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STIMULATED BIODEGRADATION OF OIL SLICKS
USING OLEOPHILIC FERTILIZERS

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<p>Biodegradation of polluting oil at sea is seriously limited by the scarcity of nitrogen and phosphorus. Since water soluble sources of these elements would be ineffective in the ocean, oleophilic compounds were screened to serve as fertilizers for oil slicks. A combination of paraffinized urea and octylphosphate was found to promote oil biodegradation both in laboratory experiments and in field trials to an extent that the practical application of this principle to oil cleanup appears feasible. The tested oleophilic fertilizer supplies nutrients to hydrocarbon-degrading microorganisms selectively, and in contrast to nitrate and phosphate salts it does not trigger algal blooms.</p>			

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The oceans of the world are currently being burdened by an estimated oil pollution load of 5 to 10 million metric tons per year (Blumer, 1973) and the resulting damage has caused widespread concern. Considerable research effort is being devoted to the prevention and control of oil spills (Joint Conferences on Prevention and Control of Oil Spills, 1969, 1971), but spills continue to occur. Theoretically, spilt oil can be cleaned up by various skimming devices, but prevailing weather conditions seldom allow this approach to succeed. Ignition is rarely feasible both because of safety considerations and because of the rapid evaporative loss of the low flashpoint components. The two remaining conventional measures, dispersion or sinking, are essentially cosmetic in nature. They merely render the oil less offensive, but in fact increase the exposure of marine life to the pollutant and are for this reason best avoided (Blumer, 1969).

A novel approach to the cleanup of spilt oil attempts to stimulate its biodegradation. This process is the major natural mechanism for elimination of oil from the marine environment, but it occurs at a distressingly slow rate. To speed up this process is the ultimate aim of ongoing research projects in various laboratories (Chem. Eng. News, 1970). Several commercial

microbial inocula are being marketed for the purpose of oil biodegradation but, to date, no controlled studies have been published on the effectiveness of these products.

In the course of our studies on the limiting factors of oil biodegradation in natural sea water we were impressed by the magnitude of the positive response to the addition of nitrate and phosphate supplements (Atlas and Bartha, 1972a). . Sea water contains very low concentrations of nitrogen and phosphorus, and petroleum or petroleum products are also deficient in these essential nutrients. Nitrate and phosphate are excellent sources of these elements as long as the sea water is enclosed in a laboratory flask, but they may be useless or worse when applied in the open ocean. Due to their solubility in water these salts can be expected to separate rapidly from the floating oil causing little or no benefit to oil degrading microorganisms. In addition, these mineral nutrients may trigger algal blooms and cause eutrophication problems. Application in the open ocean clearly requires the selection of nitrogen and phosphorous sources that physically adhere to the oil slick and benefit oil-degrading microorganisms selectively. This paper describes an effort to find such compounds and to put them to practical use in the stimulated biodegradation of marine oil slicks.

Materials and Methods

Evaluation of Oleophilic N- and P-sources. One hundred ml aliquots of a fresh sea water sample, collected in December off the east coast of Sandy Hook, N. J. and containing its indigenous microbial

population, were introduced into the flasks of a gas train arrangement described previously (Atlas and Bartha, 1972b). Each flask received 1 ml Sweden crude oil plus various nitrogen and phosphorus supplements. Sweden crude oil is a paraffinic petroleum and is described in greater detail in a previous publication (Atlas and Bartha 1972b). The additives were initially selected on the basis of their described physical and chemical properties (Stecher, 1968; Weast, 1967-68). The desired properties were: low C:N or C:P ratio to assure low BOD; oleophilic property, or the possibility of being rendered oleophilic; likelihood of being biodegradable and of low toxicity; and ready availability at a reasonable price. Compounds that had these attributes were subjected to a preliminary screening as to their behavior in an agitated two-phase system consisting of *n*-hexadecane and H₂O. Compounds that sank or dissolved in the water were excluded. Those that dissolved in the *n*-hexadecane phase or positioned themselves on the hexadecane - H₂O interface were selected for further testing. As sources of nitrogen were added dodecyl urea (Eastman) trimethylenetetraurea (Allied Chem.) and Sun Oil CRNF (a slow-release paraffinized urea agricultural fertilizer, N-content 26.8%, a gift of Sun Oil Co.). As water-soluble control KNO₃ was used. All of the nitrogen sources were added in amounts to give a final concentration of 10 mM N.

