ABSTRACT: Oil exploration in arctic regions will require special oil spill cleanup methods for shorelines. The application of fertilizers to speed biodegradation of oil may hold promise. INIPOL EAP22, an oil-soluble fertilizer that is nontoxic and biodegradable, was tested in a series of experiments on Spitsbergen, Norway, to determine its effectiveness under various conditions. The fertilizer was applied to floating oil and to oil on shoreline sediments. Effectiveness of the treatments varied. The oil-on-water experiments showed virtually no signs of biodegradation, perhaps because photo-oxidation products and low-boiling-point hydrocarbons interfered. Experiments with oil in sediments showed mixed results: oil in finer sediments showed little or no biodegradation, but oil in coarser sediments was significantly biodegraded. Uptake and exchange of nutrients depends on the complex growth kinetics of the bacteria involved, and requires detailed study.

Material and methods

Description of the fertilizer. INIPOL EAP22 can be described as a microemulsion with an optimal nitrogen/phosphorus ratio. The source of fertilizer is urea (SNEA(P) 1980). This product is oleophilic and shows a delayed release of nitrogen. INIPOL EAP22 has been tested for toxicity on different levels of marine food chains, and has been found to be nontoxic and biodegradable.1,2

Sampling. Samples from fine-grained shoreline sediments were taken with disposable 5 mL polyethylene syringes with their tips detached. Samples were then transferred to clean glass vials and transported to the laboratory, where they were kept at 3°C until processing. Samples for microbial work were processed within five hours after sampling. Samples for hydrocarbon analysis were extracted in dichloromethane and stored in the dark in brown sample vessels.

Microbial techniques. Total bacteria were enumerated after staining with acridine orange (AO), using a modification of the method of Trolldenier.3 The number of metabolically active bacteria were enumerated as the number of fluorescein diacetate hydrolyzing cells with a modification of the method described by Lundgren.4 For both enumerations approximately 5 grams of the substrate (i.e., sediment) were weighed and suspended in a sterile mixture of water, proteose peptone, and sodium hexametaphosphate. The solution was then homogenized for 2 minutes with a Waring blender at full speed, and left for 5 minutes to allow coarse particles to sink. An aliquot of 1 mL was then transferred to a disposable glass vessel that contained 10 mL of the buffer solution, which were then processed according to the two methods of staining. One was allowed to rest for 3 minutes, and was then filtered through an 0.22 μm prestained (black) polycarbonate filter (Nucleopore). The other was stained with 25 μL AO and was allowed to rest for approximately 10 minutes before being filtered as described above. The preparations were then examined for enumeration in a Leitz Dialux 20 Research Microscope with a Ploemopac fluorescence device.

Chemical analytical techniques. The aliphatic fraction was analyzed with gas chromatography, with special reference to the n-C17, pristane and the n-C15 phytane ratios.
Results

Three experiments involved fertilizer application on water. Others involved four different shoreline spill situations. The selection of experiments was based on input to the conceptual model that will be presented at the end of the paper. All experiments were carried out using INIPOL EAP22 under arctic conditions on Spitsbergen, in connection with the SINTEF field laboratories in Ny Ålesund.

Oil-on-water experiments. In Experiments 1, 2, and 3 with oil on water, Statfjord A + B crude oil was used. Experiment 1 was carried out in 100 L plastic containers on the shoreline of Kings Bay, Spitsbergen. Water supply from the adjacent water supply was provided through a pump, with semicontinuous run. Experiment 2 was carried out on the shoreline of a lagoon system on the coast of Kings Bay, Spitsbergen. Water was circulated from the lagoon to large mesocosm vessels with a water volume of 2,250 L. The water was exchanged once every 24 hours. Experiment 3 was carried out in the lagoon. The water exchange of the lagoon with Kings Bay was satisfactory during the experiment (salinity was 15 % to 30 %). Oil was confined in booms. The boom for the fertilized slick enclosed 3,250 m²; that for the unfertilized slick enclosed 800 m².

In both experiments involving microbial activity, bacterial numbers in the oil increased over time (Figures 1a and 1b). The total number of bacteria increased in both cases much more than the number of active, metabolizing cells. This result implies that there was considerable immobilizing of bacterial cells, and that parts of the added nutrients were kept within the system in a form that was not able to support further growth. Compared to the fertilized oil, the unfertilized oil yielded a smaller increase in bacterial number, and a stronger agreement in increase between the total number and the number of metabolizing cells (Figures 1c and 1d).

The nutrient added to the oil was leached relatively rapidly from the oil slick; however, the level of nitrogen in the oil was higher in the treated oil than in untreated oil throughout the experiments (Figures 2a, 2b, and 2c).

