

Bioremediation of Marine Oil Spills

Matt Radermacher

ABSTRACT

Bioremediation of marine oil spills has become a very practical approach to oil spill cleanup efforts in recent years. The tragic Exxon Valdez oil spill in 1989, informed the United States of the lack of preparedness the oil industry and country had in such remediation situations. This catastrophe did however spark further research into the use of bioremediation for marine oil spills, which has proved to be an effective method. Bioremediation for marine oil spills can be approached in two different ways depending on the case at hand. This includes bioaugmentation which involves introducing oil degrading microorganisms to the affected site, and also biostimulation which involves adding supplemental nutrients to the affected site to aid the existing oil degrading microorganisms. Although bioremediation is a new technology, there is continuous research underway which is investigating the practicality and efficiency of this process. There have been downsides found for using bioremediation for marine oil spills, however there are many benefits which make this a feasible technology.

KEYWORDS

Bioremediation, bioaugmentation, biostimulation, oil spill, oil degrading microorganism, hydrocarbon

INTRODUCTION

Marine oil spills are very catastrophic events which pose a great threat on the affected environment. Although it is often believed that oil spills affecting marine environments are primarily the result of large oil tanker spills such as the Exxon Valdez incident, most of the oil contamination that occurs is due to surface runoff, the transportation of oil, port activity, and illegal bilge water discharges (Suni et al., 2007). Oil is comprised of many different toxic compounds which endanger the natural habitat involved in the spill, however there are many natural, native microorganisms which are not only capable, but thrive on the decomposition of these toxic compounds. This process of using microorganisms for such cleanup efforts is known as bioremediation and this has proven to be a successful method for the cleanup of marine areas affected by oil spills (Coulon et al., 2006).

There are many different approaches which can be taken in the cleanup process, with bioremediation being a very beneficial method. Traditional methods of oil spill cleanup include using mechanical devices such as skimmers and oil booms, however these are very expensive and labor intensive processes. It is found that combining these traditional approaches with bioremediation can allow for a much more successful cleanup process while also reducing cost, as well as man hours. Bioremediation can consist of adding these hydrocarbon degrading organisms to the affected environment, which is known as bioaugmentation, and it can also consist of adding nutrients to the affected site which enhances the biodegradation process of the existing hydrocarbon degraders, and this is known as biostimulation.

Bioremediation is a new process and is often questioned as reliable due to uncontrollable variables in an oil spill, such as the composition of the oil, the indigenous microorganisms present at the site, the water characteristics such as temperature and energy, and also the available nutrients at the affected site. Research has been conducted and is still underway, regarding how these different parameters affect bioremediation and its practicality. Research, as well as actual applications to marine oil spills, has shown that bioremediation has many advantages and great potential for many different oil spill cases, however there are also disadvantages, making bioremediation not the best method of cleanup for all marine oil spills.

BIOREMEDIATION

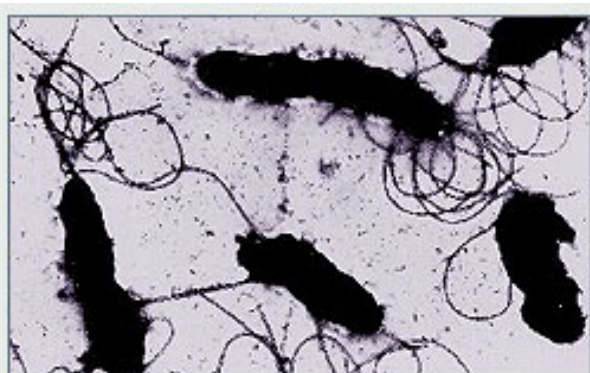
Bioremediation is defined as the use of natural microorganisms, plants, or fungi in the correction of a contaminated or altered environment. Bioremediation for the use of oil spill cleanup is either undergone by bioaugmentation or biostimulation. Bioaugmentation is the addition of microorganism capable of degrading the toxic hydrocarbons, in order to achieve a reduction of the pollutants. Biostimulation is the addition of nutrients needed by indigenous hydrocarbon degrading microorganisms in order to achieve maximum degradation of toxic compounds present in the oil. The degradation of hydrocarbons begins by the conversion of the alkane chain or polycyclic aromatic hydrocarbon (PAH) into an alcohol. Oxidation then converts the compound to an aldehyde and then into an acid and eventually into water, carbon dioxide, and biomass. In the case of the PAH, fission occurs which ultimately leads to mineralization (Venosa).