Phosphorus was added as octylphosphate, *isooctylphosphate* (both gifts of Stauffer Chem. Co.) and hexamethylphosphoramide (Aldrich Chem. Co.). As water-soluble control Na₂HPO₄ was used. The phosphorous sources were added in amounts to give a final concentration of 0.5 mM P, except for hexamethylphosphoramide, which was added to give the proper N concentration. The nitrogen sources

except for hexamethylphosphoramide were added in combination with either Na_2HPO_4 or octylphosphate. *Iso*-octylphosphate was added in combination with KNO_3 . Hexamethylphosphoramide was added alone as a source of both nitrogen and phosphorus. Two types of controls were included: sea water with Sweden crude oil but no other supplements and sea water with each tested combination of nitrogen and phosphorous but without Sweden crude oil. All treatments were done in duplicate.

The gas train apparatus was mounted on a rotary shaker and agitated at 200 rpm. Carbon dioxide-free air was passed through the flasks at the approximate rate of 15 ml per minute. Mineralization (conversion to CO_2) of the crude oil was measured in weekly intervals. After 4 weeks of incubation at 28°C all flasks were extracted with diethyl ether and the residual oil was measured by gas chromatography (Atlas and Bartha, 1972b). Sterilized controls served to correct biodegradation data for non-biological losses.

Simulated Field Tests. Because of the prevailing wave action and tidal conditions, it was not feasible to conduct the experiment in the open sea. Instead, the test was conducted in tanks with flow-through sea water at the Sandy Hook Marine Laboratory. The tests were conducted during the months of May and June in 113 liter aquarium tanks fitted with overflow outlets. Sea water filtered through cotton gauze and crushed sea shells to remove larger suspended particles was flowing through the tanks at the rate of 450 liter per day. An artificial "sea breeze" was created by an electric fan. Glass tubes 5 cm in diameter and 30 cm long were inserted into styrofoam floats as shown in Figure 1. Twenty-four tubes were floated in each

