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Bioremediation of Petroleum Pollutants in Cold Environments

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Introduction

Over the last half-century, our society's increased reliance on petroleum as a source of energy has led to a parallel increase in the amount of oil transported by pipeline, land, air, and sea. It has also brought about an increased need for storage in containers both above and below ground. Oil spills resulting from accidental groundings or collisions of ocean-going tankers are well publicized due to their magnitude, but smaller scale spills and leaks from other sources of transport and storage occur much more frequently. The ability to contain the spills and clean up the aftermath has dramatically improved since the ill-conceived treatment of the 1967 *Torrey Canyon* spill off the coast of England (Laws 1993), yet there is still much to learn.

After a large marine spill, petroleum rises to the water surface and begins to spread. Technological advances have increased the effectiveness of chemical dispersants, mechanical containment, and absorption as first-response techniques (Laws 1993), but if seas are rough or response time is slow, the spill becomes nearly impossible to contain. When spills occur near land, oil usually washes up and contaminates near-shore and on-shore sediments. In these beach sediments, as well as in terrestrial leaks and spills, physical removal of the petroleum is difficult and costly (Atlas 1995). Bioremediation, or the artificial acceleration of microbial breakdown of pollutants, is proving ever more effective in the cleanup of petroleum-contaminated sediments, and potentially open waters as well.

Bioremediation

Although bioremediation of petroleum pollutants is a slow process, often requiring many months to degrade the majority of the oil, it is relatively inexpensive and seemingly harmless to the surrounding environment. The process generally involves (1) biostimulation - the addition of nutrients to increase the metabolic rate of indigenous microorganisms and/or (2) bioaugmentation - the introduction of oil-degrading microbes, either from naturally-occurring populations or from the test tubes of genetic engineering labs (Laws 1993, Margesin and Schinner 1999). Adjustments of substrate toxicity, pH, and oxygen and moisture content of sediments can also influence the success of bioremediation efforts (Walworth and Reynolds 1995). While the oil breakdown rate can be increased by adding nutrients and microorganisms, other factors that limit the rate of microbial oil degradation are nearly impossible to control. Temperature is one of these limiting factors, and it is often positively correlated with microbial oil breakdown rate (Laws 1993). For this reason, bioremediation has been historically more challenging in cold environments than in temperate or tropical ones. This paper provides an overview of progress thus far in cold-climate bioremediation of petroleum pollutants.

Life in the Cold

Petroleum is a complex mixture of many different hydrocarbon compounds, and the properties of at least some of these compounds depend on the ambient temperature. For instance, short-chain alkanes become less volatile and more water-soluble at low temperatures. This results in slower evaporation and a decreased probability that the microbes will come into contact with the compounds, both of which delay degradation. Cold conditions may cause other alkanes to precipitate from crude oil as waxes, rendering them inaccessible to microbes. Temperature can also affect hydrocarbon utilization: bacteria readily metabolize isoprenoids at 30° C but have difficulty doing so at 4° C. Although many species can withstand freezing and thawing (Wardell 1995), bacteria cease growth and metabolism altogether at temperatures below -12° C due to the formation of intracellular ice (Margesin and Schinner 1999). Low temperatures are, however, not always detrimental. Some hydrocarbons become less water-soluble at lower temperatures. Their lower solubility may reduce the potential toxicity of those particular compounds to members of the microbial community and could explain observations of slower but greater overall biodegradation at low temperatures (Margesin and Schinner 1999).

Despite these challenges, cold-adapted, hydrocarbon-degrading microorganisms occur in most, if not all, cold climate waters and sediments. In some cases, these are the same species that occur in temperate climates, but local selection has yielded populations that exist at lower optimal temperatures (Wardell 1995). Bradley and Chapelle (1995) suggest that selection for low-temperature strains should be especially intense in groundwater aquifers, where seasonal temperature fluctuations are slight and competition for resources is great. The same argument, at least with respect to low variation in seasonal temperature, can be made for cold ocean waters and shoreline sediments.

The two genera that are typically well represented at cold, petroleum-contaminated sites are *Acinetobacter* and *Pseudomonas*, both of which contain numerous species that can survive solely on hydrocarbon compounds (Rosenberg et al. 1992, MacCormack and Fraile 1997). Colonies of hydrocarbon-degrading species usually exist in very low abundance before the site becomes polluted. Upon exposure to oil, these colonies thrive and can expand to nearly complete dominance of the viable microbial community during the span of contamination (Margesin and Schinner 1999). The amount of time between contamination and microbial "bloom" will be greater if the population or species needs to acclimate to the pollutant, which is often the case for populations in previously uncontaminated sites (Wardell 1995).