Table 2.5 Representative microorganisms capable of degrading petroleum hydrocarbons (Based on Atlas, 1984; Focht and Westlake, 1987; Jordan and Payne, 1980; Leahy and Colwell, 1990)

Bacteria	Yeast and Fungi
<i>Achromobacter</i> <i>Acinetobacter</i> <i>Alcaligenes</i> <i>Arthrobacter</i> <i>Bacillus</i> <i>Brevibacterium</i> <i>Corynebacterium</i> <i>Flavobacterium</i> <i>Nocardia</i> <i>Pseudimonas</i> <i>Vibrio</i>	<i>Aspergillus</i> <i>Candida</i> <i>Cladosporium</i> <i>Penicillium</i> <i>Rhodotorula</i> <i>Sporobolomyces</i> <i>Trichoderma</i>

Figure 1: Hydrocarbon Degrading Microorganisms (Zhu et al., 2001)

Biostimulation, the addition of nutrients, is practiced for marine oil spill cleanup when there is an existing population of oil degrading microbes present. When an oil spill occurs, the result is a large increase in carbon and this also stimulates the growth of the already present oil degrading microorganisms. However, these microorganisms are limited in the amount of growth and remediation that can occur by



A number of naturally occurring marine sediment microbes, such as *Pseudomonas fluorescens* shown in this micrograph, have been shown to be capable degraders of oil and other and other hydrocarbons.

Photo courtesy of Agriculture and Agri-Food Canada, FRDC.

Figure 2: *Pseudomonas Fluorescens* (Marinebiotech.org)

the amount of available nitrogen and phosphorus. By adding these supplemental nutrients in the proper concentrations, the hydrocarbon degrading microbes are capable of achieving their maximum growth rate and hence the maximum rate of pollutant uptake. It has been found that when using nitrogen for the supplemental nutrient, a maximum growth rate is achieved by the oil degrading microorganisms at a concentration of 2.0 mg/L (Boufadel et al., 2006). Biostimulation has been proven to be an effective way of achieving increased hydrocarbon degradation by the indigenous microbial population (Coulon et al., 2006).

An important factor in achieving successful biostimulation, is obtaining this ideal concentration of nutrients needed for maximum growth of the

organisms, and keeping this concentration present for the organisms as long as possible. This can become a difficult situation based upon where the nutrients are applied and also because of physical influences, such as differences in densities, wave movements, and tidal influences (Boufadel et al., 2006). When the applied nutrients are dissolved in the water, the nutrients move along with the water and the sand and this movement can vary on the conditions of the site. Tracer studies are often used to examine how the motion of the water and nutrients are influenced under different situations.

To investigate the role of tides, experiments were conducted by Boufadel et al., (2006), which showed that the supplemental nutrients tended to move downward during rising tides and seaward during falling tides. This is very useful information in determining the proper timing to add nutrients in order to allow for the maximum residence time of the nutrients in the contaminated areas. The results of this experiment concluded that the nutrients should be applied during low tide at the high tide line, which results in maximum contact time of the nutrients with the oil and hydrocarbon degrading microorganisms.

Waves also have an affect on the distribution and movement of the water and dissolved nutrients, which determine the residence time of the nutrients in the oil affected area. The role of waves on solute movement varies whether there is a tide or not. Research performed by Boufadel et al., (2007), showed that when a wave is present there is a sharp seaward hydraulic gradient in the backwash zone, and a gentle gradient landward of this area. It was also found that the contact time of the nutrients is increased when the waves break seaward of their location. This research also investigated the role of waves in the presence of a tide which concluded that the waves increased the dispersion and washout of the nutrients in the tidal zone, and residence time was approximately 75% when a wave was present with a tide, as compared to a tide with no waves.

Bioaugmentation, the addition of microbes capable of degrading hydrocarbons, is used when there are pollutants types which are unable to be degraded by the existing microorganisms. There are many different types of hydrocarbon degrading microorganisms, with more than 200 known species of bacteria, fungi, and yeast which are capable of degrading compounds as simple as methane to compounds with more than 40 carbon atoms (Zobell, 1973 as referenced by Zhu et al., 2001).

Since most environments naturally contain hydrocarbon degrading microorganisms, bioaugmentation is not used very often. Bioaugmentation is also not as effective for use in oil spill cleanup situations because the addition of these non-native organisms will often cause competition with the existing beneficial microorganisms. These difficulties are the reasoning behind why bioaugmentation has never been shown to have a successful impact on marine oil spill cleanup (Venosa). Bioaugmentation is not only ineffective, but it has also been looked upon negatively in the public view. There has been criticism whether it is safe to introduce a new species to an environment that it is not native to.

