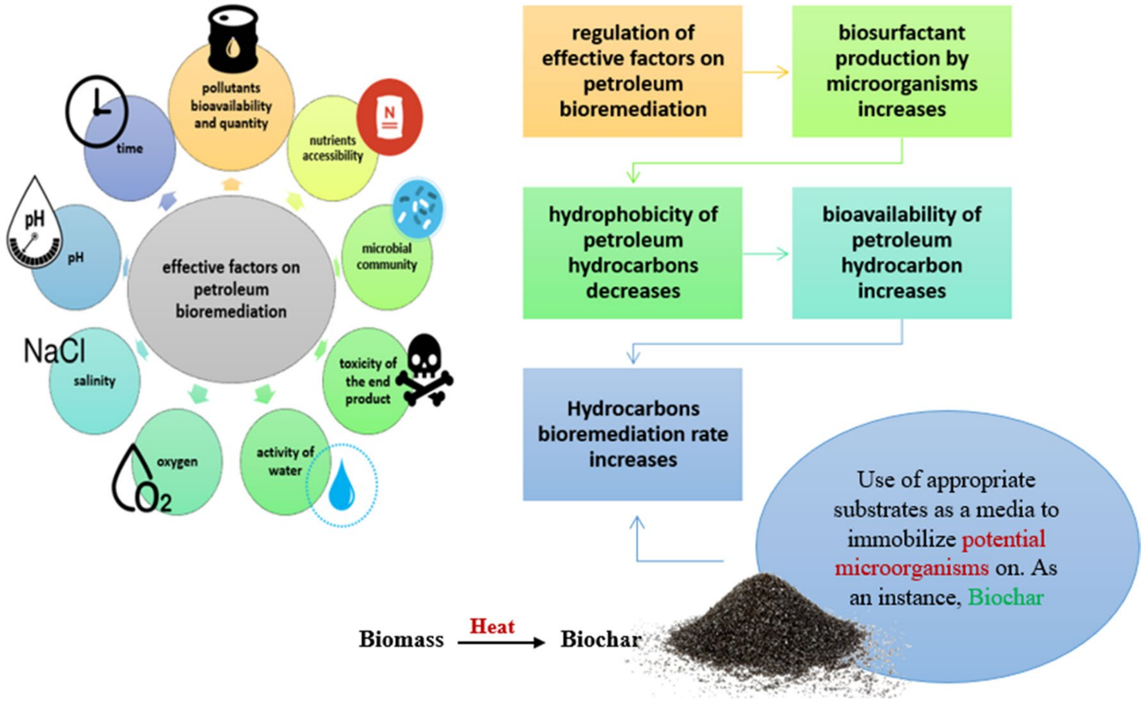
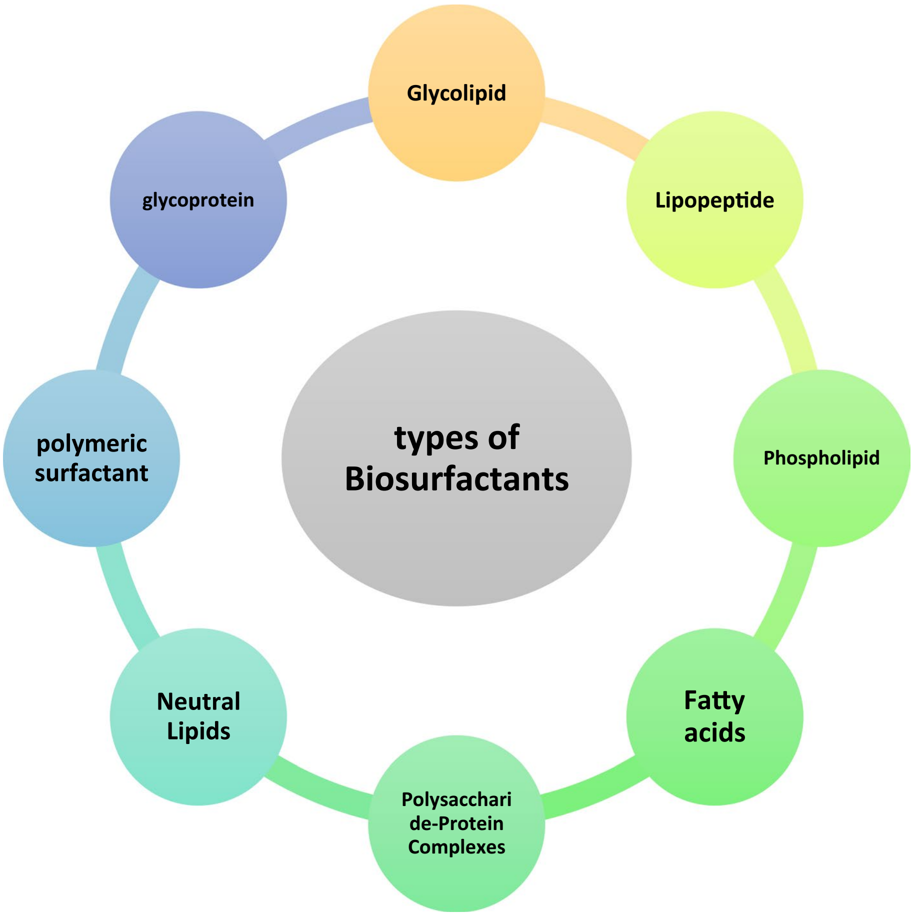


