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Enhanced bioremediation of oil spills in the sea

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Hydrocarbon-degrading bacteria are ubiquitous in the sea, including hydrocarbonoclastic bacteria that utilize hydrocarbons almost exclusively as carbon and energy sources. However, the rates at which they naturally degrade petroleum following an oil spill appear to be too slow to prevent oil from reaching the shore and causing environmental damage, as has been documented in the Exxon Valdez and Gulf of Mexico disasters. Unfortunately, there is, at present, no experimentally demonstrated methodology for accelerating the degradation of hydrocarbons in the sea. The rate-limiting factor for petroleum degradation in the sea is availability of nitrogen and phosphorus. Oleophilic fertilizers, such as Inipol EAP 22 and urea-formaldehyde polymers, have stimulated hydrocarbon degradation on shorelines but are less effective in open systems. We suggest uric acid as a potentially useful fertilizer enhancing bioremediation at sea.

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Introduction

Oil spills from the Exxon Valdez disaster and the blowout of the Deepwater Horizon drilling rig accentuate the need for effective bioremediation methods for treating oil spills in the sea. Although technologies for enhancing bioremediation of petroleum pollution in soil and reactors have been known for several years [1^{*}], there is, at present, no experimentally demonstrated methodology for accelerating the degradation of hydrocarbons in the sea.

The breakdown of hydrocarbons requires an appropriate consortium of hydrocarbon-degrading microorganisms and conditions that allow these microorganisms to grow and multiply. Two essential characteristics of hydrocarbon-oxidizing microorganisms are (a) membrane-bound, compound class-specific dioxygenases [2] and (b) mechanisms for optimizing contact between the

microbes and the water-insoluble hydrocarbons [3]. Because the oxygenases are group specific, for example, some degrade specific fractions of alkanes, others work on aromatics or cyclic hydrocarbons, etc., it follows that only a mixture of different microorganisms can efficiently degrade crude oil and petroleum fractions. Hydrocarbon oxidizers are located in virtually all natural environments, although with large variations in cell concentration [4]. This is not surprising because hydrocarbons have been present on land and in water for millions of years as a result of natural oil seeps and plant synthesis [5].

Hydrocarbon-degrading bacteria in the marine environment

Biodegradation mediated by indigenous microbial communities is the ultimate fate of the majority of oil hydrocarbon that enters the marine environment [6], where hydrocarbon-degrading microorganisms are ubiquitous [7^{**}]. Microbial biodegradation was shown to occur naturally in remediating oil contamination associated with several oil spills [8,9]. However, the rates of biodegradation are slow, and consequentially there are serious toxic effects on marine life in the water and on the shoreline.

There is a growing body of data indicating which particular microorganisms are involved under *in situ* conditions at oil spill sites. Of particular interest is the group of microorganisms, referred to as hydrocarbonoclastic bacteria [7^{**}] that utilize hydrocarbons and hydrocarbon degradation products almost exclusively as carbon and energy sources [10]. They include strains of *Alcanivorax* [11,12^{**}], *Cycloclasticus* [13], *Oleiphilus* [14], *Oleispira* [15] *Thalassolituus* [16] and *Planomicrobium* [17]. *Alcanivorax* strains were most abundant following an oil spill as determined by 16S rRNA gene analysis [18]. In our opinion, research should be directed to establishing *in situ* conditions for rapid growth of the oil-degrading bacteria. Consideration should be given to ‘seeding’ oil spills near shore with appropriate strains of *Cycloclasticus* because these bacteria degrade the toxic polycyclic aromatic hydrocarbons.

It is difficult to estimate the natural rates at which hydrocarbons are degraded in seawater following petroleum oil spills because much of the hydrocarbon is removed by chemical and physical processes. Some of the oil is photo-oxidized, the low molecular weight fractions are volatilized, and currents move the oil to shore and/or out to sea. The fact that the highly branched hydrocarbons, such as hopane, which are difficult to biodegrade, accumulate relative to straight chain hydrocarbons of the same molecular weight, suggests that

microbial degradation plays a significant role in removing the oil [19].

The need for physical interactions between bacteria and hydrocarbons

The low solubility of hydrocarbons in water, coupled to the fact that the first step in hydrocarbon degradation involves membrane-bound oxygenases, makes it essential for bacteria to come into direct contact with their hydrocarbon substrates. Two general biological strategies have been suggested for enhancing contact between bacteria and water-insoluble hydrocarbons: adhesion mechanisms and emulsification of the hydrocarbon [4].

