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Bioremediation of Oil Spill an Invasion by Bacteria to a Safe Environment

KEYWORDS: Bioremediation, Biodegradation, Alcanivorax borkumensis, Spectrophotometer.

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ABSTRACT *During an Oil spill, liquid petroleum is released into the environment and the black sticky substance covers everything it touches: soil, water and wildlife. Between 1970 to 2007 nearly 6,062,712 tonnes of oil were spilled in incidents around the world. The costs associated with each clean-up are very expensive and sometimes it is out of maintenance.*

An Oil spill from a tanker, the fallout has been environmental damage. Heralding an environmentally friendly technique, bioremediation treats pollutants with bacteria bioengineered to break down contaminants . A bacteria that degrade oil and other environmental pollutants seeping from pipes or storage tanks into water or soil. Bacteria has shown its ability to clean up oil spills more rapidly than traditional techniques and reduce damage to ecosystems in the process.

The purpose of this Research work is how rapidly & eco-friendly a Bacteria clean up the Oil and in what duration of time and also the Biodegradation capacity of the Bacteria. Hydrocarbon utilizing Microorganism are ubiquitously disturbed in the marine environment following Oil Spill. These Microorganisms are naturally degrade numerous contaminating petroleum hydrocarbon & cleansing the Oceans of Oil pollutants. Bioremediation, which is accomplished by adding exogenous microbial populations or stimulating indigenous ones, attempt to raise the rates of degradation found naturally to significantly higher rates. Here we carried out the experiment by using Pure culture of the bacteria Alcanivorax borkumensis grown on ONR7a medium.

Here by determining the amount of Oil in oil spill water by Ultraviolet Spectrophotometer method, we are estimating the Biodegrading capacity of this Bacterium. This approach is quite economic and eco-friendly.

Introduction:
Massive amount of petroleum is transported from oil producing countries to various parts of the world. Very often, transportation of crude oil and petroleum products is carried out in big ships (supertankers), which may accidentally spill the cargo of oil in the sea. Blowouts of oil platform and pipelines may also spill oil into the sea. The situation becomes worse of oil spill occurs close to the coast. For instance, the supertankers "Torrey Canyon" wrecked off the southwest coast of England in 1967 spilling 1,20,000 tons of crude oil in the sea. The largest oil spill so far occurred during Gulf War in1997 when Iraqi forces deliberately opened valves at an offshore terminal releasing oil from several tankers in the Persian Gulf. Thousands of birds and mammals lost their lives during incident.

Petroleum is a highly complex mixture of thousand of compounds, many of which cause toxicity to living organisms. Petroleum causes a variety of impacts on animals, including impaired reproduction, decreased resistance to disease, anaemia, cancer, neurological damage, and birth defects in offspring. Petroleum impairs photosynthesis, and many physiological processes of Phytoplankton and plants cumulating in inhibition of growth or death.

Birds and mammals become coated with oil, and their feathers and fur lost property of insulation. Feathers and fur provide insulation by trapping a layer of air between the skin and the external environment, and thus protects animals from cold. In extremely cold regions such as Arctic, this situation may cause Hypothermia and death of animals. The oil-coated birds are too heavy and can't fly to search food. The birds then eat the oil to clean their feathers and poison themselves.

Objectives:
The clean-up Oil & Grease so many methods were used before which are economically very costly and will affect the environment by adding some other pollutant to the Sea water.

Mechanical containment such as booms, barriers and skimmers.
Booms are floating, physical barriers to oil, made of plastic, metal, or other materials, which slow the spread of oil and keep it contained. Skilled teams deploy booms using mooring systems, such as anchors and land lines. They commonly place boom:

Skimmers are boats and other devices that can remove oil from the sea surface before it reaches sensitive areas along a coastline. In the photo below, oil is being skimmed from the sea surface by a "vessel of opportunity." Sometimes, two boats will tow a collection boom, allowing oil to concentrate within the boom, where it is then picked up by a skimmer.

Physical methods- such as pressure washing.
Chemical and biological methods- include dispersants to break up the oil.
Oil dispersant is a mixture of surfactants and solvents that break up an oil spill into droplets. By breaking it up, microbes and the environment can more easily biodegrade the oil. A mixture of oil and water is normally unstable, but can be stabilized with the addition of surfactants. Surfactants improve interaction at the oil-water junction, decreasing surface energy. Dispersants have had negative environmental effects due to their toxicity; however, reformulated dispersants have been accepted by the United States Environmental Protection Agency (EPA).

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Here is a method which is an environmentally friendly techniques: Bioremediation through biological remedial agents. Bioremediation treats pollutants (such as an oil spill and contaminated ground water) with bacteria bioengineered to break down contaminants. There is a bacterium called Alcanivorax borkumensis that not only eats oil but also eats up other environmental pollutants seeping from pipes or storage tanks into water or soil.

Alcanivorax borkumensis - oil-eating bacteria, where are you?

Given that there are hundreds of natural oil seeps in the northern Gulf, spewing out an estimated 70,000 tonnes (roughly equivalent to 20 million US gallons) of oil every year, why do we not see a more oil-polluted Gulf in normal circumstances? One big reason is the natural activity of bacteria like Alcanivorax borkumensis. One fact is that natural processes can help in the kind of catastrophe we are facing. But those natural processes don't have the critical mass to deal with events on this scale - they need help. The use of oil-eating microbes has been the subject of research around the world for many years, and it is, of course, complex - different bacteria have a liking for different hydrocarbons and graze the ocean buffet with discrimination; their activity levels vary with conditions of ocean chemistry and temperature. "The potential for 'bioremediation' as this technique is called is huge. It is, I believe, the only natural technique that would effectively remove oil that is distributed over such large distances as are being seen in the current Gulf of Mexico oil spill."