In Experiment 1, an equilibrium was obtained with approximately 20 percent nitrogen of the added amount. In Experiments 2 and 3, equilibrium was reached at approximately 10 percent.

Biodegradation of oil was evaluated through the commonly used ratio between branched and unbranched alkanes. No significant biodegradation was found in either of the experiments (Figures 3a, 3b, and 3c). The low level of biodegradation can be explained by the possible effect of toxic photochemical products and low-boiling hydrocarbons, with low evaporation rates.

A common feature of experiments with treated and untreated oil on the water surface is the high degree of photo-oxidation, demonstrated through the increase in oxygen content of the oil (Figures 4a, 4b, and 4c). In Experiment 1, an oxygen content of 5–6% was obtained during 10 days, compared with 0.1% in the original oil. In Experiments 2 and 3, the level was even higher (max 10%). The rapid rate of photooxidation is easily explained by the 24 hours of intensive sunlight under arctic conditions.

Oil-on-shore experiments. Experiments 4, 5, 6, and 7 covered a range of shoreline types, from very fine-grained sediments to coarse...
gravel beaches. In Experiment 4, a crude oil was used, while in the other experiments diesel oil was used.

Experiment 4 was conducted in shoreline sediments kept in plastic containers on the shoreline of Kings Bay, Spitsbergen. The plastic containers were about 0.5 m² in area with depths of 20 cm. Tidal water flow was simulated with seawater from the adjacent fjord. Water was renewed every week. The experimental oil was Statfjord A + B (10 L·m⁻²). Experiment 5 was conducted in connection with an accidental oil spill in Kings Bay, in which 88,000 L of gas oil were spilled on a gravel beach, which was treated with INIPOL EAP22.

Experiments 6 and 7 were carried out on lagoon shoreline sediments. In both, sediments were fine grained. The experimentally spilled gas oil (6 L·m⁻²) was fertilized on an ebb tide.

Results from the shoreline experiments can be divided into two groups, those showing a positive effect from the INIPOL EAP22
Inhibiting ureolytic activity by biological and photochemical products

The transformation of urea nitrogen to a form that is microbially available depends on the type of bacteria present. Ureolytic bacteria—bacteria producing the enzyme urease—are found in the genera Bacillus and Pseudomonas. These genera are represented in most experiments with fuel oil on fine-grained sediments (Experiments 6 and 7), virtually no biodegradation could be found (Figures 6e and 6d). However, when the fertilizer was applied on coarser shoreline sediments (Experiments 4 and 5), a high degree of biodegradation was found (Figures 6a and 6b).

In Experiment 4, with sandy beach sediment, an increase in biodegradation of approximately 60 percent was found compared to untreated oil. In Experiment 5, with a mixture of sandy and gravel beach, the enhancement reached about 90 percent during the arctic summer. However, for the two finest grained sediments, in Experiments 6 and 7, biodegradation was found to be insignificant. This result might be explained by the interactions of the fertilizer with the various types of sediment, an effect that will be discussed in more detail below.

Discussion

The present studies indicate very complex microbial growth kinetics. The increased accumulation of an inactive microbial necromass in the presence of increasing amounts of available nutrients is one of the aspects of fertilization that requires an explanation. The concept of an inactive microbial biomass is not only related to situations with artificial increases in nutrients. Natural systems often consist of as much as 40 percent inactive cells.

The Monod type of kinetics is often used to describe microbial growth. This type of growth is based on concentrations of unbound nutrients. It has become clear, however, that in many situations microbial growth deviates from this simple type of kinetics. The transfer of nutrients from free solution to incorporation in the microbial biomass involves more than a single stage process. The process of nutrient uptake and growth involves many pathways, which can be described as complex transport processes involving both convective transport and diffusion. Complete understanding of this type of nutrient uptake requires detailed studies on both the biochemical pathways, which include the nutrient uptake itself, and ecological issues, such as light, temperature, and factors influencing external nutrient concentrations (e.g., grazing by microfauna and evaporation).

Caperon and Droop both introduced nutrient limiting models, which described the growth of cells as a function of the internal nutrient concentration rather than the external nutrient concentration. Their models imply the presence of an internal unassimilated pool of nutrient, which is available for biosynthesis. As a result, the growth of the microbial community can proceed even when the external nutrient concentration decreases below the level required for the actual growth. This can be described by a two-stage kinetics: a first stage including nutrient uptake and establishment of an internal nutrient pool, and a second stage including the assimilation of nutrients in new cell biomass.

