Marine Ecosystem Enclosed Experiments

Proceedings of a symposium held in Beijing, People's Republic of China, 9-14 May 1987
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Abstract

This symposium on marine ecosystem enclosed experiments (MEEE) consists of nine review papers that describe various types of ecosystem enclosures and a series of papers resulting from enclosure experiments in Xiamen, People’s Republic of China, and Saanich Inlet, BC, Canada. The reviews on types of enclosures include benthic enclosures for rocky and sandy shores and the effects of pollutants (primarily hydrocarbons) on bacteria, macroalgae, and invertebrates. The pelagic enclosures were used to study the control of phytoplankton blooms, the uptake and release of dissolved organic substances, and the effects of pesticides on freshwater ecosystems.

Six enclosure experiments were conducted in China and Canada from 1986–87. Some of these experiments examined the effects of contaminated sediments, primarily heavy metals, on bacteria, phytoplankton, and zooplankton and the pathways and fates of these heavy metals in the seawater. Other experiments studied the chemistry and biological effects of chemically dispersed oil.

Résumé

Ce compte rendu du symposium sur les expériences faites en écosystèmes marins comprend neuf communications qui décrivent les écosystèmes retenus et les expériences faites à Xiamen en République populaire de Chine et à Saanich Inlet, C.-B., au Canada. Les communications portent, notamment, sur les écosystèmes benthiques des litoraux rocheux et sablonneux et sur les effets des polluants (surtout les hydrocarbures) sur les bactéries, les grandes algues et les invertébrés. Les expériences sur le contrôle des brutales pullulations ("blooms") du phytoplancton furent menées dans les écosystèmes pélagiques, ainsi que l’absorption et le dégagement des substances organiques dissoutes et les effets des pesticides sur les écosystèmes d’eau douce.


Resumen

Este simposio sobre Experimentos Marinos en Ecosistemas Cerrados (MEEE) consistió en nueve trabajos de análisis que describen varios tipos de enclaustramientos ecosistémicos y una serie de trabajos derivados de experimentos con estos enclaustramientos en Xiamen, República Popular de China, y en Saanich Inlet, Canadá. Los estudios incluyen enclaustramientos bentónicos para costas rocosas y arenosas, y los efectos de los contaminantes (fundamentalmente hidrocarburos) sobre bacterias, macroalgas e invertebrados. Los enclaustramientos pelágicos se utilizaron para estudiar el control de la reproducción del fitoplancton, la ingestión y expulsión de substancias orgánicas disueltas y los efectos de pesticidas en los ecosistemas de agua dulce.

Se realizaron seis experimentos en ecosistemas cerrados en China y Canadá, de 1983 a 1987. Algunos de estos experimentos examinaron los efectos que ejercen los sedimentos contaminados, fundamentalmente los metales pesados, sobre bacterias, fitoplancton y zooplancton, y el ciclo y destino final de estos metales pesados en el agua de mar. Otros experimentos estudiaron los efectos químicos y biológicos de los aceites crudos dispersados por medios químicos.
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In this review, stimulation of the growth of native microbial populations through the introduction of crude oils, refined oils, and individual hydrocarbons into marine enclosures is examined. Also, the involvement of microbes in the degradation of alkanes and aromatic hydrocarbons is assessed. Finally, a case is presented for a possible pivotal role for microbial flocs in biodegradation of petroleum hydrocarbons at sea.

In the late 1960s and early 1970s, the continuing increase in oil-tanker traffic, reports of floating tar balls far out at sea, evidence of chronic oil releases in marine operations, the potential for catastrophic oil spills, and a lack of knowledge concerning the fragility of the marine biosphere pressed the scientific community to gather evidence on the fate and effects of petroleum hydrocarbons in marine ecosystems. Pursuit of this evidence continues to this day. The marine enclosure now being used in its many forms by researchers around the world has afforded an opportunity to study the fate and effects of petroleum and other pollutants on marine communities under controlled conditions.

This paper focuses on three aspects of the fate and effects of petroleum hydrocarbons in marine enclosures: stimulation of microbial growth, biodegradation of dispersants and petroleum hydrocarbons, and the role of bioflocs in oil biodegradation and sedimentation. In addition, this paper examines the consequences of using a chemical dispersant, specifically Corexit 9527, to facilitate the transport of oil into the water column.

Stimulation of microbial growth

In one of the first oil pollution experiments (Hodson et al. 1977) carried out in a Controlled Ecosystem Pollution Experiment (CEPEX) enclosure in August 1974, No. 2 fuel oil at an initial concentration of 0.01 mg-L\(^{-1}\) caused neither an increase nor a decrease in the microbial population over a 30-d experiment. At that concentration, the fuel oil did not stimulate or inhibit bacterial growth. In contrast, the
addition of Cu\textsuperscript{2+} at 0.01 and 0.05 mg L\textsuperscript{-1} resulted in a dramatic increase in bacterial numbers. The preferred explanation for the increase was that population growth was stimulated by the rapid release of heterotrophic substances resulting from increased algal excretion and mortality (Vaccaro et al. 1977).

