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Bioremediation: A Developing Technology for Waste Management

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BIOREMEDIATION:
A DEVELOPING TECHNOLOGY FOR WASTE MANAGEMENT

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A DEVELOPING TECHNOLOGY FOR WASTE MANAGEMENT**

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I. Introduction

The existing crisis of waste management is not just an issue for environmental activists and environmental planners but it is also important to politicians, policy makers, community and urban planners. Without the combination of their expertise no solutions will be found for the pollution problems plaguing the world today.

This paper will discuss a technology which has been used in Europe for many years but is only now being researched in the United States. Bioremediation may not be the panacea to all pollution problems but its many benefits should be examined along with current waste management practices; landfilling, incineration and recycling.

Bioremediation and recycling are both under utilized technologies and there is a definite need for these nondestructive types of solutions. Both of these technologies have the benefit of not trading one source of pollution for another.

"Microbes given encouragement can do most of the reclamation work for us." ¹ This philosophy has been used in experiments involving the purification of contaminated bodies of water such as lakes and rivers. This principal of microbial degradation should be extended to include contaminated soil, landfills, old quarries, gravel pits and many other previously unusable areas.

DEFINITION OF BIOREMEDIATION

Bioremediation is the process by which microbes are used to eliminate contaminants by ingestion and degradation. These microbes are indigenous to the contaminated area and are non pathogenic. ²

The most commonly used microbes are aerobic microbes since they are very effective and easier to isolate and control during the process of biodegradation. The only disadvantage to aerobic microbes is that as depth of soil increases the amount of oxygen decreases limiting their effectiveness and the microbe's chance for survival. With aeration, water, and nutrients added these microbes will continue to degrade the contaminants. ³

Providing additional nutrients and oxygen is accomplished by using closed loop systems to circulate oxygen and nutrient enriched water through the saturated and unsaturated subsurface. The resulting increase in degradation rate and decreasing contaminant concentration is dramatic. ⁴

Bacteria, Actinomycetes, fungi, and algae naturally present in soil are all common in bioremediation. Yeast are occasionally used, which are larger than bacteria, and emit a more pleasant aroma in the degradation process. They are more commonly used in various fermentation processes in food production. ⁵

PROCESS OF BIOREMEDIATION

To obtain the correct clean up microbes, scientists take soil or water samples from the contaminated site. The microbiologists breed strains that depend on a contaminant to live and which will die off when the food source (the contaminant) is gone.

One of the quickest ways to treat contaminated soil is to excavate it, mix it up with water, nutrients, and bacteria on a plastic sheet and pump air through it. Groundwater contaminated with oil, chemicals or other substances can be treated in a bioreactor, a tank containing specially selected microorganisms.

There are several problems associated with the process of bioremediation. Bacteria often take longer to remove contaminants from soil than the process of excavation and trucking of contaminated soil to landfills. Microbes often stop degrading the contaminant before it is eliminated, due to lack of oxygen and nutrients. Another drawback is that microbes can usually only attack one contaminant at a time. This is a problem for sites contaminated by more than one toxin. Scientists are researching genetically engineered organisms for these sites, but all of these microbes must undergo strict scrutiny by state and federal regulators. ⁶

Before bioremediation can be used on a site the history of the site must be fully researched for information

concerning past uses (industrial, commercial, or residential) to search for all possible contaminants. Two studies performed include a microbial profile which includes a count of all indigenous microbes on the site and a feasibility study to determine whether bioremediation is the best solution to the cleanup of the contaminated site.

Microbes are extracted from samples taken at the contaminated site. Next the microbes which break down the contaminant(s) naturally are isolated. The amount of these indigenous microbes are then increased through the addition of fertilizers, water and aeration or by the reproduction of these microbes in laboratories. When microbes are stored for a long time, they are kept with the contaminant for which they have been isolated to attack. For example the particular organism which breaks down hydrocarbons (oil and gas compounds) is stored within a gas and oil mixture so it won't lose its attraction for these contaminants. If the microbes are synthesized in laboratories they can be applied to the contaminated site through injection, spraying, gravity drip or mixing into the soil. ⁷

During the degradation process, the soil has no prevalent odor, but out of soil the microbe and water mixture is pungent in odor. The soil during this process is harmless, since non pathogenic organisms are used.

This process speeds up the natural degradation, caused by indigenous microbes in soil, from a 40 year cycle to a 90-150 day cycle of contaminated to clean soil.

Biostabilization is the process following the introduction of the microbes into the contaminated soil and involves the feeding of the organisms with fertilizers, continuous irrigation and aeration. This process enables the microbes to continue to ingest and degrade the contaminant. Once the contaminant has been degraded the amount of microbes is reduced to the amount naturally found in the soil. Periodic monitoring of the cleaned site is necessary to ensure the complete eradication of the contaminant.⁸

Bioremediation can be performed in situ, above ground, or in specially constructed in ground cells. The key to in situ bioremediation is the transportation of oxygen and nutrients to contaminated areas. Since normal ground water flows from 10 to 200 feet per year nutrients could take years to traverse a small site. By increasing the gradient through pumping (draw down) and injection (mounding), transport times are reduced from years to weeks and days.⁹

If soils are too impermeable for in situ transport of nutrients and oxygen, other on site treatment options may be used such as oxidation or excavation of soil in situ or above ground. Above ground treatment is not

as desirable due to increased cost. In situ processes are less expensive and therefore preferred.¹⁰ (Refer to the Technical Appendix for more information on in situ microbial treatment of contaminated soil as provided by Affordable Technology, Inc.)

EFFECTIVENESS OF BIOREMEDIATION

Currently the Trent River in Great Britain is considered to be an unpurifiable water source due to pollutants caused by heavy industries such as coal mining. Researchers have conducted trials to see the effectiveness of microbes on "unpurifiable" waters such as the Trent using artificial lakes, such as the one located in an abandoned gravel pit at Lea Marton. This gravel pit was flooded with river water which was then siphoned through (3) large settling tanks. Cloudy water ran to a depth of 2 meters in the pit and there was no artificial stirring or agitation.

Naturally occurring microbes began to break down phenols and other poisonous chemicals. Soon algae appeared (on sunny afternoons) which bloomed. Within months, as the water was purified, crustaceans, water fleas (Daphnia), Beetles, fly larvae, and other aquatic creatures began to increase in numbers.¹¹

During the first year a stable community had evolved; fungi and bacteria were disposing of chemicals while taking

in the past through the sinking of the oil with ash or sand. ¹³

Due to the problems associated with traditional methods of oil spill cleanup, bioremediation has become a popular alternative. Microbes break down the smaller oil molecules easily but as the size of molecule increases degradation becomes more difficult and takes a longer period of time for microbes to break these molecules down. ¹⁴

"Just after midnight on March 24, 1989, the Exxon Valdez super tanker ran aground in Alaska's Prince William Sound, spilling 10.1 million gallons of crude oil fouling 368 miles of shore line in the sound alone." ¹⁵ The Environmental Protection Agency enlisted the help of microbiologists and a new technique called Bioremediation to aid in the breakdown of the crude oil. Six oil stained beach plots were chosen and treated with nitrogen and phosphorus fertilizers to stimulate the naturally occurring bacteria to continue the natural degradation of the oil. Detected in these beach areas were air breathing bacteria (aerobes) with the ability to break down slowly volatilizing alkanes and simple aromatic hydrocarbons.

The first 90 days tests compared two nutrient formulations created to help increase the indigenous bacteria's growth and rate of reproduction. One formulation incorporated oleic acid (best known as the primary fatty acid in olive oil). Researchers think this fatty acid

could glue the mixed in bacterial nutrients to any crude oil on which they're sprayed.

The second nutrient formula is an off-the-shelf fertilizer "brickette". These brickettes are packed into biodegradable plastic sacks and are tied to pipes anchored to the beach. Tidal action during the test period should flush the dissolving fertilizer back and forth across the shoreline rock and sand. ¹⁶

There are drawbacks to using fertilizers, such as potential risks to sea life, especially where tidal flushing is minimal. The most seriously affected sea life would be the larvae of sea urchins, oysters and mussels. Environmental Protection Agency officials say it could take 5-7 years for the Prince William Sound area to naturally return to its former "pristine" state. This natural process could be increased with fertilizers and reduce contaminant degradation to 2-5 years. ¹⁷ (For more information on the use of bioremediation to cleanup the Prince William Sound area refer to the Technical Appendix.)

Bioremediation has also been shown to have an effect on Selenium contamination. Selenium has severely contaminated the vegetation and soil of the 1,000 acre Kesterson National Wildlife Refuge in Central California. According to state officials the only feasible solution is to bulldoze off a 6" layer of top soil from the

contaminated area and dump it into a plastic lined 45 acre landfill. Researchers William Frankenberger and Ulrich Karlson (University of CA, Riverside) have found that fungus indigenous to the site have been naturally converting Selenium into a gas which is dispersed quickly into the air. The fungi do this for their own survival, to prevent Selenium from reaching lethal doses in the soil. Frankenberger and Karlson have created a hospitable environment for the fungi to encourage increasing growth therefore increasing the speed of biodegradation.

Another common wood rotting fungus may perform a similar job in treating waste water from chemical plants. The enzymes that allow these fungi to breakdown lignin (the chemical that holds wood fibers together), may also digest some hazardous and decay resistant pollutants.

Microbiologist Dunja Grbic-Galic of Stanford University also recently demonstrated a type of bacteria which can be cultivated for the purpose of cleaning soil and groundwater contaminated with trichloroethylene, a widely used industrial solvent. ¹⁸ (Refer to the article "Toxic Wastes? A Little Fungus May Help" in the Technical Appendix for more information on the effect of fungus on selenium and trichlorethylene contaminated sites .)

The Rhine River in Germany is another heavily polluted water body currently being researched for the

effectiveness of bioremediation. These polluted waters are allowed to run into lakes where they are naturally undergoing bioremedial processes through indigenous microbes. Eventually, these waters would be rejuvenated if not for the continuous flow of contaminants into them. To speed the process of pollutant degradation, contaminants are removed by chemical oxidation (in small portions) and by bioremediation.¹⁹ (For more examples of the effectiveness of in situ bioremediation on other contaminants refer to the Technical Appendix.)

II. Waste Management

DEFINITION OF WASTE MANAGEMENT

The Resource Conservation and Recovery Act (RCRA) which was passed in 1976 defines solid waste as including garbage, refuse, sludge, solids and liquids from industrial, commercial, mining, agriculture, and community activities but excluding solid or dissolved material in domestic sewage.²⁰ World-wide urban residents produce between 1 and 4 pounds of solid waste per person per day. The United States generates at least 140 million tons per year, 80 percent of which is landfilled. In 1978 there were approximately 20,000 landfills in the United States. Now there are only 6,000 and by the year 1993 it has been determined that 2,000 more of these landfills will reach their capacity and close.²¹

To manage the growing volume of solid waste, a combination of waste reduction, recycling, composting, use of landfills, incineration and bioremediation will be needed.²² To develop a solid waste management plan, a planning commission or some other appointed committee should define its objectives clearly.²³ The objectives should include the amount of waste to be handled by each waste disposal option, disposal method of priority (recycling or incineration), and the implementation of the plan.²⁴

Once the objectives of the waste management plan have been identified all pertinent information must be gathered and reviewed. All existing methods must be evaluated for effectiveness. The evaluation should take into account current practices within the community, the capacity, the remaining useful life and the budget of each disposal facility. The final part of the waste management plan should describe projected growth patterns for the community and the effect a future increase in population could have on existing waste disposal facilities. ²⁵

CURRENT TECHNOLOGY

Incineration is the generation of energy from waste.²⁶ These waste to energy burners which are also called resource recovery plants, use solid waste as fuel to produce nearly 35 million watts of electricity and reduce garbage to ash in the process. Supposedly, these plants should be able to pay for their operation with money from tipping fees charged to garbage haulers and fees received from the sale of electricity. ²⁷

Unfortunately, this technology allows the introduction of natural and artificial gaseous and particulate contaminants into the atmosphere, thus creating the dilemma of trading one source of pollution for another. ²⁸ These pollutants range from heavy metals to acid gases to dioxins. ²⁹ The ash remaining from the incineration process

is also a concern due to the high ratio of toxic metals to harmless substances which makes this residue a hazardous waste. ³⁰

Most communities had turned to landfill dumping in the 1950's and 1960's, but there were some incinerators still operating. These older incinerators still in use were responsible for disposing of about 10 percent of the waste stream during that period. The 1967 Air Quality Act placed stricter regulations on incinerator emissions increasing the need for the addition of expensive air pollution controls. The added devices increased the cost of operating incinerators to the point where the costs outweighed the benefits. ³¹

With the incineration industry declining, public officials and others concerned with the growing problems of solid waste management and disposal once again sought new technology from Europe. One of these new technologies was to serve as a model for restructuring the American incineration industry. This model, an electricity generating system, referred to as the water wall combustion unit had been used in Europe for more than 15 years. ³²

By the mid 1970's several mass burn systems had been built and others were in the planning or construction phases. During this period of time three other technologies emerged which competed with the mass burn incinerators. ³³

The small modular combustion system is used in industrial, large retail and commercial complexes and apartment houses.³⁴ These mass fired furnaces require the shortest construction time and have the lowest capital costs, therefore having the greatest number of domestic installations of all incineration technologies.³⁵

Unfortunately one drawback to this incineration method is that modular furnaces are less thermally efficient due to high excess air requirements, high radiant heat loss, and a high percentage of unburned char in residue. These units are not often used to produce high pressure and temperature steam desired for efficient cogeneration of electric power with steam and heated water.³⁶ Heat recovery units are added to these systems to allow recovery of energy either as hot water, steam or hot air.³⁷

Another incineration system is based on the Pyrolysis method which involves an endothermic (heat absorbing) process rather than an exothermic (heat generating) process. The endothermic reaction occurs when organic material in the waste is exposed to heat in the absence or near absence of oxygen. Heat is then recovered as energy by the transformation of the solid waste into steam or a gaseous or liquid fuel.³⁸

The third system which competes with the mass burn incineration process is called the refuse derived fuel

method (RDF). This method involves the shredding and / or separation of waste to reduce particle size and remove certain components of the waste stream such as ferrous metals. The more combustible fraction of the waste is ground into dense, pellet-like units suitable as fuel in a stoker feed fuel burning system such as those using coal. The RDF technology is effective both as a method of waste disposal and in providing a fuel supplement for other electricity generating systems. ³⁹

Regardless of combustion technology, resource recovery facilities produce steam or hot water, electricity or a combination of the two called cogeneration. Steam can be used for heating and cooling systems, industrial thermal processes, or mechanical driven power. Hot water can be used for heating and cooling systems and lower temperature industrial thermal processes. ⁴⁰

Electrical energy can be used in the facility itself, sold to local utility companies or nearby industries, or sent to other utilities. The electricity is produced by passing steam produced in the incinerator's boiler system through a turbine generator. The steam turbine's rotary motion turns a generator to produce the electricity. ⁴¹

The late 1970's became a time of crisis in waste management as landfills began to be filled to capacity and incinerators with their high costs and constant

breakdowns did not meet expectations for their effectiveness. Even with the stricter air quality standards the need for incineration continued to increase. Resource recovery plants were being promoted as energy suppliers rather than as garbage burners. ⁴²

By 1980 sixty plants were either on line, proposed, or under construction throughout the United States. Many municipalities began to move toward incineration. Within five years there were approximately 200 incinerator plants built or under construction. The increased interest in waste to energy technology spread throughout the country, forty four (44) out of fifty (50) states had at least one facility planned or under construction. ⁴³

As of the late 1980's the United States was spending approximately \$17 billion on the incineration industry. During this time period experts began to consider incineration risky and a potential problem maker. They felt that incineration was simply trading one pollution source for another. ⁴⁴

Despite decades of failures and costly repairs, incinerators are still a popular alternative to landfilling. One study estimates that by 1992 115 new plants will open. Incinerators built today are costing an average of over \$200 million. This cost is being absorbed by taxpayers through taxes on garbage disposal. ⁴⁵

There are several differences between the incineration technology of Europe and the United States. The differences may explain why there are fewer problems with European incinerator plants. In Europe, garbage burned consists of different products which are less damaging to the incinerator plant. American garbage produces substances that cause more corrosion due to the higher temperatures needed to generate electricity. The product of the smaller European incinerator is steam rather than electricity.

Another difference in the incineration technology is that in Europe there is no sorting of garbage before burning.⁴⁶ European incineration industry requires daily reportings of air pollution emissions from every incinerator whereas American operators are allowed to continue burning garbage with very little if any regulations on emissions.

As for health risks, the EPA has estimated the increase in cancer due to incinerators will be 4 to 60 cases nationwide annually once the 200 proposed plants are opened by 1992. The EPA has not estimated health risks caused by other methods of exposure such as absorption of contaminants through skin and ingestion of contaminated food.

A Newsday survey of 56 states and territories has revealed that more than 2,000 landfills have been closed since 1982 for environmental reasons and another 700 have

closed after reaching capacity. With an approximate 230 million tons of garbage produced per year these closures have ensured an increasing problem of burial of garbage and have forced scientists, public officials and others to look for other methods of waste disposal.⁴⁷ According to the Environmental Protection Agency, within 8 years more than one half of all of America's landfills will reach their capacity.⁴⁸

As landfills are closed on the east coast, cities are transporting their garbage west at tremendous costs to taxpayers and are spreading pollution rather than eliminating it. One of the worst problems in the trucking of waste products is the lack of regulation. Trucks which haul garbage to the midwest from the east coast are allowed to return with perishable goods for consumers.

Even though truckers say they sanitize their trucks between hauls, health officials warn that waste being carted can carry infectious diseases not easily eliminated from the trucks. One of the only ways to ensure proper cleansing of these trucks is steam cleaning which is both timely and expensive therefore it is seldom utilized by truckers between hauls.⁴⁹ "As legal disposal grows more difficult, some private waste haulers simply unload their fetid cargo anywhere, from ghetto streets to forests."⁵⁰

To minimize the problems of landfills there are several practices that should be enforced. Landfills should not be located over groundwater recharge areas especially where groundwater serves as a source of drinking water. New landfills should be lined with a synthetic material and two layers of clay which minimizes movement of contaminated water through and out of the landfill into the surrounding groundwater. Contaminated water should be collected in pipes and drawn into treatment areas. Another practice which can minimize water contamination caused by landfills is the continuous monitoring of groundwater in test wells. Air pollution can be limited by the installation of a system which recovers methane. ⁵¹

Unfortunately for all alternatives mentioned, none will replace the need for landfills. They will continue to be necessary for materials which can not be recycled and for the ash produced during incineration. Any new technology such as bioremediation is therefore important because it will be able to reduce the burden on landfills. Microbes degrade the contaminants in soil leached from landfills and reduce the amount of waste in the landfill thereby solving two problems associated with landfilling. ⁵²

Since 1960 the amount of packaging in garbage has increased 80% and now makes up one third of the waste stream

in America. Unfortunately 30 - 40% of the waste stream today is not recyclable and will cause problems in landfills.⁵³ Recycling is a means of reworking a specific product, for example paper is reprocessed as paper and plastics into plastics.⁵⁴

Waste reduction programs can help lessen the burden on landfills and incinerators. Glass, aluminum, and iron do not burn when put in incinerators thus they lessen its efficiency by lowering the temperature and causing the production of more toxic materials in the leftover ash. This toxic ash has caused many problems in disposal especially where leaching can occur from landfills.⁵⁵

One of the advantages that recycling has over incineration is in the production of energy. Incineration produces 500 BTU's steam from 1 lb. of paper whereas recycling that same lb. of paper conserves 2,000 BTU's in energy required to produce paper from virgin pulp.⁵⁶ Another incentive to recycling is the reduction in the amount of garbage that has to be incinerated or landfilled.

