Use of microorganisms to subvert oil spills and their implications for animal and human health

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REVIEW

ABSTRACT

Bioremediation is the process by which toxic compounds are removed from the environment by using microorganisms. Cleaning up of oil spills in soil and water environments require particular bioremediation strategies. Biotechnology and genetic engineering techniques, used to confer a set of genes, has also proven to be useful to increase degradation rates by existing microorganisms. The microorganism-mediated seeding process begins with the isolation of oil-degrading strains from old oil spills, which are further subjected to multiple tests to assess their degradation capacity. In spite of oil spills being primarily dominated by γ-proteobacteria, in particular Pseudomonadales, bacteria, yeast, and fungi are all potential oil degrading microorganisms. Therefore, the present review is aimed at exploring the bioremediation capacity of a number of strains isolated as potential oil degrading microorganism, along with their implications for human and animal health.

Keywords: oil spills, bioremediation, biotechnology, oil-degrading microorganisms, biodegradación de petróleo, ingeniería genética, *Pseudomonas*

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RESUMEN

El uso de microorganismos para eliminar los derrames de petróleo y sus implicaciones para la salud animal y humana. La bioremediación es el proceso mediante el cual se eliminan compuestos tóxicos del medioambiente con el uso de microorganismos. Debido a sus efectos contaminantes, los derrames de petróleo en suelos y en ambientes acuíferos requieren estrategias de bioremediación específicas. Notablemente, la biotecnología y las técnicas de ingeniería genética han sido útiles para incrementar los procesos de biodegradación mediados por los microorganismos y para incorporarles a estos genes necesarios para determinados procesos de biodegradación. El proceso de biorremediación comienza con el aislamiento de cepas de micoorganismos capaces de biodegradar el crudo y sus derivados, a partir de derrames antiguos, y continua con la evaluación de la capacidad biodegradativa de estos y su posibilidad de potenciación. A pesar de que las γ-proteobacteria predominan entre las poblaciones microbianas identificadas en los derrames de petróleo, en especial las Pseudomonadales, las bacterias, hongos y levaduras muestran gran potencialidad. Por tales razones, en el presente artículo de revisión se aborda la capacidad biorremediadora de diversas cepas microbianas con potencial para tales propósitos, así como sus posibles implicaciones para la salud humana y animal.

Palabras clave: derrames de petróleo, biorremediación, biotecnología, microorganismos degradadores de petróleo, biodegradación de petróleo, ingeniería genética, *Pseudomonas*

*I*ntroduction

Bioremediation is the process by which contaminating material is removed from a medium (soil, surface water, or groundwater), preferentially using living microorganisms to degrade toxic compounds into innocuous ones. One of the most serious sources of adverse effects in human health addressed by bioremediation strategies is environmental oil contamination [1-3].

Oil spills are dangerous for a number of reasons: flammability, explosive vapours, toxicity, hydrogen sulphide, and oxygen exclusion. Explosive vapours following oil spills can be displaced by air to nearby cities where they can be easily ignite; emission of hydrogen sulphide gas can affect the airways of residents near spills. In humans, the effects of oil translate into inhibition of protein synthesis and nerve synapse function, impairment of plasma membrane and interruption of membrane transport systems; in fact, hydrocarbons can damage DNA leading to mutagenesis, carcinogenesis, and decreased reproductive capacity [3]. In the case of animal health, oil stains on the feathers of birds can decrease insulation, and result in death during the winter; oil stains on plants results in decreased metabolism, and suffocation [4]; fish can suffocate when their gills are covered with oil [5].

