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Review

Biological approach to oil spills remediation in the soil

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Perhaps there is no any other raw material that has impacted so much, and found wide application on human civilization than petroleum hydrocarbon (PHC). The paradox is that it is this same black gold that threatens human environment. PHC pollution in the environment, as well as the importance of natural interactions amongst living entities to arrest the problems hitherto caused by oil spills are hereby examined. Biological approaches to pollution remediation, which include phytoremediation, bioremediation, and application of biosurfactant, are discussed. Two angles of approach to bioremediation of PHC spills in the soil are identified; the bioaugmentation and biostimulation. The use of surfactants of microbial origin has been found to be environmentally friendly, naturally selective and stable at elevated temperatures, $_{P}$H and salt concentration. Similarly, genetic methods have been found to be overwhelmingly promising in detecting as well as assessing PHC soil pollution, and clean up.

Key words: Oil spill, bioremediation, phytoremediation, biosurfactants.

INTRODUCTION

Natural processes, such as volcanic eruptions and erosion, produce pollution, sometimes quite acutely, but the primary environmental concern today is with anthropogenic pollution. Since the Industrial Revolution, human activities have produced pollution at a dramatically acelerating pace, and the pollution produced is increasingly toxic and persistent. "Man-made" substances of extreme toxicity, such as pesticides, plastics, synthetic chemical products, and radioactive wastes represent an increasing preponderance of the pollutants that are being released into the environment. While petroleum hydrocarbon (PHC) serves as raw material for the manufacture of goods and services, which are symbols of our civilization, its fundamental components are sources of energy that human development is based. Crude oil and its products, though not man-made, but largely manipulated by man to satisfy, as well as to provide the ingredients for thousands of pro-ducts we use every day has become a major source of environmental pollution. Oil spills affect many species of plants and animals in the environment, as well as hum-ans (Plohl et al., 2002). Thus oil pollution has remained a considerable environmental problem.

SOURCES OF OIL SPILLS

Oil spills may occur for numerous reasons such as equipment failure, disasters, deliberate acts, or human error (Anderson and LaBelle, 2000). Figure 1 shows the percentage each subcategory has contributed to the total number of oil spills in Nigeria in the past five years (Shekwolo, 2005). Crude oils are exclusively natural products, most of which are produced from artificial wells. Natural seepage of crude oils occurs in various parts of the world, not only on land, but also on the seabed. Seeps emerge through fractures in the crests of folds in rock formations beneath the sea floor that contain oil and gas deposits. Oil and gas tend to rise and become trapped in anticlinal folds in sub sea rock strata. Seepage occurs through fracture zones where the folds are truncated at the sea floor. Seeps may emanate from a single point or as many as 3×10^4 individual seepage signals may be merged onto a high resolution profile record (Clark et al., 2000).

Human sources and their impacts

During production there could be blowout from the oil wells. Blowouts represent several potential hazards. There is the danger of fatalities or serious injury to workers, equipment loss, and pollution. Blowouts usually are gas and oil combinations, but each type can occur separately. Gas blowouts are more dangerous and explosive but generally have a lesser effect on the environment compared to crude-oil blowouts (Anderson and LaBelle, 2000). The present technology associated with blowout prevention and effective oil-well control has reduced the incidence of well blowouts. Oil prospecting is