Observing other countries such as Japan where recycling is widespread reinforces this idea. While garbage production is on the rise in the United States, Japan has actually maintained a constant level of production from 1976 through 1986. Residents of Japan recycle an estimated 50% of their wastes. Education is a key factor, each year

Japan's officials explain to residents the recycling program and its' benefits. ⁵⁷

Unfortunately there are also many roadblocks to the recycling process. The United States government has not committed sufficient money or people to the recycling industry. In comparison approximately \$305 million was spent on promoting the incineration industry whereas only \$8 million was spent on promoting recycling. The American people are not enthused with the idea of recycling and many complain that there is too much extra work. Education programs for the public are necessary to make recycling a simpler and a less grueling process. Communities must be shown the benefits of recycling in comparison to other methods of waste disposal.

Another reason recycling has not become popular is that the economy has been slow to adjust to the reuse of household trash. Radical swings in prices paid for materials from natural resources and the products from recycling have discouraged some recycling programs. Incentive is also down due to the increasing need for paper refuse by the incineration industry. ⁵⁸ A major problem with the recycling industry is that it is not a supply driven business. ⁵⁹

Aluminum recycling is more promising than plastics and paper. The production of sheet aluminum for new cans

from old cans consumes less energy and is cheaper due to an effective and efficient system which separates the aluminum from the solid waste stream. There will always be a market for recycled aluminum.⁶⁰ The American Iron and Steel Institute estimates that about two-thirds of the scrap steel in the United States is recycled and most of that comes from junk cars and used appliances.⁶¹

III. Impacts of Bioremediation

BENEFITS AND FUTURE USES OF BIOREMEDIATION

The Environmental Protection Agency estimates that the cleanup of 1,200 superfund sites (areas of extreme contamination) throughout the United States would cost an estimated \$24 billion, using technologies currently available. These technologies include incineration of contaminated soil which can run up to \$1,000 per ton.

By contrast the process of bioremediation typically costs less than \$100 per ton and offers the big advantage of restoring land and water thought to be unusable due to being polluted. Microbes have the ability to ingest and destroy the contaminants without leaving any toxic residue behind unlike other waste processing technologies such as incineration. ⁶²

Microbial treatment of contaminated soil can remove the burdens of incineration and landfilling. Most naturally occurring compounds and many synthetic compounds, Xenobiotics, can be eliminated by microbes. ⁶³ Bioremediation has already been shown to significantly reduce the amount of pollution caused by large oil tanker spills such as the Exxon Valdez disaster. This technology is needed in such cases to reduce the cleanup time of oil spills and the negative impacts to plant and animal life caused

by the harsh detergents used in traditional cleaning methods. (See the Technical Appendix for other uses and benefits of bioremediation.)

Currently Americans generate approximately 160 million tons of garbage per year.⁶⁴ The landfill and incineration technologies together can barely meet the need for waste management at present and each year the amount of garbage produced by Americans will continue to increase. Bioremediation may be able to solve not only the problems of land reclamation but also the crisis of solid waste management.

Researcher William Rathje, of the University of Arizona in Tucson, has found that biodegradation does not significantly occur at present in landfills. Under normal conditions in the landfill the lack of nutrients and aeration significantly affect the efficiency of microbes in the landfill.⁶⁵ The process of bioremediation involves the addition of larger amounts of these biodegrading microbes, aeration, and fertilizers thus increasing the effectiveness of the degradation process causing a faster breakdown of the mounds of waste overflowing the landfills. Bioremediation also has the benefit of reducing waste without introducing another source of pollution such as ash and other toxic residues into the environment.

Bioremediation could also play an important part in the cleanup of oil spills from leaking underground storage tanks. As states become increasingly aware of the contamination caused by older storage tanks to soil and ground water, they are requiring environmental assessments of areas containing these tanks.

In Connecticut tank removal and excavation companies are gaining business as state regulations governing underground storage tanks become stricter. This is especially true of older gasoline stations undergoing renovations and / or sale. The tanks and soils surrounding these tanks are tested for leaching contaminants. If any evidence is found of leakage the tanks must be excavated and removed. The contaminated soil is then carted to a landfill. New soil is carted in and new tanks replace the old ones.

The major problem with tank removals is the process of carting contaminated soil to the landfill which has the potential of contaminating ground water if it should leach from the landfill. Treating the soil on site with microbes may take longer than carting it away but bioremediation is cheaper and eliminates the problem of future soil and ground water contamination.

DISADVANTAGES OF BIOREMEDIATION

There are a few disadvantages of bioremediation including length of degradation time and effectiveness of microbes. Even under ideal conditions the process of bioremediation takes longer than excavating and carting the contaminated area to a landfill. Environmental companies under contract deadlines may choose faster methods of waste disposal even if they are shorter term solutions.

Another problem with bioremediation is that there are many variables affecting each contaminated site therefore they will not all respond to microbial treatment in the same manner. Since there so many unknown variables including millions of different microbes which are affecting the contaminant(s) on the site it is not easy to isolate one microbe which will perform the degradation the most efficiently.

It can be very time consuming identifying the proper microbes to use on a contaminated site especially if there are multiple pollutants involved. To keep the microbes degrading the contaminant(s), continuous monitoring is required. The monitoring of the site shows if the microbes are functioning efficiently and whether or not additional nutrients (air, water and fertilizers) will be needed.

Unfortunately as in any new technology, there are no definites as to how long the process will take or how

effective it will be. Bioremediation is still an evolving technology and as such it can not be relied on as the sole answer to the waste management crisis facing our world today.

POTENTIAL ABUSES OF TECHNOLOGY

With all the benefits of Bioremediation there is also potential for misuse and abuse of this technology. As people find it easier and cheaper to use this method of cleanup there could be a tendency to continue to pollute and contaminate the earth's natural resources at the same rate as present. If the public is not educated properly about this technology it may be used as a panacea for all the world's pollution problems. Bioremediation has many benefits over other methods of waste management and reduction but it is most effective when used along with other technologies.

One country which utilizes all methods of solid waste management fairly efficiently is Japan. Unlike the United States, the Japanese realize that burning, burying, recycling and reducing are all important parts of solid waste management. Of all these methods though, recycling is the solution of choice in Japan. Approximately 40% of their solid waste is recycled comprised of one half of the paper, 55% of glass bottles and 66% of food and beverage cans. ⁶⁶

Since the early 1970's officials in Japan have strictly enforced mandatory separation of burnable from non-combustible trash. To burn the combustible trash there are 1,899 refuse burning plants in Japan. This is one draw back to Japanese waste management since there are so many incinerators for a small country and few regulations governing their operation. The officials only monitor four types of emissions therefore there is increasing concern over air quality. ⁶⁷

Like the United States, Japan has not completely conquered their garbage disposal problems. In Japan a major problem of public officials is keeping the incentive to recycle high. Even now little used appliances and furniture can be found in Japan's landfills. Another problem with Japanese society is that everything bought is wrapped in paper even a pencil. ⁶⁸ Japan's obsession with wrapping everything is also an example of how technology can be abused. By not reducing the amount of waste produced Japan is only temporarily putting off their waste crisis. As long as they continue to wrap everything sold and discard barely used appliances their recycling and incineration programs will not reach their optimum efficiency.

IV. Conclusion

RECOMMENDATIONS

The public must be educated about each of these technologies their benefits and drawbacks. It is important for public officials, environmental planners and environmental activists to work together to present all waste management and reduction programs. There is a need to formulate a comprehensive plan concerning methods of waste management for the United States.

In a comprehensive plan; source reduction, recycling, landfilling, incineration and bioremediation need to be addressed as they are all important methods of disposal and management. Increasing public concern on problems associated with soil, water and land contamination, have caused increasing awareness leading to new reasearch in waste management planning.

There are several important steps in any management plan. First standards for quality (air, water, soil) must be set which represent a desirable or at least an acceptable level to the society affected. Next the existing quality must be determined by monitoring. Once this quality is known plans must be developed which include the standards set and a permitting process.

There are several important parts to creating a recycling plan which could help solve current problems

with burying and burning waste as a component part of the overall waste management plan. Mandatory recycling plays an important part by increasing the participation by the public in any recycling program. With proper education, mandatory recycling programs throughout the United States have a 90% participation rate. Economic incentives to haulers and households is another method of soliciting participation in recycling programs. ⁶⁹

One of the most effective methods of educating the public is through the public school system. Teaching younger people the values of recycling in school is important since as these children grow they will not only continue to practice what they have learned but will also pass on this information to other generations. ⁷⁰

Another important part of the recycling plan is the need for local, state, and federal governments to buy products made with recycled materials. The Environmental Protection Agency and the United States Department of Commerce must purchase recycled products made in the United States to make its industry more competitive with other countries. Other economic incentives must be made to ensure the marketability of recycled materials especially to manufacturers. Some incentives for manufacturers are below market rate loans to encourage them to locate in a particular area and create markets for locally recovered

materials. This scrap based manufacturing creates jobs and new skills, encourages investment and enlarges the manufacturing tax base of the local economy. ⁷¹

Each method of waste processing and disposal has a place in the waste management plan. Every technology available must be examined to help define the problems of waste management and each should be utilized to reach a solution to this environmental crisis. Incineration alone may not be the solution to a community's problems, especially if there is already concern over air quality in that area. That community may be better off adding a recycling program and utilizing bioremedial technology to reduce their landfills in size and to protect the surrounding soil and groundwater from leaching contaminants.

Educational programs should be designed for all ages concerning the options of waste management. Public involvement can be crucial in finding solutions to problems which affect the whole community. As stated earlier in the recycling plan, school can provide an important median for training children the importance of waste management and their future lifestyles.

The government should take an active role in creating public information films, seminars, and booklets concerning the waste management crisis facing the United States and other countries today. Information highlighted should

include summaries each waste processing and disposal option and any new technology such as bioremediation which is being researched.

The research and development of new technologies such as bioremediation should also be aided by the United States Government since waste management is a concern for the whole country. A national commission involving specialists on waste management should be developed which studies the pros and cons of current methods and potentials of new technology.

SUMMARY

The crisis of waste management has created an increase in public awareness. Unfortunately this awareness may be too late and the public has little knowledge of the impact their waste has had on the environment. Although there are several technologies practiced in waste management many of them create other types of pollution.

This paper has summarized the current technologies available in waste management and the potential uses of a newer method called Bioremediation. With increasing research this method can provide a solution to the waste management crisis if it is used in conjunction with current methods. Bioremediation is also an important method of land and water reclamation. Sites previously thought to

be unpurifiable have the potential for reuse once they are treated with microbes. Bioremediation has been shown to be an effective method of decontamination without leaving any toxic residues.

Microbes used in this process die off as the pollutant is degraded and return to their normal population size. Once treatment of the site is complete, continuous monitoring is necessary to ensure that all traces of the contaminant have been eradicated.

Of the current technologies recycling has been proven to be the most effective method of waste reduction and the least damaging to the environment. Landfilling and incineration have been shown to be the most damaging and have not created a solution to the waste management crisis.

This paper has also attempted to explain waste management planning and the necessity of each method in such a plan. One method alone will not solve our waste disposal problems. The key to the management plan is waste reduction, recycling, and incineration. New methods such as bioremediation are also important in the waste management plan since they will provide future alternatives to landfilling and incineration. Continued studies of new technology should be encouraged as supplemental methods of waste disposal and processing. They should not be sought as a substitute to existing

methods until they are proven to be better than older technology. As new treatments are proposed, tested and proved reliable they can be integrated in the overall management scheme.

A solid plan for educating the public on waste management and methods should be of primary concern to any government agencies (state and federal) devising waste management plans. Proper education on the crisis existing today in waste management will help the public understand why cooperation in waste reduction and recycling programs is necessary.

Earlier education on waste management may also drive children to seek higher education and positions in environmental fields as they get older, thus ensuring new voices in the fight to save our environment. The sooner the public becomes aware of the growing problems associated with landfills, incinerators and other pollutant sources, the sooner a solution can be found to alleviate them.

Although Bioremediation may not be the ultimate solution to the problems of waste management it is a step in the right direction. Instead of sitting and waiting for the environment to clean itself, scientists are testing the potential of microbes to do the reclamation work faster. From the shores of Prince William Sound, Alaska to the East Coast of the United States researchers are learning

all they can about the microbes which inhabit our soils. Some enterprising scientists have already begun to market this technology on small sites in need of detoxification. Hopefully this technology will be effective on larger superfund sites throughout the United States. If this becomes a regular practice, billions of dollars saved in the cleanup of these site could be used to develop other methods of waste management.

V. Technical Appendix

IN SITU
MICROBIAL TREATMENT
OF
CONTAMINATED SOIL
(BIOREMEDIATION)

AFFORDABLE TECHNOLOGY, INC.
3179 BABCOCK BOULEVARD
PITTSBURGH, PENNSYLVANIA 15237
(412) 364-9005

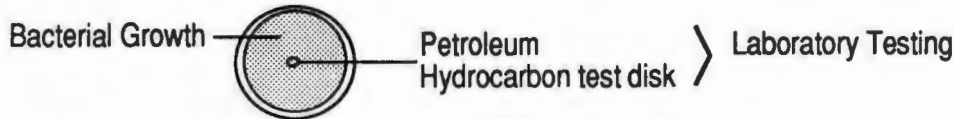
ATI Bioremediation, Inc.

"Environmental Bioremediation" by ATI using state of the art technology.

Is bioremediation feasible at your petroleum fuel contaminated site?

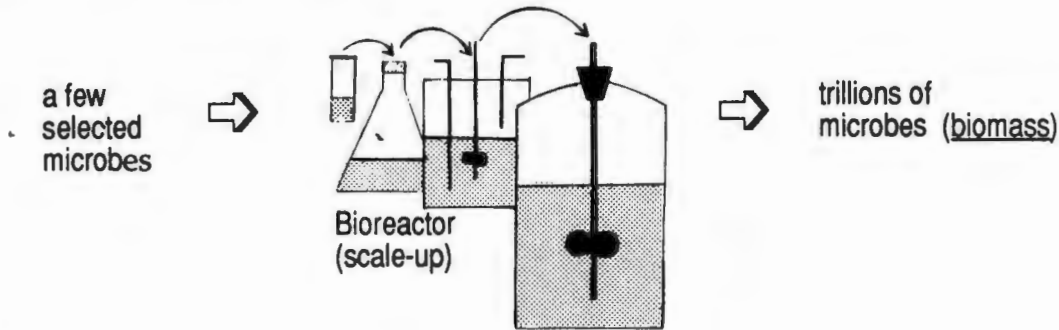
This question can be answered by an ATI microbial/soil profile (study). This profile includes:

- Isolation of natural soil microbes from the contaminated site.
- Identification of specific microbes, e.g. Pseudomonas species, that are resistant to and decompose the petroleum hydrocarbon.



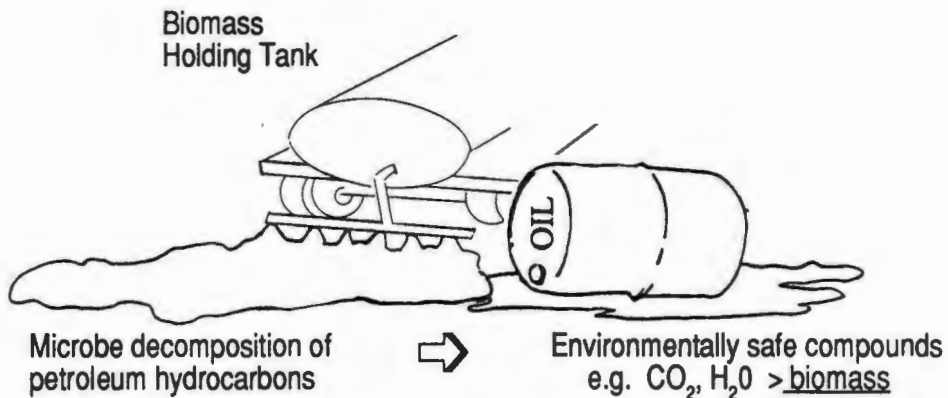
Is it feasible to proceed? What do we need?

- If it is feasible ATI will provide a biomass (increase the population) of the identified petroleum hydrocarbon decomposing microbes.



What is the next step?

- ATI will apply the biomass to the contaminated soil in situ using a gravity drip, injection or spray technology.



ATI Bioremediation efficiently eliminates petroleum hydrocarbon spills rapidly in situ (90 to 150 days) at low cost (\$25 to \$85/yd³)



AFFORDABLE TECHNOLOGY, INC.

We have enclosed information on bioremediation technology that is being used to renew contaminated soil.

This process may be new to your firm, but it is state-of-the-art technology that is being employed worldwide.

If interested, please contact us so that we may establish a time and date for our bioremediation presentation in order to interact with your firm regarding environmental remediation.

Respectfully submitted,

AFFORDABLE TECHNOLOGY, INC.

Dave A. Wheeler,
Vice President

DAW:jds
(enc.)

ENVIRONMENTAL BIOREMEDIATION

Support for engineered "Environmental Remediation" by providing:

- Microbial profile (bioassay).
- Microbial candidates for specific chemical decomposition.
- Microbial BIOMASS for application to contaminated project site.
- Biostabilization chemicals.
- Bioremediation project site monitoring.
- Pathogenic microbe isolation/identification/irradication.



AFFORDABLE TECHNOLOGY, INC.

3179 Babcock Blvd. • Pittsburgh, PA 15237

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AFFORDABLE ENVIRONMENTAL BIOREMEDIATION

We at AFFORDABLE TECHNOLOGY, INC., can expand your firms capabilities into the world of bioremediation through microbiology.

AFFORDABLE TECHNOLOGY, INC., attacks your soil contamination problem efficiently and safely with naturally-selected microbes.

AFFORDABLE TECHNOLOGY, INC., microbial bioremediation services are competitive and in fact can provide large savings over incineration or off-site disposal of contaminated soil.

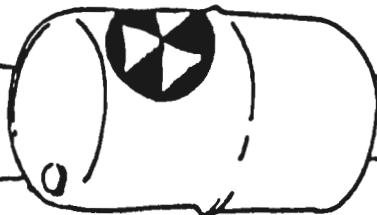
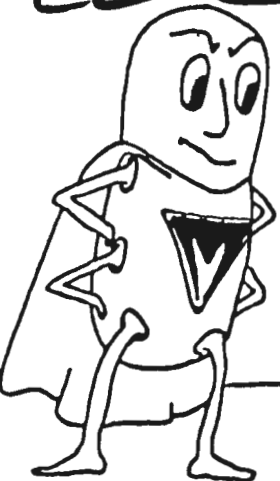
AFFORDABLE TECHNOLOGY, INC., provides your firm with technical consultation and bioremediation microorganisms to solve your soil contamination problem.