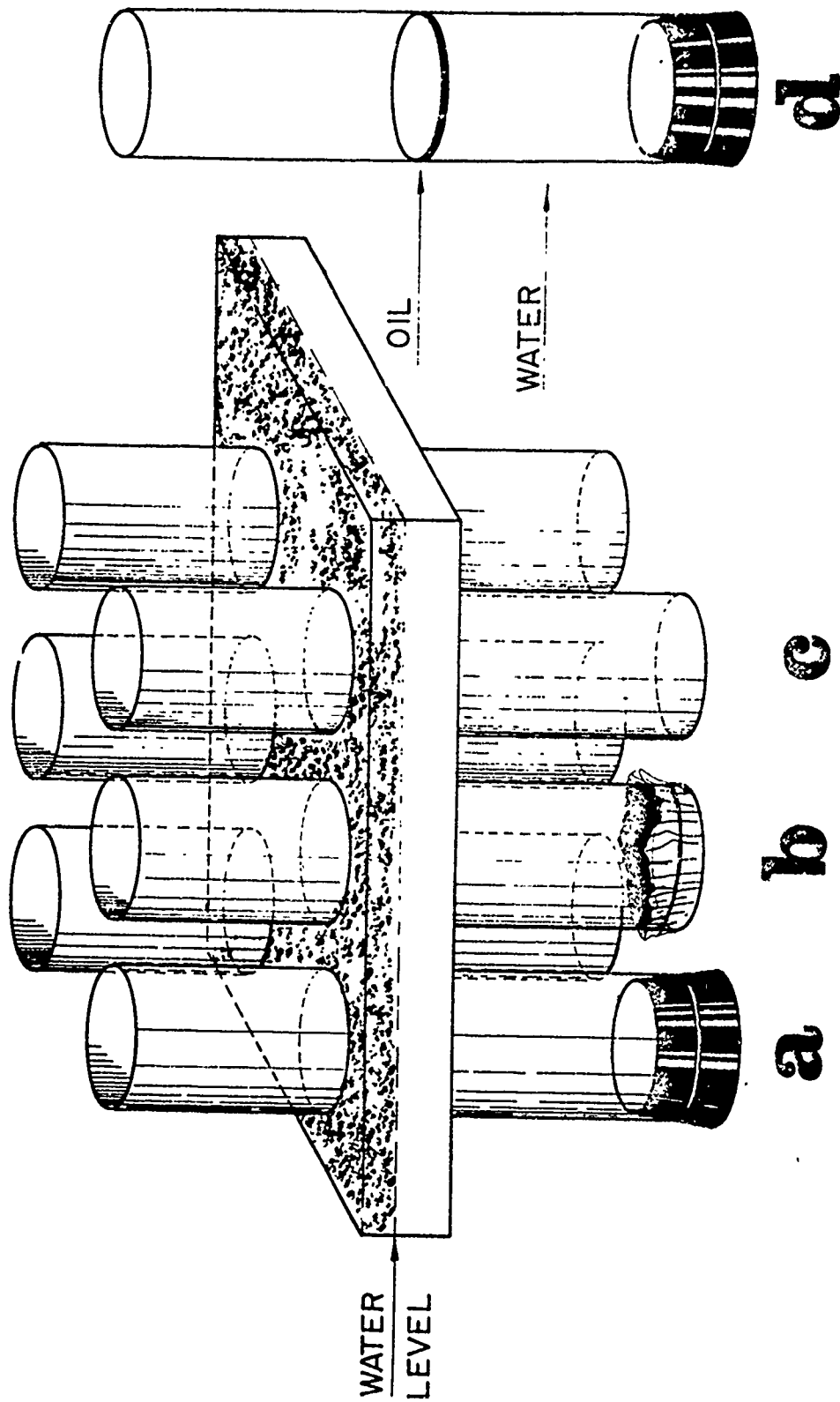


Figure 1. Diagram of an apparatus employed for petroleum degradation tests in flow-through sea water tanks. For explanation see text.

of two tanks. Some tubes were closed on their lower ends with a rubber stopper and were poisoned with 1% HgCl_2 to serve as controls for evaporative losses (a). Others were closed with a Visking cellulose dialysis membrane to allow the exchange of ions and gases but to prevent any loss of undegraded oil by sinking (b). A third type of tube was left open (c). Prior to removal for analysis, each type of tube was closed under water by a rubber stopper and was lifted out with its entire contents.

One ml of Sweden crude oil was added to each cylinder. Some of the miniature oil slicks received no supplementation. Others were supplemented with either 101 mg of KNO_3 and 7.5 mg of Na_2HPO_4 or with 62 mg of CRNF fertilizer and 7 mg of octylphosphate. Each treatment was tested with both membrane-closed and open cylinders.

Water temperature was measured daily throughout the experiment. Tubes of each type were removed for analysis weekly. The sea water and the residual oil in the tubes were transferred to separatory funnels. The tubes were rinsed with diethyl ether to insure complete transfer of the oil. The oil was then extracted using two 100 ml portions of diethyl ether. The extracts were combined, dried with anhydrous Na_2SO_4 and the residual oil was determined by gas chromatography (Atlas and Bartha, 1972b).

Algal Growth. The sea water for this experiment was collected in May off the east coast of Sandy Hook, N. J. Aliquots of sea water (150 ml) were added to sterile 1 liter culture bottles (64 x 99 x 299 mm). The sea water aliquots were either left untreated, were supplemented with KNO_3 and Na_2HPO_4 , or were supplemented with CRNF and octylphosphate. The nitrogen

supplements were added in amounts to give a final concentration of 10 mM N. The phosphorus supplements were added to give a final concentration of 0.5 mM P. The bottles were incubated flat on their sides under a bank of fluorescent lights (2 F40 cool white fluorescent tubes located 40 cm above the culture bottles; light intensity 220 foot-candles). Algal growth was measured as chlorophyll according to the method of Warburg (Vishniac, 1957). One hundred ml of the sea water was used for the chlorophyll assay.

