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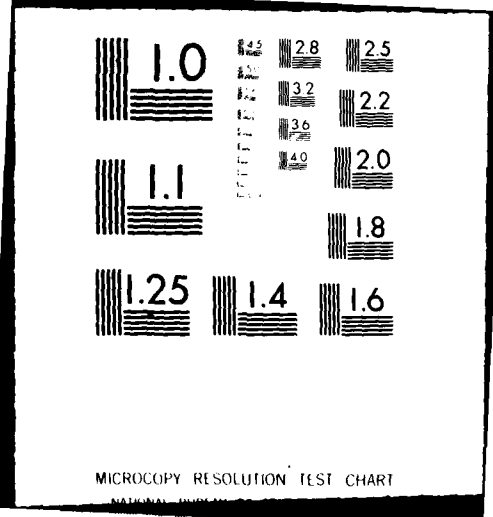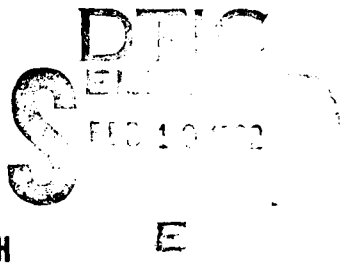
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THE BIOLOGICAL DEGRADATION OF SPILLED JET FUELS: A LITERATURE REVIEW

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Biodegradation of many of the components of Air Force fuels does occur, although most studies have been done under laboratory conditions, and the extrapolation of the findings to natural rates of biodegradation is premature. Many factors affect biodegradation rates, including the nature and concentration of the specific hydrocarbon compound, the species of bacteria present and their quantity, and environmental factors such as nutrient availability, temperature, and oxygen concentrations. Initial concerns should be first, the

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determination of the importance of biodegradation relative to other loss factors such as volatilization and sediment sorption, and second, the determination of the ultimate fate of recalcitrant compounds and their metabolites.

PREFACE

This report was prepared by Dr. Robert Carlson who worked at Headquarters Air Force Engineering and Services Center, (Hq AFESC) Engineering and Services Laboratory during the summer of 1981. The work was sponsored by the Air Force Office of Scientific Research and conducted by the Southeastern Center for Electrical Engineering Education (SCEEE). The report was published in the SCEEE annual report and republished as a Hq AFESC Technical Report to insure wider distribution and use of Dr. Carlson's work.

This report has been reviewed by the Public Affairs Office (PA) and is releaseable to the National Technical Information Service (NTIS). At NTIS it will be available to the general public, including foreign nationals.

The technical report has be reviewed and is approved for publication.

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I. INTRODUCTION:

Accidental spills or leakage of commercial or Air Force jet fuels into surface waters or ground water have happened and will happen again. Jet fuels contain a number of highly toxic and even carcinogenic compounds and their potential effect on natural ecosystems and water supplies could be serious. Research is presently underway to broaden the specifications of the currently used jet fuels so that alternate sources such as shale oil and coal could be used. These alternate source fuels may have as much as 10 percent more aromatic hydrocarbons than present fuels, some of which will be polycyclic aromatic hydrocarbons (PAH). Several PAH are potent carcinogens. The fate in and effect on the environment of the present and proposed jet fuels is currently being investigated by the Air Force.

As most of the constituents of jet fuel have a limited solubility in water and are volatile, it might be expected that evaporation from the water surface may be the major mode of loss of a spilled fuel. However, a finite amount of many of the compounds may enter the water, either as small droplets or in solution. The fate of these compounds is the concern of this review.

Soluble hydrocarbons may sorb onto the surface of any organic particle in the water.[1] Some of these organic surfaces will be suspended and settled sediments, while other surfaces will be living: bacteria, algae, invertebrates, and fish. To some of these organisms the hydrocarbons will be toxic, affecting behavior, growth, reproduction and survival. Other organisms will utilize the hydrocarbons as a source of energy and carbon, degrading the compounds into simpler organic molecules, and ultimately into carbon dioxide and water.

Volatilization, abiotic and biotic sorption, and degradation are probably the major pathways of removal of jet fuel hydrocarbons from the open water. Of these, only volatilization and biodegradation represent true losses from the aquatic habitat. Sorbed hydrocarbons, unless sorbed irreversibly, may continue to be released into the water for some time, especially if initially

buried in the sub-surface sediments by burrowing invertebrates and bottom-feeding fish. The ability of the biota of aquatic environments to degrade hydrocarbons is therefore an important consideration in the possible effect of spilled fuels.

II. OBJECTIVES

The objective of this study is to review the literature pertaining to the factors influencing the rate of degradation of jet fuels by living organisms. It became apparent early in the study that no research has been done specifically with jet fuels, and only a limited number of studies have dealt with fuel components such as gasoline, kerosene, and diesel fuel. The vast majority of the research has been done using crude and refined oils. Research has also largely been confined to studies on marine bacteria and fungi. Little work has been done in fresh water, and the possibility that organisms other than bacteria may contribute to degradation has been virtually ignored. Finally, a wide diversity of methods has been used to study biodegradation, making comparison of degradation rates obtained in separate studies impossible. For these reasons, the following was done:

1. A review of the methodologies used in biodegradation research in order to provide a perspective of the methodological problems and possible solutions.

2. A review of the factors that have been identified as affecting the rate of biodegradation. From this review, the dominant factors are identified and areas where limited knowledge is available are identified.

The review does not cover all of the vast amount of literature on hydrocarbon biodegradation, but it reviews a sampling of the relevant literature published in English in the past five years.

III. THE METHODOLOGY OF BIODEGRADATION

Microcosms

In 1941 hydrocarbons were believed to be biologically inert or highly refractory to enzymatic attack.[2] By 1972 ZoBell[2] could report that virtually all kinds of hydrocarbons were susceptable to microbial degradation. In the 31 years since 1941 a great deal of research had been done on microbial degradation of hydrocarbons, much of it being the tedious task of demonstrating degradability of each individual hydrocarbon and then isolating and identifying the degrading organism. It is now obvious from ZoBell's 1972 review that although biodegradation of hydrocarbons had been adequately demonstrated, considerable research was needed in elucidating the factors that affected degradation rates. At the same symposium, Floodgate[3] lamented the lack of an ecological approach to degradation research. He decried the tendency to use biochemical approaches to basically ecological problems. His paper is a discussion of the problems and of the possibilities that would allow the microbial ecologist to "mimic the natural environment as closely as possible."