Thus, it appeared that No. 2 fuel oil had little effect on populations growth, either directly by affecting bacteria or indirectly through algal toxicity, at a concentration of 0.01 mg L\textsuperscript{-1}. That concentration of fuel oil appeared not to increase the potential of the indigenous microbial population to degrade hydrocarbons either, based on the ability to oxidize in vitro \([\text{n}^{14}\text{C}]\text{fluorene}\) and \([\text{n}^{14}\text{C}]\text{hexadecane}\). Furthermore, with D-glucose assimilation as an index, the data obtained using several different oils and water samples drawn from control and oil enclosures after 30 d indicated that a concentration of oil greater than about 0.3 mg L\textsuperscript{-1} would inhibit the activity of natural marine bacterial populations.

In apparent contrast to the results of the early CEPEX experiment, Hagstrom (1977) reported greater than 100-fold increases in bacterial numbers over 10–15 days in a 4.2-m\textsuperscript{3} enclosure equipped with a flow-through system delivering Baltic seawater (7%o salinity) under a slick of light fuel oil. The concentration of oil under the slick varied from about 1–9 mg L\textsuperscript{-1} depending on weather conditions. Although a number of differences, such as salinity, volume, water source, and method of conducting the experiment, distinguished this experiment from the CEPEX experiment, temperatures were at least comparable and the oils were similar in that No. 2 fuel oil is a light fuel oil. The major difference appears to have been the concentration of oil in the water, i.e., 0.01 mg L\textsuperscript{-1} initially for the CEPEX experiment and 1–9 mg L\textsuperscript{-1} for the Baltic seawater study.

Another enclosure study showing microbial growth in response to oil was conducted in France. In this particular study (Marty et al. 1979), topped Saudi Arabian crude oil was mixed into 15 m\textsuperscript{3} of seawater in 20-m\textsuperscript{2} circular basins with the aid of chemical dispersants. The dispersants were Corexit 9527, Hydrogamosol LT, and OSR LT 126. The concentrations of the oil over the course of the experiment were not reported. Given complete dispersion, the initial concentration could have been over 600 mg L\textsuperscript{-1}. The concentration of oil would have fallen rapidly, however, as the dispersant could not have maintained such a high concentration for very long. By the next day, a fairly long-lasting dispersion in water (in the 1–10 mg L\textsuperscript{-1} range) would have been likely (Cretney et al. 1981; MacNeill et al. 1985).

Bacterial growth during the first 15 d was observed in all treatment tanks, whereas none was seen in the seawater control. There was a varying lag period of 1–3 d with respect to bacterial growth for the treatments involving oil and dispersant mixtures. In the case of the Corexit 9527-generated oil dispersions, after a 1-d lag in one experiment and a 3-d lag in another, increases were about 100-fold and 10-fold, respectively, after 15 d. In the case of treatments with oil or dispersants only, there was no lag in bacterial growth, so the most rapid growth was obtained during the first 24 h. In the case of Corexit 9527, a 40–120-fold increase in population was observed during this initial period. The causes of the bacterial population increase may have been multifold, arising from utilization of the carbon sources presented by the dispersant or oil or both, utilization of algal products released under dispersant or oil stress, or elimination of bacterial predators. The lag phase in the case of the mixed oil and dispersant treatments may have reflected toxicity to microbes of high initial concentrations of oil in the seawater. The absence of a lag in the growth of bacteria in the tank treated only with oil may have resulted from
inefficient transfer of the topped oil to the water column and the short period of exposure to the toxic substance.

In an experiment carried out in 1977 in Patricia Bay, Saanich Inlet, British Columbia, using 66-m³ enclosures of the CEPEX design (Cretney et al. 1981), immediate logarithmic bacterial growth was observed 2 m beneath a slick of Prudhoe Bay crude oil that was premixed with Corexit 9527 (20:1). The same phenomenon also occurred when a weathered slick in a second enclosure was sprayed with Corexit 9527. The increase in bacteria was about 100-fold whereas the presence of an oil slick itself resulted in less than a fivefold increase. The chemically dispersed oil was carried into the water column by turbulent diffusion, which generated approximately Gaussian concentration profiles. For most of the experimental period, the concentrations were in the range of 1–20 mg-L⁻¹. As in the other studies cited, the reason for the bacterial growth was not elucidated. Before the oil was placed in the enclosures, however, they were allowed to stand for 5 d. During this time, a phytoplankton bloom occurred and crashed, after which the settled material was removed at the bottom. The seawater in the enclosure was then thought to be nutrient depleted without a significant phytoplankton population. Although bacterial growth following dispersion of the oil could have been stimulated by the presence of a carbon source in the form of oil hydrocarbons or the dispersant, elimination of bacterial grazers was a more likely reason.