Results

Evaluation of Hydrophobic N- and P-sources. No measurable mineralization (conversion to CO₂) of the added petroleum occurred within 4 weeks in sea water samples that did not receive sources of nitrogen and phosphorus, nor was CO₂-evolution observed in flasks that received N- and P-sources but no petroleum. In the presence of a suitable P-source, the tested nitrogen compounds showed widely varying abilities to support oil mineralization (Figure 2). While the CRNF paraffinized urea fertilizer (B,E) gave as good or better results than nitrate (A,C,D), dodecylurea (F,G) proved to be inferior and trimethylenetetraurea (H,I) supported only a small fraction of the oil mineralization elicited by nitrate and CRNF. In the presence of a suitable nitrogen source, octylphosphate (C,E) and *iso*-octylphosphate (D) supported significantly more rapid oil mineralization than inorganic phosphate (A,B). Hexamethylphosphoramide (J) as a combined N- and P-source performed poorly.

The gas chromatographic measurement of residual petroleum at the conclusion of the experiment (Table I) confirmed the conclusions

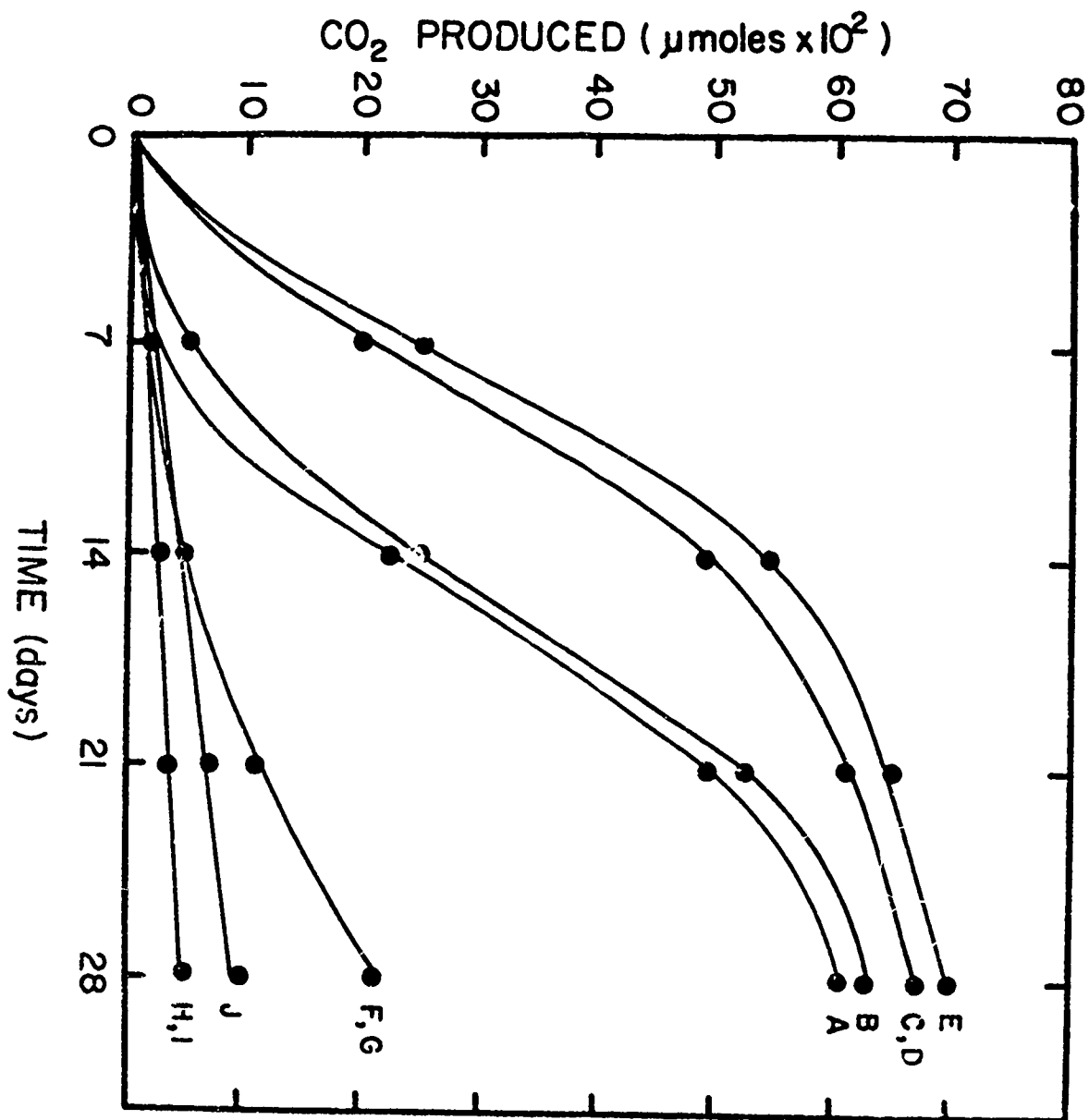


Figure 3. Influence of various nitrogen and phosphorous additives on oil biodegradation in flow-through sea water tanks. Symbols with prime indicate membrane-closed sample tubes. Symbols without prime indicate open tubes. Additives: (A,A') none, (B,B') $\text{KNO}_3 + \text{Na}_2\text{HPO}_4$, (C,C') CRIF-fertilizer + octylphosphate.

Table I. Ability of Various Nitrogen and Phosphorous Sources to Support Oil Biodegradation

Nitrogen Source	Phosphorous Source	Percent Oil Biodegraded
KNO ₃	Na ₂ HPO ₄	70
KNO ₃	Octylphosphate	72
KNO ₃	<i>Iso</i> -octylphosphate	70
CRNF	Na ₂ HPO ₄	70
CRNF	Octylphosphate	72
Dodecylurea	Na ₂ HPO ₄	37
Dodecylurea	Octylphosphate	35
Trimethylenetetraurea	Na ₂ HPO ₄	15