Considering the complexity of the natural environment relative to the simplicity of the uni-species laboratory cultures, the transferral of laboratory data to field situations should be viewed with suspicion. The microcosm is one approach to providing some measure of the complexity of the natural system while maintaining the control obtainable in the laboratory environment. Although some would regard both the uni-species flask and the mathematical model as forms of microcosms,[4] the definition of a microcosm is better limited to living multi-species micro-systems. The microcosm is used because it allows for species interactions, the one factor that cannot be obtained with a single species culture. It is also used because all possible interactions cannot be known and therefore cannot be modeled mathematically. The microcosm is a living model of a natural system. It is assumed that a multi-species system will exhibit a behavior which is a function of the quantity and quality of species within the system. Therefore the more species and functional groups represented, the more the microcosm behavior would deviate from the unispecies culture, and the more it would mimic the behavior of the natural world.

The utility of multi-species cultures is essentia' in measuring biodegradation because the complete degradation of some hydrocarbons cannot be accomplished unless several microbial species are present.[3,4,5] In such cases a uni-species culture would give an erroneous picture of the microbial community's capability to degrade the compound.

Microcosms can be classified either on the basis of their openness to inputs and outputs of nutrients and water or on the basis of the degree of definition of the species within.[7] A closed or static system assumes that the internal recyling of nutrients will provide adequate nutrients to maintain the system for the duration of the experiment. It is equivalent to a batch culture. In open systems nutrients and water are added and removed either continuously or discont'nuously. The characteristics of the static and continuous-flow systems will be discussed later.

An undefined microcosm may be no more than a grab sample of water and mud from a pond,[8,9] while in a defined system the species are all known and were deliberately added to the system. The assumption of the undefined microcosm is that knowledge of all possible species interactions in a system is impossible, and therefore a sample of the natural system, containing as complete a community as deemed necessary will be the best laboratory representation of the natural ecosystem. Often knowledge of the species or their interactions within the microcosm is de-emphasized, and the microcosm is treated as a black box, with an emphasis on total system function rather than on component behavior.[9] Critics of this approach consider the undefined microcosms to be "dirty aquaria," where the sacrifice of knowledge of specific mechanisms and interactions is not compensated by the system's naturalness.

The defined microcosm usually has a different research purpose than the undefined system. It may be a closed micro-ecosystem, open only to light and gas exchange, or it may represent only a portion of a system, as for example, a model predator-prey system. It is not meant to be a mimic of any specific natural system and is therefore often used to explore general ecosystem behavior. Since all the components are known, the system can by constructed at any time. It also has the major advantage of a reproducible behavior. It

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can and has been used to predict the effects of xenobiotics on system behavior and can provide valuable information on the possible toxicity responses of a multi-species system. As such it can be a powerful screening technique for possible toxic substances. Because it does not mimic any particular system and because its bacterial community is probably limited in numbers of species, it has little use in estimating natural degradation rates.

In biodegradation research microcosms used range in size from 20 ml scintillation vials[10] to 1500 liter plastic bags.[11] The systems themselves may consist of grab samples of mud and/or water or may be attempts to take intact segments of the natural system.[12] Many studies use only the water or sediment-water components, while others attempt to include as much of natural system as possible.[9] The exclusion of fish and invertebrates may be justified as they are thought to not contribute significantly to the total biodegradation of hydrocarbons, but restriction to small volumes simply because bacteria are small could increase the variability of replicate microcosms and miss some possible system matrix effects.

Although microcosms theoretically provide unique information because of the potential for interspecific interactions, microcosms at present have certain drawbacks that require that caution be taken when attempting to extrapolate microcosm-derived data to field situations. Some of the problems of the use of microcosms are listed below:

1. Microcosm results are specific to the type of microcosm used.[4] Microcosms have inherent design features (size, S/V ratio, use of sediments, etc.[13]) that will affect the outcome of the experiment. The use of reference compounds would allow comparisons to be made between different microcosms.[4] Certainly this problem is no different from the lack of uniformity between natural ecosystems, and is an implicit recognition that the problems of microcosm design are similar to those of field comparisons. The use of microcosms simply has not advanced to the state where the effect of microcosm design is considered.

2. A microcosm should give reproducible data within a given set of experiments.[4] This requrement is achievable in defined microcosms, but in

these systems replicability is gained at the expense of the ability to extrapolate the findings to any specific ecosystem. Replicability is difficult to achieve in an undefined microcosm. Species in natural systems change both spatially and temporally, and a microcosm may not respond in exactly the same manner if the initial samples are taken on different days[11] or at different locations within the same ecosystem. Bourquin et al.[14] achieved replicable microcosms by first mixing estuarine sediments in an aquarium and then sampling from this homogeneous system. In field work variability is handled by the use of multiple samples, both spatially and temporally. Such replication becomes logistically difficult with microcosms. Without at least temporal replication, extrapolation of microcosm results will be difficult.

3.   When microcosms are used to mimic natural systems, there is a problem of scaling.[4] If the assumption is that the richer the biotic community, the better the duplication of real-world events, then the inclusion of all or most of the natural functional groups should be necessary. To achieve this goal without putting large organisms into small microcosms, microcosms have grown in size, becoming field ecosystems in themselves. De Kreuk and Hanstveit[11] found, for example, higher degradation rates of 4-chlorophenol in larger enclosures because of the presence of a richer bacterial flora. With the achievement of the reality assumed to be gained with increasing enclosure size, comes a loss in the amount of control over and understanding of the dynamics of individual components. A trade-off exists between "reality" and control over the system.