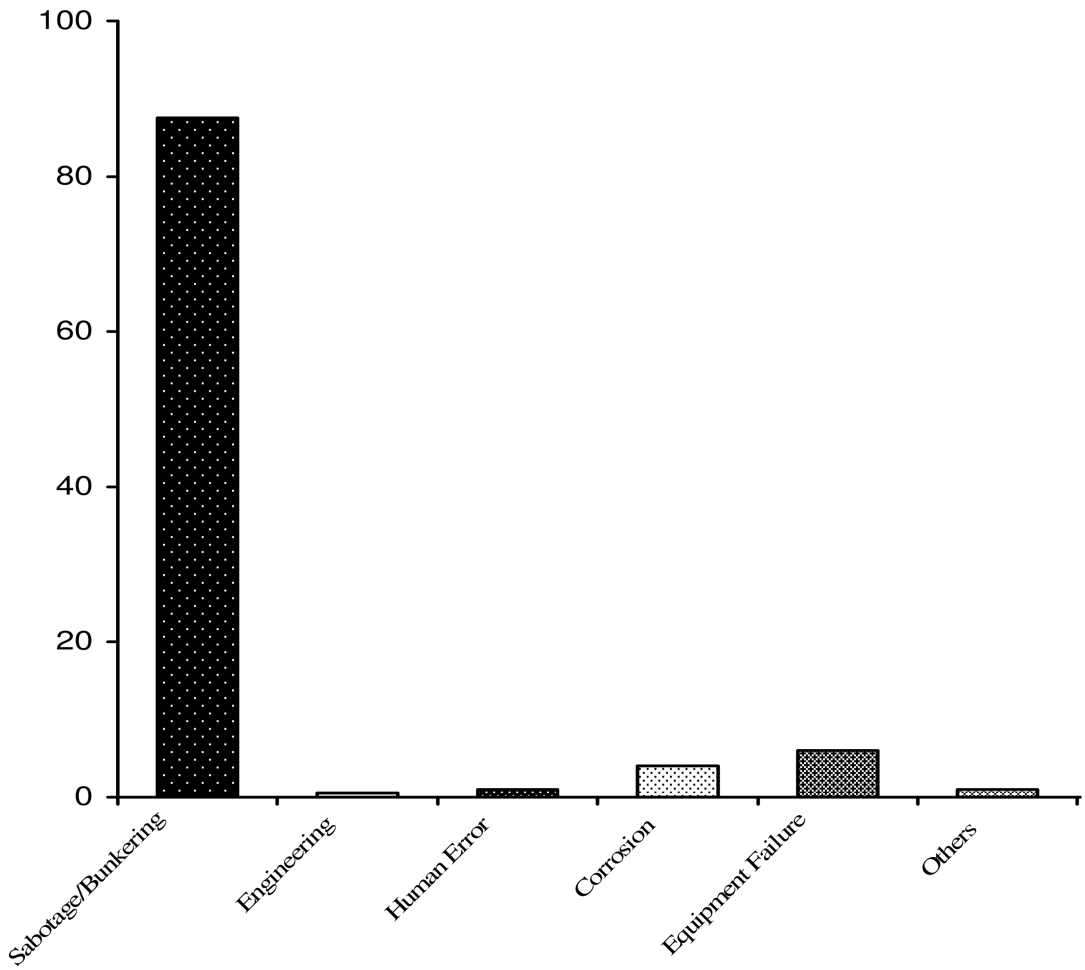

Figure 1. Causes (in %) of oil spills in Nigeria (2000 - 2004). Source: Shekwolo (2005).

sometimes accompanied with spillage.

The places where crude oil is found are not always the places where it is refined and needed. Therefore, there is major international trade in oil. While getting the oil out of the ground may seem complicated, moving it from the point of production to the final consumer is just as complex. Sabotage and oil theft have been of major concern to pollution experts, because the people involved in the nefarious activities are not skilled personnel, and tend to use crude methods, which have devastating consequences on the environment. Tanker accidents make newspaper and television headlines but, in fact, oil spilt from tanker accident is estimated to be 5% of all the oil, which goes into the sea (Clark et al., 2000). Modern technology of pipeline construction and exploitation under different natural conditions achieved indisputable successes. Examples of large spills occurred in the Guanabara Bay, off the Brazilian coast in 2000, when about 1,300 tons of oil was released into the sea, and in Nigeria in 1998, when a pipeline broke and 14,300 tons of oil were spilt (Anderson and LaBelle, 2000).

In refining process, the release of oil into refinery effluxents, as waste disposal, is practically negligible and of a lower order of magnitude to tanker washings in tankers that do not use the "load-on-top" system. Discharge and wash waters from the tankers and vessels are a kind of oil pollution that is fairly unnoticed, but it is common. Waste gas in production fields is generally burnt on the spot. In refineries and chemical plants, it may be necessary to burn some gas at a flare for reasons of safety, and some oil and gas are consumed as refinery fuel.