A much more complete study of the fate and effects of dispersion of Prudhoe Bay crude oil by Corexit 9527 in Patricia Bay using 66-m³ enclosures was carried out in July–August 1983 (Parsons et al. 1984; Wong et al. 1984; Lee et al. 1985; Harrison et al. 1986). In this experiment, the oil–dispersant mix was injected at a depth of between 2 and 4 m to generate an initial concentrations of oil at 20 mg-L⁻¹ and of dispersant at 2 mg-L⁻¹. In another enclosure, a similar injection was made with dispersant only. A third enclosure was used as a control. Inorganic nutrients were added to all enclosures to stimulate phytoplankton growth in a departure from the 1977 study. Primary production was stimulated immediately in the control and Corexit-only enclosures, but was much delayed in the oil–dispersant enclosure. The oil–dispersant enclosure showed an almost immediate increase in bacterial standing stock and heterotrophic bacterial production, each reaching a maximum after about 5 d. The other enclosures showed similar increases in heterotrophic potential, but without the increases in standing stock. This apparent discrepancy was most likely caused by grazing pressure of bacterivores that were controlling the population in the control and Corexit enclosures, but were eliminated or incapacitated in the Corexit–oil enclosure (Lee et al. 1985). Phasing of bacterial growth and the reduction of zooflagellates in each of the three enclosures was consistent with this grazing hypothesis. In general, the control enclosure and the enclosure treated only with Corexit behaved in a similar manner with respect to plankton dynamics. The small quantitative differences observed were within the range of natural variability.

In March and April 1979, at Rosfjord in southern Norway, an enclosure study similar to the 1977 Patricia Bay study was carried out using Ekofisk crude oil (Laake et al. 1984). No chemical dispersant was used, but natural mixing action generated oil concentrations in the 1–20 mg-L⁻¹ range. Bacterial growth was observed and the population reached a maximum at 6 m after 4 d. The approximately 10-fold increase in bacterial population gradually diminished in succeeding days to a minimum between days 9 and 10 before once again increasing. The bacterial maximum followed the initial oiling and subsequent crash of the resident
phytoplankton population. The minimum after 9 d was attributed to a low concentration of dissolved nitrogen and phosphorus. An increase in bacterial numbers after day 9 coincided with the mixing in of nutrients from below 10 m on days 10 and 11. No information on zooplankton dynamics was presented, so that the probable influence of grazing pressure cannot be determined.

In general, marine enclosure work indicates that stimulation of bacterial growth will occur with oil concentrations in the low-milligrams per litre range. Concentrations in the low-micrograms per litre and high-milligrams per litre ranges seem to elicit "no response" and "inhibition of growth" respectively. If nutrient supply from affected phytoplankton and elimination of bacteriovores are important general consequences of oil that is naturally, mechanically, or chemically dispersed, as indicated by the enclosure studies, bacterial growth in the wake of oil slicks would seem to be a reasonable consequence of oil spills at sea. In this regard, it is worth noting the results of a 100-t experimental oil spill that was conducted in 1982 at Halten Bank, off Norway (Lange 1985). The oil appeared to inhibit bacterial growth under the thickest part of the slick. The bacterial concentration under the tail of the slick, however, was about 10 times the concentration found in unoiled seawater at the same depth. Lange speculated that the exposure time of 1.5–3 d may have been sufficient to result in the observed bacterial increase.

Biodegradation of oil dispersants and oils

Oil dispersant biodegradation

The formulation of oil-spill dispersants tends to be proprietary information that is not readily available from manufacturers. The basic formulation of Corexit 9527 (Table 1, Fig.1), manufactured by Exxon (Canevari and Cranford 1974), is probably typical of most dispersants, however, and is available to researchers (Wells et al. 1985).

Oleic acid (I) is a natural fatty acid with a central olefinic bond and 18 carbon atoms. It is the most common fatty acid constituent of olive oil, peanut oil, beef tallow, and whale blubber (Fieser and Fieser 1961). Sorbitan monooleate (II), also known as Span 80, is used in pharmaceutical formulations. The sorbitan monooleate ethylene oxide adduct (20 mol) is also known as Tween 80. This compound is used as an emulsifier and dispersant in pharmaceutical products that are meant to be taken internally. The fatty acid component of Tween 80 and Span 80 is oleic acid. Sodium dioctylsulfosuccinate or Aerosol OT is a typical wetting agent (Fieser and Fieser 1961). The glycol ether may be a cellusolve, such as Cellusolve (2-ethoxyethanol) (IV, R=CH3CH2) or Butylcellusolve (2-butoxyethanol) (IV, R=CH3(CH2)3), which are common industrial solvents.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oleic acid (I) (and/or sorbitan monooleate (II))</td>
<td>16</td>
</tr>
<tr>
<td>Sorbitan monooleate ethylene oxide adduct (20 mol)</td>
<td>32</td>
</tr>
<tr>
<td>Sodium dioctylsulfosuccinate (III)</td>
<td>35</td>
</tr>
<tr>
<td>Glycol ether</td>
<td>17</td>
</tr>
</tbody>
</table>

Table 1. Composition of Corexit 9527.
Regarding biodegradation of compounds such as oleic acid, Tween 80, and Span 80, an interesting paper by Una and Garcia (1983) is available. They studied biodegradation of nonionic dispersants in seawater in the laboratory using microorganisms obtained from an oil-spill site. They proposed a kinetic model of enzyme action based on their results that involved hydrolysis of the ester, followed by cleavage of the double bond in oleic acid to give nonanoic acid (CH$_3$(CH$_2$)$_7$CO$_2$H) and azaleic acid (HO$_2$C(CH$_2$)$_7$CO$_2$H). These two acids are further oxidized by β-oxidation in a cascade to acetate. Based on the kinetics they observed, Una and Garcia proposed a model with two remarkable attributes:

- The hydrolysis of the ester is sterically impeded by the ethylene oxide residues or hindered by their promoting the formation of micelles.
- Once cleavage has occurred, however, the ethylene oxide-containing hydrolysis product promotes oxidative cleavage of the oleic acid.