4.   In attempts to make microcosms more realistic, the use of a continuous culture system has been recommended.[3,15] This provides a semblance of realism in regard to nutrient input, but ignores the importance of species introductions.[11] Natural systems have continual inputs of species as well as nutrients and these inputs may be responsible for the observed species richness. Cessation of immigration could result in an increasingly simpler community. This consideration becomes especially important when microcosms are used to screen toxic substances. The initial contact with a toxic compound may cause species extinctions. The resulting recovery-response trajectory of the microcosm may differ considerably from an open-species system. If realism is

desired, periodic re-seeding with field samples is recommended. Experiments where continual re-seeding was done are those of Horowitz and Atlas[16] and of de Kreuk and Hanstveit.[11]

Continuous Flow and Static Cultures

A static culture is open only to inputs of light and gasses. No effort is made to replenish nutrients or to remove metabolic by-products. Typically the growth within such a system would be initially a sigmoidal increase in bio-mass, followed by a definite period of relatively stable biomass, followed by a gradual decline of the sytem as nutrients become sequestered in internal sinks. The period of stable biomass is dependent on the degree of internal nutrient re-cycling. The advantage of the batch system is its simplicity; it requires little equipment other than the culture container, and it requires little or no maintenance once the experiment is initiated. Its disadvantages include (1) a lack of reality with the exclusion of inputs and outputs, (2) the possible buildup of toxic metabolic intermediates, (3) the time depen-dence of the results, and (4) the difficulty of monitoring changes over time.[3,15]

The alternative to the static system is the continuous culture system, where inputs of water and nutrients enter and leave the reaction vessel. A specialized form of the continuous flow system is the chemostat, where the growth of the culture is limited by the rate of input of a nutrient. In the chemostat the growth rate can be regulated by varying the dilution rate of the system.

The claim that continuous flow techniques produce more realistic systems is lessened by the use of unrealistic dilution rates. At the dilution rates usually used, nutrient inputs and dilution losses are much higher than found in lentic ecosystems, and the system selects for species growing at the rate of dilution. Slower growing species are washed out of the cultures.[11] Pritchard and Starr[15] found that manipulation of the dilution rate selected for different species of bacteria that degraded octane at different rates

and 16.5 ug/hr). Such selection would seriously hinder the extrapola-
f the results to natural systems. As mentioned earlier, most existing
continuous flow systems do not continuously introduce species to the
osm. The exceptions are many lotic microcosms. In these systems, often
put water is natural stream water, allowing a continual seeding of
:s. In these systems, the organisms usually are attached to the sub-
:, minimizing the effect of dilution rate on species selection, although
still possible that the assemblage that is established will be a func-
of the flow rate.

major drawback of the continuous flow system is the amount of ancillary
atus needed to maintain a constant flow through the system. The expense
aintenance time involved with the continuous flow systems limit the
r of replications that can be done at one time. This could add substan-
y to the expense of the project. De Kreuk and Hanstveit[11] found that,
ugh continuous flow systems appeared theoretically to be a better
ach to replicating the real environment, test results obtained in both
ms were very similar. The choice between the systems for them was guided
e basis of the relative simplicity of the method or the requirements dic-
l by the analytical procedures.

:gradation Techniques

lthough biochemical oxygen demand,[17] manometric respirometry, as well as
ncrease in optical density of a fat soluble dye[15] have been used to
re hydrocarbon degradation, the most common techniques are the direct
rement of hydrocarbon loss, usually using gas liquid chromatography as
nalytical technique, or the measurement of carbon dioxide evolution,
using a ^{14}C-labeled compound.

irect measurement of hydrocarbon loss involves the innoculation of the
re or microcosm with a hydrocarbon or hydrocarbon mix and subsequent
rement of changes in concentration with time. I samples are repeatedly
from the same culture vessel, Mrsnv et al.[18] recommended the addition of
-biodegradable internal standard, hexachloroethane, to correct for

error. Prior to analysis the hydrocarbons are extracted from the
ium with hexane,[19] diethyl ether,[20] carbon tetrachloride,[21] or
.[22] The extracts are concentrated, dried and either directly sub-
analysis by gas chromatography or other appropriate techniques, or a
eparation may be used prior to analysis. Horowitz and Atlas[16] used a
umina gel column to separate the hydrocarbons from Prudhoe crude oil
rates, monoaromatics, diaromatics and polycyclic aromatic fractions.
al.[23] used a mixture of methylene chloride and methanol (9:1) to
il hydrocarbons from sediments and subsequently fractionated the
into aliphatic and aromatic fractions in an silica-alumina gel column
xane and hexane-methylene chloride elutants.

advantage of using chemical analysis lies in its directness of
. Change in the absolute concentration can be measured, and if there
bolic by-products, they can be quantified and identified if necessary.
nique is also amendable to the use of hydrocarbon mixes such as jet
ind if the analytical technique is sufficiently sensitive, degradation
in be simultaneously obtained for each compound within the mix.

problems with the technique are those related to the sensitivity of
lytical techniques involved. Biologically significant concentrations
carbons may be less than 100 ug/l and the normal lower limits of
in of gas chromatography may be 4 to 10 times higher. Some concentra-
p is usually necessary which adds to the time of analysis and to the
lity of the results. As a considerable amount of hydrocarbon will be
y for analysis, either whole microcosms would have to be sacrificed or
intainers used to accommodate repeated sampling. Interference of
bons present initially in the sample or those produced by the organ-
mselves may also be a problem.

measurement of carbon dioxide release represents a measure of the
degradation of the original compound to carbon dioxide and water, not
loss of the original compound,[24]. This distinction is important
(1) CO_2 release is not necessarily related to the original substrate
ion by a 1:1 relationship, as the method does not account for the

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production of other non-biodegradable metabolic by-products or incorporation of the labeled carbon into cellular material (yield), and (2) the loss of the original substrate does not necessarily mean that all toxic forms have been removed. The metabolic breakdown products may be more toxic than the original substrate. Both breakdown rate of the original substrate and the evolution of CO_2 are important and not necessarily correlated measurements.

In measuring CO_2 evolution, a ^{14}C-labeled substrate is often used. The technique involves the injection of the labeled substrate into a culture vessel or microcosm, and, after a time, the sacrifice of the culture and the subsequent counting by liquid scintillation of the radioactivity of the labeled CO_2 produced, and, in some cases the radioactivity of all the components of the system. Usually a control, killed by sterilization, formaldehyde, or mercuric chloride, is used to measure volatilization losses and abiotic uptake of the compound. Often the experiments are performed in sealed containers, and no volatilization is permitted. In these cases, the rates obtained represent biodegradation only, and not rates of total loss found in open containers. Carbon dioxide is captured in KOH, NaOH, phenethyl-amine,[17,25,26,27] hyamine hydoxide,[20] enthanolamine,[28] ethanolamine and methanahol,[29] or Oxiflor-CO_2.[23] CO_2 trapping, especially with the organic solvents, may also capture volatilized initial substrates or metabolic by-products. This possibility is either corrected using the measurement of apparent CO_2 trapped in the poisoned control or the insertion of an organic trap such as a cold Tenax column[39] or XAD resin,[12] or a vial containing a toluene or xylene base scintillation cocktail.[26] Removal of the CO_2 from the culture usually is accomplished by the addition of a small amount of acid, but Walker and Colwell[27] found that acidification caused the release of the label from the cells. Rather than acidification, stripping the CO_2 from the water with nitrogen gas[30] or air[12,25] may be a more benign removal technique. The technique of air stripping of CO_2 also allows for continual CO_2 measurements without the sacrifice of the culture.