OIL SPILLS IN THE ENVIRONMENT

It is often assumed that only those oil spills, which occur near coastlines, cause any damage. These spills do of course, have the largest immediate and economic impacts, although the world's oceans are large, no oil can be spilled without harming local ecosystems (Clester et al., 1996). Air and ocean currents can also transport pollutants for thousands of kilometers; therefore oil spills affect more than just isolated locations. In many spills involving tankers or offshore oil wells, some of the oil spilled initially catches fire. When crude oil burns, the combustion results in atmospheric emissions of gasses, which contribute to global warming (CO_2) and acid rain (SO_2, NO_x), as well as large quantities of toxic ash. The toxic ash is made up of microscopic particles, which can travel for hundreds of kilometers. Humans inhaling these particles may experience allergic reactions, which result in sore throats and breathing problems.

The less dense (lighter) components of the spilled oil are more volatile and eventually evaporate into the atmosphere. The PHC then reacts with sunlight and oxygen to form greenhouse and acidic gasses similar to those from the combustion of oil. The negative impacts of oil that burns or evaporates is more diffuse (spread out) than that of oil which ends up on shore but still causes appreciable damage to the natural environment. Kvenvolden et al. (2000) reported that the oil mixes with sediments on the ocean floor and turns into a thick tar-like mass, which can destroy the habitat of many bottom dwelling organisms. These tar-like clumps can also drift with tides and currents, eventually washing up on beaches far away from the spill. If a spill occurs near a coastline, beached oil can leak into fresh groundwater reservoirs that often extend under beaches, contaminating local wells.

PHC BIODEGRADATION

A diverse group of microorganisms have been implicated in PHC biodegradation in both aquatic and terrestrial habitats (Coronelli, 1996; Ijah and Antai, 2003). For every organic compound produced by a living organism, there is an enzyme in the ecosystem that makes that substance biodegradable. The enzyme will break the substance down and recycle its components in the biochemistry of life. The biosphere has evolved as a self-consistent but limited array of substances and reactions that are internally harmonious. The attendant negative consequences of physicochemical methods of oil degradation make biological alternative or biodegradation more attractive (Okoh, 2003). It is cost-effective and does not involve high skilled technology, making it acceptable and suitable for all countries of the world.

The biodegradation of oil leads to a systematic decrease in paraffin content and an increase in oil density, sulphur content, acidity and viscosity. As such, it has negative economic consequences on oil production and refining operations. Hence, prediction of the degree of biodegradation of oil is important for assessing the risk of finding degraded oils in an exploration target before drilling (Wilhems et al., 2001). Biodegradation research has two spin-offs: many of the enzymes used in the pathways for degrading unusual substrates catalyze novel reactions often non-specifically and have potential use as biocatalysts in industry for the production of novel fine chemicals which are otherwise difficult to synthesize; and an understanding of the pathways and their genes can lead to the use of recombinant strains carrying genetically hybrid pathways, which can degrade compounds that are other-wise resistant to natural bacteria. Biodegradation, the most significant and ultimate natural mechanism for the abatement of oil spills therefore has essentially two approaches – phytoremediation and bioremediation.

Plants involvement

Phytoremediation, the use of vegetation for the *in situ* treatment of contaminated soil and sediment, promises effective and inexpensive cleanup of certain hazardous wastes. It applies to all plant-influenced biological, chemical, and physical processes that aid in remediation of contaminated substrates. It has become a very active field of study for the treatment of contaminated sites in the petroleum industry. A green plant is a "solar-driven, pumping, and filtering system that has measurable loading, degradative, and fouling capacities" (Salt et al., 1995). Types of phytoremediation include enhanced rhizosphere biodegradation, phytoextraction, phytodegradation, and volatilization. Grasses are becoming remediators of choice for spilled hydrocarbons at petroleum sites (Kim, 1996). Phytoremediation takes longer, five to seven years, but can be accomplished at a fraction of the cost. By using the more economical phytoremediation method, oil producers can reduce clean up costs and remove many sources of potential environmental pollution.