Formulation of Corexit 9527 by accident or design thus seems ideally suited to biodegradation of its principal components.

Using 38-L aquaria in a laboratory study, Traxler and Bhattacharya (1978) obtained evidence of the biodegradation of Corexit 9527 in the presence and absence of crude oils by unaltered microbial populations of seawater from Narragansett Bay, RI, USA. Oxygen depletion by microorganisms occurred in the presence of Corexit 9527 as the only carbon source. In a series of experiments using n-[1,14C]hexadecane in Kuwait crude oil, these workers observed a 20-h lag in test systems containing dispersant before the labeled n-hexadecane was oxidized to carbon dioxide. This lag corresponded to the time previously found necessary for
biodegradation of the dispersant. They concluded that Corexit 9527 was biodegradable and was not toxic to hydrocarbon degraders.

Another laboratory study (Foght and Westlake 1982) showing temporary retardation of oil degradation by Corexit 9527 also established that the length of the lag period depended on the amount of Corexit 9527 present. The length of the lag also depended on the nutrient state. In the case of no nutrient supplementation, in flasks with 10% Corexit 9527, there was essentially no degradation of n-alkanes after 12 weeks; whereas in flasks with 0 or 1% dispersant, degradation of these components was complete.

**Hydrocarbon biodegradation**

In this section, those enclosure studies in which a direct measure of hydrocarbon biodegradation in the water column has been made are specifically mentioned. Such direct measures include determination of ratios of n-alkane to isoalkane, $^{14}$CO$_2$ activity, and $^{14}$C activity incorporated into biota other than in the form of the originally labeled hydrocarbon.

**Biodegradation of aromatic hydrocarbons**

In a CEPEX enclosure experiment carried out during the summer of 1976, Lee et al. (1978) added radioactive $[^3]$H[benzo[a]pyrene (Fig.2: V) to determine its fate in the controlled ecosystem. About 40% of the labeled benzo[a]pyrene was found in the bottom sediments. Evaporation was discounted as a possible reason for the loss of the remainder. Incomplete recovery from the sediments and some loss to the enclosure walls probably occurred, but these losses were not considered to be important enough to account for the missing radioactivity. During the experiment, however, 70–90% of the radioactivity in the water was removed by filtration using glassfibre filters. Of the nonfilterable radioactivity, a portion from 0 to 67% could be attributed to hydroxylated derivatives and quinones. Based on measurements of biodegradation potential using water samples from the enclosures, biodegradation was considered to be unimportant as a removal process for benzo[a]pyrene. The researchers concluded that photochemical oxidation, rather than biochemical oxidation, probably accounted for the removal of up to 50% of the labeled benzo[a]pyrene.

In a 1978 MERL experiment (Hinga et al. 1980), the biogeochemical fate of $^{14}$C-labeled benz[a]anthracene (VI) was determined. The radiotracer label was followed for 230 d. The production of $^{14}$CO$_2$, and concentrations of labeled benzo[a]anthracene and its oxidative products were determined. Hinga et al. (1980) proposed that a combination of microbial and photochemical processes gave rise to the labeled products found in the water and sediments. They also suggested that animal metabolism might play a degradative role in sediments. These authors did not attempt to estimate the relative importance of photooxidation and biooxidation in the degradation of the hydrocarbon. In this regard, it should be noted that the benz[a]anthracenes were transported to the sediments within the first few days of the start of the experiment and that most of the mineralization of the compound occurred there. The authors calculated that the half-life of benzo[a]anthracene fell within the range of 1.2–3 years. In a later experiment using 7,12-dimethylbenz[a]anthracene, Hinga et al. (1986) found that photodegradation probably accounted for the initial
transformation of that compound in the water column, with biodegradation probably being responsible for mineralization.

In an earlier series of three experiments beginning in December 1976 and running with intervals in between through to July 1978 (Gearing et al. 1979), evidence of biodegradation of naphthalenes (VII) was obtained. In the latter two experiments, semiweekly oil additions were carried out for several months. The degradation potential for the naphthalenes was found to be enhanced by the semiweekly additions of oil. This finding was backed up by depletion of naphthalenes in the water column, attributable to biodegradation, which correlated nicely with the biodegradation potentials determined. No rates were calculated, but significant changes, due to biodegradation, were observed in 3–4 d.