Should you be interested in information about our service, please drop us a line or give us a call at:

AFFORDABLE TECHNOLOGY, INC.
3179 Babcock Boulevard
Pittsburgh, Pennsylvania 15237
(412) 364-9005

NOTHING CAN TOP THE CLEAN-UP POWERS OF...

"Super Microbe"



SCOPE AND MAGNITUDE OF THE PROBLEM

- . APPROXIMATELY 10,000 MAJOR SITES NATIONWIDE REQUIRING RESTORATION - ESTIMATED 100 BILLION DOLLAR COST
- . 400-800 DOD SITES - ESTIMATED 5-10 BILLION DOLLAR COST
- . 32 MILITARY SITES ALREADY ON NATIONAL PRIORITIES LIST
- . MULTITUDES OF PRIVATELY-OWNED RESIDENTIAL PROPERTIES CONTAMINATED WITH TOXIC WASTE

CURRENT TECHNOLOGIES

- . INCINERATION
- . LAND-FILL
- . AIR STRIPPING
- . ADSORPTION
- . BIODEGRADATION

DISADVANTAGES OF FREQUENTLY USED CURRENT TECHNOLOGIES

- LANDFILLS

A NON-PERMANENT SOLUTION FOR MANY
HAZARDOUS WASTES AND THEREFORE,
PRESENTS A FUTURE LIABILITY

- INCINERATION

MANY ORGANICS ARE DIFFICULT TO BURN
AND PRODUCE TOXIC AIR POLLUTANTS

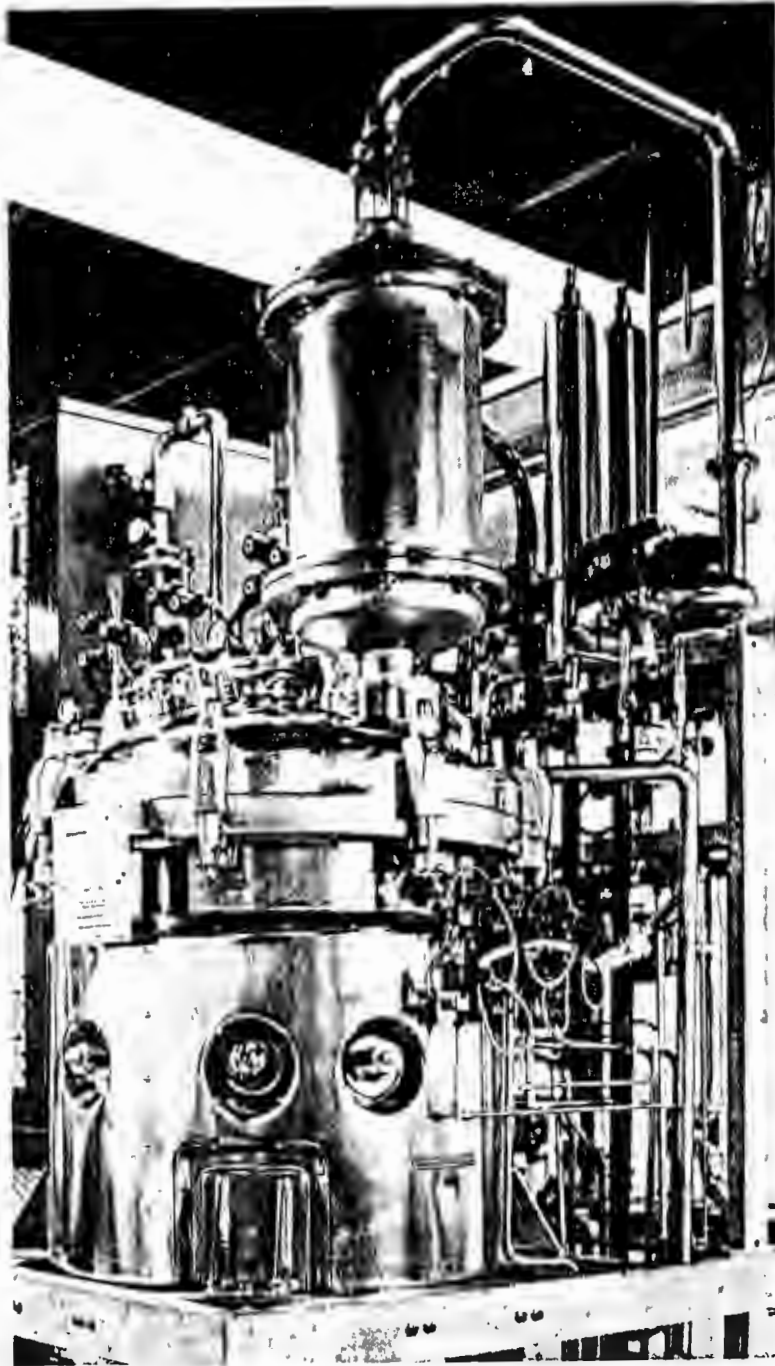
BIODEGRADATION

BIODEGRADATION IS THE USE OF MICROORGANISMS (BACTERIA, MOLDS, ALGAE) TO DEGRADE OR DETOXYIFY HAZARDOUS CHEMICALS* THAT PERSIST IN THE ENVIRONMENT

*NATURAL (OCCURRING IN NATURE) OR XENOBIOTIC (SYNTHESIZED BY MAN, NEVER FOUND IN NATURE)

STEPS IN ENVIRONMENTAL BIOREMEDIATION

- 1) HISTORY OF CONTAMINATED SITE
- 2) MICROBIAL PROFILE OF AREA
- 3) NATURAL SELECTION OF MICROBES THAT METABOLIZE CONTAMINATING CHEMICALS
- 4) QUANTITATIVE INCREASE OF SELECTED MICROBE (A FERMENTER PRODUCED BIOMASS)
- 5) APPLICATION OF THE MICROBE BIOMASS TO THE CONTAMINATED SITE
- 6) BIOSTABILIZATION
- 7) PERIODIC MONITORING OF BIOREMEDIATION SITE



500 LITER COMPUTER CONTROLLED FERMENTATION SYSTEM
USED TO PRODUCE OUR BIOMASS

BIOSTABILIZATION PROVIDES:

- . AERATION
- . NITROGEN
- . PHOSPHORUS
- . TRACE ELEMENTS

ADVANTAGES OF BIODEGRADATION (BIOREMEDIATION)

- . NATURAL "LOW-TECH" SOLUTION
- . PERMANENT SOLUTION TO PROBLEM
- . MAY OBTAIN NEED FOR EXCAVATION
- . NO RELEASE OF TOXIC EMISSIONS
- . EFFECTIVE FOR DECOMPOSITION OF A VARIETY OF CONTAMINANTS IN MANY ENVIRONMENTS
- . EASILY INTEGRATED WITH CONVENTIONAL PROCESSING

ADDITIONAL "BIODEGRADATION" FACTS

- . BIOREMEDIATION RESULTS IN DETOXIFICATION

- . NATURAL COMPOUNDS ARE DEGRADABLE

- . MANY XENOBIOTICS (MAN-MADE COMPOUNDS NOT FOUND IN NATURE) ARE DEGRADABLE

- . HYDROCARBONS ARE PARTICULARLY SUSCEPTIBLE TO BIODEGRADATION

- . MICROBES OF THE GENERA PSEUDOMONAS, NOCARDIA AND STREPTOMYCES DEGRADE HYDROCARBONS

WHY AFFORDABLE TECHNOLOGY, INC., DOES NOT
EMPLOY GENETICALLY-ENGINEERED MICROBES

- . MAY CONVERT TO ORIGINAL GENETIC STATE

- . NOT READILY CERTIFIED BY FEDERAL OR
STATE GOVERNMENTS FOR USE

- . DO NOT COMPETE WELL WITH NATURAL SOIL
MICROORGANISMS

- . MAY BE HARMFUL TO ENVIRONMENT

AT AFFORDABLE TECHNOLOGY, INC., WE
EMPLOY NATURAL-SELECTED SOIL MICROBES
THAT ARE TAILORED TO EACH PROJECT SITE

ADVANTAGES OF NATURAL-SELECTED MICROBES

- . THEY ARE DEPENDABLE FOR DECOMPOSING A SPECIFIC CHEMICAL

- . DER/EPA ACCEPTABLE

- . THEY COMPETE WELL WITH OTHER MICROBIAL FLORA

- . ADJUSTED TO LOCAL ENVIRONMENTAL CONDITIONS

- . THEY ARE NOT HARMFUL TO ENVIRONMENT

- . SELF-LIMITING

E F F E C T I V E N E S S

- BIOREMEDIATION OF HYDROCARBONS-CONTAMINATED SOIL RESULTS IN ACCEPTABLE LEVELS IN 90 TO 120 TREATMENT DAYS
- BIOREMEDIATION OF OTHER TOXIC SOIL CONTAMINANTS CAN RESULT IN A SATISFACTORY REDUCTION IN 120 TO 150 TREATMENT DAYS
- ENVIRONMENTAL BIOREMEDIATION CONTINUES EVEN AFTER ACTIVE TREATMENT OF CONTAMINATED SITE HAS ENDED

C O S T

BIOREMEDIATION COST* OF SOIL WILL VARY FROM \$20.00 TO \$80.00 PER TON (2,000 LBS) DEPENDING ON THE SIZE AND VOLUME OF THE CONTAMINATED SITE.

*COST WILL BE CALCULATED ON A DIFFERENT SCHEDULE IN THE CASE OF A MULTI-CHEMICAL CONTAMINATED SITE.

Aerobic Biodegradation of Vinyl Chloride in Groundwater Samples

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Received 30 July 1990/Accepted 8 October 1990

Studies were conducted to examine the biodegradation of ^{14}C -labeled vinyl chloride in samples taken from a shallow aquifer. Under aerobic conditions, vinyl chloride was readily degraded, with greater than 99% of the labeled material being degraded after 108 days and approximately 65% being mineralized to $^{14}\text{CO}_2$.

Widespread use of chlorinated aliphatic hydrocarbons has stimulated considerable interest in the processes which determine the environmental fate of these compounds. Because of their relatively high aqueous solubilities and persistence in soil, chlorinated aliphatic hydrocarbons have been detected in groundwater (15). In particular, vinyl chloride has received increased attention as a groundwater contaminant, since it is both toxic and carcinogenic to humans (13). The presence of vinyl chloride in groundwater has been reported and was proposed to originate from degradation of higher chlorinated aliphatic hydrocarbons, such as trichloroethylene and tetrachloroethylene (7-9). Additional laboratory studies have firmly established that vinyl chloride can result from reductive dechlorination of trichloroethylene, tetrachloroethylene, and dichloroethylene (4, 14).

Biotransformation of vinyl chloride under methanogenic conditions has been reported, although degradation was shown to be relatively slow and incomplete (1, 4). Less information is available on the aerobic biodegradation of vinyl chloride. Hartman et al. (6) isolated a *Mycobacterium* strain which used vinyl chloride as the sole carbon and energy source for growth. Roberts et al. (11) have observed degradation of vinyl chloride in an aquifer enriched with methane and oxygen. However, no degradation occurred without methane enrichment.

The purpose of this study was to examine the aerobic degradation of vinyl chloride by naturally occurring microorganisms in groundwater. Laboratory studies were conducted with soil-water microcosms prepared from authentic aquifer material.

Aquifer material was obtained from a site located on the northern bank of the South Canadian River from an area bordering the municipal landfill in Norman, Okla. (2). The water table at the site has been reported to be quite shallow and ranges from approximately 0.6 to 1.5 m below the surface (12). The sample site did not receive leachate from the adjacent landfill and was chosen to represent an aerobic portion of the aquifer. Subsurface soil samples were obtained from approximately 0.5 to 1.0 m below the surface and transferred to sterile glass jars. Groundwater was collected by digging a hole approximately 1 to 2 m deep and allowing the hole to fill. Groundwater was then haled into sterile one-gallon (3.784-liter) glass bottles. The samples were chilled and sent to the laboratory in Midland, Mich.

Analysis of the subsurface soil for organic and inorganic contents and soil texture was performed by A & L Midwest Laboratories, Inc. (Omaha, Nebr.), by standard methods

(3). The total number of bacterial cells and the number of viable microorganisms associated with the subsurface soil were determined by the method of Ghiorse and Blackwell (5).

Biodegradation of ^{14}C -labeled vinyl chloride was examined in microcosms prepared with the subsurface soil and groundwater. Samples were prepared in 30-ml serum bottles by combining 20 g (wet weight) of solids with 20 ml of groundwater which had been sterilized by filtration through a 0.45- μm -pore-size filter. To ensure aerobic conditions, the microcosms were sparged for 5 min with 100% O_2 before addition of the labeled material. The bottles were then supplemented with an aqueous solution of ^{14}C -labeled vinyl chloride (specific activity, 0.53 mCi/mmol; Dupont, NEN Research Products, Boston, Mass.) to yield a final concentration of either 0.1 or 1.0 ppm (wt/wt or grams of vinyl chloride per gram of soil and water) and sealed with a Teflon-faced butyl rubber septum and an aluminum crimp seal. Reaction mixtures also contained resazurin (0.002%) as a redox indicator. Autoclaved controls were included to monitor abiotic degradation, as well as loss of test material from the microcosms. All samples were incubated at 20°C in the dark and agitated on a tissue culture rotator which continually rolled the bottles at 1 rpm.

Analysis for ^{14}C -labeled vinyl chloride in the aqueous fraction was performed by high-performance liquid chromatography. Before analysis, the samples were chilled on ice for approximately 30 min. Chromatography was performed with a ZORBAX octyldecylsilane column (4.6 mm by 25 cm, Dupont) with acetonitrile-water (50:50) as the mobile phase delivered at 1.0 ml/min by a Waters 510 solvent delivery system. Radioactive compounds were detected by an on-line radioactivity monitor unit (Berthold 506A).

Total radioactivity in the aqueous fraction was determined by liquid scintillation counting. Triplicate samples of the aqueous fraction (200 μl) were counted in 10 ml of liquid scintillation counting cocktail (AquaSol; Dupont, NEN).

Mineralization of ^{14}C -labeled vinyl chloride to $^{14}\text{CO}_2$ in the reaction mixtures was determined during the study. $^{14}\text{CO}_2$ was collected by passing N_2 gas (250 to 350 ml/min) through the slurry mixtures, which had been acidified with 200 μl of concentrated phosphoric acid. The purged gas was collected in a series of two traps, each containing 10 ml of a 1 N potassium hydroxide solution. One-milliliter portions from the combined traps were analyzed by liquid scintillation counting. $^{14}\text{CO}_2$ production was confirmed by adding barium nitrate to the trap solution, mixing it for 30 min, and determining the radioactivity in the solution after removal of the precipitate (10).[‡]

The physical, chemical, and biological characteristics of the subsurface soil and groundwater are summarized in

* Corresponding author.

TABLE 1. Subsurface soil and groundwater characteristics

Parameter	Subsurface soil	Groundwater
Texture	Sand	
Composition (%)		
Sand	93	
Silt	1	
Clay	6	
pH	8.6	7.6
% Organic carbon	0.24	
Dissolved organic carbon concn (ppm)		48
Sulfate concn (ppm)		27
Nitrate nitrogen concn (ppm)	6	<0.2
No. of bacteria/g (dry wt) by:		
Direct count (acridine orange)	9.77×10^7	
Total heterotrophic count (plate count)	3.01×10^4	

Table 1. The solids, which contained relatively little organic carbon, were classified as a sand on the basis of low levels of silt and clay. The total number of microorganisms associated with the solids, as determined by acridine orange direct counting, was similar to that observed by Beeman and Sulflita (2) in solids from anaerobic areas within the same aquifer. The level of viable microorganisms associated with the solids determined by a standard plate count (5) was several orders of magnitude lower than that determined by direct counting.

Biotransformation of ^{14}C -labeled vinyl chloride at two different concentrations was examined under aerobic conditions in the groundwater microcosms. ^{14}C -labeled vinyl chloride (1.0 ppm) was readily degraded in the groundwater samples (Fig. 1). No adaptation or observable lag occurred before the transformation of vinyl chloride, and approximately 25% of the test material was degraded during week 1

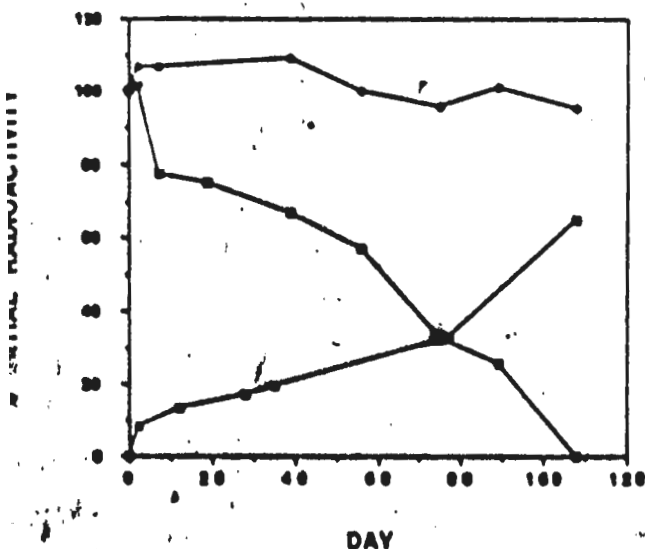


FIG. 1. Biodegradation of ^{14}C -labeled vinyl chloride at 1 ppm in aquifer microcosms. Symbols: \bullet , sterile samples; \square , viable samples; \blacktriangle , $^{14}\text{CO}_2$ produced by the viable samples.

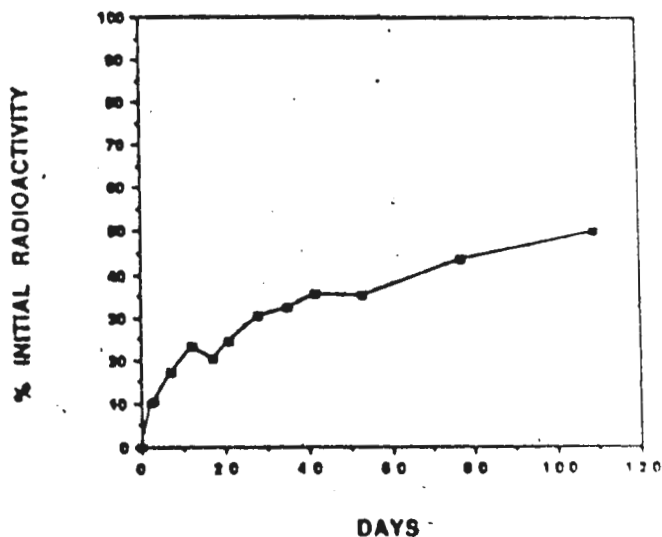


FIG. 2. Biodegradation of ^{14}C -labeled vinyl chloride at 0.1 ppm in aquifer microcosms. The amounts of $^{14}\text{CO}_2$ produced by the viable samples are shown. Less than 1% $^{14}\text{CO}_2$ was detected in the sterile controls.

of incubation. After 108 days, greater than 99% of the test material was degraded in the biologically active samples. Vinyl chloride degradation was biologically mediated, since greater than 95% of the labeled material was recovered from the aqueous fraction in the sterile controls. Mineralization accounted for much of the loss, since approximately 65% of the labeled material was recovered as $^{14}\text{CO}_2$ after 108 days of incubation.