Microbial approach

This is when biotechnology is used to speed up the process of oil-spill clean up. Governments and industries are being forced to create clean alternatives for manufacturing, and to research into efficient remediation methods for polluted sites. Bioremediation's role is to optimize conditions for natural microbes to degrade environmental pollutant (Peng et al., 2003). In recent years, it had been established that bioremediation is a safe and effective oil-removal option. It was used in the 1970s for the in situ (in place) clean up of fuel contaminated soil and groundwater of mild magnitude.

It has since developed into a popular method of pollution remediation and has expanded to such areas as sludge, surface waters and process waters contaminated with pesticides, metals, crude oil, and industrial solvents. It is the process of using microorganisms to convert petroleum hydrocarbon and hazardous pollutants into less toxic compounds, or even water and carbon dioxide, a process called mineralization. It involves the manipulation of the process of molecular degradation of compounds through biological activity. Several aspects of bioremediation make it an appealing choice.

Advantages of bioremediation

It is a natural process; microbes work to break down practically all hydrocarbon contaminant in the natural environment. It transforms pollutants, and not changes them from one medium to another. Also, it degrades a wide range of different pollutants. It has simultaneous multiple activity and some degree of toxin resistance. It is essentially local, and does not require imported foreign substances. A final and often most convincing factor is cost. Relative to other methods of pollutant removal, bioremediation is of reduced risk and higher degree of safety, reduced labor and very cost effective (Wagenet and Bouma, 1996).

Approaches to bioremediation

The two main approaches to oil spill bioremediation are bioaugmentation and biostimulation. Bioaugmentation involves the use of beneficial microbes that have an affinity towards a specific pollutant. Typically, these microbes are suspended by a stabilizing agent and lie dormant until activated in solution and applied together with micro-nutrients and stimulants. Bioaugmentation allows one to control the nature of the biomass. It ensures that the proper team of microbes is present in the spilled soil in sufficient type, number, and compatibility to attack the constituents effectively and break them into their most basic compounds (Venosa et al., 1996; McKee and Mendelssohn, 1995). Biostimulation on the other hand involves aeration and the addition of selected micronutrients and sometimes topsoil in appropriate quantities (Shekwolo, 2005; Swannell et al., 1999). It is only effective when indigenous microbial populations, present in the substrate, are high enough to degrade the PHC, and when these microbes can readily adapt to foreign substrates.

Mishra et al. (2001) argued that since microorganisms capable of degrading oil usually grow at the expense of one or more components of crude oil, and these bacteria are ubiquitous, there is usually no reason to add PHC biodegraders unless the indigenous bacteria are incapable of degrading one or more important components. The size of the hydrocarbon degrading bacteria population usually increases rapidly in response to oil contamination, and it is very difficult, if not impossible to increase the microbial population over that which can be achieved by biostimulation alone (Lin and Mendelssohn, 1998). The carrying capacity of most environments is probably determined by factors such as predation by protozoan, the oil surface area, or scouring of attached biomass by wind activity that are not affected by bioaugmentation, and added bacteria seem to compete poorly with the indigenous population. Therefore, it is unlikely that they will persist in a contaminated soil even when they are added in high numbers. Hence, bioaugmentation may not have any long term beneficial effects in clean up operations.

Biostimulation involves the addition of rate-limiting nutrients to accelerate degradation by indigenous microbes. This assumes that every organism needed to accomplish the desired treatment-results is present (Mishra et al., 2001). Though it is not certain that those organisms present are the most suitable to degrade the pollutant. When an oil spill occurs, it results in a huge influx of carbon into the impacted environment. Supplying enough nitrogennous nutrients to the cultures to enable their proliferation present serious problems, since sea, river, or lake water and soil have little nitrogen and phosphorus, rapid oil utilization will require external supplies of these elements for the oil-consuming microorganisms. Carbon is the basic structural component of living matter, and in order for the indigenous microorganisms to be able to convert this carbon into more biomass, they need significantly more nitrogen and phosphorous than is normally present in the soil (Wright et al., 1997). Both of these essential elements are ingredients of protein and nucleic acids of living organisms, and therefore should be adequately provided.