FACTORS AFFECTING BIOREMEDIATION

The conditions of the contaminated area plays a major role on whether bioremediation is the appropriate method of cleanup for the given oil spill. The success of bioremediation is dependent upon physical conditions and chemical conditions. Physical parameters include temperature, surface area of the oil, and the energy of the water. Chemical parameters include oxygen and nutrient content, pH, and the composition of the oil. Temperature affects bioremediation by changing the properties of the oil and also by influencing the oil degrading microbes (Nedwell, 1999). When the temperature is lowered, the viscosity of the oil is increased which changes the toxicity and solubility of the oil, depending upon its composition (Zhu et al., 2001). Temperature also has an affect on the growth rate of the microorganisms, as well as the degradation rate of the hydrocarbons, depending upon their characteristics. In a study conducted by Coulon, 2006, it was found that when crude oil was added to the test the presence of oil

degrading microbes increased by two orders of magnitude at 4°C, more than three orders of magnitude at 12°C, and more than four orders of magnitude at 20°C. The surface area of the oil is also a significant parameter in the success of bioremediation because the growth of oil degrading microorganisms occurs at the interface of the water and oil. The larger the surface area of the oil results in a larger area for growth and hence larger numbers of microbes (Venosa). The energy of the water is important because rough waters will disperse and dilute essential nutrients for the microorganisms and also spread the oil, contaminating more areas.

Appropriate levels of oxygen, nutrients, and pH are factors which will directly influence whether or not the microorganisms are capable of surviving in the environment.

Oxygen is required for the survival of many microorganisms and also drives the reactions for the degradation of the hydrocarbons. The necessary nutrients, particularly nitrogen and phosphorus are also needed for the growth of the microorganisms and also for the conversion of the excess carbon present from the oil. The chemical composition of the oil is another parameter which affects whether bioremediation is a possible alternative. Unlike many of the other requirements needed for successful bioremediation, the chemical composition of the oil is a factor which cannot be altered. If the oil is a heavy crude oil which contains resins and asphaltene compounds, it is very difficult for microorganisms to degrade compared to lighter crude oils (Venosa).

ALTERNATIVES TO BIOREMEDIATION

The more traditional methods which have been practiced for marine oil spill cleanup include physical, chemical, and natural methods. Typically the type of cleanup process decided upon is influenced by the conditions of the oil spill and the environment in which it takes place. The cleanup method can also be chosen due to public opinion and political decisions.

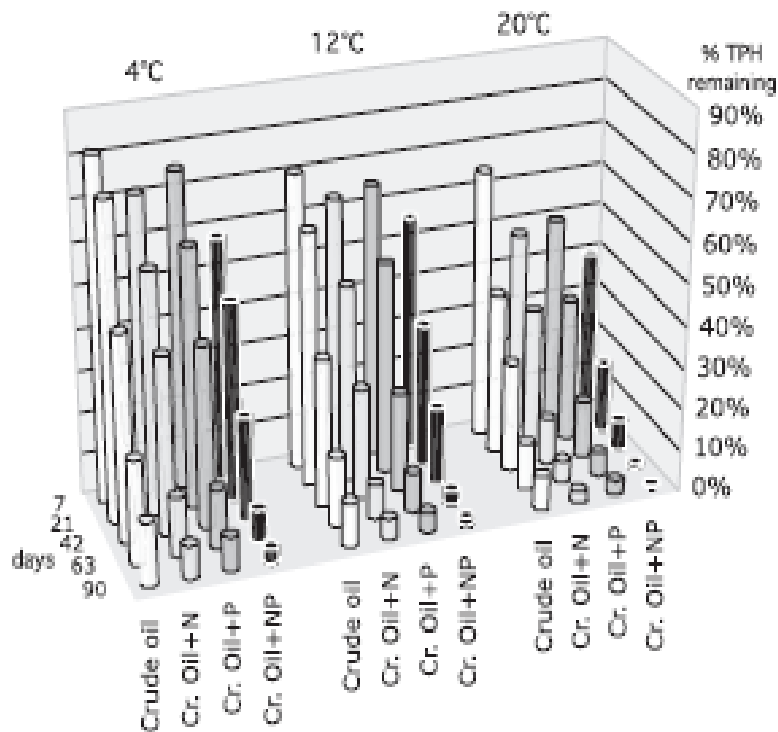


Fig. 2. Percentage remaining of total petroleum hydrocarbons (TPH) during microcosm experiment at three temperatures and with four nutrient regimes (N, NH_4NO_3 ; P, KH_2PO_4 ; NP, both together). Each value represents the mean amount of hydrocarbon groups from duplicate samples and the standard error calculated for all

Figure 3: Hydrocarbon removal with respect to temperature, nutrients, and time (Coulon et al., 2006)