To understand the special cell surface properties of bacteria that allow them to grow on hydrocarbons, it is necessary to consider the dynamics of petroleum degradation in natural environments. Following an oil spill in the sea, hydrocarbons rise to the surface and come into contact with air. Some of the low molecular weight hydrocarbons volatilize; the remainder is metabolized relatively rapidly by microorganisms, which take up soluble hydrocarbons. These bacteria do not adhere to oil and do not have high cell-surface hydrophobicity. The next stage of degradation involves microorganisms with high-cell-surface hydrophobicity, which can adhere to the residual high molecular-weight hydrocarbons. Adherence is due to hydrophobic fimbriae, fibrils, outer membrane lipids and proteins and certain small cell-surface molecules, such as gramicidin S and prodigiosin. Bacterial capsules and other anionic exopolysaccharides appear to inhibit adhesion to hydrocarbons.

Hydrocarbon-degrading bacteria often produce bioemulsifiers [20]. These can be divided into low-molecular-weight molecules that lower surface and interfacial tensions efficiently and high-molecular-weight polymers that bind tightly to surfaces. Some bioemulsifiers enhance the growth of bacteria on hydrophobic water-insoluble substrates by increasing their bioavailability, presumably by increasing their surface area, desorbing them from surfaces and increasing their apparent solubility. Bioemulsifiers also play an important role in regulating the attachment–detachment of microorganisms to and from surfaces. Addition of artificial chemical emulsifiers, as was performed in the Deepwater Horizon blowout, can have negative effects. More than 700 000 gallons of the dispersant Corexit were applied directly at the wellhead. Two years after the spill, studies showed Corexit increased the toxicity of the oil by 52 times [21].

Nutritional requirements for the growth of hydrocarbon-degrading bacteria

Hydrocarbons, as the name implies, contain only hydrogen and carbon. Thus, for bacteria to grow and multiply on hydrocarbons in seawater they require utilizable adequate sources of nitrogen, phosphorus, sulfur, iron and

oxygen (anaerobic degradation of hydrocarbons can also occur at slow rates). Since seawater is a desert when it comes to nitrogen and phosphorus, these two elements must be supplied for efficient biodegradation of hydrocarbons. In fact, the rate-limiting process in bioremediation of petroleum pollution in the sea is provision of nitrogen and phosphorus [3]. In principle, approximately 150 g of nitrogen and 30 g of phosphorus are consumed in the conversion of 1 kg of hydrocarbon to cell material. The nitrogen and phosphorus requirement can be met with fertilizers, such as ammonium phosphate, nitrates, phosphates and urea. However, all of these compounds have a high water solubility which reduces their effectiveness in open systems, such as the sea, because of rapid dilution. The first attempt to overcome this problem was the use of paraffinized urea and octyl phosphate [22**]. Although this mixture was effective, it is not a practical solution to the problem because of cost and the high ratio of C to N. Subsequently, there have been several other attempts to provide oleophilic fertilizers for the treatment of oil slicks.

An oleophilic fertilizer (Inipol EAP 22 = oleic acid, urea, lauryl phosphate) was used in the bioremediation of polluted shorelines following the Exxon Valdez spill [23]. Initial reports of success [24] have been challenged [25]. There are at least three problems with Inipol EAP 22. First, it contains large amounts of oleic acid, which serve as an alternative carbon source, thereby increasing the C:N ratio in the environment. Second, it contains toxic compounds. Third, as soon as the fertilizer comes into contact with water, the emulsion breaks, releasing urea into the water phase where it is not available for the microorganism.

Another approach has been the use of water-insoluble polymers, based on urea-formaldehyde formulations that adhere to oil [26]. This latter fertilizer was used successfully to bioremediate a heavily oil-contaminated sandy beach, but the polymer would not be effective in the sea because its high density causes it to sink. Other slow-release or oleophilic fertilizers that have been considered include chitosan [27], Customblen [28**] and lecithin [29]. Although these fertilizers stimulate petroleum bioremediation to a more or less extent on oil-contaminated shorelines, none of them are effective in treating oil at sea. The utilization of chitosan and urea-formaldehyde polymers requires the production of extracellular hydrolyses. Thus, when using these fertilizers it may be advantageous to ‘seed’ oil slicks with hydrocarbon-degrading bacteria that contain the appropriate depolymerase. The interesting possibility of using nitrogen-fixing, hydrocarbon-oxidizers has been considered [30–32]. Since nitrogen is often the limiting factor for growth of microorganisms following an oil spill, one might expect a strong selection for nitrogen-fixing, hydrocarbon-oxidizers following an oil spill. In 1967, Cody (325) claimed to have isolated microorganisms, from

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