If other radioactive system components are counted, this is usually accomplished by extraction of the remaining hydrocarbons and the subsequent measurement of the radioactivity in the extract. Hexane or diethyl ether are

commonly used as a solvent,[16,27] although ethyl acetate[24] has been used. Herbes and Schwall[10] first used acetone to extract the labeled compounds from sediments and then combusted the sediments to obtain a bound-^{14}C fraction. They used thin layer silica gel chromatography on the acetone extract to separate polar ^{14}C compounds from the unaltered substrates. Herbes et al.[24] and Herbes and Schwall[10] used silica gel column chromatography on ethyl acetate extracts evaporated to near dryness and subsequently redissolved in benzene to separate metabolites from unaltered PAH substrates.

There are several advantages to using a radio-labeled substrate.

1. Using labeled compounds with high specific activities, very small concentrations of the hydrocarbon can be detected without the analytical problems associated with direct chemical analysis.

2. Compounds can be added and detected at levels that would actually be found in the environment. There is no need to use high concentrations simply to make the procedure analytically tractable. High concentrations may activate dormant bacteria, be toxic, or mask cometabolic reactions,[31] and should be avoided if the concentrations would not be found in either natural or spill conditions.

3. As only the original compound and its metabolic by-products will be labeled, the fate of the compound can be traced throughout the system without interference from naturally occuring hydrocarbons.

4. If parts of the system, including outputs, are sampled, a mass balance can be calculated, identifying both problems of technique and ultimate fate of the compound.

5. The sensitivity of the technique allows the measurement of small changes in substrate or CO_2 concentrations, allowing both the study of degradation over very short intervals of time and the measurement of extremely low degradation rates.[24]

6. Labeled CO_2 evolution avoids possible errors associated with enhance-
ment of microbial respiration and consequent increased unlabeled CO_2 evolution
by hydrocarbon addition, and, if all the labeled CO_2 is driven from the water
by acidification or air stripping, problems related to CO_2 incorporation into
the bicarbonate system.[32]

The technique does have some drawbacks.

1. As compounds can only be individually labeled and each is labeled with
^{14}C, the degradation of only one compound can be studied at a time, although
it should be possible to study its degradation within a hydrocarbon mix.

2. As the ^{14}C atom occupies a specific site on the hydrocarbon molecule,
the apparent degradation of the molecule will be a function of the ease with
which that labeled location on the molecule comes under enzymatic attack.[30]
The substrate-CO_2 balance could give the impression that the compound was
completely degraded while in truth its breakdown products could still be
within the system.

3. Because the labeled carbon can be incorporated into bacterial cellular
material and the $^{14}CO_2$ can be taken up by algae, the tracer can remain in the
system long after the original substrate is degraded and its metabolic by-
products metabolized. This could give a false impression of resistance to
degradation.

The Units of Degradation

One of the most frustrating aspects of this review was the impossibility
of comparing degradation rates gathered in separate studies. This is largely
because of a lack of uniformity in the units used to report degradation rates.
Below are the enumerated and evaluated the units commonly used.

1. "Amount/unit volume/time" is a commonly used unit, especially when the
methodology involves direct analysis of the hydrocarbon loss. A similar
measure is "Amount/time" which is more an indicator of how fast an initial

dose or spill would last in the environment. These units assume a linear (zero-order) decrease in concentration with time. This assumption may be incorrect except at higher hydrocarbon concentrations and probably incorrect at concentrations of biological interest. If incorrect, the error will be greatest at the lower concentrations where the accuracy is necessary.

2. A variation of #1 is the use of the fraction degraded or the percent degraded per time. This is computed using the initial and final hydrocarbon concentrations, or the fraction of the initial labeled substrate evolved as labeled[14] CO_2 at the termination of the experiment, or it may be a least square fit from a series of measurements over time. When the percentage is calculated using only initial and final values, a zero-order decay is still being assumed. As shown by Walker et al.[33] this assumption may be correct for saturated hydrocarbons, as least at the concentrations that he was using.

3. Degradation potential or heterotrophic potential are terms commonly used to report degradation as the percentage of radioactivity in the original substrate that is recovered as CO_2. As mentioned in a pervious section CO_2 evolution is not necessarily a measure of substrate loss, although it is sometimes reported as such. Only Button et al.[30] have compensated for cellular incorporation in order to use CO_2 data to calculate substrate loss.

4. If degradation rates are assumed or found to be substrate concentration dependent, then a first-order decay rate is used. The rate constant (1/time) is reported in the same units as #2, but the decrease in concentration is assumed to be exponential. Some papers reported degradation rates in terms of half-life, the time necessary for one-half of the original substrate to be degraded.

5. The fraction of original substrate degraded per unit time is termed turnover rate.[34] Both uptake rate and substrate concentration affect turnover rates,[35] but it is a convenient measure if the naturally occuring concentration cannot be determined. The inverse of turnover rate is termed turnover time. Uptake rate (or negative degradation rate) is calculated by multiplying the turnover time by the concentration. If the labeled substrate is added in

very small quantities relative to the naturally occurring substrate, the
natural uptake rate and the natural turnover times can be calculated.[35] In
the case of hydrocarbon additions, the naturally occurring concentrations may
not be a significant consideration except in polluted areas, and degradation
rates could be calculated using only the added concentrations.

6. Since the degradation rate should be a function of the number or activity of the bacteria in the environment, it is quite possible that the degradation rate is second-order rather than first-order decay. If the incubation time is short relative to the growth rate of the bacteria, the number of bacteria may be relatively constant and a first-order rate may be obtained. In this case, the degradation rate obtained will be dependent on the number of bacteria present, and the rate constant should be standardized to the number of degraders present, termed the specific degradation rate. Paris et al.[36] have shown that the use of the specific degradation rate can produce similar decomposition rates over widely varying first-order constants. The use of these units assumes that an accurate method exists to quantify the number of degraders present.