Typically, contaminated media are usually relocated to a landfill, capped, or contained; incineration and chemical degradation of toxic compounds are considered as well. In this case, bioremediation is a safer alternative, in spite of it being slower. Bioremediation is cheap, a powerful reason to use it as an alternative to physical methods, since physical washing was found to cost approximately $1 million per day, and the rates of natural biodegradation can vary from 0.03 to 50 g/m^3•day [6]. Particularly, oil is composed of alkanes, branched alkanes, cycloalkanes, olefins, monoaromatics, polyaromatics and phenols [3]. Biodegradation potential decreases with increasing molecular complexity; in other words, alkanes are

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easily degraded while complex molecules remain undegraded for a longer period of time [6]; the reported order of degradation is as follows: C15 > C20 > (Pristane, Phytane) > C25 > C30 > C35 > ploy aromatic hydrocarbons (PAHs: fluorine, dibenzothiothene, phenanthrene and chrysene) [7].

Another limitation of bioremediation is that it is not applicable to every toxic compound (e.g.: cadmium, and lead), and may leave residuals. Most compounds which are naturally present in the environment are degraded by a microorganism for it which would have evolved to tackle it. Petroleum, for instance, is a naturally occurring compound, and as such, there exists microorganisms capable of degrading it [2]. Furthermore, even when microorganisms can degrade synthetic compounds, not all compounds are potential targets for microorganisms. Synthetic compounds, in most cases, require biotechnological strategies to be applied, to enable or bestow particular microorganisms with the ability to degrade them. In general, microorganisms are sensitive to temperature, pH, nutrients, soil structure, and moisture, and under non-optimal conditions, degradation rates are lowered due to decreased microbial growth rates [2]. Similarly, biodegradation is dependent upon abiotic factors (oxygen, phosphate, nitrogen). However, the safety concerns to human, animal, and plant health from using said microorganisms have not thus far been addressed.

In this work, the potential effects of using said microorganisms in subverting oil spills is examined, not arguing against bioaugmentation but rather to discuss on the selection of safe microorganisms for seeding, genetic alteration, and/or strain improvements, which would be more appropriate for the purpose of bioremediation.

Discovery of target-compound degrading microorganisms for use in bioremediation

A large number of microorganisms isolated in oil as potential agents for bioremediation were found to be opportunistic human pathogens. As such, in all cases, the massive growth of microorganisms can be harmful to humans, animals, or plants. This implies that selection of safe microorganisms would be advisable. Furthermore, non-addition of microorganisms to oil, or not resorting to bioremediation, is not a solution either. In fact, oil spills are the perfect microenvironment for the propagation of microorganisms such as *Pseudomonas putida*, where it grows rapidly. The latter also implies that with time, the density of said microorganisms increases and causes damage. The addition of more microorganisms, or even the promotion of their population density by increasing the surface area of the spill to make it more accessible to microorganisms involved in bioremediation, increases the chances of damage to the ecosystem. These given that the presence of the microorganism is accompanied by health effects to organisms. The argument is not against the use of microorganisms for bioremediation but rather the selection of a safe organism which might be beneficial when all aspects are considered (economy, health and others) or at least with the safest bioremediation/potential harm balance.

Nearly 79 bacterial, 9 cyanobacterial, 103 fungal and 14 algal genera have been characterized as having the ability to degrade hydrocarbons. For the process of bioremediation, microorganisms can be selected from various sources (including extremophiles). Identification of microorganisms that are capable of degrading the target compound begins with the collection of microorganisms from various sources such as old oil spills, and standard culture tests to determine whether the target compound can be utilized by the microorganisms [8, 9].

Once a potential microorganism is identified, it is subjected to various tests to assess optimal growth conditions (pH, temperature, salinity and others), and to determine the microorganism's ability to degrade the target compound as well as other compounds of interest [8]. The growth rate of said organisms is also measured [10-12]. Since classical techniques are time consuming, novel molecular methods have been applied to achieve the same goal. DNA probes, generated by examining the genotype of the microorganisms that are capable of degrading target compounds, are used to identify other microorganisms with the genetic profile required to degrade those or similar compounds [13, 14]. The extraction of DNA from a particular microorganism followed by PCR amplification of a particular gene, and identification with particular probes can speed up the process [13-15].