Biostimulation

Biostimulation is the most widely practiced form of petroleum bioremediation. Its goal is to greatly increase the metabolism and population growth of indigenous, hydrocarbon-degrading microorganisms through *in-situ* addition of limiting factors. Inorganic nutrients, primarily nitrogen (N) and phosphorous (P), are in short supply immediately after an oil spill; the huge influx of petroleum hydrocarbons drastically inflates the C:N and C:P ratios (Margesin and Schinner 1999). If the spill is oceanic or the oil has washed ashore, natural sources of N (ammonia) and P (phosphate) in seawater will slowly replenish these nutrient pools through water exchange (Pritchard 1991). However, the longer the oil persists in the environment, the more resistant to biodegradation certain components of the petroleum mixture become (Margesin and Schinner 1997b, Mitchell 1999). It follows that a major goal of petroleum bioremediation is to increase biodegradation rates as soon as possible following a spill. In addition, because oil on beaches and sediment surfaces is detrimental to wildlife and unsightly (to humans), faster and more complete microbial degradation than would occur without manipulation is desired. This can be attained through the addition of various forms of N and P.

Numerous low temperature lab studies and a few field trials in cold climates have demonstrated positive correlations between nutrient addition and hydrocarbon degradation by indigenous microbes (Pritchard 1991, Rosenberg et al. 1992, Atlas 1995, Walworth and Reynolds 1995, Wardell 1995, Margesin and Schinner 1997a, 1998, 1999). Nutrients are generally added in the form of fertilizer solutions. One set of lab studies showed that adding N fertilizer to a diesel-oil contaminated alpine soil at 10° C led to 43% decontamination in 30 days (compared to 9% with no added N) and greater than 90% in 155 days (Margesin and Schinner 1997a, 1997b). They attributed approximately two-thirds of the decontamination to microbial degradation and the other third to abiotic processes. In another lab study, Walworth and Reynolds (1995) found an interaction between temperature and the degree of microbial stimulation after P addition – stimulation was only greater after added P at the higher temperature treatment. However, the fact that the contaminated soils used in their study were actually fairly nutrient rich, as well as the possibility that the soil microbes were better-adapted to one temperature treatment than the other, could have confounded their results.

It is extremely difficult to measure biodegradation rates in the field due to the patchiness of the contamination and the fact that hydrocarbons can be lost to the system through other paths, such as evaporation, dissolution, and adsorption to detritus or soil particles (Margesin and Schinner 1999). Methods of quantification of hydrocarbon loss to microbial degradation involve the use of internal standards: components of the petroleum that are degraded much more slowly than others. For instance, if one knows the initial ratio of straight chain alkanes (degraded quickly) to branched alkanes or hopanes (degraded slowly), the ratio of these two types of compounds at a later time should give a reasonable estimate of biodegradation rate (Atlas 1995). These ratio-based techniques have enabled workers to observe biodegradation rates in the field following nutrient addition of two to six times natural rates of degradation (Atlas 1995, Wardell 1995). Margesin and Schinner (1999) reported a 65% reduction of an unnamed petroleum compound after one year in a fertilized, Antarctic soil.

The tragic *Exxon Valdez* spill in 1989 in Prince William Sound, Alaska, provided an opportunity to field test these nutrient addition techniques in a cold climate. Three different types of fertilizers (hydrophilic, oleophilic, and slow-release) were applied to contaminated sections of shoreline. The oleophilic and slow-release fertilizers led to an increased rate of petroleum degradation on the beach surfaces, yielding close to 90% reduction of "total resolvable hydrocarbons" after 120 days. Similar oil degradation rates were observed within coarse shoreline sediments, but not within beaches composed of fine sand. This was probably due to the restricted movement of fertilizers into interstitial water and low oxygen availability associated with the finer beach sediments (Laws 1993, Atlas 1995).

Rosenberg et al. (1992) point out that water-soluble fertilizers will not be very effective in ocean systems and moist environments because they will become rapidly diluted. They also criticize the use of the particular oleophilic fertilizer (Inipol EAP 22) that was employed in the *Valdez* cleanup. Evidently, Inipol EAP 22 contains a large proportion of oleic acid, which, because it is an alternative carbon source, will cause the C:N ratio to rise. They also suggest that the emulsifier it contains is potentially harmful to the environment. Instead, they have developed a hydrophobic fertilizer, F-1, which should efficiently and safely accomplish the goals of biostimulation through nutrient addition. Indeed, after fertilization with F-1 and daily watering and plowing of the beach sediments, they found 86% hydrocarbon degradation after 28 days in a contaminated beach plot in Israel. Perhaps because of the warm climate of their study site, there is no mention of their experiments and fertilizer in the current literature on cold-climate bioremediation. The development of appropriate biostimulation fertilizers will be an ongoing process in this emerging technology.