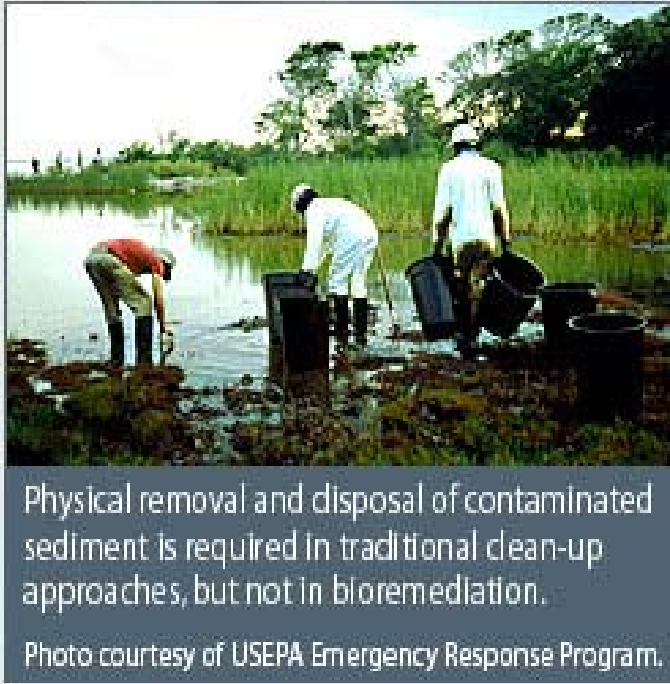


Figure 4: Physical Cleaning (Marinebiotech.org)

The physical process for the cleanup of oil spills in marine environments is the primary method used in the United States (Zhu et al., 2001). Physical methods include using booms, washing, soil movement and tilling, and mechanical removal. The use of booms is combined with skimmers to control the flow and movement of the oil and then collect the contained oil. This is a very effective method of cleaning marine oil spills, as well as collecting the spilled oil, and this also allows for minimal damage to the natural environment if the process is carried out properly (Zhu et al., 2001). Washing is another physical method that is practiced, however this can be more time consuming. Washing is used on the coastal areas affected by oil spills. Washing can be performed with the use of hot or cold water and high or low pressure depending on the situation. Absorbent materials can also be used, which utilizes

a hydrophobic material to aid the removal of oil along the coast. Another method is to relocate the contaminated soil to a site that is less threatened and to also till the soil which helps degrade the oil naturally. Mechanical methods of oil cleanup can also be used, but this should only be used when the site is not vulnerable to disruptions or erosion. Mechanical methods are utilized when the amount of oil contaminated shore is minimal, and a mechanical machine is capable of removing the polluted soil (Zhu et al., 2001).

Chemical methods for oil cleanup have not been used much in the United States, however areas with rougher shorelines, such as the United Kingdom, use this alternative for marine oil spill remediation (Lessard and Demarco, 2000 as referenced by Zhu et al., 2001). The United States has been hesitant to use chemical methods because of the possibility of toxic compounds being introduced to the natural environment (Zhu et al., 2001). The most widely used chemical method is the use of dispersants, which helps to disperse the oil from the water surface and into the water which allows for dilution to occur and avoids coasts and shorelines from becoming affected. Other chemical methods include using demulsifiers, solidifiers, and surface film chemicals which allow for easier physical cleaning of shorelines (Zhu et al., 2001).

Natural methods of marine oil spill remediation is a slower process, which does not utilize any supplemental actions to aid in the cleaning process. It can be used when the environment affected is not extremely vulnerable, or if the spill is in a location which will have little to no impact on the site. Natural methods are also preferred for certain locations that are very sensitive and could be more impacted and affected by physical and chemical methods than to be left alone and allowed for nature to run its course. Natural methods of marine oil spill cleanup primarily include evaporation of pollutants, photooxidation by sunlight, dispersion, dissolution, emulsification, adsorption, and biodegradation by the indigenous microorganisms. These processes, as well as others are seen in figure 5.