USE OF BIOSURFACTANTS

These are surface-active-agents produced by microorganisms, which emulsify hydrocarbon in growth medium and reduce surface and interfacial tensions. Biosurfactants are compounds with vast potential for use in environment, petroleum, food, pharmaceutical and other Industries. Their range of possible application in oil industry processes includes cleaning up of oil spills (land and marine), removal of oily sludge from oil storage tanks through increased mobility, as well as general enhancement of oil recovery processes from reservoirs and the level of the production well. The ability of a biosurfactant to emulsify hydrocarbon water mixtures have been widely reported (Borjana et al., 2001; Kvenvolden et al., 2000). These emulsification properties have also been demonstrated to enhance hydrocarbon degradation in the environment; hence making them potentially useful tools for oil spill pollution control (Cameotra and Pruthi, 1997). Most surfactants currently being used are chemically derived from petroleum. Interest in microbial surfactants has however steadily increased in recent years due to desirable characteristics such as their selectivity, environmentally friendly nature, and stability at elevated temperatures, pH and salt concentrations (Borjana et al., 2001). In addition, biosurfactants have increased versatility in comparison to many synthetic surfactants and are suitable for pollution control through biodegradation.

GENETIC INVOLVEMENT

Biologists are now to employ genetics to develop a simple, quick method for assessing the progress of environmental clean-up efforts at polluted sites. The principle involves screening soil for genes that reveal the presence of an enzyme produced by pollution-busting bacteria. If the enzyme is detected, that means bacteria probably are cleaning the soil. Information about the bacteria's presence and concentration might then be used to assess the progress of efforts to remove pollutant from the contaminated soil. It is like a direct biochemical method that takes the attendance of the bacterial organisms in the soil (Watanabe, 2002; Matthew et al., 2000).

The method could be used to test soil at oil-contaminated sites within a few hours. Conventional methods require that soil samples be taken to a laboratory, where bacteria are cultured on a growth medium. However, that can take days and is not always effective because some microorganisms will not grow under laboratory conditions. The technique works by detecting the enzyme catechol 2,3-dioxygenase, which several types of bacteria produce when soil is contaminated by petroleum-based com-pounds such as benzene, toluene and xylenes. DNA is first extracted from polluted soil samples and then specific genes that reveal the enzyme's presence are searched. Detecting the enzyme is made possible by using primer that matches the gene sequence indicating the enzyme's presence. The primer is specially designed to detect only catechol 2, 3-dioxygenase, which neutralizes the petroleum-based contaminants. After the primer finds and attaches to a matching piece of DNA, thousands of copies are produced through a well-known method known as polymerase chain reaction, which enables scientists to make billions of copies of a single strand of DNA (Watanabe, 2002). Catechol 2,3-dioxygenase is present in several types of bacteria that degrade environmental pollutants. The toxins typically get into soil, and they are absorbed by the bacteria and broken down by enzymes into less toxic compounds, such as methyl catechol, which are further degraded into harmless chemicals by catechol 2,3-dioxygenase. While this is not the only enzyme used by microorganisms to neutralize petroleum-based compounds, it is one of the key biochemical "pathways" through which the contaminants are degraded.

CONCLUSION

Oil spills are caused by human errors and carelessness, but sometimes by natural disasters such as hurricanes or earthquakes. Deliberate acts by terrorists, countries at war, sabotage and bunkering, or illegal dumpers however, prove that oil spills are not always accidents. Some oil from any spill is degraded into simpler substances naturally by microorganisms. Use of biosurfactants have been shown to be of desirable characteristics, therefore it is one of the best approaches suitable for emulsification of crude oil spills for biodegradation. The biodegradation process is relatively slow, and when an oil spill occurs, workers must act fast to protect the environment. Most crude oils are inherently biodegradable, but they contain essentially no nitrogen, phosphorous or other trace elements that are necessary to stimulate the microbes that degrade the oil.

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