Fig. 1 Diagram of the optimization process of petroleum hydrocarbon bioremediation via regulation of effective factors in water

Fig. 2 classification of biosurfactants



decreased 32.3%, 73.2%, and 80.9% of total petroleum hydrocarbons (TPHs). BC improved adsorption of aromatic compounds and rhamnolipid biosurfactant plus nitrogen increased remediation rate of heavy and light aliphatic compounds and decreased diversity of the microbial community and respectively shifted it to the greater abundance of *Proteobacteria and Bacteroidetes*. Results show that plants can tolerate an extra amount of rhamnolipids in association with BC and this association leads to amelioration of petroleum remediation rate [169, 170]. The combination of BC and rhamnolipids can accelerate the rate of petroleum hydrocarbons bioremediation in support of phytoremediation [171]. Magnetic BC is also potent for adsorption and removal of contaminants including petroleum hydrocarbons in aqueous media and in recent years widely used [172, 173]. Since hybridization of different bioremediation approaches may lead to an optimized and promising bioremediation method and based on former studies, this paper suggests a new bioremediation system integrated with a potential BC as a nutrient source for microorganisms to thrive on, crude oil adsorbent, and a material that potential microorganisms can immobilize on and calls it "potential-microorganisms-immobilized-on-biochar system" (PMIBC system) which its theory elucidated in the following figure by the authors (Fig. 3) [174]. This system can be categorized as a microbial enhanced oil recovery (MEOR) method integrating with biosurfactant in which biosurfactant production will be in-situ and make the bioremediation process more cost-effective compared to the ex-situ production of biosurfactants. Using PMIBC system saves biosurfactant production, purification, and transportation expenses and make bioremediation of hydrocarbons including petroleum hydrocarbons more economical and can be used in the decontamination of groundwater, lakes, marshes, etc. [163, 175].

9 Conclusion

Biosurfactants have various industrial applications like in medicine, food, cosmetics, agriculture, bioremediation, and they are more biodegradable, less toxic, and in some cases more effective rather than their chemical counterparts, still the gap between the final cost of biosurfactants and chemical surfactants generation is considerable, as the statistics demonstrate the final production cost of biosurfactants is approximately 12 times higher than chemical surfactants. To overcome this enormous gap to the goal of popularizing the use of biosurfactants in the bioremediation of petroleum in various environments such as aquatic environments, researchers must develop new and effective biosurfactant-based bioremediation systems and solutions to achieve a cost-effective bioremediation method. As the best bioremediation method is the in-situ bioremediation and the most effective and productive biosurfactant producers are microorganisms,