Trimethylenetetraurea	Octylphosphate	15
Hexamethylphosphoramide	Hexamethylphosphoramide	15
None added	None added	5

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drawn from the CO₂-evolution patterns. On the basis of this experiment CRNF fertilizer with octylphosphate was chosen as the most promising nutrient combination to be tested in a field-like situation.

Simulated Field Test. This experiment was designed to answer two critical questions: are nitrogen and phosphorous limiting factors in oil biodegradation when the oil slick is not enclosed in a laboratory flask with a limited volume but is floating on an essentially infinite amount of sea water, and, is a hydrophobic nitrogen and phosphorous fertilizer able to benefit selectively the oil-degrading microorganisms under the above conditions?

During the 42-day period of the experiment (May 9 - June 20) the temperature of the sea water rose linearly from 15°C to 20°C. The biodegradation of unsupplemented oil slicks was very slow in the circulating sea water system (Figure 3, A and A') and amounted to only 10% oil biodegradation in 42 days. No difference was observed between the open (A) and the membrane-closed tubes (A'), indicating that no losses had occurred through sinking of oil droplets from the open tubes. When the oil slicks in the open tubes were treated with nitrate and phosphate salts, only a slight and temporary increase in oil biodegradation was observed (B). In contrast, the same treatment resulted in a large early increase in the membrane-closed tubes (B') followed by a rate that became similar to the unsupplemented tubes (A and A'). Apparently, the dialysis membrane delayed the loss of nitrate and phosphate salts from these tubes. The greatest increase in oil biodegradation occurred, however, in the CRNF- and octylphosphate-treated slicks (C and C'). There was no