Working principle of the Bacteria:

"The microbe used in the experiments -- Alcanivorax borkumensis - is extremely well adapted to oil degradation. It lives solely on oil and dies after consuming all oil in its surrounding. Although it is effectively able to survive and function in a range of temperatures above 5° C, there are bacteria which perform this job in the Polar Zones, too.

A. borkumensis is a native species and is adapted to living in oil-contaminated aquatic environments. Its genome encodes for a broad spectrum of efficient oil-degrading enzymes that can be used in bioremediation of oil spills. This provides A. borkumensis with a competitive advantage in that it can consume a wider variety of alkanes than other known species and thus become the dominant population in an oil-contaminated area.

Although little is known about the exact mechanism used by A. borkumensis to biodegrade oil, a hypothesis summarizes the method with the following steps:

1.) Oil leakage into aquatic environments causes an increase in phosphorus and nitrogen concentrations.
2.) Increased nutrient availability causes A.borkumensis to metabolize and grow faster; population increases.
3.) A.borkumensis attaches and forms a biofilm around the oil droplet. The biofilm aids in the recruitment of additional bacteria to the site of contamination.
4.) AlkB1 and AlkB2 enzymes synthesized and are used to oxidize C-alkanes, thereby catalyzing the degradation of oil.
5.) Biosurfactant produced and breaks oil and water emulsions to make oil more readily available for A.borkumensis.

Research Methodology:

Here we carried out the experiment by using Pure culture of the bacteria Alcanivorax borkumensis grown on ONR7a medium.

ONR7a MEDIUM

Solution 1:

NaCl 22.79 g
Na2SO4 3.98 g
KCl 0.72 g
NaBr 83.00 mg
NaHCO3 31.00 mg
H3BO3 27.00 mg
NaF 2.60 mg
NH4Cl 0.27 g
Na2HPO4 x 7 H2O 89.00 mg
TAPSO 1.30 g adjust pH to 7.6 with NaOH

Solution 2:

MgCl2 x 6 H2O 11.18 g
CaCl2 x 2 H2O 1.46 g
SrCl2 x 6 H2O 24.00 mg

Solution 3:

FeCl2 x 4 H2O 2.00 mg

Autoclave the solutions separately. All solutions should be mixed after autoclaving when they have cooled to at least 50°C. For solid media add to the first solution 15 g/l of Bacto agar or 12 g/l of agarose.

We have Cultured the Bacteria both in solid and in broth culture. The bacterial Colony characteristics and Grams staining was done to confirm the cultured bacteria is A.borkumensis.

(ONR7a medium)

The Sea water sample is collected from Arabian Sea (Malpae beach) Udupi. The PH of the Sea water is 6. 50ml of 5 Sea water sample is taken in a 5 different conical flask and known volume of oil & grease is added to each sample. Here we have added 1ml, 2ml,3ml,4ml and 5ml of Oil & Grease to the 5 different sample of Sea water taken in a conical flask respectively. Then the A. borkumensis Bacterial culture is inoculated to each of the sample and kept for one week incubation.

The bacteria in this period of duration degraded the Oil & Grease present in the sample. After One week the sample is centrifuged in a Normal Centrifuge, where the oil is separated from the water and the volume of oil is measured and it is noted. Then the Graph is plotted to know amount of degradation capacity of this Bacteria

Result & Discussion:

The Table is showing the amount of Oil degraded by bacteria in terms of ml.

	Amount of Oil added before inoculation of the bacteria	Amount of Oil left out after inoculation of bacteria	% of Recovery
Sample 1	1 ml	0.7 ml	70

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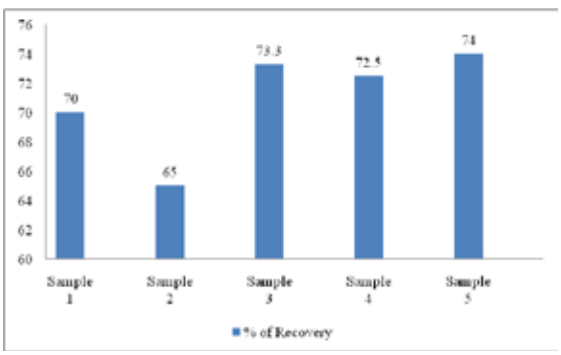
	Amount of Oil added before inoculation of the bacteria	Amount of Oil left out after inoculation of bacteria	% of Recovery
Sample 2	2ml	1.3ml	65
Sample 3	3ml	2.2ml	73.3
Sample 4	4ml	2.9ml	72.5
Sample 5	5ml	3.7ml	74

The Overall percentage of Recovery= 70.96 %.



The microbes that degrade oil are part of the local food web; they are consumed by other microbes that are, in turn, consumed by larger predators. When the oil is gone, the oil-degrading microbes, with less food available, stop dividing so rapidly and eventually return to pre-spill abundance. Like this the method what we opted for Biodegradation is an ecofriendly and natural one and the Bacteria is itself will grow whenever there is an Oil spill, because this oil eating bacteria will grow and multiply only in the presence of oil. As soon as all the Oil is degraded and the amount of oil is decreased the Bacteria by itself stop multiplying and at last no more bacteria will be seen in the Sea water.

Conclusions:

The method which we suggested here is a very easy method of detecting the oil in the laboratory and ecofriendly , economically friendly technique. In this method the Bacteria what we have used is played a very important role This method shows a good linearity and precision. Finally by giving a wonderful technique of cleaning the Oil & Grease in Sea water we are challenging the environment that we are ready to face any problem without harming you and also without harming ourselves i.e mankind.

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