A few of the noted strains that can degrade oil are: *Pseudomonas* strains, *Yokenella* sp., *Alcaligenes* sp., *Roseomonas* sp., *Stenotrophomonas* sp., *Acinetobacter* sp., *Flavobacter* sp., *Corynebacterium* sp., *Streptococcus* sp., *Providencia* sp., *Sphingobacterium* sp., *Capnocytophaga* sp., *Moraxella* sp., *Bacillus* sp., and *Enterobacter* sp. [4]. Moreover, a number of microorganisms found in oil spills are harmful to humans, animals, and/or plants (Table). Although the harmful effect of oil is immediate, microorganisms are contagious and can remain in the flora for longer periods of time, potentially resulting in bacterial or fungal infections and producing long lasting symptoms. A closer look at the microorganisms used for bioremediation and their potential health risks are presented below.

Candida sp.

Candida tropicalis, a human pathogen, can cause symptoms similar to those by *C. albicans* [45]. Infections caused by *C. albicans* in immunocompromised patients can trigger an immediate overgrowth of *C. albicans* fungal cells which can lead to a myriad of unspecific symptoms such as panic attacks, poor concentration, brain fog, memory loss, diarrhea/constipation, abdominal cramps, irritable bowel syndrome, heart burn, extreme lethargy/fatigue, bad breath, eye fatigue, headaches, premenstrual syndrome, poor libido, frequent yeast infections, and mood swings amongst others; all these ultimately leading to death. Further, immunocompromised adults are not the only possible victims, babies, especially those premature with an incompletely developed immune system, could be potential ones. Current drugs used to treat infection by *C. albicans* face the issue of resistance, this microorganism can evolve to become resistant to currently available drugs.

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Table. Microorganisms used to degrade oil and their potential pathogenic effects on humans, animals or plants

Oil degrading microorganism [source]	Safety status for humans	Symptoms [source]
Pseudomonas aeruginosa [16-20]	Opportunistic human, animal and plant pathogen	Location dependent but usually involve high fever, chills, confusion, shock, pus, ear aches, can be fatal, pneumonia, enteritis, vaginitis, mastitis, necrotizing bronchopneumonia [21]
Pseudomonas stutzeri [22, 23]	Opportunistic human pathogen	Location dependent but usually involve high fever, chills, confusion, shock, pus, ear aches, can be fatal, pneumonia, enteritis, vaginitis, mastitis, necrotizing bronchopneumonia [24]
Pseudomonas putida [19, 23, 26]	Fish pathogen, opportunistic human pathogen	Dorsal ulceration of rainbow trouts, bacteremia, sepsis, urinary tract infections in humans [21]
Bacillus thuringiensis, Bacillus cereus [9, 27]	Human pathogen	Food poisoning [28, 29]
*Candida tropicalis** [30]	Opportunistic human pathogen	Skin lesions, polyarthralgias, polymyalgias, loss of renal function [31]
Acinetobacter calcoaceticus, Acinetobacter johnsonii [32, 33]	Human pathogen	Pneumonia, bronchopneumonia, high mortality rate, tracheobronchitis, urinary tract infections, skin infection, fatal cellulitis, septicaemia, pancytopenia [34]
Pseudomonas stutzeri [35]	Opportunistic human pathogen	Location dependent but usually involve high fever, chills, confusion, shock, pus, ear aches, can be fatal, pneumonia, enteritis, vaginitis, mastitis, necrotizing bronchopneumonia [24]
Aspergillus fumigatus [36]	Human and animal pathogen	Invasive aspergillosis, allergic bronchopulmonary, aspergillosis, rhinitis, Farmers's lung, cutaneous infections, otomycosis, tracheobronchitis, aspergilloma, osteomyelitis, invasive pulmonary, extrapulmonary infections [37]. Produces gliotoxin, an immunosuppressant
Penicillium notatum [38]	Opportunistic human pathogen	Invasive pulmonary mycosis [39]
Rhizopus stolonifer [40]	Plant pathogen	*Rhizopus* soft rot [41]
Aeromonas hydrophila [19]	Opportunistic human, and fish pathogen	Hemolytic syndrome, kidney disease, cellulitis, wound, soft-tissue infection, meningitis, bacteremia, septicaemia, ocular infections, pneumonia, respiratory tract infections, urinary tract infection, osteomyelitis, peritonitis, acute cholecystitis [42]
Acinetobacter baumannii [43]	Opportunistic pathogen	Pneumonia, blood infection, meningitis, urinary tract infection, skin or wound infection
Corynebacterium aquaticum [20]	Human pathogen	Multiple infections [44]
Pseudomonas mallei [16]	Human and animal pathogen	Cough, skin abscesses, fever, chills, prostration, death [45]
Rhodococcus equi [46]	Opportunistic animal and human pathogen	Fever, cough, malaise, chest pain, dyspnea, hemoptysis, weight loss, lymphadenopathy, eye drainage, pain, joint pain, altered level of consciousness, bloody diarrhea [47]