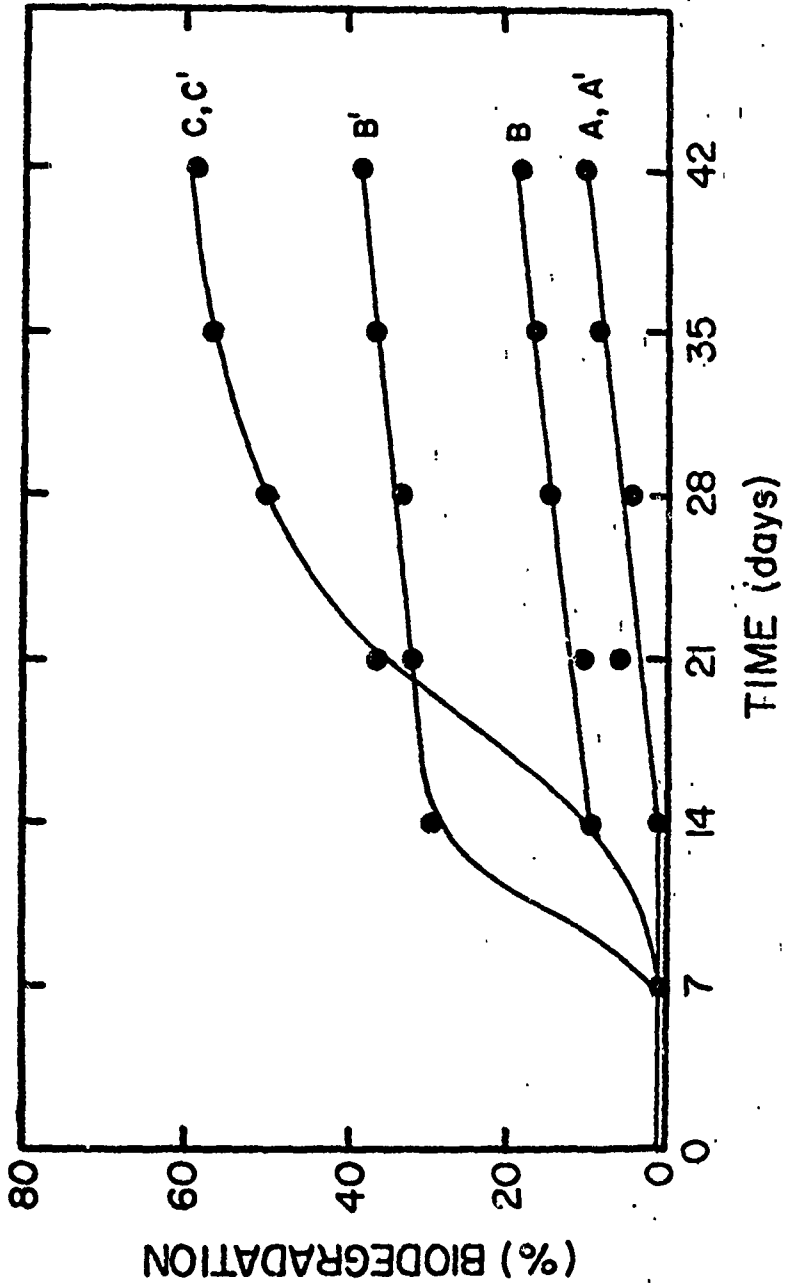


Figure 2. Influence of various nitrogen and phosphorous additives

on the microbial CO₂-production from *P. putrescens* on sea water. Additives: (A) KNO₃ + Na₂HPO₄, (B) CRNF-fertilizer + Na₂HPO₄, (C) KNO₃ + octylphosphate, (D) KNO₃ + iso-octylphosphate, (E) CRNF-fertilizer + octylphosphate, (F) dodecylurea + Na₂HPO₄, (G) dodecylurea + octylphosphate, (H) trimethylenetetraurea + Na₂HPO₄, (I) trimethylenetetraurea + octylphosphate, (J) hexamethylphosphoramide.

difference between the stimulation in the open (C) and membrane-closed (C') tubes, showing that the fertilizer adhered strongly to the oil and was not eluted by circulating sea water.