From March 1982 to September 1983, a series of 10 radiotracer experiments were conducted using the MERL system (Wakeham et al. 1986a). All but three of the experiments involved aromatic hydrocarbons or halogenated aromatic hydro-
carbons. Removal rates were determined for the compounds. First-order rate constants for biodegradation of benzene (VIII), toluene (IX), and naphthalene (VII, \( R_i = H \) for \( i = 1 \ldots 8 \)) were determined. The rate constant's values depended on the time of the year for which they were determined, the method of calculation, and also on the time interval from the start of the experiment over which they were calculated. The maximum rates, however, were 1.67, 1.07, and 1.17 \( \text{d}^{-1} \) for benzene, toluene, and naphthalene respectively.

**Biodegradation of n-alkanes**

During the series of experiments (Gearing et al. 1979) described above in which evidence of naphthalene biodegradation was obtained, biodegradation of n-alkanes was also evident from the changing ratios of n-alkanes to isoprenoids in water samples collected from the enclosures. Adaptation of the microbial population to oil was also indicated. For example, in a comparison of the hydrocarbon distributions determined for water samples obtained 90 h after the 3rd and 33rd oil additions, the n-alkane to isoprenoid ratios were much reduced after the 33rd addition, but only slightly reduced after the 3rd addition compared with those ratios observed when No. 2 fuel oil was added to the ecosystem. The authors did not attempt to determine rate constants or half lives for the n-alkanes. Given that there was no degradation of the isoprenoids and that simple first-order kinetics apply, a rough estimate of 0.17 \( \text{d}^{-1} \) for the rate constant can be made using the n-heptadecane to pristane ratio (Table 2).

In a series of experiments designed to examine transportation of No. 2 fuel oil between the water column, surface microlayer, and atmosphere in a MERL enclosure, the relative extents of biodegradation occurring in the water column and surface microlayer were compared (Gearing and Gearing 1982). No evidence of heightened microbial oxidation of oil in the surface microlayer was obtained. A temperature effect was observed, however, so that enhanced degradation was observed in the warm waters of the July 1978 and September 1979 experiments compared with the cold water of the March 1978 experiments.

Although the data are perhaps unsuitable for rate-constant determinations, a crude calculation, to provide a basis for comparison, gives a first-order rate constant of 0.05 \( \text{d}^{-1} \) for the March (0–2°C) water and constants of 0.32 and 0.19 \( \text{d}^{-1} \) for the warmer waters of July (20–21°C) and September (17–19°C) respectively. These estimates are calculated from the ratios of n-hexadecane to norpristane and are subject to error arising through evaporation and dissolution during the course of the experiment. Norpristane has a higher vapour pressure and greater solubility than n-hexadecane at ambient temperature, so these processes tend to increase the ratio of n-hexadecane to norpristane with time as biodegradation reduces it. The same problem exists for the other n-alkane to isoprenoid ratios used; so with all other factors being equal, the rate constants calculated would tend to underestimate the actual values.

In the March 1982 to September 1983 series of radiotracer experiments on biodegradation of aromatic hydrocarbons using MERL enclosures, biodegradation of n-decane and n-octadecane was also studied (Wakeham et al. 1986b). Depending on whether the rate constant was determined from \( ^{14} \text{CO}_2 \) production in situ or loss of radioactivity in situ that was not accounted for by evaporation or sedimentation or loss mechanisms other than mineralization, rate constant values were 1.21 or 1.26 \( \text{d}^{-1} \) for n-decane and 0.66 or 0.34 \( \text{d}^{-1} \) for n-octadecane.
<table>
<thead>
<tr>
<th>Site</th>
<th>Reference</th>
<th>Date</th>
<th>Water temperature (°C)</th>
<th>Hydrocarbon source</th>
<th>Means of dispersion</th>
<th>Biodegradation rate (per day)</th>
<th>Means of dispersion</th>
<th>Biodegradation rate (per day)</th>
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<td>May/June 1978</td>
<td>8-10</td>
<td>No. 2 fuel oil</td>
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<td>May 1978</td>
<td>0-2</td>
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<td>17-19</td>
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<td>11-14</td>
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<td>13-18</td>
<td>n-octadecane</td>
<td>Mechanical</td>
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<td>Prudhoe Bay crude oil</td>
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<td>Li W. et al. (this volume)</td>
<td>June 1986</td>
<td>20-26</td>
<td>Shengli crude oil</td>
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</tbody>
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# Marine Ecosystems Research Laboratory, University of Rhode Island, USA.

* Calculated from n-heptadecane to pristane ratio.
* Calculated from n-hexadecane to norpristane ratio.
* Calculated from n-octadecane to phytane ratio.
* Calculated from n-octadecane to phytane ratio.
* Calculated from n-heptadecane to pristane ratio.
* Calculated from n-heptadecane to pristane ratio.
* Calculated from n-heptadecane to pristane ratio.
* Calculated from n-heptadecane to pristane ratio.
* Calculated from n-heptadecane to pristane ratio.
In the 1977 experiment conducted in Patricia Bay in which dispersion of Prudhoe Bay crude oil with Corexit 9527 was studied, the ratio of n-octadecane to phytane was monitored as the means of detecting microbial degradation (Cretney et al. 1981). This ratio remained essentially constant for a period of at least 4 d. Between day 4 and day 8, the ratio decreased to almost one-third of its initial value and continued to decrease to the end of the experiment. The total bacterial population apparently increased by more than an order of magnitude up to day 4 and by more than another order of magnitude by day 8 before the population entered senescence. Thus, most of the biodegradation appeared to occur during the logarithmic growth phase of the bacteria. Of course, total bacterial dynamics do not necessarily reflect those of the oil-degrading microbial population. Indeed, an extended linear growth phase is often observed for microorganisms growing on low-solubility hydrocarbons such as n-hexadecane (Mallee and Blanch 1977).