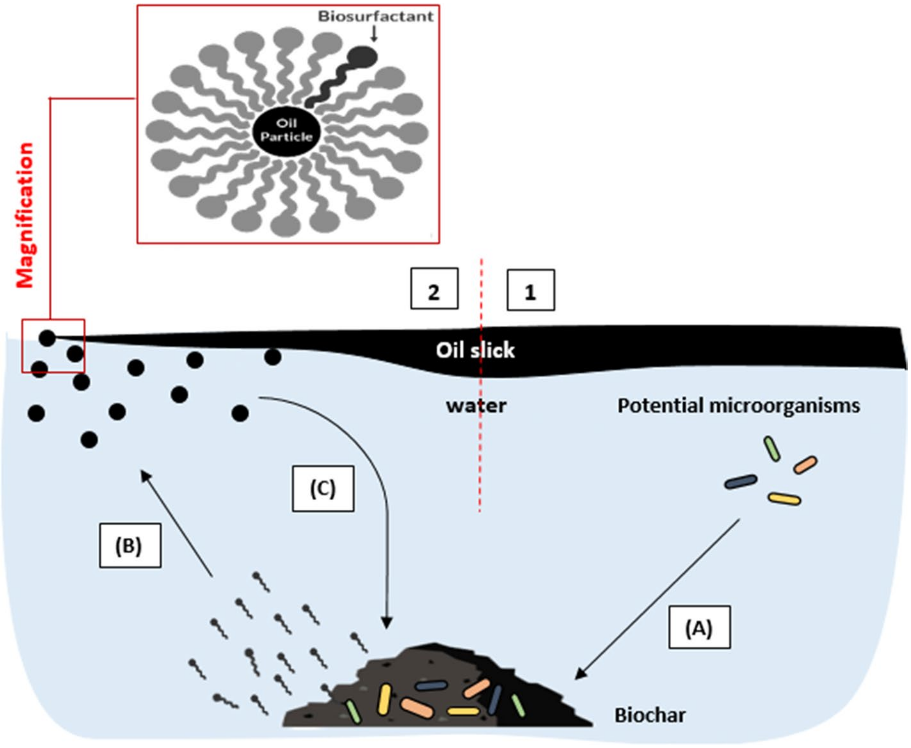


Fig. 3 Illustration of PMIBC system mainstream: **A**: immobilization of potential microorganisms on BC as a sustainable source of energy and porous media, **B** biosurfactants produced by potential microorganisms transform large oil slick to oil-microdroplets and make it more bioavailable for potential microorganisms (emulsification), **C**: BC absorbs oil-microdroplets and potential microorganisms utilize these bioavailable petroleum hydrocarbons which lead to the greater growth of potential microorganisms. This cycle between steps B and C remains until the bioremediation and biodegradation rate of the intended polluted environment reaches a satisfying level. (1): oil slick before the effect of PMIBC system, (2): oil slick after the effect of PMIBC system

the ideal biosurfactant-based bioremediation systems are in-situ and dependent upon microorganisms' activity with an appropriate and stable source of energy, which, as an example, this article based on former studies suggested PMIBC system. Hence, this article deduces that the most potent microorganisms in bioremediation of petroleum, which can be used in bioremediation systems, are biosurfactant producing and hydrocarbon utilizing microorganisms that the article listed as "potential microorganisms". Since an increase in biosurfactant production will lead to an increase in the rate of petroleum hydrocarbon bioremediation, progression in the development of the mentioned bioremediation systems requires further studies based on earlier data about potential microorganisms. Also, great headways in molecular biology, genetic engineering like CRISPR-Cas systems, bioinformatics, and big data about the genome sequence of potential microorganisms and their enzymatic pathways connected to petroleum degradation and biosurfactant production, promise researchers to overproduce recombinant strains more efficient rather than wild-type strains to use them in their designed biosurfactant-based bioremediation systems.

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Declarations

Competing interests The authors declare no competing interests.

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