To determine whether biodegradation would occur at lower concentrations of vinyl chloride, additional microcosms were prepared as previously described and spiked with the ^{14}C -labeled material at 0.1 ppm. Degradation of the test material at this concentration was monitored by $^{14}\text{CO}_2$ production only. The microorganisms present in the aquifer material were capable of mineralizing vinyl chloride at concentrations of 100 ppb and below (Fig. 2). After 109 days, approximately 50% of the labeled material was recovered as $^{14}\text{CO}_2$. Mineralization was not observed in the sterile controls.

The results of this study demonstrate that vinyl chloride can be rapidly degraded under aerobic conditions. The absence of an observable lag or adaptation period was unexpected, since the aquifer at this site had no known previous exposure to vinyl chloride or other chlorinated solvents. Thus, the ability of microorganisms associated with soil and groundwater to degrade vinyl chloride may be widespread. These results are consistent with those of other investigators (6) who have reported the occurrence of vinyl chloride-degrading microorganisms associated with soil.

This investigation is the first report of aerobic biodegradation of vinyl chloride in environmental samples. Although there have been several reports on vinyl chloride biodegradation (1, 11), previous studies have relied on addition of exogenous nutrients, such as methane, to demonstrate degradation.

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Over California Seismic Hot Spot

...ked, doors swung open and
...one woke with a start last
...Lindh's home south of San
...was the best earthquake we've
...Lindh of the Lake Elsman
...seismologist such as Lindh,
...5.2 earthquake and its near
...k nearby last year are also the
...earthquakes to have shaken
...portion of the San Andreas
...San Francisco quake of 1906.
...aken that segment of the San
...more seriously than other
...Lindh, who lives a half
...the fault, 60 kilometers south
...and 20 kilometers to the
...Elsman. "Now we've had two
...mide 5's where we didn't have
...thought it was a dangerous
...anything happened; I can
...feel so that feeling now."

...e that these events may be a
...warning magnitude 6.5 quake
...of a kilometer section of the
...south of Lake Elsman. This
...crosses through the southern
...Sonoma, a sparsely populated
...to the south of Silicon
...San Jose and Santa Cruz.
......of the southern
...ment have alerted researchers
...California seismic danger.
......ment's role in the great
...earthquake. It was here that
...preparing rupture of the
...out of steam and came to a
...The amount of slippage be-
...miles of the fault during the
...ed from more than 4 meters
......meter on this section to

zero at its southern end, near San Juan Bautista. But every part of the fault must slip several meters every few hundred years on average; the drifting of the continents requires it. So, seismologists expect one or more moderate earthquakes on such terminal segments to make up for the shortfall in fault slip in 1906.

Another worrisome circumstance is the length of fault that would break in any quakes that help the slippage catch up. If the southern end of the 1906 break were subdivided into a series of segments, each only a few kilometers long, the quakes would be small and relatively harmless. But in 1983 Lindh, who works at the U.S. Geological Survey office in Menlo Park, decided that the entire 45 kilometers of fault south of Lake Elsman was a single segment that would break all at once. If so, a respectable earthquake of magnitude 6.5 would result.

Then there are the Lake Elsman quakes. They are two of the three largest earthquakes since 1914 on the 420 kilometers of the northern San Andreas. Each broke about a 1-kilometer patch of fault at depths of 14 kilometers within a kilometer or two of the intersection of the San Andreas and a side fault called the Sargent, right at Lake Elsman. That puts these quakes precisely at one end of Lindh's segment. Often a fault section first shows signs of imminent rupture at its ends.

With more and more signs that the southern Santa Cruz Mountains segment is dangerous, seismologists are starting to give it the attention an immediate threat deserves. A USGS working group estimated last year, before the first Lake Elsman earthquake that the southern Santa Cruz section has about a 30% probability of breaking some time during the next 30 years (*Science*, 22 July 1988, p. 413). No one is formally raising that probability, but Lindh's seismological intuition tells him they better assume the worst. ■ RICHARD A. KERR

Exxon Bets on Bugs in Alaska Cleanup

In the largest experiment of its kind, Exxon Corporation is trying to feed native Alaskan bacteria. The hungry microbes slurp oil and, if Exxon can grow lots of them, they'll help clean up beaches that were stained with the crude oil dumped by the company's tanker, the *Valdez*, last April.

By September, says Bob Mastracchio, who heads Exxon's cleanup program, the company intends to coat 70 miles of shoreline with two kinds of nitrogen- and phosphorous-bearing fertilizers to boost the indigenous bacterial populations. Exxon is so confident about the potential of the approach that it is gambling \$10 million on the effort.

But Exxon's confidence rests on a limited experimental base. The company, in conjunction with the Environmental Protection Agency (EPA), began experiments with the fertilizers in early June on four test plots measuring 30 meters by 12 meters. Two more plots were used as controls and were not treated. "Within 10 days a clear rectangle appeared against a background of oil-contaminated beach material," says Hap H. Pritchard, a microbial ecologist in EPA's Office of Research and Development.

But Pritchard, who normally is stationed at EPA's Gulf Breeze, Florida, research laboratory, says it is hard to quantify just how effective the fertilizer really is. Even without chemical treatment, "there is significant biodegradation going on," he says. Another variable, he adds, is the uncertainty about

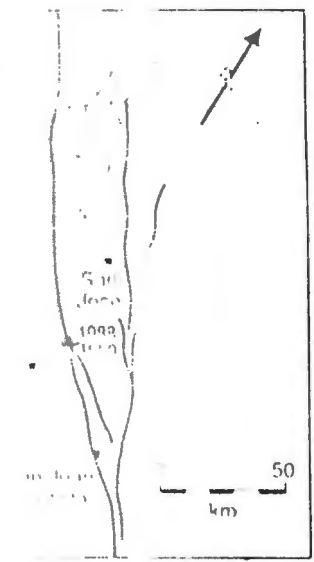
how long elevated bacterial populations will be sustained with just one application. A reduction in air and water temperatures also is expected to reduce bacterial activity.

Curiously, neither EPA or Exxon have done much work to identify the different species of bacteria at work on the beaches. Nor have they sought to gauge their appetite for oil. With a narrow time frame for executing the plan before winter closes in, Mastracchio says there has not been time for such basic research.

One of the chemicals to be used is "Impol EAP 22," a special fertilizer created for the very same purpose in the early 1980s by the French petroleum concern, Elf Aquitaine. But the chemical's only major deployment occurred in 1985 to help mop up a far smaller spill of refined marine oil.

The use of the fertilizers, EPA and Exxon officials acknowledge, may pose a risk to some sea life. Lab studies suggest that where tidal flushing is minimal, nitrogen-leaching fertilizers could be toxic to the larvae of sea urchins, oysters, and mussels. For this reason, EPA is monitoring shellfish, but so far there is no evidence that they are being affected.

Even if there were some damage, experts view the risk as small relative to the potential benefit. Pritchard says that it could take 5 to 7 years for oil on beaches to break down under natural conditions. With the fertilization program that time could be reduced to 2 to 5 years. ■ MARK CRAWFORD



Bacteria Effective in Alaska Cleanup

A year after the *Exxon Valdez* dumped its cargo into Prince William Sound, oil-soaked beaches that were treated with an experimental cleanup technique are beginning to return to normal. Indeed, the technique has turned out to be so effective that even some of the scientists who helped develop it are expressing surprise.

Last summer, in a \$10-million experiment, Exxon researchers sprayed some 70 miles of beaches around Prince William Sound with a fertilizer called Inipol that was developed in the early 1980s by the French petroleum company Elf Aquitaine. The goal: to stimulate the growth of naturally occurring bacteria known to have an appetite for hydrocarbons. It was the biggest test ever conducted of the use of bacteria to clean up an oil spill (*Science*, 18 August 1989, p. 704).

Though nobody is touting the technique as a cure for every oil-fouled beach, preliminary surveys conducted last summer by the Environmental Protection Agency (EPA), which is participating in the test, indicated that the sprayed beaches showed dramatic improvement compared to untreated areas—usually within 15 days. Now laboratory tests performed this winter have provided detailed support for these observations.

For example, scientists found two orders of magnitude greater microbial counts on beaches soon after they were treated than existed in untreated areas. And the effect lasted, with elevated levels of oil-degrading bacteria persisting 5 months after spraying, according to Russell R. Chianelli, senior research associate at Exxon Research and Engineering Company. Best of all, the bacteria turned out to have a much greater appetite for oil than anyone had imagined, says Chianelli. In fact, EPA's and Exxon's data collection efforts were initially hampered because the organisms even attacked some compounds in the crude oil that researchers were hoping to use as long-term markers for statistical analyses.

Still to be determined is how effective the technique is in digesting oil that has penetrated porous stone or migrated below the surface of pebble beaches. EPA and Exxon researchers say the fertilizer seems to be stimulating increased degradation to depths of about 1 foot, but biological activity there may occur at a slower rate. Chianelli reports, however, that preliminary tests indicate that oil beneath surface rocks was consumed by microorganisms in about 40 to 50 days.

As for toxic effects, so far no significant impact has been seen in mussel larvae and oyster larvae, says Hap H. Pritchard, a microbial ecologist with EPA. Nor did the chemicals simply dissolve oil on the beaches and cause it to run off into the sound, as some researchers had feared, says Pritchard.

All this makes one of the originators of the technique—Ronald M. Atlas, a professor of biology at the University of Louisville, who first experimented with fertilizer formulations similar to Inipol in the late 1960s—ecstatic. Currently working as a consultant for Exxon on the cleanup, Atlas says "There were more dramatic surface results than anyone had predicted."

But while these results are encouraging, EPA officials are quick to point out that the method is not a magic, cheap solution nor a cure-all for oil spills. Every beach that was treated first had to be hosed down to disperse the oil across the surface of the beach before the fertilizer was applied. The technique also is not likely to be useful on rocky portions of the 1089 miles of Alaska shoreline contaminated by oil because the fertilizer solution will not cling to vertical surfaces. This could also limit the usefulness of the technique on steeply sloping beaches, EPA officials say. Furthermore, the level of biological activity declines with cold weather—by some 75% just as biologists expected. Quantifying the effects of biological degradation particularly in winter is difficult because of increased physical washing that results from wave action.

Nevertheless, the treatment strategy has worked well enough for Exxon to continue to experiment. This summer the company is expected to expand the use of fertilizers into additional parts of Prince William Sound. It also has spurred the American Petroleum Institute to step up research on bacterial scouring. Moreover, Atlas predicts that the results will trigger a wave of new research by oil companies, EPA, and universities to better understand how shoreline microorganisms break down oil. It could also stimulate interest in developing more advanced bioremediation methods for dealing with oil spills on land as well as along coastlines. ■ MARK CRAWFORD

Australia during the cold period and moved south over thousands of years as the climate warmed. Alternatively, they may have remained in the south through the frigid times.

Researchers cannot yet determine whether dinosaurs survived cold winters in Australia, Rich says. The newest isotope samples come from rock layers nearer the bone beds, so they should give a better picture of the dinosaurs' habitat than did previous isotope data. However, even the closest samples are not contemporaneous with the bone beds. "They are separated by 2 to 3 meters vertically," he says. "That could still be a couple of millennia" — R. Monastersky

Valdez 'bugs' chomp away

Last month, Environmental Protection Agency biologists initiated experiments on a soiled beach in Alaska's Prince William Sound to see whether treating its sandy and rocky shoreline with either of two types of fertilizer would enhance the natural detoxification of crude-oil residues (SN: 6/17/89, p.383). Preliminary data from those tests, released late last week, indicate that fertilizing indigenous aquatic bacteria indeed appears to accelerate the breakdown of oil spilled from the *Exxon Valdez* supertanker.

According to the EPA report, "natural biodegradation of the oil was already well underway... by the time the fertilizer was applied." This, it says, explains why the researchers found so many oil-degrading microbes at the start of their study. Just one week after beach fertilization began, however, the microbial communities had expanded measurably. And, as suspected, the fertilizers' formulations appeared to influence their efficacy. Sites treated with the water-soluble fertilizer contained 30 times more oil-degrading bacteria than did untreated beach plots. Sites sprayed with a fertilizer incorporating a vegetable oil to help it bind to the crude oil, however, housed 100 times more of the beneficial bacteria than nearby untreated zones.

Observation of the oil/fertilizer-treatment areas "clearly shows a striking disappearance of the [tanker] oil from rock surfaces," the report says. While EPA scientists haven't established that the bacteria ate the oil rather than simply loosening it to be washed back into the sound, such analyses are underway. Early data do indicate that the fertilizers have not collected in near-shore waters or overfed offshore algae, contributing to an oxygen-depleting algal bloom.

Noting the preliminary success of these bacterial-feeding regimes, EPA has expanded its Valdez microbial-detoxification program to include studies of nutrient movement within the beach and to monitor for adverse effects of the nutrient releases. □

Deadly RSV may fall to improved vaccines

In the alphabet soup of childhood vaccines, pediatricians hope the letters RSV will someday become as familiar as DPT. Diphtheria, pertussis and tetanus — once major causes of illness and death among children — have all but disappeared in the United States since development of the DPT vaccine. Not so with RSV.

RSV stands for respiratory syncytial virus, an influenza-like virus and the single most important cause of lower respiratory tract infection in infants and children. In the United States, RSV kills about 2,000 infants each year and hospitalizes an additional 55,000. Despite decades of attempts, major problems have stymied scientists' efforts to develop a vaccine against RSV, which spreads through close contact with infected children and adults and blooms in epidemic proportions each winter.

At this week's annual meeting of the American Society of Virology in London, Ontario, scientists provided some encouraging reports of RSV vaccine progress. Researchers estimate a commercially available vaccine remains three to five years away. But ongoing trials in animals and small numbers of humans now suggest they have overcome the major obstacles of previous years. An experimental vaccine in the 1960s enhanced the disease in some children, leading to some deaths and a strategy change among RSV vaccine researchers. Rather than working with inactivated whole viruses, scientists today use purified, antibody-provoking RSV proteins.

After years of tests in rodents and primates, scientists from Praxis Biologics in Rochester, N.Y., say they have immunized 40 adults and 23 toddlers 2 to 4 years old with a purified protein from the RSV outer jacket. They find high levels of protective antibodies, no disease enhancement and no notable adverse reactions, says Praxis researcher Thomas Kostyk. Pending Food and Drug Administration review of the data, Praxis hopes to begin tests in younger children.

Michael W. Wathen and his colleagues at the Upjohn Co. in Kalamazoo, Mich., report their creation of a genetically engineered vaccine made from a combination of two RSV proteins. They say tests in rats suggest their "chimeric protein" triggers a stronger immune response than does the single protein Praxis uses. Inoculation with the engineered protein protected rats from infection when they were challenged with a nasal spray of RSV. The company plans to expand to primate trials and expects to vaccinate humans within two years, says Upjohn virologist Roger J. Brideau.

— R. Weiss

Science & Society

Presented at the annual meeting of

Diabetic eye-disease risk

Diabetics face an increased risk of developing eye disease called retinopathy. The finding highlights the need for regular eye exams and prompt treatment of any problems.

Researchers at the University of Wisconsin-Madison studied 891 people with Type 1 diabetes. They found that systolic blood pressure was a "predictor" of whether these people developed retinopathy. The study found that systolic blood pressure readings — not mercury — at the start of the study predicted the development of retinopathy during the study. Systolic readings of 110 mm

or higher were associated with retinopathy. Very high blood pressure of the eye. Retinopathy occurs when the blood vessels in the retina, causing blurry vision, become blocked. In most diabetics, but in some, it leads to scar tissue, causing blindness.

Diabetics with Type 1 or insulin-dependent diabetes are at a higher risk of the disease.

Developing children

Diabetes has not been associated with cognitive impairment in childhood. But a three-year Canadian study hints that children with diabetes may have deficits in cognitive function.

Researchers at the Hospital for Sick Children in Toronto studied a three-year prospective study of 100 children with diabetes. At the start of the study, they gave the children a battery of tests measuring spatial and verbal abilities.

The children have completed the study. The results show that cognitive impairment was not found at the time of diagnosis. For the first time, researchers found that compared with non-diabetic children, children with diabetes performed poorly on tests measuring spatial and verbal abilities.

Microbes recruited in Valdez cleanup

Just after midnight on March 24, the *Exxon Valdez* supertanker ran aground in Alaska's Prince William Sound, spilling 10.1 million gallons of crude oil and fouling 368 miles of shoreline in that sound alone. Roughly 2,500 people have already enlisted in the manual cleanup of area beaches and wildlife. But the newest recruits in the cleanup are local communities of bacteria that specialize in detoxification.

Last week, Environmental Protection Agency scientists began seeding six oil-stained beach plots with nitrogen and phosphorus fertilizer in a 2-acre experiment at Alaska's Snug Harbor. Previous studies had detected air-breathing bacteria in Prince William Sound and nearby beach waters with the ability to break down slowly volatilizing alkanes (straight-chained compounds) and simple aromatic (ring-shaped) hydrocarbons. Together, these compounds represent about half the oil left on the beaches. Moreover, says EPA's Hap Pritchard in Valdez, Alaska, they account for most of that oil's toxicity.

The 90-day test will compare two nutrient formulations aimed at spurring the yet-unidentified bacteria's growth and alkane/aromatic degradation. One formulation incorporates oleic acid, best known as the primary fatty acid in olive oil. Researchers conducting the test think this "fat" will glue the mixed-in bacterial nutrients to any crude oil on which they're sprayed. The other formulation is an off-the-shelf fertilizer "brickette." The researchers are packing several brickettes into biodegradable plastic sacks and tying the sacks to pipes anchored in the beach. Over the course of a month, they expect wave and tidal action to flush the slowly dissolving fertilizer back and forth across the shoreline's rocks and sand.

The team will use its preliminary data, available by early July, to determine whether either formulation offers enough promise for widespread treatment of Alaska's beaches. Neither regimen, however, can restore affected beaches to their former, nearly pristine state. Because these bacteria ignore asphalt-like oil constituents, a tarry residue will remain.

NAS suspends collaboration with China

The U.S. National Academy of Sciences (NAS) is "shocked and dismayed by the action of Chinese government troops against peaceful demonstrators in Tiananmen Square and elsewhere in Beijing, with such great loss of life," NAS President Frank Press said in a telex to Chinese officials last week. "While we earnestly hope to maintain our cooperation with ... Chinese institutions," he added, "we must suspend all activities for the time being. We do so in outrage and sadness."

According to Robert B. Geyer, who heads NAS' China office in Washington, D.C., those most strongly affected by the NAS suspension are 25 U.S. nationals in China — a mix of doctoral

Science & Technology

ENVIRONMENT

THE TINIEST TOXIC AVENGERS

More cleanup companies are using bacteria that gobble up wastes

Of the 1,100 miles of Alaskan shoreline fouled last year by the Exxon Valdez, few places suffered more than Passage Cove on Prince William Sound's Knight Island. Within days, oil seeped nearly two feet into its gravel-and-sand beaches. Even hot-water sprays didn't help much. By mid-July, says Robert L. Mastracchio, Exxon Corp.'s technical manager in Alaska, the shore was still "black and gooey."