* Pathogenicity reported for strains different from the one having oil degrading activity.

Pseudomonas sp.

In the case of *Pseudomonas*, it is a major fish pathogen causing ulcerative syndrome, bacteria haemorrhagic septicaemia, tail and fin rot, bacteria gill rot, and dropsy. Infection requires contact and attachment with dermal tissue of fish. In fact, major economic losses, and decrease in quality of fish results from infections by *Pseudomonas*. *P. aeruginosa* (bacterial dosage LD_{50}: 2.4×10^8 c.f.u./mL), injected into *Oreochromis niloticus* was found to be highly virulent, resulting in 70-95 % mortality within 48 h [46]. Further, infections of *P. aeruginosa* and *Aeromonas* resulted in the following symptoms: darkness of skin, scale detachment, hemorrhages on body, necrotizing ulcers on skin, fin necrosis, inflamed vent, exophthalmia, blindness, and eye cataract/trachoma [46].

This is relevant since the most common microorganism found growing in oil spills is *Pseudomonas putida*, a major opportunistic human pathogen, which is a common pathogen rainbrow trout, Oncorhynchus mykiss. In experimental settings, *P. putida* was shown to cause dermal ulceration, and necrosis of muscles of the rainbrow trout [21]. The ulceration was quite severe, resulting in kidney swelling and 35 % mortality [21]. In another experiment, 20 % of rainbow trouts injected with *P. putida* (5×10^5 cells) died; the injected fish displaying the following symptoms: dark skin pigmentation, pale gills and liver, erosion of pectoral and caudal fins, skin haemorrhages and ulcers [47]. Moreover, rainbow trout infected with *P. putida* was found to have lower levels of erythrocytes, and hemoglobin and much higher levels of mean corpuscular volume and hemoglobin (MCV and MCH, respectively) [47].

P. aeruginosa is commonly found in soil and water, and requires a minimal nutritional load for growth and propagation; it can propagate in at least seventy-five compounds and can grow under multiple conditions [48]. It can even grow in distilled water and even better in poor quality water, and it is recognized as hazardous since contamination of distilled water with *P. aeruginosa* is not visible [49, 50]. In theory, this implies that *P. aeruginosa* can survive in water once the oil spill has been cleaned. Therefore, it is plausible that infection and their accompanying diseases could occur, particularly in immunocompromised patients when open wounds are present [49, 50]. Moreover, only three antibiotics are effective against *P. aeruginosa*,

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making its containment quite difficult. Among its related symptoms in humans [51] are:

General manifestations: bacteremia, sepsis, febrile neutropenia, bone/joint infection (i.e., osteochondritis, osteomyelitis, pyarthrosis.

Skin and tissue infections: burn wound sepsis, dermatitis, ecthyma gangrenosum, pyoderma, cellulitis, hot tub folliculitis, necrotizing fasciitis, chronic paronychia.