7. Kinetic models assume that degradation rate is dependent on the concentration of the substrate relative to the uptake abilities of the bacteria. Bacterial uptake and growth is often represented by a Monod equation, and the kinetic variables measured are V_{max}, the maximum uptake rate, and k_s, the concentration of substrate at $1/2$ V_{max}. Paris et al.[36] have shown that at substrate concentrations less than k_s, uptake is a linear function of concentration, and a second order decay rate should be expected, which would produce a pseudo-first-order decay constant if the bacteria numbers are constant. At concentrations greater than k_s, the decay will be zero-order with respect to substrate concentration if the bacterial numbers are constant. Obtaining kinetic parameters requires the calculation of uptake or degradation at several substrate concentrations. The validity of the use of kinetic variables obtained from mixed bacterial populations has been questioned.[37]

8. Representing the decomposition rate from cultures containing sediments is a special problem. Roubal and Atlas[20] added volumetric amounts of diluted

sediments to their culture vessels as did Wyndham and Costerton[29]. Wyndham and Costerton[29] reported their results as ug degraded/ml of sediment/day. Herbes and Schwall[10] reported PAH degradation in sediments as rate constants (1/hr), turnover time (hr) (both of which are dependent on the concentration of sediment used), and transformation rate (ug PAH degraded/gm sediment/hr). Representation of rates per gram sediment may not be appropriate unless the sediments are completely stirred. If the sediments are allowed to settle, only a small fraction of the sediments will actually be in contact with the hydrocarbons and the degradation rate will be underestimated. In this case, representation of the rates on an areal basis (amt degraded/cm^2 of sediment/time) would seem more appropriate.

All of the above units and more are found in the degradation literature, making comparison of rates between studies impossible. Certainly each study had its own objectives and therefore used appropriate techniques and terminology, but the lack of conformity has lead to duplication of effort. Some possibilities do exist to bring about some conformity.

1. As CO_2 evolution is not necessarily equivalent to substrate loss, both should be measured. If only CO_2 is measured, the results should be corrected for non-CO_2 losses if the data is used to represent degradation rate.

2. Degradation rate will probably be a function of substrate concentration and bacterial numbers, and a second-order decomposition model should be assumed unless demonstrated otherwise. Results should be standardized to the number of degraders present.

3. Because of the non-linearity of uptake kinetics, the degradation rate per bacterium may be zero-order at high concentrations, changing to first-order as the concentration falls below k_s. Obtaining kinetic parameters would be desirable, but the necessity of using several substrate concentrations for each compound would make the work difficult, unless k_m and V_{max} can be calculated from time course data.[38]

4. The introduction of spilled hydrocarbons will be largely a surface phenomenon. No matter where the point of entry, the majority of the fuel will

be at the water surface. An areal rather than volumetric degradation rate may be appropriate. If settled sediments or intact cores are used, again an areal representation of degradation could be used.

Probably no single unit of degradation will be appropriate in every study, but units should be used that could be utilized in a degradation model. If rate constants were used, comparisons could be made not only with other degradation studies but also with studies of volatilization and sorption losses. It is quite possible that different models are appropriate for different hydrocarbons.[33]

Enumeration of Microorganisms

If it is necessary to obtain decomposition rates special : . number of active degrading bacteria, then sensitive and accurate estin . of bacterial numbers are needed. Various techniques are used to enumerate the bacteria responsible for hydrocarbon degradation. It has been suggested that the ratio of hydrocarbon degraders to total heterotrophs is a better indicate f the hydrocarbon pollution in any environment than is the count of hydrogen degraders alone,[39] and usually both total heterotrophic bacteria and hydrocarbon-degrading bacteria are counted.

Total heterotrophic bacteria are usually enumerated with a plate count method using a wide variety of freshwater and marine media. Most probable number (MPN) techniques are occasionally used. Because of the selective nature of plate culturing, alternative techniques such as direct counting using epifluorescence have been recommended.[40] Epifluorescence counts however do not distinguish between living, dormant, and dead bacteria,[35] and auto-radiography could be combined with the counts to determine activity.[41]

Hydrocarbon degrading bacteria are usually enumerated using a plate count technique in which a specific hydrocarbon or hydrocarbon mixture has been added as the sole carbon source. Recently a MPN technique using radiolabeled hydrocarbon substrates has been tried.[20,29] The labeled $^{14}CO_2$ evolved is used

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as the indicator of bacterial activity. Lehmicke et al.[31] advocate this technique because it uses substrate concentrations much closer to natural levels, thus avoiding errors associated with high substrate levels such as toxicity, or the activation of dormant bacteria or enzymes. The technique, however, is specific to the one labeled substrate, and could be difficult to use in a mix unless the single labeled compound was an adequate indicator of bacterial activity on the total hydrocarbon mix or unless a number of labeled compounds were used simultaneously.

IV. FACTORS AFFECTING BIODEGRADATION

Many factors can affect degradation rates. Rates can be affected by (1) the nature and concentration of the specific hydrocarbon, (2) the species and quantity of the bacteria present in a given environment, (3) environmental factors that affect the metabolism and the growth rate of the bacteria, and (4) indirect effects such as the presence of other toxics, alternate carbon sources and cometabolic substrates. This section is a summary of some of the research related to the effect of these factors on hydrocabron degradation.

Hydrocarbon Type and Concentration

Considerable research has been done on the relative degradability of various hydrocarbon compounds, and the bulk of this literature is not reported here. Degradability appears to be related to the cyclicity of the compound, the degree of branching, and the particular arrangement of the carbon atoms attached to a ring.

According to Bartha and Atlas[42] the following summary can be made of the relative biodegradability of hydrocarbons.

1. n-Alkanes, especially those between C10 and C25, are the most widely and readily utilized hydrocarbons.

2. Iso-alkanes are generally degraded slower than n-alkanes, especially if branching is extensive or creates quarternary carbon atoms.

3. Olefins are less readily utilized than alkanes.

4. Low-molecular-weight aromatic hydrocarbons can be metabolized when present in low, non-toxic concentrations.

5. Polycyclic aromatic hydrocarbons are metabolized only rarely and at low rates.

6. Cycloalkanes serve as growth substrates for isolated organisms only in exceptional cases, but may be degraded by cometabolism.

