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A Review on Bioremediation of bulk oil in Sea Waters and Shoreline

Jina Tanzadeh[1], Mohammad Faezi Ghasemi[2]

[1]*Environmental Research Institue Academic Center for Education culture and Research, Rasht,Iran*
[2]*Faculty of basic sciences, Islamic Azad University, Lahijan branch, Lahijan Iran*
E-mail: Jina_tanzadeh@yahoo.com
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Abstract: Bioremediation, which is done by adding exogenous or indigenous microbial populations, attempts to raise the levels of degradation found naturally to considerable higher rates. Oil spills are some of the sources of hydrocarbon oil pollution in marine environments. Hydrocarbon oils are difficult to remove from water because of their oily nature and poor water solubility. The oil contamination presists in nature for long period of time and Water and soil pollution with oil spills is a serious problem that is very noticeable because this contaminaton is a serious threat for water, soil and human health. Oil spills severely reduces oxygen to water And the mortality of fish .Among the different techniques to enhance natural biodegradation by native microorganisms, seeding of new bacteria and fertilizing the indigenous consotium have attracted the most interest. Bioremediation has been reported to describe the process of utilizing biological agents to remove toxic waste from environment. it is the most effective management instrument, to manage the polluted environment. Bioremediation is simpler and public attitude toward bioremediation are generally favorable, the lack of knowledge about microorganisms and their natural role in the environment could affect the acceptability of their use. However, bioremediation can be considered one of the best technologies to deal with oil spills. In this research, will focus on the use of bioremediation for marine (surface water) oil spills.

Keywords: bioremedation, soil, oil spills, microorganism, water.

Introduction

The Promise of Bioremediation

Different methods have been used for removal of oil contamination from soil. One of these methods is using of microorganisms to bioremediate of oil which is very considered in recent years.(21) Advantages of this method are ease of use, suitable effeicieny and in-situ operating.In the last 20 years, many ways to accelerate the biodegradation of oil spills in sea level and ocean and beaches studied.(7). Bioremediation is aninnovative technology of

using living microorganisms metabolic activities, to degrade the environmental Polluters into lower toxic forms and Bioremediation of crude oil spill has been accepted as a remediation methods(15). It uses biological agents such as bacteria, fungi or plants to degrade or neutralize hazardous pollutants**.** (13)Bioremediation is not a new notion. Microbiologists have studied the methods since the 1940s. However, it became known to a broader public in the U.S. only in the late 1980s as a technology for cleanup of shorelines contaminated with spilled oil. Bioremediation is a process by which chemical substances are degraded by bacteria and other microorganisms.(17)Bioremediation is an attractive approach of cleaning up oil spills because it is simple to maintain,cost effective,use over large areas and leads to the complete destruction of the contaminant(3)it is a normal process and is therfore perceived by the public az an acceptable waste treatment method for contaminant material such az soil. microorganisms able to degrade the contaminant increase in numbers when it is present; when the contaminant is deraded , the biodegradative population declines. The dregs for the treatment are usually harmless products and include carbon,water,and cell biomass.Crude oil mostly compoed of mixture of thousands of different chemical compounds, that includes some of the groups that contain only hydrogen and carbon molecular structure of various types of hydrocarbons include: paraffin hydrocarbon-hydrocarbon such as cyclohexane and aromatic hydrocarbons such as benzene, paraffins As the compound of each type of oil is unique, there are different ways to deal with them through microbes and flora. Bioremediation can happen naturally or can be naturally or can be encourage with addition of microbes and fertilizers.The option of bioremediation, defined as the use of microorganisms to detoxify or remove pollutants owing to their diverse metabolic capabilities is an Expanding method for the removal and degradation of many environmental pollutantsincluding the products of petroleum industry(15).also, bioremediation technology is believed to be cost-effective And non-invasivemethod.(3,14)

What an oil spills?

One of the major sources of water contamination is oil spills.oil and petroleum products can contaminate sources of water such as seas,rivers,oceans or underground waters. (1) the spilled oil eventually enters toxicity components into the human food chain and affects our health.(11,18)oil spillsare the exit of liquidpetroleumhydrocarbons into the environment, especially marine areas, due to human activity, and is a form of pollution. Oil Spills can harm living things because its chemical constituents are poisonous. This can Trace organisms both from internal exposure to oil through ingestion or inhalation and from external exposure through eye and skin irritation. Oil can also smother some small species of fish or invertebrates and fur and coat feathers, reducing birds, and mammals, ability to maintain their body temperatures.A tarball is a blob of petroleum and remnants of oil pollution which has been weathered after floating in the ocean. Tarballs are an aquatic pollutant in further environments, although they can happen naturally and as such are not always associated with oil spills(13,16,20)Tarballs slowly parseed by microorganisms such as *Chromobacterium violaceum*, *Cladosporium resinae*, *Micrococcus varians*, *Candida marina*,*Pseudomonas aeruginosa,Bacillus submainus*and *Saccharomyces estuari*. pollution with oil spills is the main global concern today. Soil contaminated with oil spills has a major hazard to human health [13], causes organic pollution of ground water which limits its use, environmental problems,economic lossand decreases the agricultural fertility of the soil [2]. The concern base, primarily from health risks, from direct contact with the contaminated