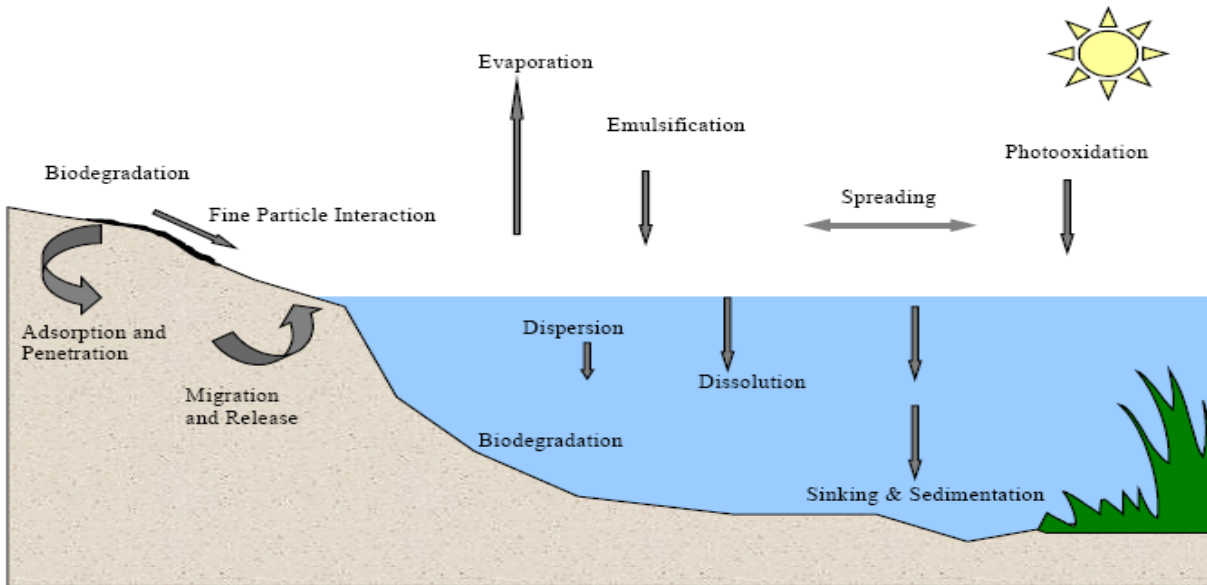


Figure 2.2 Major weathering processes after an oil spill

Figure 5: (Zhu et al., 2001)

ADVANTAGES

Bioremediation has many advantages over traditional cleanup methods of marine oil spills. One of the major advantages of bioremediation is the savings in cost and also the savings in the time put forth by workers to clean a contaminated site. The financial savings of bioremediation, when used properly, have tremendous benefits compared to traditional cleanup processes. After the Exxon Valdez spill, the cost to clean 120 km of shoreline by bioremediation was less than cost to provide physical washing of the shore for one day (Atlas, 1995 as referenced by Zhu et al., 2001). Another way that bioremediation allows for savings, is that unlike traditional methods, bioremediation continues to clean the contaminated site without the constant need of workers. This saves a great deal of money which would be spent on labor hours, and it also allows for time to be spent performing further research on bioremediation.

Bioremediation is also advantageous due to its environmentally friendly approach. Unlike chemical methods, no foreign or toxic chemicals are added to the site. It is also environmentally friendly because it does not require any disruption to the natural habitat which often occurs from physical and chemical methods of cleanup. Bioremediation allows for natural organisms to degrade the toxic hydrocarbons into simple compounds which pose no threat to the environment, and this also eliminates the need to remove and transport the toxic compounds to another site (Venosa). This loss of a need to transport the oil and contaminated soils lowers further risk of additional oil spills, and also saves energy and money which would be put forth in the transportation process. These environmental benefits also make bioremediation a positively viewed method by the general public. With the limited resources in today's world, this is a very much supported technology, which pleases the public and hence is given political support and funding for further research.

DISADVANTAGES

One of the greater downsides of bioremediation for marine oil spills is that it is a slow process (Venosa). Oil spills can pose a great threat to many different habitats, environments, and industries, and depending

upon the urgency of cleanup, bioremediation may not always be the best available option. Also there are many variables that affect whether bioremediation is capable and practical for the cleanup of different oil spills. Depending on where the spill takes place and the conditions of the water there, it may be very difficult to provide proper nutrient concentrations to the oil degrading microorganisms. If an oil spill occurs offshore, there is typically much more energy and waves, and this can cause for the quick loss and dilution of nutrients provided by biostimulation (Venosa). In the case of bioaugmentation, there are problems which occur, particularly the competition that will develop between the native and foreign microbes, making this an unsuccessful method of bioremediation. Another disadvantage of bioremediation is that it is a very difficult process to conduct field tests on. This is due to the many factors and conditions which can not be controlled in the field, but only in laboratory tests.

EXXON VALDEZ & BIOREMEDIATION

The Exxon Valdez catastrophe occurred in 1989, after the tanker hit the Bligh Reef which is located in Northern Prince William Sound. This accident resulted in the tanker dumping 20% of its Prudhoe Bay Oil, 42 million liters, into the sea off the coast of Alaska. This enormous amount of oil spread along the coast, contaminating more than 1900 km of shoreline (Peterson et al., 2003). This had a horrific effect on the natural habitat involved and resulted in the death of numerous animals. The cleanup process for this spill had many complications due to the remoteness of the location which only allowed access for boats and helicopters (EPA, 2006).

The first stage of cleanup following the Exxon Valdez spill was the use of burning the oil and a fire-resistant boom. This method however, was quickly abandoned due to rough weather. Following the attempt to burn the oil, mechanical methods were tried with the use of a skimmer and boom. This method was also unsuccessful due to the nature of the oil which was very dense, and easily clogged the skimmers. The density of the oil also created problems and difficulties in transferring the collected oil. As well as using mechanical methods, chemical dispersants were also used for cleanup. Like the previously attempted methods, dispersants were also unsuccessful. This controversial method failed due to the lack of waves needed in order to provide proper mixing of the chemicals with the sea (EPA, 2006).