Central nervous system infections: brain abscess, meningitis.

Ear infections: otitis media, chronic suppurative otitis media, otitis externa, malignant external otitis.

Eye infections: endophthalmitis, keratitis, ophthalmia neonatorum, blepharoconjunctivitis, scleral abscess, orbital cellulitis, corneal ulcers in humans, and treatments rarely aid in restoring vision.

Gastrointestinal infections: epidemic diarrhea, necrotizing enterocolitis, typhlitis, rectal abscess, Shanghai fever.

Genitourinary infections: epididymitis, prostatitis, urethritis.

*Cardiovascular infection*s: endocarditis, pericarditis, cardiac tamponade.

Respiratory infections: primary or nonbacteremic, bacteremic, colonization, and nosocomial pneumonia, lower respiratory tract infections of cystic fibrosis, ventilator-associated pneumonia.

Pseudomonas also causes major economic losses on the giant freshwater prawn *Macrobrachium rosenbergii*, causing degeneration of the body muscle and the hepatopancreas. This has been observed 96 hours post-inoculation with *P. aeruginosa* MTCC 1688, with tissue destruction and multiple lesions also present.

Aspergillus sp.

Aspergillus fumigatus, *Aspergillus flavus*, and *Aspergillus niger* are potential bioremediation agents. Particularly, *A. fumigatus* can produce aflatoxins and cause aspergillosis. This can result in the dispersion of the microorganism's spores during its growth and sporulation phase and subsequent inhalation by animals such as chickens (commercial poultry) [52]; it is allergenic to humans to say the least. In fact, *A. fumigatus* is a harsh respiratory pathogen in birds, with high mortality rates among young animals and chronic infections in adults. This microorganism also secretes a gliotoxin which functions as an immunosuppressive agent, making it problematic when coinfections are present. Currently, there are no treatments available for aspergillosis in humans [53].

Moraxellaceae sp.

Moraxellaceae is another microorganism considered for bioremediation. Data on its potential harmful effects comes mostly from experimental settings and case reports. The injection of *Moraxellaceae bovis* into cattle has resulted in rapid shallow breathing mydriases, increased serous lacrimination, mouth frothing, hacking cough, micturition, blood in nasal and oral area; subsequent injection of epinephrine and antihistamines induced a moribund state and ultimately death [46]. Injection of *M. bovis* in mice led to lethargy, diarrhea, moribundity, and death [46]. *M. bovis* can also induce keratoconjuntavitis in cattle, and mice. *M. bovis* does not require a compromised immune system for appearance of clinical symptoms. Another species, *Moraxella catarrhalis* can cause acute otitis media, chronic or serious otitis media, acute/chronic sinusitis, upper/lower respiratory tract infections/systemic infections, meningitis, bacteraemia, endocarditis, keratitis, and suppurative arthritis [54]. Other species have been reported as causing conjunctivitis, keratitis, meningitis, arthiritis, endocarditis, osteomyelitis, as well as upper/lower respiratory tract and otolaryngologic infections [54].

Acinetobacter sp.

Acinetobacter baumannii is another bacterium affecting healthy animals, is contagious and preferentially affects the blood, brain or lungs. *A. baumannii* infections can cause pneumonia, blood infection, meningitis, and urinary tract, skin or wound infections [55].

Aeromonas sp.

Aeromonas hydrophila has been shown to affect the loach (*Misgurnus anguillicaudatus*) and *Aeromonas salmonicida* infects the Brook trout (*Salvelinus fontinalis*), the Rainbow trout (*Oncorhynchus mykiss*) and the Atlantic salmon (*Salmo salar*), resulting in hemorrhagic septicemia, tail and fin rot, red-fin disease and fuminculosis in the three species [56].