soil, vapors from the pullutions, and from secondary pullution of water supplies within and underlying the soil.

How formed oil tallballs ?

Bullet oil, are small, dark oil, that feet the skin of human when walking on the beach.they are very stable In the pelagic environment and can be toted hundreds of kilometers.Lakes, beaches, piers and harbors on oil samples from areas that are encapsulated in it, Will infect areas. Changing the oil on the beaches spread to a large extent depends on the type and sex beach. Wind currents, waves and currents cause the oil to become, instead of being continuous coverage in the form of a lump or Bands along the coast precipitate.Weathering processes cause oil balls Have finally dry and brittle on the outside and soft and gooey inside. Temperature affects the viscosity of oil pellets. With increasing temperature, weather, oil balls are more soft.A second factor affecting the adhesion bullets, particles in the Water or beach that balls that stick.

Oil spills in Iran

Iran is OPEC's largest oil producer and contain 9% of the world's oil reserves and 15% of its gas reserves .iran designs wide development of exiting offshore fields.the attack to develop oil and gas resources in the caspian sea makes oil contamination in the caspian a real enviromental threat. Main increases in energy concumption over the past 20years have contributed greatly to contamination levels as Iran's carbon emissions have nearly tripled over the same time period. Also , Iran's affluenceof fossil fuel resources has tended to discourage the incentive to shift to cleaner alternnative energy sources for energy needs.the caspian sea is the largest inland water body on earth,has low salinity ,and unique yet vulnerable marine ecology has developed.main commerical species contain herring,mullet,sturgeon,carp bream,pike- perch and salmon.the The most valuable species commercially, Caspiansturgeon, has suffered substantial declines in community due to over-exploitation. The freshwater Caspianseal is an endemic species and has also decreased substantially in numbers in recent decades. Tehran Refinery is one of the largest refineries in Iran and Middle East, with a substantival capacity of 225,000 tanks per day and an operational capacity of 240,000 barrels per day (5)that contain liquid gas, ordinary gasoline, light and heavy naphtha, kerosene, gas oil, furnace oil, mineral oil, and sulfur and its wastewater has 900 mg/L chemical organic demand (COD). The recent increases in the activities of Tehran Refinery, notwithstanding the old transmission lines and tanks, have resulted in the oil spills leakage and surefire, feasibility of soil and following underground water contamination with oil spills compounds.

Figure1. Photograph showing tarball morphology typically found from shoreline of Anzali beach in Iran

Microorganisms in Bioremedation

Many microorganisms can degrading crude oil. They first attack these organic chemicals by the enzymatic apparatus acquired during the cycle of enrichment, when they are exposed to these specific or structurally related compounds. Presence of these pollutants in the environment either induces or depresses the enzymatic function of microorganisms. Scientists found that biodegradation of complex hydrocarbon usually reguires the assistance of more than a single species. single microorganisms lonely cannot mineralize most hazardous compounds into water and CO2 as final product. However, for better bio-degradable, the method of cooperation between the microorganisms can be utilized.Most bioremediation systems running under aerobic conditions, but running a system under anaerobicconditions (8)

may permit microbial organisms to degrade otherwise recalcitrantmolecules.The most common microorganisms that carry out biodegradation in most different environments are identified as active members of microbial consortiums. These microorganisms include*Moraxella , Aeromonas,Beijerinckia, Pseudomonas, F lavobacteria, chrobacteria, Nocardia Atinetobacter, Mycobactena, ,Modococci , Streptomyces, ,Corynebacteria, Bacilli, Arthrobacter,Aeromonas, Aspergillus ,Cyanobacteria,, Mucor,Penicillium, Fusarium, Rhodotorula, Sporobolomyces and Candida.(6)*. Microorganism need carbon and macronutrient like nitrogen and phosphorous to snsure effective degradation of the oil.(2)

Table1.compound of a microbial cell(13)