The threshold of an increased level of nutrients in the oil slick depends, as pointed out above, on microbial immobilization. The ecological function of immobilization, and thus of fertilization with urea-based fertilizers, may be described by a multistage kinetic model similar to those of Caperon and Droop. Bacterial cell density is maintained over a long time at the maximum level. As can be seen from the size distribution of cells in the experiments, several taxa are involved. If the size classes remain more or less constant, several growth curves are involved, and the time development of the different taxa represents a succession related to the change in oil properties. A model of this type allows for a more complex explanation of nutrient growth dynamics than when bacteria are only regarded as bulk. This model further makes it possible to assume that the immobilization of nutrients is less extensive over time, and that nutrients observed to be immobilized are used dynamically in microbial succession.

Inhibiting ureolytic activity by biological and photochemical products

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Figure 5. Microbial cell development in the oil on shoreline experiments (TNC, total number of cells; ANC, active number of cells; 5a, 5c, 5e, 5f, references without oil; while other figures show cell development in oil with INIPOL EAP22)
Figure 6. Biodegradation of oil in the shoreline experiments (data for fertilized and unfertilized oil given in the same figure, with 6a through 6d showing results from Experiments 4 through 7)

habits. We can thus assume that ureolytic activity is also present in arctic waters and at the experimental site.

Many slow-releasing fertilizers have been produced for agricultural use. Some contain urea as a source of nitrogen. The main problem of slow-releasing urea fertilizers has been the speed at which the transformation from urea to ammonium takes place, owing to the high ureolytic activity within the soil.5 The oil-soluble properties of the nitrogen of INIPOL EAP22 are related to the urea state of the nitrogen. Once mineralized to ammonium, the difference in solubility of the ion in oil and water, or the microbial fate of the ammonium ions, will determine the nutritional status of the oil slick or droplets. As the solubility will be much greater in water, osmotic forces will decrease the concentration of the ammonium ions in the vicinity of the oil. The only way to maintain a nitrogen concentration that is higher than that in the water is by microbial immobilization.

In the present experiments it has been demonstrated that a large inactive microbial biomass is developed. Thus, large quantities of the applied nutrients will be immobilized.

Fertilization efficiency will depend partly on whether the bacteria immobilizing the mineralized urea nitrogen are able to metabolize oil components, or whether there is one group of ureolytic bacteria and one hydrocarbonoclastic group, with little or no overlap between them.

Another issue is the spatial location of the fertilizer relative to the available urea fertilizer. If we consider the oil-fertilizer mixture as droplets, the arrangement of the fertilizer will determine whether a bacterial cell colonizing the droplet is able to metabolize both the oil components and the urea source of nitrogen, as urea.

In agriculture, urea is often used together with an inhibitor, to diminish the level of ureolytic activity. The most used commercial inhibitor is a mixture of catecol and p-benzoquinone. Catecol is a common intermediate product of most metabolic pathways, including those for aromatics.

We have no experimental indices on the presence of p-benzoquinone or control in these experiments. However, little is known of the photochemical fate of oil mixtures, and there is no reason to believe that similar products might not occur. On the other hand, there is no reason to believe that the available commercial mixture used for inhibition is unique, and other effect mixtures or components might possess the same inhibitory properties.

The results obtained until now with INIPOL EAP22 are of two types: those from oil-on-water experiments, where little or no biodegradation has been recorded; and those from experiments on oil in solid substrate, where significant biodegradation has been recorded for coarse sediments. The main differences between the abiotic forces exerted on either the oil or nutrients in these two situations are the following:

- Less light is available in the solid substrate than on water. Susceptibility to photochemical degradation is thus lower in solid substrate.
- Diluting action is less in solid substrate than in water. Mineralized nutrients might thus be less susceptible to leaching. Nutrients in ionic form might therefore be adsorbed.

The lower photochemical degradation that probably occurs in solid
that the inhibition of ureolytic activity can be a problem in water substrate, where biodegradation is recorded, might support the idea that the inhibition of ureolytic activity can be a problem in water habitats in the presence of oil. However, chemical degradation of oil can occur from radiation and, in solid substrate sediments, by the surface production of oxidants. Though results from the shoreline sediments vary depending on the sediment particle size, this may be explained by the fertilizer’s partitioning within the sediment. In coarse sediment the water saturation will decrease more during each tidal cycle, and the relative amount of air-filled space will be greater. The oleophilic fertilizer will thus have a greater ability to penetrate into the sediment and partition with the oil. In fine sediment, the fertilizer will only partition on the surface and will be removed by water as it rises.