Rosenberg et al. (1992) do, however, raise the important question of environmental safety. Part of the argument against pumping huge quantities of fertilizers into an oil-contaminated area is that nutrients that are useful to one type of organism may be toxic to others when present in large quantities. The extensive monitoring that accompanied the *Exxon Valdez* cleanup effort, however, showed that the quantities of fertilizers used had no toxic effect on sensitive marine species. There is also the potential for eutrophication from algal growth, but again this was not observed in Prince William Sound (Atlas 1995). So far, biostimulation appears to be a relatively "green" long-term oil spill cleanup method, but these potential dangers should always be considered during site assessment and monitoring of every project.

Although no research on microbial oil degradation in cold-climate open waters has been reported, data from temperate climates indicate that biostimulation could be an effective strategy. In fact, bacteria consumed an estimated 73,000 barrels of oil in the nutrient-rich waters off the coast of France after the *Amoco Cadiz* accident in 1978 (Laws 1993). While bioremediation is generally considered a long-term strategy, there is also the potential to supplement standard first-response techniques with nutrient addition. More research is needed to assess the success rate, cost-effectiveness, and environmental safety of extensive nutrient addition in open waters.

Bioaugmentation

Bioaugmentation is the addition (seeding) of non-indigenous, petroleum-degrading microorganisms into contaminated sites. Unlike biostimulation, it has met with minimal success in both temperate and cold environments (Goldstein et al. 1985, Atlas 1995, Margesin and Schinner 1997a, 1997b, 1998, 1999). The premise behind the procedure is that the indigenous microbes may not be able to degrade all of the compounds in a given petroleum mixture. If the non-native or genetically modified microbes can survive in the novel habitat and outcompete the indigenous microbes, they should bring about efficient degradation of the petroleum (Atlas 1995). In the lab, cold-adapted microbes may efficiently degrade target hydrocarbon compounds over wide ranges of temperature and nutrient level. The *in situ* competitive interaction between novel and introduced species generally seems, however, to favor the indigenous species. When the added microbes do have a favorable effect, that effect appears to lessen considerably over time (Margesin and Schinner, 1997b).

Other potential reasons for failure of bioaugmentation at any temperature include the following: (1) concentration of the carbon source may be too low to support growth, (2) introduced microbes may be adversely affected by predators or toxins in the water or sediments, (3) other available sources of food may "distract" microbes from the pollutant, and (4) microbes may have trouble moving through the soil to contaminated microsites (Goldstein et al. 1985). Despite these hurdles, there are times when bioaugmentation may be the only way to stimulate petroleum biodegradation. This is the case when the contaminant resists degradation by or is toxic to the indigenous microorganisms, and when the contamination event causes sterilization of the local area (Margesin and Schinner 1997a, 1997b).

This option is not available in every cold climate either – in Antarctica, restrictions on non-native species introductions preclude this method (MacCormack and Fraile 1997). History has shown that the risks inherent in species introductions are great, yet there is little mention of these risks in the bioaugmentation literature. Special care must be taken with genetically modified microorganisms lest efforts to clean up the environment result in ecological disaster.

Land Farming

The *in situ* bioremediation methods discussed above are generally cheaper and less disruptive to the natural landscape than off-site procedures that require excavation and transportation of sediment (Wardell 1995). Nonetheless, a study on the feasibility of land farming in cold climates was conducted in Antarctica and in the Arctic. Workers spread petroleum-laden soils over an impermeable liner and then added nutrients and water to stimulate biodegradation of the hydrocarbons by indigenous soil microbes. Moderate degradation rates were observed: 43% in 5 weeks in Antarctica, 34% in 35 weeks in an Arctic, sandy soil. However, the effort involved and low water availability (due to freezing) may keep this procedure from being as inexpensive and effective as *in situ* biostimulation (Margesin and Schinner 1999).

Ultimate Degradation Limits

Despite the advances in bioremediation of petroleum pollutants that have been made in the last few decades, even the most effective microorganisms can only metabolize 70% – 90% of the original hydrocarbon content of contaminated waters and sediments. It is difficult to completely remove the pollutant because there is often a buildup of inhibiting metabolites as well as recalcitrant "leftover" compounds. Also, as the availability of hydrocarbons diminishes, there is a corresponding reduction in the microbial community (Margesin and Schinner 1997b). Reduction to 10% of original petroleum pollutant levels through inexpensive and potentially harmless bioremediation techniques is impressive, yet there is always room for improvement.

Summary

Our society's reliance on petroleum, as well as increasing oil extraction efforts in far northern and southern territories, will ensure the continuing need for efficient and effective spill and leak cleanup techniques in cold climates. Aside from first response procedures following an oceanic spill, bioremediation is emerging as a sound long-term option for cold terrestrial sediments, beach sediments, and potentially open waters as well. Biostimulation, the artificial stimulation of indigenous, cold-adapted microorganisms through the addition of nutrients, has been particularly successful in both lab and field studies. Even though bioremediation alone may never completely remove the pollutants, it is generally clean and safe and should be given sincere consideration during assessment of any sediments or waters contaminated with petroleum.

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