That's when scientists decided to incite the natives—microorganisms that live in soil and water. They sprayed the beach with fertilizer, hoping that adding nutrients would stimulate the naturally occurring bacteria to feed on the gunk. Three weeks later, the fertilized areas were nearly clean of oil for a foot down, while untreated areas wore a sticky coat. And researchers found that the population of voracious bugs in the fertilized soil had increased a hundredfold.

THIS WORKS. The Valdez cleanup, while far from complete, is the biggest success yet for a technique called bioremediation, which uses nature's tiniest creatures to clean up mankind's biggest messes. "We've proven that this works," says scientist John A. Glaser of the Environmental Protection Agency. On May

2, Alaskan officials gave Exxon approval to fertilize 35 more miles of spoiled shoreline this summer.

Now, more than three dozen cleanup companies are turning to organisms that scarf up everything from diesel oil to highly toxic polychlorinated biphenyls (PCBs) and heavy metals, which were once thought to be impervious to decontamination. But it turns out that "there are bacteria that will eat anything," says Richard C. Cassin, founder of San Diego startup Bioremediation, Inc.

This year, the market for bioremediation products and services is only about \$30 million, says Concord (N. H.) environmental consultant William T. Lorenz. But it may be ready to bust loose. Some companies are netting contracts of more than \$1 million, far higher than the \$250,000 or so that was common just two years ago. Venture capitalists are beginning to fund a few startups, and even traditional waste-treaters such as IT Corp. are using more bioremediation.

The trend comes just in time. The EPA estimates that conventional cleanups of some 1,200 U.S. Superfund sites, areas of extreme contamination, would cost \$24 billion. Some methods, such as incinerating contaminated soil, can run up to

\$1,000 per ton—and are under attack from both regulators and the public as potentially unsafe. Bioremediation, by contrast, typically costs less than \$100 a ton. It also offers a big advantage: Instead of simply relocating the problem, bacteria eliminate it. And bioremediation may be safer: It has been used in wastewater-treatment plants—and even out-houses—for half a century.

EAT AND DIE. To obtain the right cleanup bugs, scientists typically take soil or water samples from a toxic site—Cassin has even scraped oil deposits off his driveway—and grow the microorganisms they contain in a lab. Some of these bacteria feed on the carbon atoms that make up organic chemicals, usually by breaking the chemicals down to carbon dioxide and water. Researchers can then breed strains that depend on a contaminant to live and that will die off once the food source is gone. That way, there's little risk that they'll run amok.

The quickest way to treat contaminated soil is to excavate it, mix it up with water, nutrients, and bacteria on a plastic sheet, and pump air through it. In early 1989, Groundwater Technology Inc. in Norwood, Mass., used this method to clean up an oily mess at a Texas oil-storage facility in eight weeks. Contaminated groundwater is usually treated in a "bioreactor"—a tank containing specially selected bugs. Randall J. von Wedel, president of Cytoculture International Inc. in Point Richmond, Calif., hopes his company can shave months off a two-year project by pumping bacterial-laden water back into ground that has been contaminated with diesel fuel.

For all the recent success, bioremediation faces huge obstacles before it be-

BIOREMEDIATION: A BARGAIN FOR CLEANING UP SOIL

\$50-\$100 a ton
BIOREMEDIATION

\$50-\$100 a ton
CHEMICAL STABILIZATION

\$200 a ton
THERMAL DESORPTION

\$300-\$400 a ton
INCINERATION (ON-SITE)

\$1,000 a ton
INCINERATION (OFF-SITE)

DATA REMEDIATION TECHNOLOGIES INC.



VON WEDEL OF
CYTOCULTURE:
FAST WORK

comes the preferred pollution treatment. It usually takes longer for bacteria to work than for soil to be hauled away or incinerated, and bugs often stop munching before the contaminant is gone. One problem: They need nutrients and oxygen. "You can't just take a bag of bacteria and throw it on the ground," says Roger J. Colley, president of Envirogen Inc., a Lawrenceville (N.J.) startup.

Now, scientists are finding anaerobic bacteria, which can survive without oxygen. For instance, Woods Hole Oceanographic Institution in Massachusetts has discovered anaerobic bacteria living near warm water vents 6,000 feet deep in the Gulf of California that can degrade naphthalene, a stubborn hydrocarbon. And General Electric Co. has found both anaerobic and oxygen-dependent bugs that could help clean up 500,000 pounds of PCBs in a 40-mile stretch of the Hudson River in upstate New York.

HELPFUL FUNGUS. Another limitation for today's tiny toxic avengers: Bugs usually attack only one contaminant. So they may be useless in some of the worst waste sites, which contain many different toxins. One idea is to use genetically engineered organisms for these. Envirogen is exploring the insertion of several remediation genes into *Escherichia coli* bacteria, perhaps the most common around. But regulators must approve the use of any genetically engineered organisms. The Electric Power Research Institute, looking to clean up power-plant waste, hopes to avoid that by adjusting environmental factors, such as nutrients and oxygen, to get organisms to exchange genetic material naturally.

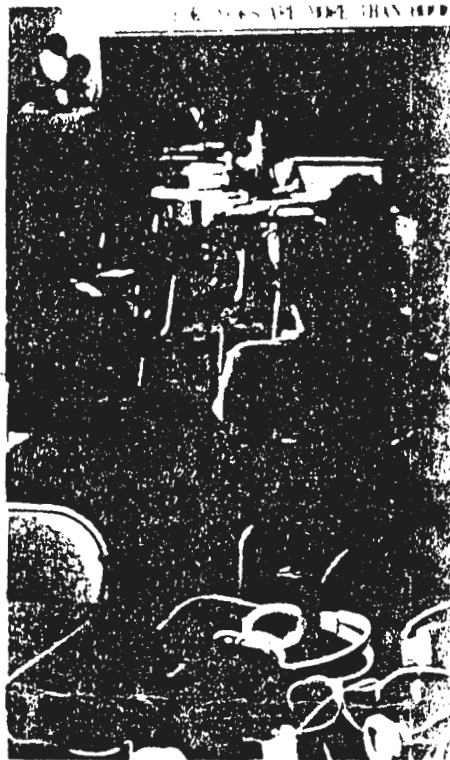
The search is also on for bioremediation bugs to tackle even tougher problems. Bacteria found at the Energy Dept.'s Hanford Reservation nuclear facility in Washington State keep radioactive materials such as cesium and uranium attached to rocks and soil—and out of groundwater. And researchers at the University of California at Riverside have found a fungus that detoxifies selenium, a metal that causes birth defects in migrating birds in California's Central Valley. Last month, a University of Illinois professor even described a bacteria-produced detergent that could be sprayed on beaches before a spill arrives, to prevent oil from sticking.

Some environmentalists still have reservations: In Alaska, they fear that fertilizers used to stimulate bugs may harm wildlife. They also worry that business may simply see bioremediation as a way to avoid more thorough cleanups. But GE scientist Daniel A. Ahramowicz disagrees: His studies show that natural bacteria are already slowly eating some PCBs in the Hudson river. The challenge now is to help them along.

By Robert D. Hof in San Francisco

Social Issues

EDUCATION



TEACHERS AT SAN ANTONIO'S EDGEWOOD HIGH

THE MONEY QUEST THAT HAVE SCIENCE

Is equal spending one cure for the

An "anything you can imagine" need," says James Vasquez, superintendent of the Edgewood independent school district in San Antonio. In this low-income, largely Hispanic district, teachers often dig into their own pockets to pay for basic supplies. Many classrooms are not air-conditioned, and temperatures can soar above 100°F in the Texas heat. High school students share a few 10-year-old computers.

Vasquez says such conditions reflect the best he can do on an annual budget of \$3,150 per student, which covers free lunches for 92% of Edgewood's students plus teachers, books, and maintenance. In contrast, upper-middle-class Clear Lake High near Houston has two libraries and science lab areas, three gymnasiums, a pool, and dozens of new computers. Clear Lake's district tax base will generate \$4,100 per student this year, and locals recently passed a \$22 million bond issue to build new school facilities.

Toxic wastes? A little fungus may help

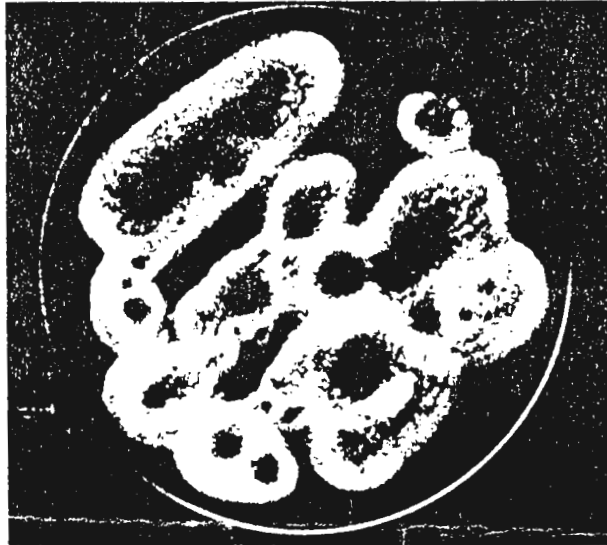
Most of the tranquility of the cattail marsh and white salt flats is jarrred by a single blast that sends a flock of birds scattering out of the reeds to the water's edge. The blasts come from a single gram, echoes of a machine gun. The effort to save water here is the 1,000-acre Kesterson National Wildlife Refuge, a former World War II California military base. The placid surface of the pond is being cleaned up, and the birds' only concern is the water. The water is contaminated with selenium and the state now has no other solution. Bulldozers will strip the top 6 inches of soil and dump it into a plastic-lined 17-acre landfill.

Meanwhile, two California biologists are racing against time to prove that a tiny organism can do a better job than a bulldozer, and at a fraction of the cost. They've found a naturally occurring microbe that can convert selenium into a harmless gas. If the project is successful, their work would mark the coming of age of a new biological cleanup. Nationwide, more than 1,000 toxic sites have been targeted for cleanup by the Environmental Protection Agency. The expensive, bulldozer approach ordered at Kesterson is only typical of the technology available to do the job. The system's shortfall

Kesterson's troubles are the result of a management that has been unannounced in farm-drainage watersheds. Usually, a system of underground drains and canals was to have carried water off when runoff from San Joaquin Valley farms into San Francisco Bay was prohibited, and the canal was to be used for a Kesterson's. Not only the salt, the selenium. Pushed out of the marshes and cattails soaked with selenium, birds ate the plants. Biologists started looking for selenium eaters. Chicks born with missing wings, swollen heads.

William Frankenberg and Karlson at the University of California at Riverside found that fungi present in soil have the ability to convert selenium into a gas that's dispersed into the air. The fungi do it for their own survival as they eat their way through the soil,

SCIENCE ■ Biologists are trying to prove that, in the cleanup of polluted soil, invisible microbes are better than bulldozers



Cheap labor: Microbes with an appetite for chemicals

they in effect spit out the selenium that would otherwise build up to a lethal dose. What Frankenberg and Karlson are trying to do is speed up the process. They studied habits of fungi with an eye to learning how to create a hospitable environment for them. "We're 'farming' for fungi—adding manure, rototilling or discing to aerate the soil, adding water," explains Frankenberg.

Biological cleanup offers some big advantages. Mainly, it's cheap. The state's bulldozing plan is estimated to cost at least \$25 million; the fungi work



Farming for fungi at a toxic-waste site

for free. And, notes Frankenberg, "scooping up soil and dumping it in a landfill is just like burying a problem under the rug. It's not a cure."

Selenium-eating microbes may play a part in the best solution to California's farm-drainage problem. It may avert another Kesterson plan, all the while the treatment of the microbes on the site will scrub out the selenium. Then would the water be dumped into agricultural ponds. The only water should be table salt.

A yen for pollutants

A common wood-rotting fungus may perform a similar job in treating wastewater from chemical plants. The enzymes the fungus uses to chew through lignin, the complex chemical that holds the wood fibers together, can also digest some hard-to-kill and otherwise resistant pollutants. At the University of California,

Gail G. Galic of Stanford University recently demonstrated that naturally occurring bacteria can be cultivated to clean up trichloroethylene, a widely used industrial solvent that has contaminated many ground-water supplies.

Frankenberg now is testing his fungus "farming" on 46 small plots at Kesterson. Although hard data will not be in until later this month, Frankenberg says that initial indications of that selenium gas is being given off from his test plots. The crucial question is whether the fungi can be stimulated to eat their way through the high concentrations of selenium in the Kesterson soil fast enough.

But even if the fungi do prove their credentials, they may not be the end of a carefully wrought compromise between environmentalists, the state and the federal government, the federal Bureau of Reclamation has already ordered to carry out the state order by August 1, 1988, and to be completed by August 1988. Moving tons of soil with a bulldozer is a dramatic effort, but the only thing's being done to prevent the site from being destroyed in the process. It may be some time for people to be convinced to the fact that the very diversity of microbes is their chief virtue.

by Stephen Budiansky, with Russell Clemings

account for the 400-km discontinuity. The seismic anomaly at 400 km is due mostly to the relatively fast transition from olivine to β -spinel (19) because transformations from pyroxene to garnet are comparatively slow and occur over much larger intervals at depth. The xenolith data do not at present permit evaluations of the degree of heterogeneity (centimeter to kilometer scale) in the mantle at these depths, but much heterogeneity is predicted if oceanic slabs pile up in the transition zone (20, 21). Kimberlites are thought to be associated with mantle plumes (21), perhaps originating in the lower mantle; therefore, xenoliths from, or with chemistries reflecting an origin deeper than, the transition zone should be anticipated.

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knowledge the comments by I. D. MacGregor and the anonymous reviewers. Supported under NSF grant EAR89-054046 (to S.E.H.) and the Centre National de la Recherche Scientifique for a grant from the INSU-DBT program, Theme 4: Fluids, minerals, and kinetics (to V.S.), contribution 252.

2 January 1991; accepted 5 March 1991

In Situ Biodegradation: Microbiological Patterns in a Contaminated Aquifer

EUGENE L. MADSEN, JAMES L. SINCLAIR,* WILLIAM C. GHIORSE

Conventional approaches for proving in situ biodegradation of organic pollutants in aquifers have severe limitations. In the approach described here, patterns in a comprehensive set of microbiological activity and distribution data were analyzed. Measurements were performed on sediment samples gathered at consistent depths in aquifer boreholes spanning a gradient of contaminant concentrations at a buried coal tar site. Microbial adaptation to polyaromatic hydrocarbons (PAHs) was demonstrated by mineralization of naphthalene and phenanthrene in samples from PAH-contaminated, but not adjacent pristine, zones. Furthermore, contaminant-stimulated in situ bacterial growth was indicated because enhanced numbers of protozoa and their bacterial prey were found exclusively in contaminated subsurface samples. The data suggest that many convergent lines of logically linked indirect evidence can effectively document in situ biodegradation of aquifer contaminants.

THE RELEASE OF ORGANIC CHEMICALS to waters and soils can have dire consequences for wildlife, ecosystem integrity, and water quality (1). Alleviation of environmental pollution by stimulating native microbiological populations to effect biodegradation processes is promising (2), but such "bioremediation technologies" are far from proven. Although indigenous microorganisms in samples from many natural settings have been shown to have the potential to effect pollutant elimination (3, 4), the extent to which biodegradation potential is expressed in situ usually is a matter for speculation. Proof of in situ biodegradation must show that the mass of pollutant compounds has decreased and that microorganisms are the causative agents. These two pieces of information are exceedingly difficult to obtain in a field setting because mass balances may be precluded by the open complexity of the site and because other abiotic attenuating processes (dilution, migration, volatilization, sorption) may occur simultaneously with biodegradation (5). In situ biodegradation has been documented successfully in field studies of ponds and soil plots (6), in which specific responses of microorganisms were distinguished from abiotic responses. In contrast, such studies

have not been possible in ground-water aquifers because their inaccessibility and variability prevent implementation of replicated, statistically valid experimental designs. Thus, studies that directly and unequivocally demonstrate in situ biodegradation in aquifers are rare (7).

The majority of attempts to document in situ biodegradation in aquifers have used indirect observations. Typically an imperfect mass balance, based on computer modeling, is cited to show loss of a pollutant in water pumped from the aquifer (8); but this approach does not distinguish unambiguously between biotic and abiotic processes. Chemical data from ground-water or sediment samples also may suggest that putative biodegradation activity is accompanied by changes in reactants (for example, oxygen and nutrients) and products (for example, CO₂ or intermediary metabolites) which are indicative of known microbiological processes (2, 9). The case is strengthened if high numbers of microorganisms, especially of pollutant-degrading bacteria are found (2, 10). Further support may be garnered from laboratory biotransformation assays indicating that the pollutant is chemically modified or converted completely to CO₂ in samples from the site (2, 4, 11). However, no established combination of these measures is robust enough to constitute absolute proof of in situ biodegradation in aquifer sediments. Methodological improvements are needed. We used patterns of microbiological activity

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14. This orientation would match the tetrahedral chains of Si in pyroxenes with the octahedral sites of garnet that contain both Si and Al under very high pressures (>80 kbar).
15. In a displacive transformation, the product phase results generally from shearing of the preexisting mineral without bond breaking as observed in reconstructive transformation. Displacive transformations are fast under a suitable driving stress and are almost independent of temperature (diffusionless).
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tribution to indicate in situ biodegradation in a shallow aquifer contaminated by buried coal tar.

We measured the potential of subsurface microorganisms to degrade organic compounds in aquifer sediment core samples obtained from a buried coal tar site (12) by aseptic subcoring procedures. Evolution of $^{14}\text{CO}_2$ from the ^{14}C -labeled PAHs (naphthalene and phenanthrene) and *p*-hydroxybenzoate (PHB) (14) was measured (15). The abundance and activity of bacteria (including actinobacteria), fungi, and protozoa were assessed using established methods (16, 17). Core samples were obtained from the unsaturated, water table, shallow saturated, and deep saturated subsurface zones in each borehole (Fig. 1). On the basis of previous site characterization data (12), borehole locations were selected for collecting sediments representing a range of contaminant conditions both horizontally and vertically. Naphthalene and phenanthrene were detected in samples taken from boreholes drilled through the plume (Fig. 1 and Table 1), but not in samples from a pristine borehole outside the plume.

Mineralization of PHB was evident in samples from all three boreholes, but the samples from the upgradient borehole near the buried coal tar were most active (Fig. 2, A to C). In the upgradient borehole samples, no detectable lag period (18) preceded the onset of biodegradation activity (Fig. 2A). Downgradient in the plume PHB mineralization also was detected in all samples; microbial metabolism was most rapid and extensive in the sample from the water table zone (Fig. 2B). A slight lag was noted in the deepest sample from the unsaturated zone. Three of the four sam-

Table 1. Concentrations of PAHs. No naphthalene or phenanthrene detected in the pristine borehole; BD, below detection.