By ignoring bacterial growth and assuming simple zero- or first-order kinetics for biodegradation after the lag phase, a rate constant of 0.13 d\(^{-1}\) (relative) or 0.27 d\(^{-1}\), respectively, can be calculated for the degradation of n-octadecane and by inference the other n-alkanes, which in the study all appeared to be used at about the same rate.

The lag phase may have been a result of earlier bacterial use of Corexit 9527, in keeping with the studies of Traxler and Bhattacharya (1978) and Foght and Westlake (1982). The lag phase may also have been a reflection of the time required for the growth of an n-alkane degrading population or the formation of bioflocs or both.

In the 1983 experiments in Patricia Bay using Prudhoe Bay crude oil and Corexit 9527 as a dispersant, n-[1\(^{14}\)C]hexadecane was added to the oil to provide a measure of biodegradation other than the ratio of n-alkane to isoprenoid. In this experiment, there was a 5- to 6-d lag before biodegradation was indicated by \(^{14}\)CO\(_2\) evolution and a decrease in the ratio of n-alkane to isoprenoid. In contrast to the previous experiment, initiation of biodegradation coincided with the maximum in bacterial standing stock and production. Furthermore, biodegradation proceeded as these two measures decreased, which was also in contrast to the previous experiment. As noted above, however, it is the standing stock and activity of the oleoclastic bacteria, not the total bacteria, that one would expect to be closely coupled with biodegradation. Production of \(^{14}\)CO\(_2\) between day 6 and day 8, after dispersion of the oil, can be used to calculate the first-order rate constant for biodegradation. This estimate is 0.34 d\(^{-1}\). The average rate constant for biodegradation calculated from the n-hexadecane to nonpristane, n-heptadecane to pristane, and n-octadecane to phytane ratios is 0.41 d\(^{-1}\).

In an experiment performed in China in May–June 1986 using 14-m\(^3\) marine enclosures in the eastern part of Xiamen Bay, n-alkane biodegradation was again observed (Li W., et al., this volume; Wu S., et al., this volume). In this experiment, Shengli crude oil was dispersed with Corexit 9527 (Zhuang et al., this volume). In contrast to the results of the two experiments conducted in Patricia Bay, biodegradation commenced with almost no lag period. The maximum rate was observed after only 1 d based on ratios of n-alkane to isoprenoid. An average rate constant of 0.46 d\(^{-1}\) was determined for the particulate (filtered) oil from n-heptadecane to pristane and n-octadecane to phytane ratios.

In many enclosure experiments in which evidence of n-alkane biodegradation
has been sought, it has been found. Remarkably, for whatever reasons, the rate constants have fallen in quite a narrow range. The consistency of the results from enclosure studies establishes that the microbial population will respond positively to oil in the water column under a variety of conditions encompassed by a variety of enclosure designs, geographical locations, oil compositions, and means of dispersion. Further work in marine enclosures should address the mechanistic aspects of degradation to increase the predictive capability of enclosure experiments with respect to bacterial response to actual oil spills.

Role of bacterial flocs in oil biodegradation

An important consideration pertaining to the mechanism of biodegradation of petroleum oil hydrocarbons at sea is the relevance of bacterial floc formation. For the purpose of this discussion, a bacterial floc is an agglomeration of bacteria, oil as discrete particles, particulate organic material (POM), and sometimes air bubbles. Several advantages may accrue to oil-degrading microorganisms through floc formation:

1. First, the hydrocarbon substrate is kept close to the bacterial cells under conditions in which global dilution, for example by diffusive mixing under an oil slick, would tend to separate unattached bacteria and substrate.

2. Second, the floc surface may concentrate inorganic nutrients essential to the microorganisms (Velankar et al. 1975; Shanks and Trent 1979; Hebel et al. 1986).

3. Third, inclusion of phytoplankton in a bacterial floc would facilitate the interchange of chemicals (Escher and Characklis 1982). For example, a close coupling would occur between O₂ released by phytoplankton and required for bacterial respiration and CO₂ released by bacteria and required for phytoplankton photosynthesis.

4. Fourth, hydrocarbon-degrading microorganisms, including bacteria, are generally capable of producing nonspecific oil-emulsifying agents and specific solubilizing agents that selectively carry particular hydrocarbons (Velankar et al. 1975; Reddy et al. 1982, 1983; Swaranjit et al. 1984). Floc formation would retard the loss of these agents to their environment (Atkinson and Rahman 1979) and create a microenvironment for hydrocarbon assimilation (Kulkarni and Barnett 1979). The floc represents a superentity in which hydrocarbon processing is effectively internalized.