Approaches for creating novel proteins to improve microorganisms for bioremediation

Given that bioaugmentation has been noted to be less effective than biostimulation, the goal behind strain improvement would be to generate 'improved' versions of the original oil-degrading microorganisms. For that purpose, protein engineering aimed at generating new proteins or modified versions of the existing ones is a promising alternative to enhance the biodegradative capacity of the available strains, its practical application still remain to be proven. Briefly, a number of techniques will be summarized, that could be applied to generate such modifications.

Common approaches to creating novel proteins are based on the generation of multiple potential novel genetic codes followed by directed evolution or selective pressure. In situ directed mutagenesis, a technique using a primer complementary to the template DNA to create a single mismatch on it at the desired location, was one of the former techniques used for generating proteins with modified structure and function. In fact, primers can be designed in such a manner to permit insertions, deletions, or mismatches [57]. Other methods, such as polymerase chain reaction (PCR) amplification to generate mutant segments, DNA shuffling (also known as in vitro recombination) used to swap segments between two homologous genes can also being used.

Typically, PCR amplification and random DNA ligation follows cleaving of the two homologous genes with DNase resulting in the generation of multiple novel potential enzymes which can be introduced into *Escherichia coli* for functional screening [58, 59].

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An alternative to DNA shuffling is in vitro random priming. In DNA shuffling, DNAse I is used to generate short segments while in in vitro random priming, PCR amplification of multiple sections is achieved using random hexanucleotide primers to generate multiple short segments of DNA from two genes. Subsequently, the parental templates are removed, and the daughter templates are denatured, re-annealed, and extended. Repetition of PCR cycles (both annealing, and extension) results in the generation of novel full length genes. In this method, both single and double stranded parental DNA molecules can be used, and extension of segments as short as 200 nucleotides can occur. Errors that occur during priming and their perpetuation further increase the diversity of final novel genes [60].

Another technique named StEP (Staggered Extension Process), uses two homologous parental DNA strands which are primed (one primer) and extended. Since the annealing-extension process is allowed to proceed only for a very short period of time, the extended segments can bind either template, a process known as template switching. The process ends when a novel gene is created [61].

In Random chimeragenesis on transient templates (RACHITT), begins with digestion of single stranded genes with DNAse I, and size fractionation. The latter is followed by segmental hybridization onto a scaffold. Strands overhangs are excised and gaps are filled in. Subsequently, the template is digested, and the created partner strand is amplified using PCR [62].

Lastly, another method known as directed evolution relies on Darwinian evolution in test tubes. The first step consists on generating a variety of codes which can be accomplished by any of the above mentioned methods. During the second step, a selection pressure is placed on the created diversity to select the most successful variants [61].

Novel proteins, generated by using the abovementioned methods could confer or enhance the bioremediation capacity of oil-degrading microorganisms. Under selective pressure, strains that outperform parental or initial strains can be further selected and engineered for a particular task, such as oil degradation. An alternative could be the application of a selective pressure over the existing oil-degrading bacterial strains to permit evolution and response to the selective pressure for the purpose of bioremediation. Those that are unfit will be a minimal population and the majority will be the evolved version of the existing strains; this method is an alternative to genetically modified organisms (GMOs). This particular strategy could replace the need for any type of genetic alteration, and as such would be adequate for generating improved strains using an ecosystem-friendly method without the ethical and biosafety concerns that govern GMOs. In either case, it is advisable that the potential impact on animal and human health be carefully considered and tested.

Oil spills in the ocean

Oil spills in watery environments, particularly in the open sea and oceanic waters results in the formation of a floating film; heavy components, on the other hand, will sink [63]. These components settle down in the sediment where it can affect the fish and the ocean bed ecosystem [63]. Further, the floating oil layer acts as a barrier between water and air, lowering oxygen inflow to the water basin that further suffocates living organisms. Particularly, long-term exposure to petroleum oil and PAHs can damage the liver, kidney, and bone marrow or even cause cancer [9] in humans. Typically, oil spills affects both land and water.