Zone	Plume			
	Upgradient		Downgradient	
	Naphthalene (mg kg ⁻¹)	Phenanthrene (mg kg ⁻¹)	Naphthalene (mg kg ⁻¹)	Phenanthrene (mg kg ⁻¹)
Unsaturated	0.06	1.6	BD	BD
Water table	BD	0.86	BD	BD
Shallow saturated	2.3	0.33	0.24	0.35
Deep saturated	0.06	BD	0.05	BD

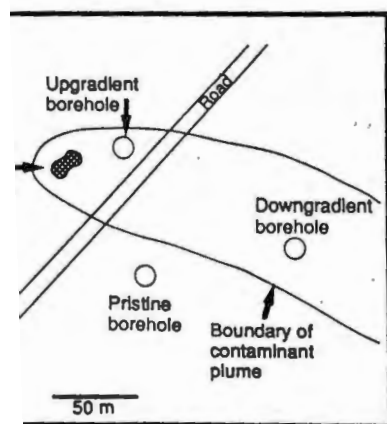
ples from the pristine borehole showed appreciable mineralization of PHB, albeit with lag periods prior to $^{14}\text{CO}_2$ evolution (Fig. 2C). The water table sample was most active. No mineralization was observed in the deepest saturated zone sample from the pristine borehole.

Mineralization of naphthalene and phenanthrene was detected only in sediments taken from inside the contaminant plume (Fig. 2, D to G). All sediment samples from the pristine borehole failed to mineralize these PAHs during the 3-week incubation period. In samples from upgradient in the plume, naphthalene and phenanthrene were mineralized in all cases. The water table and deep saturated zones were most active (Fig. 2, D and F). Naphthalene was mineralized in three of the four samples taken from the downgradient borehole; again the water table zone sample was most active (Fig. 2E). Phenanthrene was mineralized only in the water table sample

from the downgradient borehole (Fig. 2G).

There was often an inverse relation between the PAH concentration in sediments and PAH biodegradation activity for a given sample. For instance, neither of the PAHs were detected in the water table zone of the downgradient borehole (Table 1), yet PAH mineralization activity was high in these samples (Fig. 2, E and G). Furthermore, elevated levels of PAHs were detected in all shallow saturated zone samples (Table 1) where PAH mineralization activities were low (Fig. 2, D to G). These findings might be explained by small-scale sample heterogeneity or other sampling problems. However, it is also possible that the absence of detectable PAHs reflected zones of rapid in situ biodegradation (4) and that the presence of measurable PAHs reflected zones where rates of contaminant influx exceeded rates of microbiological mineralization.

Sediment samples were examined for the numbers and types of microorganisms pres-



Plan view of field site showing boundary of contaminant plume and borehole locations. The coal tar was buried 30 years ago (12). Groundwater has distributed coal tar components, including naphthalene and phenanthrene, in sedimentary strata at depths between 1 and 3 m.

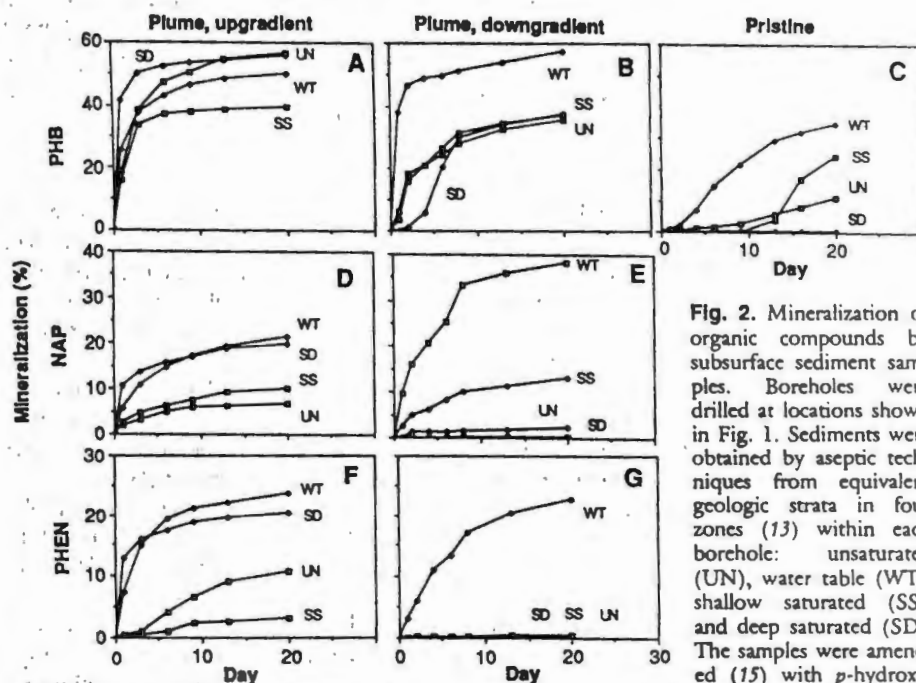
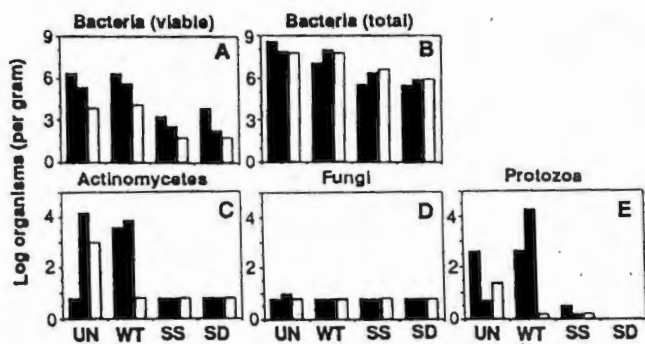


Fig. 2. Mineralization of organic compounds by subsurface sediment samples. Boreholes were drilled at locations shown in Fig. 1. Sediments were obtained by aseptic techniques from equivalent geologic strata in four zones (13) within each borehole: unsaturated (UN), water table (WT), shallow saturated (SS), and deep saturated (SD). The samples were amended (15) with *p*-hydroxybenzoate (PHB), naphthalene (NAP), and phenanthrene (PHEN). Plots (A to G) are time courses of cumulative $^{14}\text{CO}_2$ trapped in triplicate flasks. No $^{14}\text{CO}_2$ was evolved in control flasks containing autoclaved, HgCl_2 -poisoned sediments.

Fig. 3. Comparison of microbiological abundances in sediments at four depths within three boreholes. The four depths examined were the unsaturated (UN), water table (WT), shallow saturated (SS), and deep saturated (SD) zones. Each cluster of three bars represents numbers of microorganisms from samples inside the plume, upgradient (left); inside the plume, downgradient (center); and the pristine area (right). (A) Viable bacteria, (C) actinomycetes, and (D) fungi were determined by the plate-count method (16). (B) Total bacteria were determined by epifluorescence microscopy (16). (E) Protozoa were enumerated using *Enterobacter aerogenes* as prey bacteria (17).



nt (Fig. 3). Viable counts of aerobic hetero-
bacteria showed a consistent pattern;
they were highest upgradient, inside the
plume, closest to the source of contamination
and lowest in the pristine borehole (Fig. 3A).
Microscopic counts for total numbers of bac-
teria were 100- to 1000-fold higher than the
viable counts (19) and showed the same
declining trend with depth, but only small
differences were observed between pristine
and contaminated samples (Fig. 3B). Actino-
mycetes were found in significant numbers
 $\geq 10^3$ per gram of sediment; Fig. 3C) in
two-thirds of the unsaturated and water table
zone samples. Low numbers of actinomycetes
were detected in saturated zone samples. Low
numbers of fungi also were present in subsur-
face sediments; only small differences were
found between samples regardless of depth or
borehole location (Fig. 3D). Similar low
numbers of fungi and actinomycetes have
been found in other shallow and deep subsur-
face sites (16, 17). In contrast to fungi,
protozoa (amoebae and flagellates) showed a
striking range in abundance (Fig. 3E). High
protozoan numbers were found in several
samples from unsaturated and water table
zones within the contaminant plume. The
upgradient plume borehole contained more
than 400 protozoa per gram in both the
unsaturated and water table zone samples.
This is a relatively high population density for
subsurface protozoa (17). In the downgradi-
ent borehole, the water table zone sample
contained more than 19,000 protozoa per
gram, a number far above those normally
encountered in shallow aquifer sediments
(17), but comparable to those found in acti-
vated sewage sludge facilities (20). The water
table zone sample that supported a high
density of protozoa was highly active in min-
eralizing the three compounds examined
(Fig. 2, B, E, and G): Another recent study
is also reported the occurrence of large
numbers of protozoa in subsurface sediments
near jet fuel vapors commingled with atmo-
spheric oxygen (21). Typically low numbers

of protozoa (<50 per gram) were present in
all samples from the pristine borehole and in
saturated sediments from just below the wa-
ter table within the plume of contamination.
Sediments from deeper in the saturated zone
were not examined for protozoa.

The mineralization activity and microbial
distribution patterns observed in this study are
likely to be controlled by spatial heterogeneity
of sediment properties such as texture and
hydraulic conductivity (22), as well as by the
presence or absence of carbon and energy
sources provided by coal tar components in the
ground water. Even though sedimentary char-
acteristics of this study site were relatively uni-
form (12), it is impossible to be certain that the
sediment samples obtained from four depths of
each borehole were derived from hydrogeologi-
cally equivalent strata. In an attempt to sepa-
rate the influence of aquifer sediment hetero-
geneity from that of PAH contamination, we
obtained vertically and horizontally separated
samples from zones of high and low PAH
concentration and sought patterns in the mi-
crobiological data. The significant patterns
were as follows: (i) PHB mineralizing micro-
organisms were present throughout the site;
however PAH mineralization activity was re-
stricted to samples from within the plume of
contamination; (ii) samples from all depths in
the upgradient borehole mineralized both
PAHs, whereas several samples from the
downgradient borehole were inactive; (iii) lag
periods prior to mineralization were observed
only in downgradient and pristine samples; (iv)
viable bacteria were detected in greatest abun-
dance in samples from the upgradient plume
borehole, whereas the lowest abundance was
found in samples from the pristine borehole;
and (v) elevated numbers of protozoa were
found in unsaturated and water table zone
sediment samples from within the contaminant
plume which contained active populations of
PAH-degrading microorganisms. The obvious
conclusion from these results is that microbial
distribution and adaptational biodegradation
activity (23) in this polluted aquifer system

were governed by proximity of the source of
PAH contamination.

Protozoa are important predators in
aquatic and terrestrial environments (20),
but only recently has it been established that
protozoa also are widely distributed in sub-
surface sediments (17). The concept of using
protozoan abundance as an index of pollu-
tion dates from the early part of this century
(24). In fact, associations between protozo-
an abundance and high levels of organic
carbon in soil or municipal waste water are
well established (25). However, studies ex-
amining interactions between organic con-
taminants and protozoa are rare (21). Data
derived from coastal field samples and labo-
ratory-incubated soil cores indicated that
crude oil was inhibitory to protozoa (26,
27). In contrast, another laboratory study
found that a ciliate protozoan enhanced
microbial degradation of crude oil (28).
Until now field evidence for the bio-
geochemical function of subsurface proto-
zoa, which usually are found at low popula-
tion density, has not been obtained. The
population density of protozoa usually re-
flects the rate at which they are able to graze
on their bacterial prey (20). A high proto-
zoan grazing rate is indicated by a high
population density. This in turn reflects a
high bacterial growth rate rather than in-
creased bacterial biomass. The dependence
on bacterial growth rate has been shown in
sewage treatment plants where high num-
bers of protozoa are able to reduce viable
counts of bacteria while simultaneously ac-
celerating carbon cycling and increasing
their own biomass (20). In this study, ele-
vated numbers of protozoa occurred exclu-
sively in sediment samples from upper zones
of the subsurface profile where contami-
nants and oxygen would be expected to mix.
The high protozoan numbers are indicative
of rapidly growing populations of bacteria
in situ. To the extent that prey are growing
on contaminant compounds, the elevated
protozoan biomass reflects in situ biodegra-
dation activity. Thus, we have compelling
indirect evidence for in situ biodegradation
of organic contaminants in aquifer sedi-
ments: (i) protozoan biomass indicates in
situ growth of prey bacteria and (ii) adapta-
tional biodegradation activity indicates that
the prey bacteria are growing in response to
contaminant compounds.

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Coal tar is a black material produced from coal gasification processes [J. F. Villaume, in *Hazardous and Toxic Wastes: Technology Management and Health Effects*, S. K. Majumdar and E. W. Miller, Eds. (Pennsylvania Academy of Science, Easton, PA, 1984), pp. 362-375]. A single truckload of coal tar was buried in a forested area in the northeastern United States. This site has been characterized under EPRI research projects 2879-1 and 2879-12 [B. B. Taylor *et al.*, in *Proceedings: Environmental Research Conference on Groundwater Quality and Waste Disposal* (Electric Power Research Institute, EN-6749, Palo Alto, CA), pp. 26-1-26-12]. Sediments were 99% sand. Subsamples of materials used in biodegradation assays were analyzed for PAHs by gas chromatography (GC) and GC/mass spectrometry by Cambridge Analytical Associates, Inc., Boston, MA, under EPRI contract 2879-1.

Septic coring procedures followed established principles (2, 4, 16, 17). Depths selected for analyses were 1 to 2 m (unsaturated zone), 2.3 to 3 m (water table interface), 4 to 5, and 5 to 6 m (shallow and deep saturated zones). Additional details appear in EPRI Final Report RP 2879-5.

HB is an intermediary metabolite in lignin biodegradation [J. P. Martin and K. Haider, in *Lignin Biodegradation: Microbiology, Chemistry, and Potential Applications*, T. K. Kirk, T. Higuchi, H. Chang, Eds. (CRC Press, Boca Raton, FL, 1980), vol. 1, pp. 1-100] that is metabolized by many aerobic soil bacteria.

Conversion of organic compounds to inorganic compounds (mineralization) was measured by standard ¹⁴C₂ trapping methods. Radiolabeled [¹⁴C]naphthalene (80 mCi/mmol, >98% radio-purity), [¹⁴C]phenanthrene (10.4 mCi/mmol, >99% radio-purity), and *p*-hydroxybenzoate (ring I, 7.7 mCi/mmol, >99% radio-purity) were purchased from Sigma Radiochemicals (St. Louis, MO). Compounds were dissolved, filter-sterilized aseptically in acetone before being diluted 100-fold in distilled water, *p*-hydroxybenzoate in distilled water) and then added to sterile 5-ml flasks containing 4 g of aseptically distributed sediment sample. Each flask received 0.04 μCi of [¹⁴C]-labeled and unlabeled naphthalene, phenanthrene, or *p*-hydroxybenzoate at concentrations of 1 μM. An abiotic control flask was prepared in each set by autoclaving the sample for 1 hour and

adding 1 ml of 1 M HgCl₂ before addition of carbon compounds. The flasks were sealed with lids suspending a small plastic cup (containing 0.6 ml of CO₂ trapping agent) and incubated statically at 23°C. At each sampling time, trapping agent was withdrawn, counted in a scintillation counter, then replenished.

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Control of doublesex Alternative Splicing by transformer and transformer-2 in Drosophila

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Sex-specific alternative processing of *doublesex* (*dsx*) precursor messenger RNA (pre-mRNA) regulates somatic sexual differentiation in *Drosophila melanogaster*. Cotransfection analyses in which the *dsx* gene and the female-specific *transformer* (*tra*) and *transformer-2* (*tra-2*) complementary DNAs were expressed in *Drosophila* Kc cells revealed that female-specific splicing of the *dsx* transcript was positively regulated by the products of the *tra* and *tra-2* genes. Furthermore, analyses of mutant constructs of *dsx* showed that a portion of the female-specific exon sequence was required for regulation of *dsx* pre-messenger RNA splicing.

SOMATIC SEXUAL DIFFERENTIATION IN *Drosophila melanogaster* is accomplished by a hierarchy of regulatory genes that act in response to the number of X chromosomes relative to the number of sets of autosomes in a cell (the X:A ratio) (1). One of these regulatory genes, *dsx*, is required for terminal sexual differentiation in both male and female flies (2). Molecular analyses have shown that the *dsx* transcript undergoes sex-specific RNA processing (splicing and cleavage-polyadenylation reactions), which leads to the production of two distinct sex-specific polypeptides (Fig. 1A) (3). The male- and female-specific *dsx* products regulate sexual differentiation by repressing the female- and male-specific terminal differentiation functions, respectively

(2). Genetic analyses have shown that the *tra* and *tra-2* genes are required for regulation of sex-specific *dsx* expression (4). In males, *tra* produces a nonfunctional product, whereas the female-specific *tra* product is functional and is produced by alternative splicing of *tra* pre-mRNA (5, 6). The *tra-2* product is also required for proper differentiation of male germ line cells (7). The predicted polypeptide encoded by *tra-2* (8) contains a domain of 90 amino acids that is also found in RNA binding proteins (9). In addition, the predicted protein sequences encoded by *tra-2* (8) and *tra* (10) contain arginine- and serine-rich regions that are characteristic of proteins that participate in RNA processing (9). Although these findings suggest that the products of *tra* and *tra-2* function in the regulation of alternative processing of *dsx* pre-mRNA, direct evidence has been lacking.

To decipher the mechanism of alternative processing of *dsx*, we constructed a plasmid

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Aerobic Biodegradation Potential of Subsurface Microorganisms from a Jet Fuel-Contaminated Aquifer

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In 1975, a leak of 83,000 gallons (314,189 liters) of jet fuel (JP-4) contaminated a shallow water-table aquifer near North Charleston, S.C. Laboratory experiments were conducted with contaminated sediments to assess the aerobic biodegradation potential of the in situ microbial community. Sediments were incubated with ¹⁴C-labeled organic compounds, and the evolution of ¹⁴CO₂ was measured over time. Gas chromatographic analyses were used to monitor CO₂ production and O₂ consumption under aerobic conditions. Results indicated that the microbes from contaminated sediments remained active despite the potentially toxic effects of JP-4. ¹⁴CO₂ was measured from [¹⁴C]glucose respiration in unamended and nitrate-amended samples after 1 day of incubation. Total [¹⁴C]glucose metabolism was greater in 1 mM nitrate-amended than in unamended samples because of increased cellular incorporation of ¹⁴C label. [¹⁴C]benzene and [¹⁴C]toluene were not significantly respired after 3 months of incubation. With the addition of 1 mM NO₃⁻, CO₂ production measured by gas chromatographic analysis increased linearly during 2 months of incubation at a rate of 0.099 μmol g⁻¹ (dry weight) day⁻¹ while oxygen concentration decreased at a rate of 0.124 μmol g⁻¹ (dry weight) day⁻¹. With no added nitrate, CO₂ production was not different from that in metabolically inhibited control vials. From the examination of selected components of JP-4, the *n*-alkane hexane appeared to be degraded as opposed to the branched alkanes of similar molecular weight. The results suggest that the in situ microbial community is active despite the JP-4 jet fuel contamination and that biodegradation may be compound specific. Also, the community is strongly nitrogen limited, and nitrogen additions may be required to significantly enhance hydrocarbon biodegradation.

Current efforts to remediate subsurface contamination have spurred research in the application of in situ bioremediation. Depending on specific hydrogeological, microbiological, and chemical constraints, in situ biodegradation of organic contaminants has been suggested as a cost-effective and environmentally sound remediation alternative to pump-and-treat and vacuum-extraction technologies. Before an in situ bioremediation project can be implemented, a feasibility study is required to assess the extent and type of contamination, the hydrogeology of the site, and the activity of the microbial community and its capability to degrade the contaminants of concern.