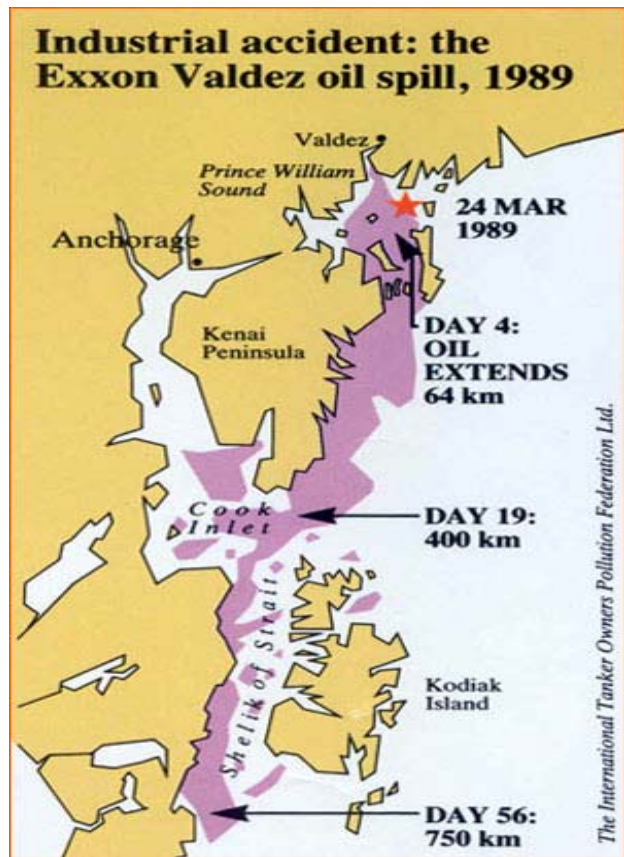


Figure 6: The spread of oil following the Exxon Valdez spill (UNESCO)



Figure 7: Water fowl killed from oil at Valdez (Accent Alaska)

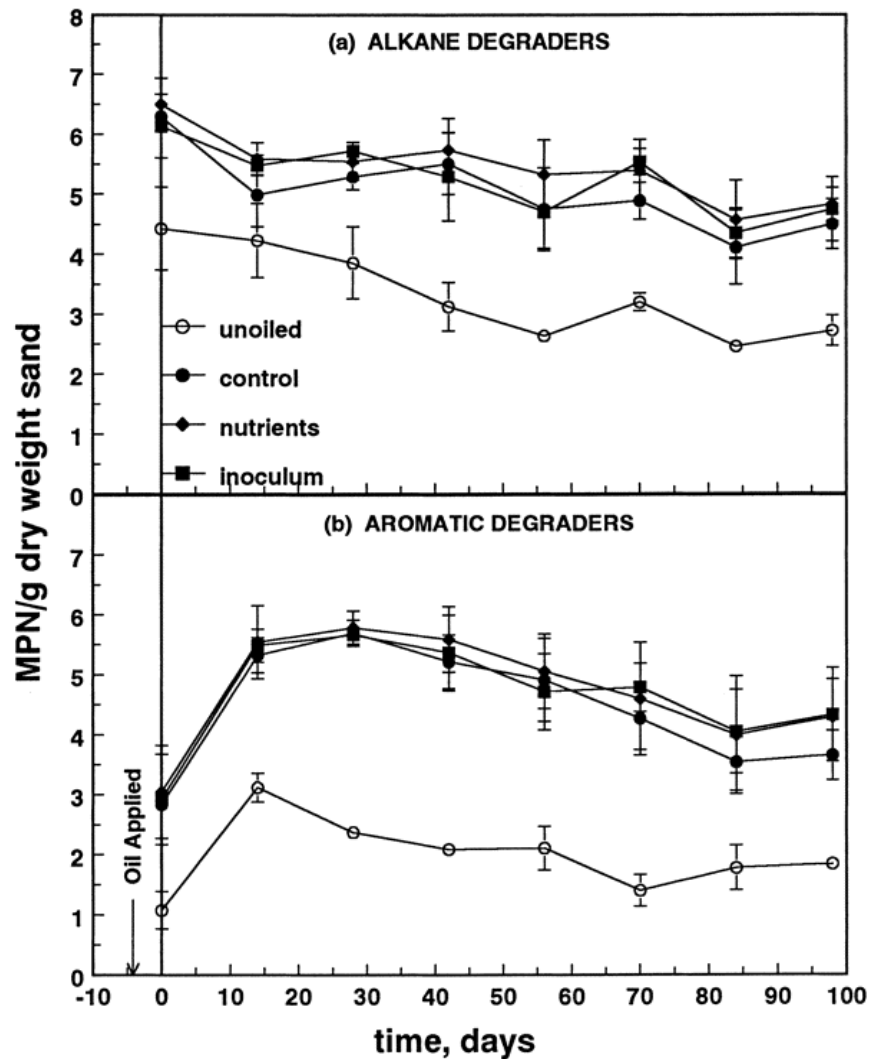
With little luck resulting from the cleanup efforts applied, researchers from the EPA felt this situation was an ideal scenario to try bioremediation. Although there had been very little experience at this time with bioremediation, experts decided that “the Alaska oil spill situation should be treated as a laboratory to increase the nation’s knowledge and readiness for action in future oil spills” and the use of fertilizers should also be utilized (McDonnell 1992, p.102 as referenced by Gordon, 1994). It was known that there were indigenous hydrocarbon degrading microorganisms present in Prince William Sound, and after the oil spill it was found that there was a 10,000-fold increase in the number of these microbes in the areas that were affected by the spill (Prichard 1992, as referenced by Gordon, 1994).