The environment onto which the oil is spilled is also a deciding factor. Spills into flowing water are less severe due to the currents permitting circulation [5]. Evaporation, oxidation, and biodegradation are three natural processes that are involved in the clean-up of oil spills. In fact, 50 % of the light weight oil components evaporate within the first 12 hours after a spill [5]. Furthermore, the light weight components are the most toxic components in a spill, and as such, the toxicity of a spill decreases with time.

Oil is ofttimes accidentally released into the ocean, as an agglomeration of multiple compounds: alkanes, cycloalkanes and aromatics. Its fate depends on the medium onto which it ends: a spill of one gram of oil into water will result in its dispersion and coverage of an area between 1 to 10 m^2, while on land, it will move vertically, penetrating the different ground layers until it reaches groundwater (see below) [64]. For land spills and spills near the shore, physical removal becomes the method of choice. Therefore, in water, dispersion of oil is the preferable method for containment; however, bioremediation strategies are also an option.

Clean-up strategies usually involve sorbents, vacuuming, low-pressure flushing, the removal of vegetation or monitoring the natural cleaning process. Another method of decontamination involves the use of dispersants to disaggregate the spill, and therefore, increase the degradation area to microorganisms. Thereafter, the scientific community realized that oil, much like any compound, is degradable but that it lacks the necessary allies to allow uptake by microorganisms; compounds were generated that could adhere to oil to fertilize the process and further accelerates degradation [65].

Two methods have been developed to deal with oil spills: addition of fertilizers (biostimulation) or oil degrading microbes (bioaugmentation) [66]. Biostimulation implies providing nutrients necessary for microbial growth to microorganisms in oil-spills, microorganisms which are not necessarily innocuous to humans, animals or plants. Hence, the massive growth of microorganisms using fertilizers (biostimulation) would be equivalent to bioaugmentation. The addition of fertilizers to increase hydrocarbon degradation has been shown to be effective [67].

A second solution that is less effective involves the addition of microorganisms (seeding) [65, 68]. Another study noted that the addition of nutrients without indigenous microorganisms (ULR: seawater + oil supplemented with uric acid, lecithin and biosurfactant (rhamnolipids)) had a higher rate of alkane degradation than when combined with microorganisms (NPKM: seawater + oil supplemented with KNO_3, K_2HPO_4 and pre-adapted indigenous cultures), the alkane degradation rates were significantly lower for NPKM than ULR [7]. Addition of biosurfactant (NPKMR: seawater + oil supplemented with KNO_3, K_2HPO_4, biosurfactant (rhamnolipids) and pre-adapted

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indigenous cultures) maximized degradation rates of alkanes. PAHs, however, were found to be degraded at high rates in the ULR treatment, and at much lower rates in the NPKM treatment [7]. Effectiveness of seeding was shown to depend on the growth rates of seed microorganisms, their response to the physicochemical environment and their competition with native microbes. If bioaugmentation is less effective, then the risk associated to health concerns might supersede the process.

It was thought that the solution to this problem would be biotechnology and GMOs. Though many have isolated a number of microorganisms that have the ability to degrade oil, it appears that safety concerns have not been addressed. Hence, the adverse long-term health effects that microorganisms can have on human health when used for bioremediation have been overlooked for the immediate disastrous effects of spills. The assumption in most cases is to increase the abundance of said bacterial population to remove contaminants. Nevertheless most of the microorganisms used could have significant pernicious effects in humans depending on the given scenario. Therefore, it would be advisable to carefully reconsider the intentional growth of such miasmatic microorganisms case by case; also, inactive microorganisms have not been shown to be an effective solution as demonstrated by using killed *Pseudomonas* [16].