Several laboratory studies have examined the capability of microorganisms to degrade organic solvents (4, 9), pesticides (16), and petroleum hydrocarbons (22). Petroleum hydrocarbons are well suited to biological treatment, and in situ bioremediation has been attempted most frequently on this type of contamination (12). Both aerobic (1, 5, 20) and anaerobic (7, 10, 14) biodegradation have been shown to reduce the concentration of several components of petroleum hydrocarbons. This is particularly encouraging in light of the potential for widespread petroleum contamination of subsurface material from leaking underground and above-ground storage tanks at sites across the United States.

The present investigation was undertaken to examine the microbial community of a shallow water-table aquifer near North Charleston, S.C., which was contaminated in 1975 when the aboveground storage tank no. 1 leaked 83,000 gallons (1 gallon = 3.785 liters) of JP-4 jet fuel (Fig. 1). Approximately 21,000 gallons of the fuel was recovered by

1976, but 75% remained in the subsurface environment, both sorbed onto sediments and dissolved in water. Dissolved concentrations of benzene, toluene, ethylbenzene, and total xylene as high as 3 mg liter⁻¹ and sorbed concentrations of total petroleum hydrocarbons of 4,000 mg per kg of dry sediment (15) have been measured at the site. Experiments were carried out to examine whether the microbial community was active despite the substantial contamination present or alternatively if the JP-4 in the heart of the plume was toxic to the microorganisms. Also, the biodegradative potential of the in situ microbial community to degrade the lower-molecular-weight components (C₄ to C₇) of the JP-4 and the influence of nitrogen additions on microbial activity and metabolism were assessed.

MATERIALS AND METHODS

Subsurface samples. The aquifer material used in this study was collected aseptically from the contaminated aquifer in North Charleston, S.C., in March 1989 and stored at 4°C until incubations were begun. The shallow water-table aquifer at the site consists of sediments of medium-grained sands with interfingering lenses of clay to a depth of approximately 20 to 35 ft (1 ft = 30.48 cm). Underlying these sediments is a formation consisting of predominantly clay material. The depth to the water table varies seasonally but is approximately 5 to 14 ft below land surface. Experiments were carried out by using sediment from the saturated zone, collected at a depth of 12 to 20 ft.

Compounds used. [U-¹⁴C]glucose, [U-¹⁴C]benzene, sodium [¹⁴C]bicarbonate (Amersham Corp., Arlington Heights, Ill.), and [ring-U-¹⁴C]toluene (Sigma Chemical Co., St. Louis, Mo.) with specific activities of 270, 121, 55, and 56.3

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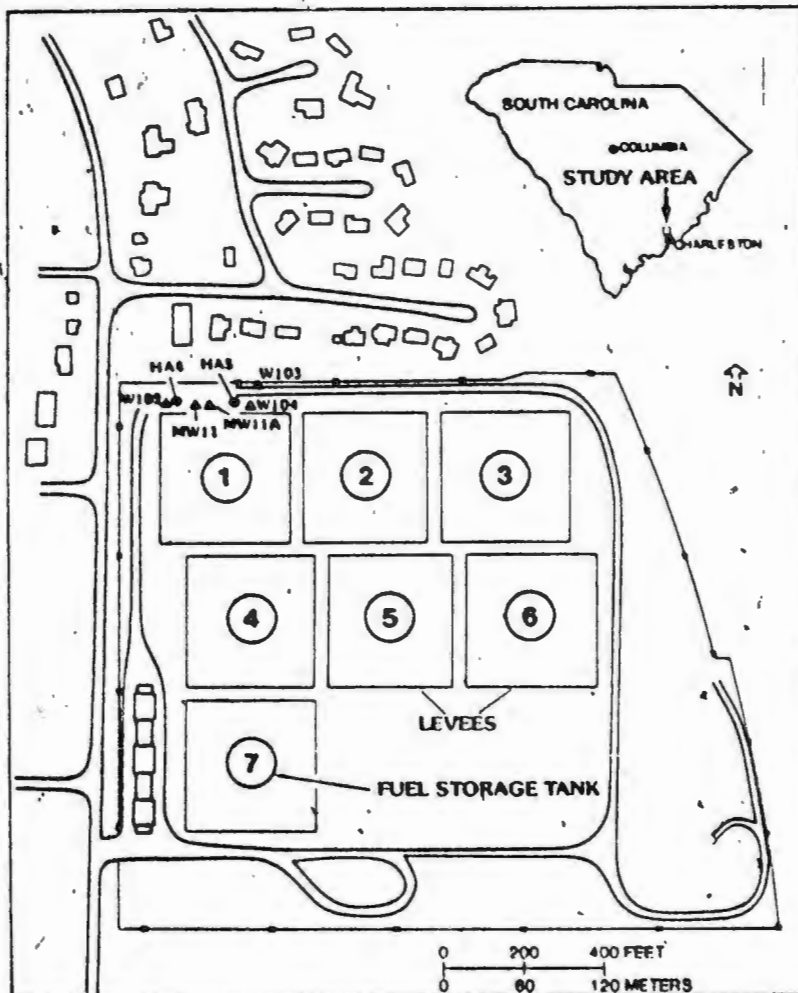


FIG. 1. Site map showing approximate location of study area and locations of monitoring well (\blacktriangle) and sediment sample (\bullet) sites.

mCi/mmol, respectively, were used in this study. Inorganic nutrients included $\text{Ca}(\text{NO}_3)_2$, NaNO_3 , and $\text{Ca}(\text{H}_2\text{PO}_4)_2$. Sodium azide (NaN_3) was used as a metabolic inhibitor of aerobic respiration (Sigma).

Fate of ^{14}C -radiolabeled isotopes. The mass balance and respiration of organic substrates were measured by using a modification of the procedure described by Dobbins and Pfander (6). For all incubations, a sample of 3 g (dry weight) of aquifer material from the boring designated HA4, from a depth of approximately 12 to 20 ft (Fig. 1), was weighed into a 20-ml glass vial (Pierce Chemical Co., Rockville, Ill.), and approximately 10 ml of sterilized, distilled water was added. Radiolabeled substrate and inorganic nutrient amendments were then added to all of the samples, and the remaining volume was filled with sterile, distilled water leaving no headspace. For [^{14}C]glucose, the vials were sealed with Teflon-lined septa and capped. Samples were inverted and incubated in the dark at room temperature. For the more volatile compounds, [^{14}C]toluene and [^{14}C]benzene, 10-ml serum vials were used and sealed with rubber butyl stoppers and aluminum crimp caps. Metabolically inhibited control vials were treated similarly to experimental vials but were amended with NaN_3 to a final concentration of 0.5%.

After incubation, the samples were transferred to 40-ml

vials by using Teflon connector caps (Wheaton Scientific Co., Millville, N.J.). The samples were acidified with H_3PO_4 to a pH of 2 and shaken overnight on a rotary shaker, and the $^{14}\text{CO}_2$ was collected in a KOH base trap in the vial headspace. Respiration values were corrected for abiotic contributions by subtracting values for the metabolically inhibited control vials. The efficiency of the $^{14}\text{CO}_2$ recovery method was measured by means of $\text{NaH}^{14}\text{CO}_3$ control vials, processed in a manner similar to that used for the sample vials but with $\text{NaH}^{14}\text{CO}_3$ added instead of the ^{14}C -labeled organic compound. After correcting for abiotic processes and $^{14}\text{CO}_2$ recovery, the percentage of the substrate mineralized was calculated.

After the $^{14}\text{CO}_2$ recovery was completed, a mass balance determination was carried out on the remaining sediment and filtrate. For this procedure, the amounts of ^{14}C measured as $^{14}\text{CO}_2$, associated with cellular biomass, associated with sediment, and present as a soluble fraction in the filtrate were determined (6). Cells were removed from sediment particles by using two washings, shaking, centrifuging, and filtering procedures. The first wash used a mixture of sodium pyrophosphate and polyvinylpyrrolidone (final concentrations, 0.1 and 1.0%, respectively), and the second used a solution of hydrogen peroxide (final concentration, 0.1%).

TABLE 1. Compounds identified in vial headspace by coelution with gas chromatographic standards

Compound	Retention time (min)
n-Pentane	4.93
2,2- and 2,3-Dimethylbutane	6.75
3-Methylpentane	10.70
2-Methylpentane	11.66
n-Hexane	17.85
2,4-Dimethylpentane	23.50
Methylcyclohexane	20.78
2,3-Dimethylpentane	24.39

Gas chromatographic analyses. CO₂ production, O₂ consumption, and hydrocarbon disappearance were monitored by using gas chromatography. For CO₂ and O₂ concentrations monitored over time, 125-ml serum vials were filled with 50 g of sediment collected from a depth of approximately 15 to 20 ft from the boring designated HA5 (Fig. 1). Two replicates were used for each condition. A 2-ml volume of autoclaved liquid was added to each vial, either as distilled water in unamended samples or as inorganic nutrient solution in nitrate-amended samples. Sodium azide was used to create duplicate metabolically inhibited control samples. Approximately 2 ml of gas in the vial headspace was withdrawn through a Teflon Mininert valve (Supelco, Bellefonte, Pa.) and injected into the gas chromatograph via a fixed-volume sample loop. The volume of gas that was withdrawn was replaced with atmospheric air, and this dilution effect was accounted for in calculations of constituent concentration.

Several compounds were identified in the gas in the vial headspace by coelution with chromatographic standards (Table 1). The range of compounds included primarily C₄ to C₆ branched and normal aliphatic compounds. The disappearance of these compounds was monitored in 125-ml serum vials containing 100 g of sediment under the following conditions: sediment metabolically inhibited with NaN₃, sediment with added nitrate (28 mM), and sediment with added nitrate (28 mM) and phosphate (4 mM) and in a JP-4 standard control consisting of 10 μ l of JP-4 jet fuel in 50 ml autoclaved, distilled water. The samples were processed as described above except that the headspace that was removed was not replaced. Reductions of individual components of JP-4 were calculated as a percentage of the ratio (C_t/C₀) of concentration at time *t* (C_t) to initial concentration (C₀). Carbon dioxide and oxygen also were measured after approximately 100 days of incubation.

All O₂ and CO₂ analyses were carried out with a Carle AGC-111 gas chromatograph (Hach Co., Anaheim, Calif.) equipped with a thermal conductivity detector and a stainless-steel (Hayesep A, 50/70) analytical column (1/8 in. [cn. 0.32 cm] by 8 ft). Helium (22 cm³ min⁻¹) was the carrier gas, and the column temperature was isothermal at 80°C. Hydrocarbon analyses were carried out with a Carle AGC-211 gas chromatograph equipped with a flame ionization detector and a glass column (1/8 in. by 6 ft) (0.1% SP-1000 on 80/100 Carbowack C). Helium (42 cm³ min⁻¹) was the carrier gas, hydrogen (25 cm³ min⁻¹) was the detector gas, and compressed air (500 cm³ min⁻¹) was the fuel gas. Column temperature was isothermal at 100°C. A digital integrator (Hewlett-Packard model 3390A) was used to quantify peak areas. Quantitative standards for hydrocarbons were purchased from Supelco, and those for CO₂, O₂, and CH₄ were

TABLE 2. Total petroleum hydrocarbon (TPH) concentrations in selected sediment samples from March 1989 (15)

Sample no.	Sampling depth (ft)	TPH concn (mg kg ⁻¹ [dry wt])
HA4-3	9	13
HA4-4	13	15
HA4-5	16	14
HA4-6	22	18
HA4-7	27	19
HA5-3	10	52
HA5-4	14	252
HA5-5	17	652
HA5-6	21	38
HA5-7	26	49

purchased from Scotty Specialty Gases (Plumsteadville, Pa.).

RESULTS

Groundwater sampled in the area of the spill (Fig. 1) contained concentrations ranging from 0.009 (well MW11) to 0.56 (well W102) mg of benzene per liter, 0.003 (well W103) to 0.51 (well W104) mg of toluene per liter, 0.003 to 0.27 (well MW11) mg of ethylbenzene per liter, and 0.008 (well W103) to 1.3 (well W102) mg of total xylene per liter (18). Values for total organic carbon ranged from 7 to 52 mg per liter, specific conductance ranged from 105 to 170 mg per liter, temperature ranged from 19 to 25°C, and biological oxygen demand measured after 5 days ranged from 4 to 10 mg per liter. Water from all wells was acidic, with pH values ranging from 4.8 to 6.0. Dissolved oxygen was present at 2.9 mg per liter in water from the shallow well (MW11) screened at 3 to 18 ft, but it was not detected in the adjacent well (MW11A) screened at 27 to 32 ft. Inorganic nutrients were measured in groundwater at concentrations of 3.4 mg of ammonia-N per liter, 0.042 mg of nitrate-N per liter, and <1.0 mg of orthophosphate per liter.

The total petroleum hydrocarbon concentrations in sediments from borings HA4 and HA5 used for these experiments ranged from 13 to 652 mg kg⁻¹ (Table 2). Sediment from other areas within the contaminant plume contained total petroleum hydrocarbon concentrations ranging from 11 to 4,487 mg kg⁻¹ (dry weight), with an average concentration of 79 mg kg⁻¹. Concentrations of alkylbenzenes in groundwater from these areas were as high as 3 mg of benzene per liter, 5 mg of toluene per liter, 1 mg of ethylbenzene per liter, and 3 mg of xylene per liter, and values for total organic carbon were as high as 600 mg per liter.

Fate of ¹⁴C-radiolabeled isotopes. The microbes from the North Charleston sediment were active despite heavy contamination with jet fuel. Respiration was measured after 24 h, and it reached a maximum after 3 days of incubation during incubations with 5 ng of [¹⁴C]glucose per g (Fig. 2). Initial respiration rates calculated over the first 3 days of incubation were on the order of 2.4% per day, and the maximum amount respired was approximately 9%. The addition of 0.1 mM NO₃ or 0.1 mM NH₄ did not significantly increase respiration rates or the maximum percentage of the glucose respired over those of the unamended samples. A mass balance of the distribution of the added [¹⁴C]glucose suggested that cellular incorporation of the ¹⁴C was not different in the nitrogen-amended and unamended samples when measured after 1 and 29 days of incubation (Table 3).

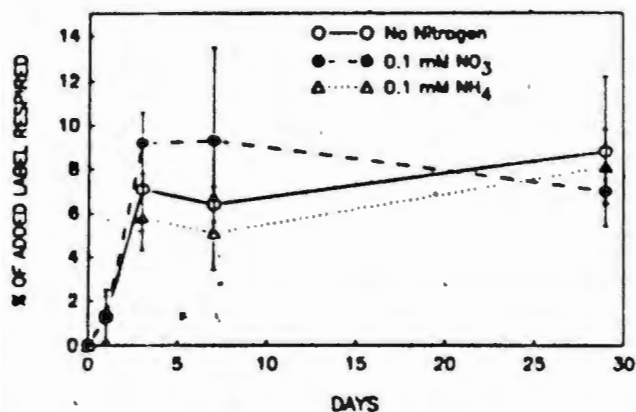


FIG. 2. [¹⁴C]glucose respiration over time in samples with no added nitrogen, and in samples amended with 0.1 mM NO₃⁻, and in samples with 0.1 mM NH₄⁺. All data are means ± standard deviations.

When [¹⁴C]glucose (4 ng g⁻¹) was incubated with a higher concentration of nitrate, 1.7 mM, a decrease in the maximum percentage of glucose respired was measured in the nitrate-amended samples as compared with that of the unamended samples, 8% versus 16%, respectively, after 8 days of incubation (Fig. 3). A mass balance procedure was performed after 26 days of incubation, at which point respiration was lower in both nitrate-amended and unamended samples than that measured after 8 days (Table 3). Although the ¹⁴CO₂ measured after 26 days was greater in the sample with no nitrate, 6% versus 4%, a significantly greater percentage of the labeled carbon added was incorporated into cellular material for the nitrate-amended samples, 24% versus 7%. The total glucose metabolized, as indicated by respiration and cellular incorporation, was approximately twice as great for the nitrate-amended samples (28%) as for the unamended samples (14%).

Results from incubations with [¹⁴C]-radiolabeled toluene (17 ng g⁻¹), benzene (7 ng g⁻¹), and benzene (7 ng g⁻¹) amended with 3 mM NO₃⁻ showed limited mineralization (less than 0.5% after 4 months of incubation) of these compounds (data

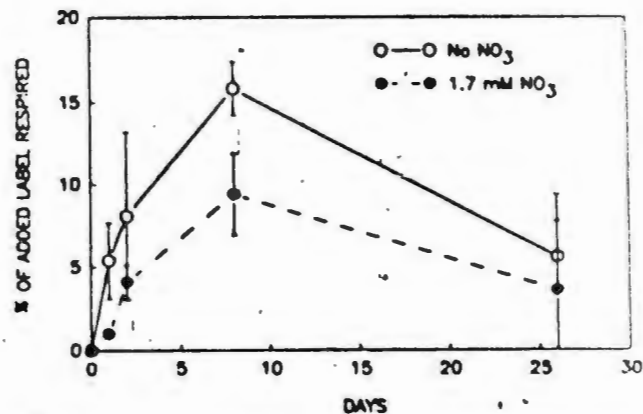


FIG. 3. [¹⁴C]glucose respiration over time in samples with no added NO₃⁻, and in samples amended with 1.7 mM NO₃⁻. All data are means ± standard deviations.

not shown). There was no significant difference in ¹⁴CO₂ evolution in experimental and control vials for any of the conditions. Because 65% of the added toluene remained dissolved in the filtrate after 3 months of incubation, it is unlikely that volatilization of toluene limited the respiration of this compound during the experiment. At three to four times higher concentrations of benzene (30 ng g⁻¹) and toluene (43 ng g⁻¹), both amended with 1.2 mM NO₃⁻, respiration was again low and less than 0.5% of the radiolabeled carbon was measured as ¹⁴CO₂ (Fig. 4). However, a distinction in mineralization between experimental and control vials was observed. Approximately three times as many disintegrations per minute were measured from ¹⁴CO₂ production in live samples than that from metabolically inhibited control vials. This difference in ¹⁴CO₂ production was measured after approximately 7 days and remained constant for the remaining 3 months of incubation.

Gas chromatographic experiments. Gas chromatographic analyses of vial headspace gases indicated high rates of CO₂ production in 1 mM nitrate-amended sediment samples (Fig. 5). CO₂ was measured after 4 days of incubation and increased linearly throughout 2 months of incubation ($r^2 = 0.902$). CO₂ production may have begun to decrease after 70 days of incubation. CO₂ production rates for these samples

TABLE 3. Metabolic fate of [¹⁴C]glucose in unamended samples and samples amended with 0.1 mM nitrate and 0.1 mM ammonium after 1 and 29 days of incubation and in samples amended with 1.7 mM nitrate after 26 days of incubation

Sample amendment (mM)	Days of incubation	Concn added (ng g ⁻¹)	[¹⁴ C]glucose		
			% Respired ^a	% Uptake ^b	% Recovered ^c
No N	1	5	1	5	84
0.1 NO ₃ ⁻	1	5	1	6	83
0.1 NH ₄ ⁺	1	5	1	3	72
No N	29	5	7	9	109
0.1 NO ₃ ⁻	29	5	7	9	98
0.1 NH ₄ ⁺	29	5	7	9	100
No N	26	4	6	7	77
1.7 NO ₃ ⁻	26	4	4	24	81

^a Net percentage of [¹⁴C]glucose mineralized.
^b Net percentage of [¹⁴C] label recovered on filters.
^c Percentage of [¹⁴C] label recovered.