Algal Growth. This experiment was conducted to test whether or not the application of a hydrophobic fertilizer triggers algal blooms. The chlorophyll concentration of unsupplemented sea water and of sea water treated with CRNF and octylphosphate was less than 0.0033 $\mu\text{g/ml}$, the limit of detection for the procedure employed. In contrast, chlorophyll rose to 0.15 $\mu\text{g/ml}$ in the flasks that were supplemented with nitrate and phosphate.

Discussion

Studies on an ecological problem, such as oil pollution, require that the natural situation be modelled as closely as possible. Laboratory tests are useful because they allow for controlled conditions, but they often radically alter the natural situation. Field experiments are often very difficult to conduct in a manner that is sufficiently controlled and quantitative to yield scientifically acceptable evidence. In some cases floating frames were used in field experiments to contain an oil slick (Chem. Eng. News, 1970) but wind or current in excess of only a few knots renders the most elaborate floating barriers ineffective (Swift *et al.*, 1969). Oil that escapes from the containment may be mistakenly considered as "degraded." Some workers have used stagnant sea water tanks of various sizes (Gunkel, 1967; Kator *et al.*, 1971) but this experimental design limits the amount of available sea water. Quantitative and representative sample removal for analysis in the systems described

above is very difficult (Kator *et al.*, 1971). We believe that our experimental design with flow-through sea water and individual sample tubes solved some of these difficulties and may prove useful in future studies of similar nature.

Stimulated oil biodegradation as compared to other methods of oil cleanup has several desirable features but also some limitations. On the positive side, biodegradation is a natural process and, therefore, can be expected to have few undesirable ecological side effects. Biodegradation is not hampered by wind or wave action, it is expected to be relatively inexpensive, and can be carried out without elaborate machinery. On the other hand, its rate is inherently slower than that of physical removal or dispersion by detergents and this rate may be further slowed by low water temperatures or by exhaustion of dissolved oxygen. Stimulated biodegradation has its proper role in an integrated oil pollution program, being applicable primarily to that portion of the spilled oil that escapes containment and spreads out too thinly to be collected by physical methods.

Most of the current effort directed towards the artificial stimulation of oil biodegradation (Chem. Eng. News, 1970) seems to be directed towards the development of proper bacterial inocula. However, it is important to remember that even the best inocula will fail in a nutritionally unfavorable environment. We suggest that such inocula should be combined with the type of fertilizers we described. Additionally, some marine environments seem to have sufficient indigenous microbial population to carry out extensive oil biodegradation if properly fertilized (Atlas and Bartha, 1973).

The CRNF fertilizer was shown here to be as good a source of nitrogen for oil degrading microorganisms as KNO_3 , and octylphosphate was actually superior to Na_2HPO_4 . The latter may be explained with the fact that phosphate tends to precipitate in sea water while octylphosphate and *iso*-octylphosphate remain available to the oil degraders. In addition, the surface active properties of octylphosphate may increase the oil surface available for microbial attack. CRNF and octylphosphate contribute relatively little to the BOD (N in CRNF = 26.8%; P in octylphosphate = 14.8%) and in preliminary tests this fertilizer combination failed to trigger algal blooms. Roughly, CRNF and octylphosphate will support the biodegradation of oil that is 10 and 100 times their own weights, respectively. The cost of such nutrient additives to treat 1 ton of spilt oil is estimated to be under \$15.00 (estimate based on manufacturers' recommended prices for CRNF and octylphosphate).

We do not claim that the described fertilizer formulation is necessarily the most effective and the most inexpensive, and practical use of the presented principle will require further commercial development, toxicity studies, and large-scale field trials. These matters are beyond our mission and facilities, and will best be handled by experienced commercial organizations. Our aim here has been to document the scientific validity of an idea, i.e., that hydrophobic fertilizers are able to promote oil biodegradation on the high seas to a degree that invites practical exploitation.

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