Land oil spills

Freshwater and marine shorelines are the natural ecosystem to many animal species, including elephant seals, sea lions and salmons, among many others which can be affected by spills on land. Light weight oil such as alkanes can evaporate and are effortlessly degraded; light weight oils are rarely found near shorelines. Heavy weight oil, on the other hand, can be labyrinthic since it can form mousses, tar balls, and asphalts which are extremely difficult to eradicate. Petroleum hydrocarbons, when spilled on land, travels vertically towards the groundwater, it may bind soil particles, further altering the properties and composition of soil [63]. Oil that is exposed to sunlight or waves is typically degraded at a faster pace. However, oil that flows vertically into the ground is harder to degrade and can remain there for longer periods.

Natural biodegradation of oil spilled onto shorelines can take numerous years. On land, the following genus have been isolated as oil-degrading microorganisms: *Pseudomonas*, *Bacillus*, *Serratia*, *Rhodococcus*, *Ralstonia*, *Cyanobacterium*, *Micrococcus*, *Proteus*, *Acinetobacter*, *Mycobacterium*, *Arthrobacter*, *Flavobacterium*, *Moraxella*, *Corynebacterium*, *Pleurotus*, *Candida*, *Eisenia*, *Allolobophora*, *Lumbricus*, *Phanerochaete*, *Coriolus*, *Bacillus*, *Arthrobacter*, *Clavibacter*, *Corynebacterium*, *Nocardia*, *Axoarcus*, *Geobacter*, and *Dechloromonas* (reviewed in [69]). Microorganisms should be halophilic and temperature resistant to be functional near the shore or in the ocean. Such bacteria are typically isolated from previously contaminated areas (i.e., an oil spill that occurred 10 years ago) [70].

A number of microorganisms have been isolated for bioremediation in land spills [9]. In one experiment, where an oil spill was simulated and microorganisms present were classified, five categories were defined: α-proteobacteria, β-proteobacteria, δ-proteobacteria, γ-proteobacteria and the CFB group [71]. Analysis of a field collected sand sample contaminated with oil showed that the most predominant microorganisms were Oceanospirilalles, Alteromonadales, Vibrionales, and Pseudomonadales, all of them belonging to γ-proteobacteria [72]. Additionally, *Alcanivorax* has been characterized as an 'obligate hydrocarbonoclastic bacterium', since it uses petroleum oil hydrocarbons as the solely carbon source [72]. A representative list of oil-degrading bacteria and fungi alongside their pathogenic effect is shown in the Table.

Conclusions

Modification of a non-flowing system is acceptable since the modification is compartmentalized, that is, adding a chemical to soil with the aim of decontamination most probably will not contaminate another part of soil in another area. However, seas are flowing systems, circulating water masses that constantly mix with the rest of the ocean. In such systems, it is not wise to add anything lest it be properly contained in spite of the associated dilution effect, especially microorganism on a large scale or GMO nearly coastal areas. The question still remains: would it be safe to use massive amounts of microorganisms of potential harmful effect on human health and the ecosystems to contain a significant oil spill? With safety in mind, the main microorganisms suggested for oil spill cleanup were analyzed herein. Most of them have some degree of potential harmful effects on humans and animal. This implies that their compelling use to control an oil spill should be carefully considered and any potential harmful effect on the long term be monitored and also mitigated, when present.

Additionally, it is necessary to recognize that oil spills are also breeding grounds for pernicious microorganisms, which is further amplified when the spill remained uncontrolled. Fortunately, only a few microorganisms selected from spills and used for bioremediation can affect healthy humans and animals, while there are others which can release airborne spores potentially affecting organisms miles away from the source of spill.

Given that both biostimulation and bioaugmentation aim to increase the density of microorganisms with the assumption that an increase in density corresponds to an increase in degradation ability, it can be stated that the resulting population could not be necessarily innocuous to nearby humans or animals. As such, the choosing of symptomless or non-pathogenic microorganisms would be advisable for massive growth to cleanse oil spills. In conjunction with directed evolution (placing a selection pressure on strains followed by selection of outperforming strains), a natural method of strain improvement could be safer to generate outperforming non-pathogenic microbial strains for bioremediation of oil spill either in water and soil.

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