The use of bioremediation was proven to be effective with the Exxon Valdez spill, and within 10 to 14 days after the application of nutrients there was a noticeable difference in the reduction of oil on the sites which had biostimulation, compared to those which were not treated. This showed that using bioremediation not only worked at cleaning up the oil, but it also worked very quickly. With the success of bioremediation after the first summer of

its use, the EPA then supported further use of bioremediation on the contaminated beaches and after more research, the EPA declared it a safe method of cleanup for marine oil spills (Gordon, 1994).

DELAWARE BAY FIELD STUDY

In 1994, an experiment was conducted on Fowler Beach which is located on Delaware Bay. In this experiment oil was intentionally distributed onto specified plots, which allowed for research and knowledge to be gained on the practicality of bioremediation for marine oil spills. The advantage of this test over past experiments was that random and independent replication was used in order to eliminate uncertainties in the results and the variables affecting the results. For the experiment, five random blocks were designated along the beach and each block was broken into four plots. The four plots were separated from each other, with three of the plots testing oil removal rates. These three plots were applied oil with one of the plots acting as a control with no supplements added, another one of the plots having water-soluble nutrients added, and the third plot having both water-soluble nutrients and also natural microbe inoculum added. The fourth plot had no oil and was not treated to act as a background control (Venosa et al., 1996).



According to Venosa et al., 1996, when the oil concentrations were tested to determine hydrocarbon removal rates, it was found that significant removal was obtained at the control plot. This substantial removal was due to the naturally present microbe degraders and particularly the naturally present high level of nitrogen. Significantly higher rates of removal were obtained at the plot containing the addition of nutrients and the plot containing the addition of nutrients and the inoculum, however the differences between these two rates were very small. The small differences found between the rates at the plots with nutrients added and the plots with both nutrients and the inoculum added, indicated that biostimulation is a much more practical and effective means of bioremediation than bioaugmentation. However, even though biostimulation did

increase the rate of hydrocarbon removal as compared to the control plot, this increase was calculated to be approximately two-fold for the alkanes and 50% for the PAHs, which could be justified as not worthy of the time, effort, and money of using any bioremediation at all (Venosa et al., 1996).

Samples were taken at the different plots to not only determine removal rates, but also to determine the population and growth rates of the microbes. Analyzing both alkane and aromatic degraders showed little differences in growth at the three contaminated plots. A sharp increase in growth was seen initially for the aromatic degraders and very little change in growth was seen at all for the alkane degraders, which possibly indicates that the alkane degraders were already at their maximum field capacity at the

beginning of the experiment (Venosa et al., 1996).

Figure 8: Growth of (a) total alkane degraders and (b) total aromatic degraders in treated, untreated, and unoiled plots. (Venosa et al., 1996)

This experiment had many significant findings and was very valuable in the understanding of bioremediation. One important insight this experiment gave was determining when bioremediation is an effective and practical method. The first step in determining whether bioremediation is the right cleanup method to is to determine the natural concentration of nutrients in the area, and then decide whether they are high enough to maximize growth of the microbes, as this will determine whether biostimulation is necessary. This experiment also

indicated the ineffectiveness of bioaugmentation which is due to the fact that the foreign microbes are not able to compete with the indigenous population naturally present and also because the population of microbes cannot surpass the carrying capacity of the area simply by adding additional microbes.

CONCLUSION

Bioremediation has proven to be a successful secondary method of treatment for marine oil spills when followed by traditional physical cleaning methods. It is capable of being used as the sole treatment method in the certain cases where cleanup of the oil is not a great urgency and if the oil is not a free product (Venosa). Being a newer technology, there is still much research to be conducted examining the benefits, limitations, and capabilities of bioremediation use for marine oil spills. The Exxon Valdez oil spill, although very tragic and devastating, shed much light on the possible benefits of bioremediation and also influenced much further research on the technology. The Exxon Valdez spill also made aware the need for improvement on further prevention of marine oil spills which resulted in congress passing the Oil Pollution Act of 1990, which strengthened regulations on oil tankers, as well as their owners and operators (EPA, 2006). The Delaware Bay field study was another very valuable research opportunity on bioremediation and this brought about a much greater understanding on the effectiveness of this technology, as well as its practicality.