5. Fifth, aggregation of bacteria increases their settling velocity and their potential for absorbing nutrients (Csanyi 1986; Logan and Hunt 1987). In this regard, collection of finely dispersed oil droplets would also be enhanced. Advection to deeper water may be controlled by complete or partial disaggregation, i.e., generation of smaller particles or more porous, less dense, particles. Some large marine snow particles (4–5 mm in diameter) have been observed to sink very slowly (1 m·d⁻¹) because of their low density (Asper 1987).

6. Sixth, in general, the ability of microorganisms to adhere to surfaces or to detach from them appears to be a survival response elicited by starvation
(Loosdrecht et al. 1987). In the case of oleoclastic bacteria presented with an insoluble hydrocarbon substrate, floc formation would seem to be a natural manifestation of this survival strategy, especially when the hydrocarbon is dispersed in a size range with an upper limit not many-fold larger than the bacteria. At the same time, once the hydrocarbon has been completely respired or assimilated, detachment would be an appropriate survival response, freeing the bacteria to find and attach to other sources of carbon. Disintegration of flocs into free cells after complete use of the hydrocarbon substrate and conversion of extracellular material into intracellular products has been observed during microbial growth in bioreactor experiments (Mallee and Blanch 1977).

- Seventh, the floc may protect the cells from predation and competition. As in the case of solubilizing and emulsifying agents, loss of protective toxins to the environment would be retarded. Loss of autotoxic wastes, however, may also be retarded. In addition, the framework of the floc may present an obstruction to some predators and toxins of competitors. Although floc size may make the bacteria susceptible to predation by higher trophic level consumers (Biddanda 1985, 1986), spreading feeding pressure among many consumers may enhance overall survivability. In this regard, cyclic aggregation and disaggregation could permit a bacterial population to stay a step ahead of consumer populations that prefer prey of specific limited size ranges.

Floc formation, however, is not without probable disadvantages for oil-degrading microorganisms:

- First, mass transport of hydrocarbons into and within flocs may be diffusion limited (Atkinson and Rahman 1979; Benefield and Molz 1983; Csanady, 1986). Because molecular diffusion varies inversely with molecular weight (Glasstone and Lewis 1960), hydrocarbons carried by solubilizing agents, which can have molecular weights in the $10^5-10^6$ Dalton range, or in colloidal-sized or larger micelles (Zosim et al. 1982; Cameotra et al. 1983; Reddy et al. 1983) may be severely diffusion-limited inside flocs. This limitation, however, assumes that the floc interior is stagnant and not influenced by the exterior flow field. There is evidence, however, that microbial aggregates may be sufficiently porous to allow fluid flow through them (Ho et al. 1984; Logan and Hunt 1987). Also, deformation of the flocs and disaggregation–reaggregation processes may serve to alter concentration gradients within them (Atkinson and Rahman 1979).

- Second, even in the presence of hydrocarbon substrate within a floc, a portion of nutrients and oxygen (which is not supplied by any associated organisms) must be obtained from the surrounding medium. If the floc is a three-dimensional ovoid or similar shape, the larger the floc, the more serious the problem of diffusive transport to its centre (Atkinson and Rahman 1979; Benefield and Molz 1983). Indeed, oxygen depletion has been observed directly in marine snow particles of 1-mm diameter or greater (Alldredge and Cohen 1987). Microbial flocs, and marine snow particles for that matter, may not have a well-defined, three-dimensional structure, however. They may have an effective or fractal dimension between 0 and 3 (Mandelbrot 1983). Once the bacteria produced an exocellular adhesive polymer, flocs would form through a random growth process of cluster-by-cluster aggregation to give scale-invariant structures with a fractal dimension (assuming no annealing) between 1.6 and 2.2 (Witten and Cates 1986). The floc structure
would be sponge-like. The highly porous flocs would be permeable to flow from the surrounding medium. External fluid would flow through them while they settled under gravity or were subjected to turbulent shear.

Evidence for possible involvement of biological floc formation during oil biodegradation in enclosures rests on experiments carried out in marine enclosures in Patricia Bay, Vancouver Island, BC, Canada. In controlled ecosystem enclosure experiments in which Prudhoe Bay crude oil augmented with several aromatic hydrocarbons was used, Lee et al. (1978) observed that naphthalene and its mono- and dimethyl derivatives were transported rapidly to the sediments during the first days of the experiment, whereas higher molecular weight compounds settled more slowly, arriving during the final days of the experiment. They proposed that the naphthalenes were actively taken up by the phytoplankton and settled out with them during the first days of the experiment. On the basis of autoradiographic studies with labeled aromatic hydrocarbons, they also concluded that higher molecular-weight hydrocarbons were associated with detrital material that was “composed of dead phytoplankton cells and bacteria,” and presumably oil particles. They also suggested that these aggregates settled more slowly than the phytoplankton cells, accounting for the slower descent to the sediments of higher molecular-weight aromatic compounds.