This summary is illustrated by the work of Wal er et al.[33] In this study it was shown that the degradability of cyclic alkanes and cyclic aromatics decreased with each additional ring on the structure. Herbes and Schwall[10] found that benz(a)pyrene was degraded nearly 5,000 times slower than naphthalene in an oil-contaminated stream. In arctic marine samples, biodegradation potential of 4 ^{14}C-labeled compounds followed the order naphthalene > hexadecane > pristane $\geq$ benzanthracene, with the potential for pristane and benzanthracene often being zero. When nitrogen and phosphorus were added to the cultures, the order was altered to hexadecane > naphthalene >> pristane > benzanthracene.[20] Roubal and Atlas[20] suggested that in the first experiments naphthalene and hexadecane degradation rates were nutrient limited, and the addition of nitrogen and phosphorous removed this limitation. They suggested that pristane and benzanthracene were limited by available degradative enzyme systems and the addition of nutrients could not stimulate degradation.

When gasoline was exposed to a mixed bacterial flora for 192 hours, the highest degradation was found for benzene, ethyl benzene, toluene, and xylene, while the least degraded were iso-alkanes.[22] They suggested that many of the degraded compounds may have been degraded by co-oxidation. Kappeler and Wuhrmann[5] found that similar compounds differing only in the arrangement of the carbon atoms attached to the benzene ring could differ significantly in their degradation rates. Rates for 1,2,4, tri-methylbenzene were much higher than for 1,2,3 tri-methylbenzene or 1,3,5 tri-methylbenzene. Ortho-xylene degraded much slower than m- and p-xylene.

Degradability is also related to hydrocarbon concentration. If degradation is a first- or second-order function, then a rate changing as a function of concentration would be expected. However, at high concentrations the compounds or their metabolic intermediates may be toxic[42] and therefore inhibit degradation. Photosynthesis in the marine diatom Cyclotella cryptica is stimulated at low concentrations of aromatics from North Sea crude oil, but is inhibited at concentrations greater than 1 mg/l.[43] Similar results of concentration-dependent stimulation or inhibition has been found in algae by others.[44,45,46]

When hydrocarbon concentrations are low, biodegradation may also cease. Boethling and Alexander[47] found little degradation of 2,4-dichlorophenoxy-acetate and l-naphthyl-N-methylcarbamate occuring if the initial concentrations were less than 2-3 ug/l. McCarty et al.[48] determined the minimum concentration of acetate that would support growth to be 0.66 mg/l. Both Boethling and Alexander[47] and McCarty et al.[48] suggested that below these limiting concentrations, insufficient energy is extracted to offset these energy demands. McCarty et al.[48] suggested a compound might be degraded at concentrations below the minimum concentration if the concentration is fluctuating (non-steady state) or if the compound were degraded by cometabolism. Spain et al.[49] found that the duration of time before a given bacterial flora began to degrade p-nitrophenol was dependent on substrate concentration. At initial level below 0.43 uM, adaptation did not occur.

Number and Species of Bacteria

Over 200 species of bacteria, yeasts, and filamentous fungi have been shown to degrade one or more hydrocarbon compounds.[50] One alga, Protothea zophi, has been shown to degrade hydrocarbons,[51] but the number of reports of stimulation of algal growth[44,46,51] when hydrocarbons are added suggest that algae may also contribute to its degradation. Algae, however, are not thought to contain the proper oxidases to permit hydrocarbon metabolism.[52] Other organisms, zooplankton, amphipods, crabs, and fish have been shown to degrade hydrocarbons to some extent.[52] Bacteria are probably considered to be the primary degraders of hydrocarbons not only because of their heterotrophic

existence, but also because of their high surface area relative to their
volume.

Not all bacteria degrade hydrocarbons. The most common genera of
hydrocarbon-degraders are Pseudomonas, Achromobacter, Arthrobacter,
Micrococcus, Nocardia, Vibrio, Acinetobacter, Brevibacterium, Corynebacterium,
and Flavobacterium.[42] Each species may not be able to degrade all hydrocarbon
compounds. Norcardia is probably responsible for n-alkane degradation while
Pseudomonas degrades aromatics.[22] Jamison et al.[ ] reported that gasoline
could only be completely degraded by a mixed bacteria flora, not by isolated
species. Similarily, Kappeler and Wuhrmann found that 12 out of 30 isolated
bacsterial strains degraded gas-oil, but also found that there were only 4
metabolically different strains represented in those 12 species. Complete
degradation of the gas-oil required the combined presence of 3 or those 4
strains.

The rate at which a hydrocarbon will disappear in a given environment will
depend in part on the number of hydrocarbon degrading bacteria present. Iden-
tification and enumeration of all the possible hydrocarbon-degrading species
would be tedious, and simpler indices have been advocated. As the isolation
of hydrocarbon-degraders is possible by the plating of water samples with
hydrocarbons used as the sole carbon source, it has been possible to enumerate
the total number of hydrocarbon degraders without further identification.
Some studies have found relationships between the number of hydrocarbon-
degraders and the amount of hydrocarbons in the environment[16,19,53] but
better correlations have been found between concentration and the ratio of
hydrocarbon-degraders to the total number of heterotrophic bacteria.[39,53,54]
Others have found little relationship between either total hydrocarbon
degraders[29,55] or hydrocarbon-degrader/total heterotroph ratios.[17,56]
Attempts at correlations of numbers of hydrocarbon-degraders or ratios with
the heterotrophic activity have also gotten mixed results. Studies by Wyndham
and Costerton,[29] Ward and Brock,[17] Roubal and Atlas,[ ] and Herbes[55] found no
relationships, but Seki,[28] Caparello and LaRock,[25] and Walker and Colwell[27]
did. In the study of Herbes[55] there was no relationship of heterotrophic
potential with ambient PAH or with bacterial numbers, but higher degradation

rates were found at sites that were formerly polluted. He suggested that either the PAH degraders remained longer than the ambient PAH or that PAH concentrations were sufficiently high to maintain the degrading enzymes in the population. There appear to be several instances where there are higher degradation potentials in polluted environments than in pristine environments, although the difference is not seen in the number of degraders. This may be a result of the techniques used for the isolation and enumeration of these bacteria. If accurate bacterial numbers are needed to obtain number-specific degradation constants, the methodology should be examined carefully.