Concerning the two methods of bioremediation, bioaugmentation and biostimulation, biostimulation is the more effective approach to take. Bioaugmentation does not allow for any greater growth of microorganisms or degradation of hydrocarbons than biostimulation alone, when there is already an indigenous oil degrading microorganism in the affected site. Depending upon the concentration of the already present natural nutrients at the site, biostimulation can have a dramatic effect on the growth rate of the hydrocarbon degrading microorganisms which allows for an inexpensive cleanup method at a much more accelerated rate than natural biodegradation alone.

References

- Atlas, R.M. (1995) Bioremediation of petroleum pollutants. *International Biodeterioration & Biodegradation*, 317-327.
- Boufadel, M.C.; Suidan, M.T.; Venosa, A.D. (2006) Tracer Studies in Laboratory Beach Simulating Tidal Influences; *Journal of Environmental Engineering*, **132** (6), 616.
- Boufadel, M.C.; Suidan, M.T.; Venosa, A.D. (2007) Tracer Studies in a Laboratory Beach Subjected to Waves; *Journal of Environmental Engineering*, **133** (7), 722.
- Coulon, F.; McKew, B.A.; Osborn, A.M.; McGenity, T.J.; Timmis, K.N. (2006) Effects of Temperature and Biostimulation on Oil-Degrading Microbial Communities in Temperate Estuarine Waters; *Environmental Microbiology*, **9** (1), 177-186.
- EPA Website; (2006) <http://www.epa.gov/oilspill/oilprof.htm>
- Figure 2, 4. Marine Biotech.; <http://www.marinebiotech.org/biorem.html>
- Figure 6. United Nations Educational, Scientific and Cultural Organization.
http://portal.unesco.org/education/en/ev.phpURL_ID=29447&URL_DO=DO_TOPIC&URL_SECTION=201.html
- Figure 7. Accent Alaska, [http://www.accentalaska.com/cgi.pan\\$enlarge&033_040?AccentLynx](http://www.accentalaska.com/cgi.pan$enlarge&033_040?AccentLynx)
- Gordon, R. (1994) *Bioremediation and its Application to Exxon Valdez Oil Spill in Alaska*.
- Lessard R.R. and Demarco G. (2000) The Significance of Oil Spill Dispersants; *Spill Science and Technology Bulletin*, **6** (1), 59-68.
- McDonnel, J.A. (1992) *The U.S. Army Corps of Engineers Response to the Exxon Valdez Oil Spill*; U.S. Army Corps of Engineers, Fort Belvoir, Virginia.
- Nedwell, D.B.; (1999) Effect of Low Temperature on Microbial Growth: Lowered Affinity for Substrates Limits Growth at Low Temperature.; *FEMS*; **30**, 101-111.
- Peterson, C.H.; Rice, S.D.; Short, J.W.; Esler, D.; Bodkin, J.L.; Ballachey, B.E.; Irons, D.B. (2003) Long-Term Ecosystem Response to the Exxon Valdez Oil Spill; *Science*.
- Pritchard, P.H.; Mueller, J.G.; Rogers, J.C.; Kremer, F.V.; Glaser, J.A.; (1992) Oil Spill Bioremediation: Experiences, Lessons and Results from the Exxon Valdez Oil Spill in Alaska. *Biodegradation*, 315-335.
- Suni, S.; Koskinen, K.; Kauppi, S.; Hannula, E.; Ryyanen, T.; Aalto, A.; Jaanheimo, J.; Ikavalko, J.; Romantschuk, M. (2007) Removal by Sorption and In Situ Biodegradation of Oil Spills Limits Damage to Marine Biota: A Laboratory Simulation; *Ambio: A Journal of the Human Environment*; **36**(2), 173-179.

Venosa, A.D. *National Response Team Fact Sheet on Bioremediation Technologies*; U.S. Environmental Protection Agency, Cincinnati, OH.

Venosa, A.D.; Suidan, M.T.; Wrenn, B.A.; Strohmeier, K.L.; Haines, J.R.; Eberhart, B.L.; King, D; Holder, E. (1996) Bioremediation of an Experimental Oil Spill on the Shoreline of Delaware Bay; *Environmental Science & Technology*, **30** (5), 1764.

Zhu, X.; Venosa, A.D.; Suidan, M.T.; Lee, K. (2001) *Guidelines for the Bioremediation of Marine Shorelines and Freshwater Wetlands*; U.S. Environmental Protection Agency, Cincinnati, OH.

Zobell, C.E. (1973) *Microbial degradation of oil: Present status, problems, and perspectives*. In Ahearn and Meyers (Eds.), *The Microbial Degradation of Oil Pollutants*, Publication No. LSU-SG-73-01, Louisiana State University, Baton Rouge, LA, pp3-16.