Element	Percentage	Element	percentage
Carbon	50	Sodium	1
Oxygen	20	Calcium	0/5
Nitrogen	14	Chloride	0/5
Hydrogen	8	Magnesium	0/5
phosphorous	3	Iron	0/2
sulfur	1	All others	0/3
Potassium	1		

MATERIALS AND METHODS

Microbiological methods for bioremediation evaluation

primary soil analyses of the total heterotrophic microbial counts and specific hydrocarbon degradingmicrobial counts in the contaminated soil can procatavide effective information on soil biological activities,and the extent to which the native microbialpopulation has acclimated to the site conditions. Theresults will also indicate whether the soil contains ahealthy native microbial population capable ofdoing bioremediation. plus the primarymicrobial assessment of the pulluted soil, being microbial populations during the soilbioremediation is a useful tool for following thechanges and observation for microbes active in hydrocarbon degradation.(3)

Table2: Environmental facros affecting degradation(16)

Factors	Condition required for microorganism activity	Optimum value bioremedation
Soil pH	5.5-8.8	
Soil moisture	25-28% of water holding capacity	30-90%
Nutrient content	P and N for microbial growth	C:N:P=100:10:1
Oxygen content	Aerobic,minimum air	10-40%
Temperature (C)	15-45	20-30
Type of soil	Low clay or silt content	
Heavy metals	2000ppm	700ppm
Contaminants	Not too toxic	

Microorganisms, Media and Culture Condition :

Native Bacterial strains were isolated from samples collected from oil spils in Sea waters and shorelline located at the oilpolluted water.10ml of each sample were washedwith 90mL saline and filtered with membrane. Incubation was carried out on nutrient agar plate for 24h at 37oC. Mineral Salts Medium (MSM)[16] was prepared by dissolving 1/8g K2HPO4, 4g NH4CI, 0/2g MgSO4.7H2O, 0/1g NaCL, 0/01g FeSO4.7H2O in1L of distilled water. Bacteriological agar was added (15g/l) to the solution where solid basal medium was required.concentrations of 0/3, 0/5, 1 and 1/50% (v/v).cultures were carried out after 5 days and the growth was measured as CFU count.

Construction of Bacterial Consortia:

some different sampels that demonstrated high growth werechosen to construct consortia of hydrocarbon degraders.generally, two different bacteria were constructedconsortia and tested for

bioremedation to confirm theirbiodegradation abilities. Use of consortia inoculum: The samples were grown separately in Nutrient bruth and processed to yield separatesuspensions with an absorbance reading of 0/5 at 550nM. Specific aliquots of the bacterial inoculum werethen separately added into normal saline solution togive a ultimate combined inoculum concentration of 10%(v/w).(2)

Microbial counts

incipient soil analyses of the total heterotrophic microbial count and specific hydrocarbon degrading microbial enumerate in the polluted soil can provideeffective information on soil biological activities,and the extent to which the native microbialpopulation has acclimated to the site conditions. Theresults will also indicate whether the soil contains ahealthy native microbial population capable ofsupporting bioremediation. also the primarymicrobial assessment of the polluted soil, monitoring microbial populations during the soilbioremediation is a effective tool for following thechanges and knowing for microbes active in hydrocarbon degradation(3) Bacterial enumerate is usually determined in representativesoil composite and oil spils in Sea waters and shorelline isolates with the standardserial dilution and nutrient agar plate-counting techniques.(14)

Characterization of Bacterial strains

Bacterial strains were characterized with traditional microbiological methods, e.g. by plating on selective microbiological media. (10)

Biosurfactant screening(15)

1. Hemolytic activity

This method described by screening Isolated strains on blood agar plates containing 5% (v/v) blood and incubated for 24–48 h at45.C and Hemolytic activity was detected as the presence of a clear zone around acolony.

2. The drop-collapse technique

In This method was added 100-ml culture supernatant to wells of a 96-well microliter plate lid, then 5 ml of oil spills was added to the surface of the culture supernatant. Biosurfactant-producing culture Showed flat drops. th from a culture of each isolate were analyzed on two separate microliter plates(4,15)

3. Stalagmometric method

In This method , The DPP and DC polarographies were used to evaluate biosurfactant adsorption was done by using Traubeg'sstalagmometer that Surfacetension was measured with a ring-tensiometer at room temperature.