Prior History of Hydrocarbon Contamination

In several instances, including the findings of Herbes,[55] mentioned in the last section, there are instances where the degradation rate of hydrocarbons is higher if the environment has been previously exposed to hydrocarbons. Roubal et al.[57] reported that gasoline was not detected in the sediments 48 hours after a major gasoline spill in the Ohio River. They attributed the rapidity of the loss of gasoline to the degrading bacteria already being present because of a prior spill. The idea that degradation rate is dependent on the prior activation of hydrocarbon-degrading bacteria fits into the larger subject category of the causes of time lags in hydrocarbon degradation.

It has been noticed in a number of studies that a period of time often passes before degradation commences. This time lag may last from a few hours to a few days. According to Spain et al.[49] these lags may be the result of the time necessary to (1) induce or de-repress specific enzymes not present before exposure, (2) select new metabolic capabilities produced by genetic changes, and (3) increase the number of organisms able to catalyze a particular transformation. Caparello and LaRock[25] determined that the duration of the time lag was dependent on the initial size of the innoculum, although the final extent of degradation was not affected. Ward and Brock[17] and Kappeler and Wuhrmann[58] observed that the initiation of decomposition commenced with the increase in bacterial numbers. Pritchard and Starr[15] found that degradation of octane commenced when one bacterial species comprised 90 percent of the total bacterial numbers. Spain et al.[49] found that adaptation (a change

adation rate) would not occur if the substrate were below some
ld concentration, or if the proper bacteria were not present in the
ment.

duration of time lags can be altered in a number of ways. Spain
[9] found that prior exposure to p-nitrophenol would significantly
se the time lag on re-exposure to the chemical. Volatile compounds of
e toxic, and a temperature-dependent lag period can be produced until
compounds evaporate.[42] Soto et al.[59] demonstrated that the volatile
ds in crude oil extracts inhibited the growth of the alga, Chlamydomonas
sa. The toxic effect was maintained as long as the culture flasks were
red. When unstoppered, the toxics evaporated and algal growth commenced.

e effect of other organic substrates on time lags is more difficult to
ret. Ward and Brock[17] found that the addition of glucose prolonged the
efore hexadecane was degraded. When the glucose was consumed, hexa-
degradation began. They suggested that hydrocarbon-degrading enzymes
uppressed during growth on a preferred substrate. In a later experiment
ound that if the samples were aged to remove BOD, the time lag was
ned. Walker and Colwell[27] found a time lag in the uptake of glucose
hey suggested was the result of the preferential utilization of hydro-
s before switching to alternate substrates. Gusev et al.[21] found that
dition of glucose to diesel fuel extracts shortened the time lag for
fuel degradation, but had no effect on the degradation rate of diesel
Both substrates were utilized simultaneously.

nt Limitation

nce the work of Atlas and Bartha[60] it has been generally recognized that
dition of nitrogen and phosphorus will often stimulate the degradation
f hydrocarbons.[32] Nitrogen and phosphorus concentrations are the most
icant factor in degradation of diesel oil.[21] Horowitz and Atlas[16] found
itrogen and phosphorus additions gave 10 percent higher losses of crude
an did the control, but if oleophilic fertilizers (paraffinized urea and
phosphate) were used, a 15 percent higher degradation loss was obtained.

and Bartha[19] have shown that the addition of iron together with nitro-
phosphorus further stimulated degradation, but only in clean, iron-
d waters. They also found that degradation was largely restricted to
lkane peaks if only nitrogen and phosphorus were added, but if iron
so supplemented, the unresolved envelope was also degraded. This may
at only some of the bacterial species were iron-limited, but the acti-
these species was necessary for the complete degradation of the oil.

a detailed study of the nutrient limitation of hexadecane and mineral
radation in Wisconsin lakes, Ward and Brock[17] found that nitrogen and
rus additions stimulated degradation rates in all of the nutrient-poor
tudied. Degradation rate was a hyperbolic function of phosphorus con-
ion. Half-saturation concentrations for growth rates on mineral oil
adecane were approximately 20 ug of phosphorus and 50 ug of nitrogen
er. They suggested that nutrient limitation of biodegradation is a
ead occurrence in freshwater systems.

istinct seasonal pattern of degradation rate that suggests the dual
 of temperature and nutrient limitation has also been noted.[11,17]
sition rates are typically low in the winter, rising as the temperature
es in the spring. In mid-summer degradation rates decrease, following
n soluble nitrogen and phosphorus concentrations. Apparently from
y to late fall, degradation rate is limited by nutrient supply.

itation of biodegradation by nutrients is not only important in the
degradation of hydrocarbons in the field, but also in the estimation of
tion rates in the laboratory. In batch systems, where nutrient inputs
ent, nutrient limitation may reach levels far above that observed in
ld. The experiments performed on such system may indicate far less
tion potential than is actually found in the field. Supplementing the
s with nitrogen and phosphorus may give an artificially high degrada-
tential relative to the natural system, but at least it could be used
ndex of potential degradation. Approximations of real-world values
equire either short-term incubations (<24 hours) or the use of large
rough systems where the enclosure effects are minimized and natural
t inputs are simulated.

Temperature

As mentioned earlier, temperature can have an important seasonal effect on the biodegradation rate of hydrocarbons. As might be expected for a factor that affects metabolic activity, degradation rate increases with a rise in temperature. Atlas and Bartha[61] reported that degradation rate roughly doubled with each 5°C temperature increase in the 5° to 20° range. Usually, the temperature response curve is sigmoidal, with the maximum rates being reached between 20° and 25°,[17,62,63] although inhibition can be sometimes seen at higher temperatures.[17] Ward and Brock[17] found that temperature response curves were similar in both summer and winter samples, suggesting that there is little low temperature adaptation.