In a subsequent experiment in Patricia Bay using Prudhoe Bay crude oil dispersed into a marine enclosure with Corexit 9527 (Creney et al. 1981), bacterial floc formation concomitant with biodegradation of the n-alkane components of the oil was observed. Toward the end of the experiment, flocs were present that were 1 cm or more in diameter. The concentration of these flocs at the end of the experiment was so high that divers cutting away samples of the enclosure walls for further analysis commented that water coming out of the enclosures “looked like a sleet storm.” It should be noted that, in this experiment, sufficient time was provided for the phytoplankton to bloom, settle out, and be removed from the bottom before the addition of oil. No nutrients were added to stimulate new phytoplankton growth. Of the crude oil that was added, most remained at the surface or in suspension. Very little sedimented out or adhered to the enclosure walls.

In an experiment conducted in 1983 using Prudhoe Bay crude oil and Corexit 9527 (Parsons et al. 1984; Wong et al. 1984; Lee et al. 1985; Harrison et al. 1986), bacterial flocs were also observed. In this experiment, nutrients were added to stimulate phytoplankton growth. Although rapid sedimentation of oil was observed, in contrast to the previous experiment, sedimentation coincided with the settling out of pennate and centric diatoms. Phase-contrast and epifluorescent microscopy of sedimenting material (Wong et al. 1984; Lee et al. 1985) showed that discrete oil particles in the ≤0.2–3 µm range were held in close association by what seemed to be an immobilizing organic matrix. These aggregates of oil particles and bacteria were also associated with centric and pennate diatoms whose siliceous exterior presumably provided ballast to permit more rapid sedimentation than would be possible for a bacteria-only aggregate. The nature of the interaction between the flocs and diatoms is unclear, but particularly in the case of the centric diatoms, where the flocs seemed to be impaled on their silica spines, the association may be purely mechanical. Thus, sedimentation of the oil-containing flocs may be attributable to a mechanical sweeping of the water column by sedimenting diatoms and to chemical adhesion through bacterial exudates (Iman et al. 1984; Lewin 1984; Paul and Jeffrey 1985; Loosdrecht et al. 1987). It should be noted, however, that diatoms
may aggregate through mucous secretions and undergo physiological changes to increase sinking rates (Smetacek 1985).

A noteworthy characteristic of the 1983 experiment was a shift in the maximum of $^{14}$C activity added in the crude oil as radiolabeled n-hexadecane from the 0.45–3 to 3–8 µm size ranges (Wong et al. 1984; Lee et al. 1985). This shift coincided with the peaks in bacterial activity, bacterial standing stock, and incorporation of activity in polysaccharide, nucleic acid, protein, and low molecular-weight components of the biological materials. Although the involvement of bacteria in the initial stages of the association of oil particles (generally in a size range of ≤0.2–3 µm) with POM is open to question, the bacteria seem likely to have been responsible for the formation of larger aggregates from smaller ones that originally contained the radiolabel. Following the rapid release of $^{14}$CO$_2$ between day 7 and day 9, the peak in $^{14}$C activity shifted back to the size range of smaller POM. This occurred even though the trend to larger particles continued (as shown by Coulter Counter results) indicating, perhaps, that respiration of $^{14}$CO$_2$ was more efficient in the larger particles. Because alternative explanations are possible, however, this explanation must await confirmation in other studies.

The mesocosm experiments conducted in Patricia Bay have indicated that, at least at this site, bacteria play an active role in the formation of an organic matrix much larger than the nominal size of bacteria or oil particles that have been generated by a chemical dispersant. The evidence has not established that the hydrocarbons in these oil particles are biodegraded by the attached or encapsulated bacteria in the floc. The flocs and associated oil particles may only act as reservoirs of substrate for free-living bacteria, which may move freely in and out of flocs. It seems clear, nevertheless, that in Patricia Bay the aggregation of finely dispersed crude oil is a sufficient, if not necessary, prerequisite for biodegradation to take place. Although the involvement of bioflocs has not been specifically addressed in other mesocosm experiments, an extensive body of literature exists that examines the propensity of bacteria to self-aggregate or attach to surfaces through the formation of exocellular adhesive polymers. Indeed, recent work by Biddanda (1985, 1986) emphasizes the importance of bacterial aggregation in carbon and energy flow and establishes that certain conversions of dissolved organic carbon (DOC) to particulate organic carbon (POC) that had formerly been considered abiological processes were actually mediated by microorganisms. Thus, it seems that attempting to describe the phenomenon of floc formation in the Patricia Bay mesocosm experiments in the context of microbial biodegradation of oil is too restrictive. In an expanded view, petroleum oil dispersions of a size class that may be considered as POC or DOC in the classical view (i.e., passing through or retained by a 0.45-µm filter) may be thought of as being a potentially exploitable carbon source by bacterial populations that are predisposed to collect or stick to it by prevailing environmental conditions. The appearance of a floc will depend on the size of the carbon source. The appearance may vary from a biofilm on a very large substrate structure to an amorphous agglomeration in the case of micron- and submicron-sized substrate particles.

Acknowledgments

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Gearing, P.J., Gearing, J.N. 1982. Transport of No. 2 fuel oil between water column, surface microlayer and atmosphere in controlled ecosystems. Marine Environmental Research, 6, 133–143.