Conceptual model of the effect of fertilizers on hydrocarbon degradation

Several matters have to be considered to evaluate whether fertilizer treatment will enhance the degradation of contaminating oil in any habitat. For instance, how available will the oil be to the applied fertilizer? In developing any fertilizer, we will have to determine the minimum criteria the fertilizer must meet in terms of physicochemical properties to ensure treatment of the contaminant, and not of organic matter in general. Practical issues, such as the possibility of spreading, will also have to be dealt with in this connection. Evaluations of such issues can be carried out with general transport models based on physical and chemical properties of the sediment.

It is also important to determine how the fertilizer and oil together interfere with the natural population of oil-degrading bacteria, and how the properties of the fertilizer affect this. When designing a fertilizer, we will have to determine the prerequisites for the fertilizer to optimize the microbial activity in relation to abiotic and biotic factors. In addition, the effect and retention time of the oil should be assessed. This type of evaluation can be accomplished by using models of generalized nutrient transformation, microbial activity, and decomposition.

The rest of this section presents a framework for such model evaluations. The models are conceptual rather than mathematical.

Aspects of fertilizer application to consider in modeling and development. Fertilizer application will depend on the available pore space, that is, total pore space excluding the total volume of substance that occupies that pore space. It will also depend on the properties of the fertilizer, such as sorption properties, density, and viscosity. The fertilizer-in-oil sorption properties will depend on the relation between fertilizer and external properties and on oil weathering. This implies that penetration of fertilizer into the sediment can have three different routes and can change from one route to the other at any time. These routes are in the gas phase, in the water phase, and in the oil phase. Besides diffusive properties of the fertilizer, the vertical movement due to gravity will be determined by the density of the fertilizer. Thus, the fertilizer INIPOL EAP22 applied might have had a density too low to enable optimal distribution. Future products for shoreline use should be developed with attention to density. Figure 7 illustrates these patterns.

Mineralization and transformation model. The mineralization and transformation model (Figure 8) describes the states of the nutrient and the substrate, and the abundance and adaptation of microbial cells. The modeling initially emphasizes the transport process that first predominates, while the transformation processes have increasing importance in the model as time proceeds. Points considered are the following:

- Substrate consumption, that is, biodegradation of oil, is divided into two flows, growth and maintenance.
- Nutrient uptake is associated with growth, not with maintenance of cell biomass, and thus only partly with oil biodegradation.
- Substrate consumption is associated with the availability of O2.
- Degradation of the surfactant matrix of the fertilizer can also occur under anoxic conditions.
- Rate of cell death is a function of nutrient conditions in relation both to biomass and to cell age. The death rate might be altered by chemical inhibitors.
- Rate of cell lysis is a function of cell age and external stress (e.g., microbial grazing).
- Transformation of organic nitrogen to available forms is an enzymatic process. Transformation may be faster in anoxic than inoxic conditions.
- There are two ways of achieving ammonification of immobilized nitrogen: by cell lysis and by faunal grazing on the biomass and necromass.
- Photochemical degradation of hydrocarbons might give products that inhibit ureolytic activity and thereby decrease the action of urea-containing fertilizers.

An increase in microbial numbers might be supported by two carbon sources: the surfactant, which is part of the nutrient, and the oil. Initiation of hydrocarbon degradation might start production of compounds that are inhibitors either alone or in mixture with photochemical or microbial products. The transformation of organic nutrients to a form available to the microbes may cease or decrease in the Arctic. Photochemical processes can therefore be a primary influence on the feasibility of enhancement after fertilizer is applied to oil.

The loss of evaporative nitrogen components can be greater in fine-grained sediments, with less oxygen exchange, than in coarse sediments.

Conclusions

It is possible to enhance the biodegradation of oils on shorelines through the application of fertilizers. The fertilizer must be adapted to the environmental constraints of the particular contaminated environment. INIPOL EAP22 has the properties necessary to support the enhancement of biodegradation under several difficult and different conditions on arctic shorelines. In Spitsbergen, the maximum en-
hancement obtained with INIPOL EAP22 during one arctic summer season (100 days) was approximately 90 percent, for marine gas oil in coarse shoreline sediments.

The experiments on water do not so far support the application of INIPOL EAP22 in open arctic marine water systems. In arctic conditions radiation is extensive, compared to more southern conditions. There are reasons to believe that when photochemical processes prevail, they are not always compatible with normal biological degradation. The interaction of components produced by the two processes may yield mixtures that inhibit the essential transformation of urea to ammonium, which can be utilized by bacteria.

The microbial kinetics of fertilizer application are complex and are of prime importance particularly with regard to the dose of fertilizer applied to the spilled oil and the limits of maximum enhancement of biodegradation.

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References