The percentage of the initial substrate degraded to CO_2 is also dependent on temperature. Dibble and Bartha[63] found that the percent of original substrate (oil sludge) evolved as CO_2 increased as a function of temperature, but Walker and Colwell[27] found that although the percentage of hexadecane degraded increased with temperature, the fraction converted to CO_2 decreased. Increased time lags at low temperatures have also been described.[17,61,63] Atlas and Bartha[61] showed that the lag periods were caused by inhibitory volatile components in crude oil and that volatilization rates of these toxics were less at lower temperatures.

pH

It is generally believed that there is an optimum pH over and under which decomposition will decrease. Hambrick[64] found that the mineralization rates of naphthalene were highest at pH 8.0, lowest at pH 5.0. Dibble and Bartha[63] found that raising the pH from 5 to 7.8 increased the mineralization rate of oil sludge. One could conclude from studies such as these that the lowest degradation rates might be found in acid environments such as strip-mine impoundments, bogs, and poorly buffered lakes stressed by acid rain. The accuracy of this conclusion depends on the assumption that no adaptation or species replacements occur in low pH environments. My laboratory has isolated species of algae that grow at pH 3.2 as rapidly as the bioassay organism,

Selanastrum *capricornutum* does at pH 7. This suggests that producing pH
curves from samples taken from one environment should not be used to predict
the degradation rates in environments of different pHs. In-situ studies
within each environment may be a better approach.

Oxidation-Reduction Conditions

Most biological degradation of hydrocarbons involves metabolic reactions
that require oxygen; anaerbolc degradation is negligible.[42] Hambrick e al.[64]
found decreasing rates of decomposition of naphthalene and octadecane as the
eH values decreased. If hydrocarbons are somehow displaced into anaerobic
hypolimnia or sediments, they probably will not be degraded further. During
periods of turnover in lakes, both the hypolimnetic waters and sediments could
be mixed with oxygenated waters, releasing the hydrocarbons for futher degra-
dation. With each seasonal mixing event, further release and degradation
would occur, producing an "echo effect" (A. Carlson, personal communication).

Salinity

Few papers have been written on the biodegradation of hydrocarbons in
freshwater,[17] and even fewer have examined rates as a function of the salinity
of the parent environment. Ward and Brock[65] showed that the degradation of
hexadecane and mineral oil decreased with increasing salinity in the Great
Salt Lake; however their lower salinity was greater than the salinity of the
oceans. Caparello and LaRock[25] reported hexadecane mineralization in fresh-
water samples to be greater than in estuarine and marine samples. Spain
et al.[49] found that a riverine sediment degraded p-nitrophenol much faster
than an estuarine sediment. They suggested that the differences were not so
much the effect of salinity itself as much as the degrading ability of the
natural flora. One study[11] found the relative rates of degradation in marine
or freshwater environments to be dependent on the compound.

V. ULTIMATE FATE

Many factors, both abiotic and biotic, can affect biodegradation rates of
hydrocarbons. The environments where biodegradation rates are potentially the

lowest can be approximated by superimposing each of factors that have been reviewed here. Degradation would be low in environments where there are few degrading organisms (which may be related to the lack of prior exposure to spills), high salinities, and cold temperatures. Such a superimposition would fit a pristine, arctic marine environment. Certainly the emphasis on possible environmental effects of oil spills in the arctic reflect such a reasoning. In temperate freshwater habitats the pristine acid bogs, as well as lakes already stressed by acid rain, may have problems degrading hydrocarbons. The interesting point of the above comparisons is that those habitats that may degrade hydrocarbons the slowest are those thought to be most ecologically susceptible to hydrocarbon toxicity.

The two other major pathways of hydrocarbon loss from the water column are volatilization and sediment sorption. Sorption by the sediments may be a complicating factor in the biodegradation of hydrocarbons. In a real sense, sediments act as competitors with the microbial flora for soluble hydrocarbons. The greater the concentration and sorption capacity of the sediments, the lower the concentration of the hydrocarbons in the water, and perhaps the greater the absolute amount of hydrocarbon that will be solubilized. As volatilization and biodegradation are concentration dependent, the net effect of sediment sorption will be a lower absolute rate of hydrocarbon loss from the water. As the hydrocarbons are lost by biodegradation and volatilization, they will be replaced to an extent by desorption from the sediments. Thus the organisms in the water will be exposed to a lower concentration than they would if sediments were not present, but the exposure will be for a longer period of time. To further complicate matters, if sediment sorption decreases the concentration below the minimum concentration for biodegradation, volatilization would be the only mode of hydrocarbon loss, further extending the exposure time of organisms to the fuel components.

It has been commonly observed that the fraction of a ^{14}C-labeled hydrocarbon that is mineralized to $^{14}CO_2$ is often much less than 100 percent. The fate of the remainder of the labeled carbon may be reversible or irreversible sorption onto the sediments, the formation of recalcitrant metabolic intermediates, or incorporation into cellular carbon. In an open system the

eventual loss of the material as CO_2 would be expected, but the studies so far reveal that these bound materials or metabolic intermediates are not easily metabolized. It may be that the hydrocarbons or their metabolic by-products, once spilled, will be around for a long time.

VI. RECOMMENDATIONS

As no research has been done so far by the Air Force in the area of the biodegradation of jet fuel components, certain priorities can be set based on existing knowledge of hydrocarbon biodegradation. The following research concerns could be considered.

1. Biological effect rather than analytical limitations must set the minimum hydrocarbon concentrations to which their biodegradation is observed. If such concentrations cannot be ascertained from existing research, then sensitive and unambiguous measures of effect should be developed.

2. The importance of biodegradation should be studied in relation to losses by volatilization. If biodegradation of hydrocarbons cannot be shown to contribute significantly to fuel component losses, it may cease to be a research concern of the Air Force.

3. The possible persistence of certain fuel components or their metabolic by-products weeks or even months after the initial spill should be investigated. The location and chemical characterization of these compounds should be studied, as well as their toxicological importance. The possible interaction with sediment sorption may play a role in the persistence of these compounds.

4. Previous experiments have been performed in the laboratory on relatively few environments. The findings of any laboratory studies should be tested in much larger field enclosures where a more complex interaction with the natural biota can be simulated. I value the use of large enclosures over the use of an entire pond, because enclosures can mimic most of the responses of the pond, but allow replication. Enclosures can also be utilized on

several types of environments, whereas the use of ponds limits the extrapolation of data to other environments.

5.   Biodegradation rates have been shown to be a function of many factors. Extrapolation from a few laboratory studies to all the possible natural environments would be unwise. Manipulation of temperature, pH, or other variables on microbial samples from a single environment may produce response curves that bear little resemblence to the response of a microbial community taken from environments where those extreme condition: actually exist. An abbreviated methodology such as heterotrophic potenti.1 could be used to rapidly census a number of environments for their rel.tive degradation rates. Special attention should be given to small, freshwater habitats because their small size relative to the size of a spill makes them more susceptible to fuel effects.

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