4. The Emulsifying activity

Emulsifying activity was determined by using a modification of the method.(17)7 ml of the culture filtrates was weard with 3 ml, petroleum oil, diesel oil, xylene and toluene, and then, vortexed for 1 minand the stability of emulsion was specified after 24 h and the emulsification index (EI-24) wasestimated by dividing of measured height of the emulemulsion layer by the height of the hydrocarbons phase . An emulsion was determined as stable if the EI-24 was 50% or better(4)

Gas Chromatography (GC) analysis for oil spills extraction

A novel experimental method has been developed for the gas chromatographic (GC) analysis of oil spills. The injection of theisolates is performedusing a thermal extraction device, directly connected to the GC. Oil spills samples are initially adsorbed on a appropriate material and ingredients their light components are thermally extracted and driven into the GC by the bearer gas flow, while the heavy end remains adsorbed on the sample probe. Factors affecting the profundity of the analysis, such as temprature, the kind of adsorbent material, and duration of sampling, were studied. The

majorpreponderance of the proposed technique is that no preliminary separation of the oil spills sample into a light and heavy part is needed prior to the GC analysis. also, the pollutions of the chromatographic system by the heavy oil spills components is avoided.Oil spills was extracted with a separating funnel(16). Then isolates(50ml), were centrifuged at4000 rpm for 15 min. The separating funnel wasstoppered with a glass stopper and it was shakenvigorously. The process of venting and vigorousshaking was repeated for several minutes and themixture was allow to divided into two phases. Afterphases have been separated in the funnel, chloroformwas collected in a 250 mL pre-cleaned bottle. Theextraction with chloroform(25 mL) twice was repeated. Then, it was dried by passing it through of3g pre-combusted Na2SO4 (150oC for 3h in incubation)and collected in a globular bottom flask. The extract wasthen evaporated with using a rotary evaporator. The oil spillsdraw out with 2 mL chloroform was washed. Thesolvents under a gentle stream for 7 days were removed. The residue was reconsttituted with cholorofom(1mL) and analyzed byGC.

Genetic Engineering

Genetic engineering improve the genetic combination of an organism using methods that remove heritable material or that display DNA supplied outside the organism either directly into the host or into a cell that is then fused or hybridized with the host(9).researcher are currently looking into certain genetically engineered microorganisms to progress their ability to degrade hydrocarbons and pesticides. The eventuality of application genetic engineering for development of bioremediation methods had an early boost in the late 1980's. Recombinant DNA techniques have been studied intensively to progress the degradation of dangerous waste under laboratory condition. The genetically engineered microorganisms have higher degradative valency and have been demonstrated successfully for the degradation of various pollutants under defined conditions. Genetic Engineering has conclude often in a wide variety of current and usefull applications for utilize in the methods of bioremediation.The genetic architecture of these microorganisms makes them precious in Biodegradation, Biosorption Biotransformation, and Bioaccumulation. The fundamental blue print of gene encoding for biodegradative enzymes is supply in chromosomal and extra-chromosomal DNA of such microorganismsthis technology Include PCR, site directed mutagenesis, anti-sense RNA technique,electroporation and particle bombardment methods. The biotechnology armed with recombinant DNA technology is now good tuning the bioremediation technology by improving contaminant degrading microorganisms via strain improvement and genetic modification of specific regulatory and metabolic.Bioremediation can be used to clean unwanted substances from water,soilair, and for the degradation of pollutants.

Conclusion

Researches by bioremedation has shown great aims to date.also latter research is certainly to study its application outside the laboratory-scale and to develop the kinetics of degradation.(14) bioremediation is the most economical at the required yield when the soil to be treated is more. Although public attitudes toward bioremediation are generally desirable, the lack of science about microorganisms and their normal role in the environment could affect the acceptability of their application. Before bioremediation techniques to be used widely, their efficacy and safety will have to be persuasiving demonstrated and communicated to the public. The Surfactant production of the isolates Done by the following methods; drop-collapse method, oil spreading and stalagmometric methods, and finally surfacetension measurements. Both the oil spreading techniques and drop-collapse can

be used as qualitative and quantitative methods. Research shows thatIsolates with emulsifying abilities and grow under extreme conditions may be suitable for MEOR as well as bore and oil spills cleanups.and the ultimate of MEOR is an attractive option that will be worth pursuing in the future.below The Advantages of bacterial consortium are:

1. In almost all cases using a consortium of microorganisms compared with single planting Show better results in biodegradation of contaminants.

2. As a result of several factors, And employing a more metabolic pathway, the risk of breakdown increases.

3. The elimination of toxic substances produced by a strain of strain or other strains, By eliminating toxic compounds,increases degradation efficiency.

4. In analyzing the mixture of pollutants, the use of effective microorganism consortium as may be several members of the consortium to break one or some of the pollutants are most effective.

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