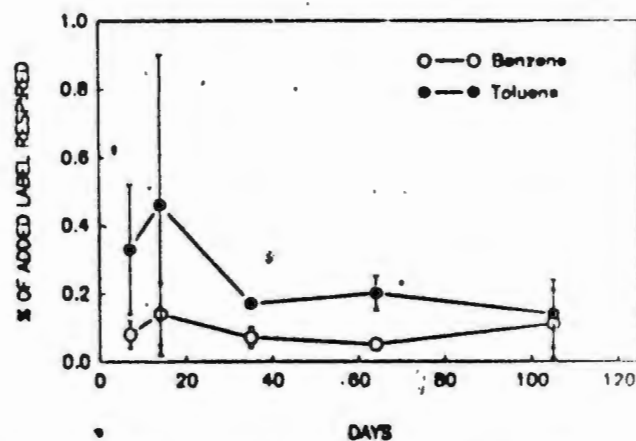


FIG. 4. [¹⁴C]benzene and [¹⁴C]toluene respiration over time in samples amended with 1.2 mM nitrate. All data are means ± standard deviations.

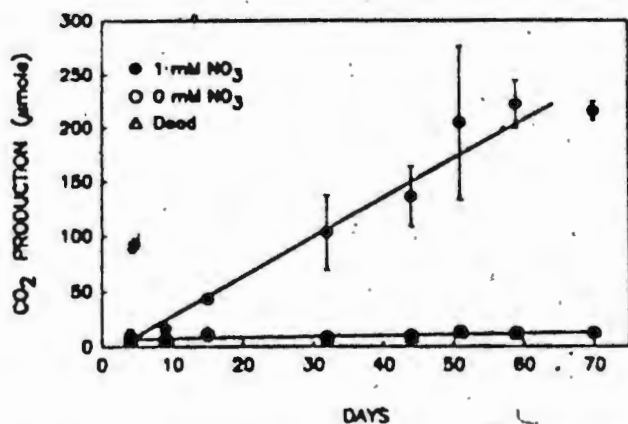


FIG. 5. CO₂ production over time in metabolically inhibited control vials (Δ), unamended samples (O), and samples amended with 1 mM nitrate (●). All data are means \pm standard deviations. Lines represent linear regressions.

of incubation. In samples with no added nitrate, no CO₂ was measured, and CO₂ production rates were not different from the abiotic control vials.

Oxygen concentrations in these same vials decreased stoichiometrically as CO₂ concentrations increased (Fig. 6). O₂ concentrations decreased during the first 4 days of incubation and continued to decrease linearly over 2 months of incubation ($r^2 = 0.972$) at a rate of $-0.124 \mu\text{m g}^{-1} \text{day}^{-1}$ over the first 59 days of incubation. As with CO₂ production rates, in samples with no added nitrate, O₂ consumption rates were not different from those in the abiotic control vials.

Several compounds identified in the vial headspace by coelution with chromatographic standards (Table 1) were monitored over time in sediment metabolically inhibited with NaN₃, sediment with added nitrate (28 mM), and sediment with added nitrate (28 mM) and phosphate (4 mM). Of the compounds identified, *n*-hexane was a major component and its concentration substantially decreased over time in the live sediment samples (Fig. 7). Samples amended with nitrate had reductions in hexane concentration of approximately 90% after 2 months of incubation. The addition of

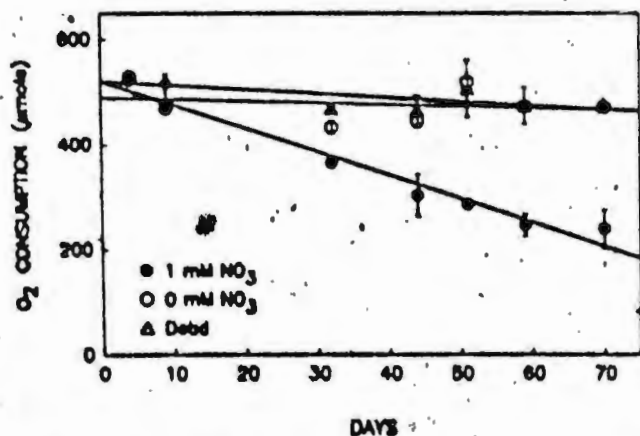


FIG. 6. O₂ consumption over time in metabolically inhibited control vials (Δ), unamended samples (O), and samples amended with 1 mM nitrate (●). All data are means \pm standard deviations. Lines represent linear regressions.

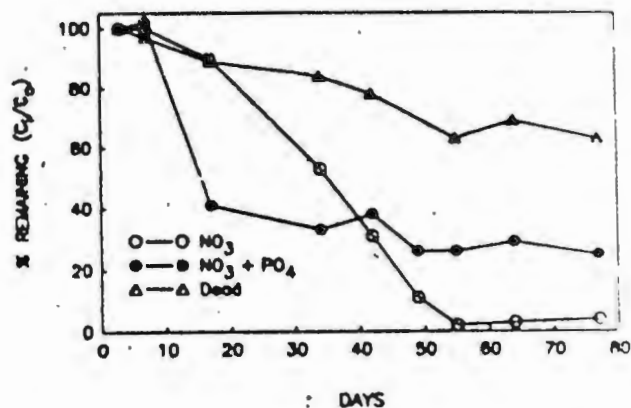


FIG. 7. Hexane disappearance over time in metabolically inhibited control vials (Δ), vials amended with 28 mM nitrate (○), and samples amended with 28 mM nitrate and 4 mM phosphate (●), expressed as the percentage ratio of C_t, the amount at time t, to C₀, the initial amount.

phosphate (4 mM) and nitrate (28 mM) together reduced hexane concentrations by approximately 70% after 2 months of incubation but did not appear to enhance reduction of hexane concentrations over that resulting from the addition of nitrate alone. Some reduction (approximately 30%) of hexane did occur in the metabolically inhibited control vial potentially because of abiotic processes or anaerobic respiration which may have occurred in anaerobic microzones in the sediment sample. The concentrations of the branched alkanes listed in Table 1 were not reduced (data not shown).

Carbon dioxide, oxygen, and methane were examined after approximately 100 days of incubation (Table 4) in all vials including a JP-4 jet fuel standard sample consisting of 10 μl of JP-4 in 50 ml of water. CO₂ concentrations in the nitrate-amended and in the nitrate-and-phosphate-amended sediment were approximately equal and were two orders of magnitude greater than those in the control vials. Similarly, oxygen concentrations were one order of magnitude lower in these live vials than in the controls. Methane was not present in the JP-4 control vial but was present in the metabolically inhibited control vial at approximately 10 $\mu\text{mol liter}^{-1}$ and in the live vials at 25 to 30 $\mu\text{mol liter}^{-1}$. Sodium azide, which was added to the control vials, is a metabolic inhibitor of aerobic respiration via the cytochrome system and therefore does not inhibit anaerobic processes such as methanogenesis.

DISCUSSION

Results from both radioisotopic incubations and gas chromatography demonstrated that the microbial community at the North Charleston site was active despite highly contam-

TABLE 4. Concentrations of CO₂, O₂, and CH₄ ($\mu\text{mol liter}^{-1}$) in vial headspace after 94 days of incubation

Sample treatment	Concn ($\mu\text{mol liter}^{-1}$) of		
	CO ₂	O ₂	CH ₄
JP-4 standard	34	7,439	0.1
Aerobic control	87	6,631	10.4
28 mM NO ₃	4,931	768	28.7
28 mM NO ₃ + 4 mM PO ₄	5,122	767	25.0

inated sediments. The microbial community was capable of respiring a readily degradable substrate (glucose) immediately. In addition, a mass balance determination indicated that, in the presence of 1 mM nitrate, the majority of ^{14}C was incorporated into cellular biomass. Therefore, the cells were actively respiring and growing. Swindoll et al. (21) found similar rates of [^{14}C]glucose metabolism, $27\% \text{ day}^{-1}$, in uncontaminated subsurface sediments and significant incorporation of ^{14}C into cellular carbon.

[^{14}C]benzene and [^{14}C]toluene did not appear to be substantially utilized by the in situ microorganisms at the North Charleston site during long-term incubations. It appeared, however, that the capability for benzene and toluene respiration existed in the microbial community from the contaminated sediment as was demonstrated by the greater generation of $^{14}\text{CO}_2$ in experimental vials than in control vials. In contrast to these findings, other researchers have found adaptation to and rapid removal of these compounds in sediment microcosms (1, 5, 22). The sediment at the North Charleston site has been contaminated for approximately 15 years, providing adequate time for microbial adaptation to the organic compounds. Because JP-4 jet fuel is a complex mixture, adaptation to selective compounds may have occurred at the North Charleston site but does not appear to have occurred for benzene and toluene.

Several possibilities exist for limited respiration of benzene and toluene in sediment microcosms in this study. Compound concentration and the interactions between components of the complex JP-4 mixture may influence biodegradation patterns (2, 11). It is possible that toxicity resulting from high contaminant levels may have affected the toluene and benzene degraders selectively and not the glucose degraders that may represent a more general and larger population of microorganisms. Alternatively, limited respiration of these compounds may be due to preferential utilization of more readily available alternative carbon sources (19, 21) or differences in bioavailability of the compounds. The aqueous solubilities of *n*-alkanes such as *n*-hexane (10 mg liter^{-1}) and *n*-octane ($0.66 \text{ mg liter}^{-1}$) are less than those for aromatic compounds such as benzene ($1,780 \text{ mg liter}^{-1}$) and toluene ($515 \text{ mg liter}^{-1}$) (13), and the octanol-water partition coefficients are greater ($\log P$: *n*-hexane = 3.0; *n*-octane = 4.0; benzene = 2.13; and toluene = 2.69) (8). In general, greater amounts of *n*-hexane would be associated with the sediment and would therefore be more available to the organisms than benzene.

Despite limited respiration of benzene and toluene, results of the gas chromatographic analyses suggested that substantial oxidation of organic contaminants occurred in the presence of nitrate. High rates of CO_2 production were measured and CO_2 production was observed within days of incubation and occurred concomitantly with the consumption of oxygen. Aerobic oxidation of the organic compounds appears to be the predominant CO_2 -producing process. However, anaerobic microzones may exist in the aquifer material because of the duration of the incubations and may have contributed to the total CO_2 produced.

Although the measurement of CO_2 by gas chromatography cannot provide direct information on the specific compounds which are being oxidized, analysis of vial headspace gases can identify which compounds are decreasing in concentration. For petroleum contamination, Atlas and Bartha (3) generalize that *n*-alkanes of intermediate length (C_{10} to C_{24}) are most rapidly degraded, aromatic compounds are degraded more slowly than alkanes, and that branching, in general, reduces biodegradability. In the present study,

high-molecular-weight organic compounds were not examined. Generally, results indicated that for the North Charleston sediment, the normal alkane *n*-hexane, appeared to be degraded more readily than the branched alkanes of comparable molecular weight.

Because of the excess organic carbon present at the Charleston site, it is not surprising that the addition of nitrogen significantly enhanced biodegradation and respiration. Just as [^{14}C]glucose metabolism was enhanced by nitrate addition, nitrate enhanced the oxidation of the organic components of the JP-4 jet fuel. The addition of nitrate at approximately 1 mM concentrations significantly increased CO_2 production from essentially undetectable levels in unamended samples to CO_2 concentrations 40 times greater in nitrate-amended samples. The addition of nitrate also increased the reduction of hexane concentrations, while the addition of nitrate and phosphate did not increase hexane degradation over the addition of nitrate alone. It has been shown previously that microbial nutritional requirements associated with petroleum hydrocarbon contamination appear to be site specific (17). In one case, nitrogen and phosphorus alone may have been sufficient to significantly increase biodegradation of contaminants and produce bacterial biomass. In a second case, nutritional requirements may have included nitrogen, phosphorus, and the addition of trace amounts of inorganic compounds for optimal growth.

This study confirms the existence of an actively respiring and growing microbial community despite heavy sediment contamination. In general, it appears that the microbial community at the North Charleston site is nitrogen limited and is capable of degrading certain components of the JP-4 jet fuel following nitrate addition. JP-4 is comprised of a wide range of constituents, from low-molecular-weight to high-molecular-weight aromatic and aliphatic compounds. More work must be done to define the capabilities of the indigenous microbial community with respect to the biodegradation of individual organic components of this complex mixture and to assess the contributions of specific microbial processes to this biodegradation.

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Bioremediation Potential of Terrestrial Fuel Spills†

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A bioremediation treatment that consisted of liming, fertilization, and tilling was evaluated on the laboratory scale for its effectiveness in cleaning up a sand, a loam, and a clay loam contaminated at 50 to 135 mg g of soil⁻¹ by gasoline, jet fuel, heating oil, diesel oil, or bunker C. Experimental variables included incubation temperatures of 17, 27, and 37°C; no treatment; bioremediation treatment; and poisoned evaporation controls. Hydrocarbon residues were determined by quantitative gas chromatography or, in the case of bunker C, by residual weight determination. Four-point depletion curves were obtained for the described experimental variables. In all cases, the disappearance of hydrocarbons was maximal at 27°C and in response to bioremediation treatment. Poisoned evaporation controls underestimated the true biodegradation contribution, but nevertheless, they showed that biodegradation makes only a modest contribution to gasoline disappearance from soil. Bunker C was found to be structurally recalcitrant, with close to 80% persisting after 1 year of incubation. The three medium distillates, jet fuel, heating oil, and diesel oil, increased in persistence in the listed order but responded well to bioremediation treatment under all test conditions. With bioremediation treatment, it should be possible to reduce hydrocarbons to insignificant levels in contaminated soils within one growing season.

Soil that is accidentally contaminated by petroleum fuel spills is classified as hazardous waste (2). When the amounts of contaminated soil are large, the currently accepted disposal methods of incineration or burial in secure chemical landfills can become prohibitively expensive. This often results in cleanup delays while the contaminated soil continues to pollute scarce groundwater resources (8). Land treatment disposal of oily refinery sludges has been practiced for decades with generally good results (1). This project was designed to test, on the laboratory scale, what type of fuel spills could be cleaned up by a cost-effective bioremediation approach based on a land treatment process optimized for oily sludges (4). In addition to five different fuels, the variables included three contamination levels, three incubation temperatures, and three different soil types. Petroleum hydrocarbon disappearance rates were compared in contaminated but otherwise untreated soil, in bioremediation-treated soil, and in soil poisoned in order to suppress biodegradation (6).

MATERIALS AND METHODS

Fuel products. The following fuel products were selected for use in this study: as a low-boiling-point distillate, unleaded gasoline; as a medium-boiling-point distillates, jet fuel, heating oil (no. 2 fuel oil), and diesel oil; and as a high-boiling-point distillate, bunker C (residual fuel oil). The bunker C sample contained 15 to 20% (by volume) of a medium distillate, which is commonly added to lower the otherwise very high pour point of this product. All products were supplied by the Bayway Refinery, N.J. (Exxon USA). The fuel products were initially characterized as to their class composition and carbon range.

Preparation and incubation of fuel-contaminated soil sam-

ples. Soils were selected to include light, medium, and heavy textured ones. Their textures, organic matter contents, and pHs were determined (7). Soils were freshly collected for each experiment. They were partially but not completely air dried to allow sieving (2-mm-diameter openings) for uniform consistency, but without damaging their biological activity. The sieved soils were packed into glass columns (outer diameter, 25 mm; length, 250 mm) at the bulk density of cores collected from the field. The resulting columns were 60 g (dry weight), 22 mm in diameter, and 150 mm in length. The lower ends of the columns were closed with a Teflon (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.)-wrapped plug and a closable drain spout. After packing, water was added to the top of the column to adjust the moisture content of the soil to 50% of its holding capacity. Lime (CaCO₃) was added to semidry soil prior to column packing. The amount was based on liming curves, and the lowest amount of CaCO₃ sufficient to raise the pH to 7.5 to 7.6 (7) was added. For all three soils, this was 10 mg of CaCO₃ g of soil⁻¹.

Nitrogen and phosphorus fertilizers, unless noted otherwise, were 60 μmol of N as NH₄NO₃ and 5 μmol of P as K₂HPO₄ g of soil⁻¹ (4). They were dissolved in the water that was used for adjusting the moisture content. Soil columns were contaminated with fuel products by placing the fuel products on top of the columns and allowing them to infiltrate by gravity flow. The maximal application rate (135 mg g of soil⁻¹) was chosen so that it would not result in either fuel or water flowing out from the soil column. Bunker C was too viscous to be applied in this manner. It was mixed with semidry soil and was packed and subsequently moistened. The evaporation of water during incubation was compensated for by weighing the prepared soil columns and adding distilled water to compensate for any weight loss during incubation. Weekly tilling of the soil columns was performed by inserting a stainless steel wire into the soil columns 15 times. This treatment, which was forced by the constraints of the incubation system, was much less effective in aerating the soil than conventional tilling in the field is

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Biologically inactive poisoned controls (2% HgCl_2) were used to differentiate evaporative losses from biodegradative losses (6). The poisoned controls showed the maximal evaporative loss that may occur under the incubation conditions. In fact, however, biodegradation and evaporation compete in the removal of petroleum hydrocarbon, and subtracting the loss of hydrocarbons from poisoned controls from the loss observed in active soil samples strongly underestimates the true contribution of biodegradation. This fact should be kept in mind when interpreting the results.

Analytical methods. For each point of analysis, the fuel in the soil of an entire column was extracted. Gasoline was extracted from soil with cold Freon 11 (fluorotrichloromethane; E. I. du Pont de Nemours & Co., Inc.). Anhydrous sodium sulfate (equal in weight to the weight of the soil) and 150 ml of Freon 11 were sealed with the soil into a 500 ml Teflon-lined screw-cap flask and shaken at 17°C at 200 rpm for 24 h. The sample was filtered in a cold room, the soil residue was washed, and the total Freon 11 extract was brought to volume. Bromooctane was used as the internal standard.

Jet fuel, heating oil, diesel oil, and bunker C were Soxhlet extracted for 6 h by using methylene chloride. Anhydrous sodium sulfate was added to the extraction thimble to absorb sample moisture. After the extraction of bunker C, which has no highly volatile components, the solvent was evaporated in a preweighed dish and the residual weight was determined gravimetrically. Extracts of the medium distillates were brought to volume, internal standards (octadecane for jet fuel; tetracosane for heating oil and diesel oil) were added, and the extracts were analyzed by gas chromatography by using an instrument (Model 5890; Hewlett-Packard Co., Palo Alto, Calif.) with a 10-m macrobore (0.53-mm diameter) fused-silica capillary column with an immobilized polydimethyl siloxane phase (Alltech Associates, Inc., Deerfield, Ill.). The nitrogen carrier flow rate was 30 ml min^{-1} ; hydrogen and air for the flame ionization detector had flow rates of 40 and 200 ml min^{-1} , respectively. Temperatures for gasoline analysis were as follows: injection port, 150°C; flame ionization detector, 250°C; oven, initially 35°C and programmed to reach 150°C at 4°C min^{-1} . For jet fuel, the initial oven temperature was 50°C and was programmed to reach 200°C at 4°C min^{-1} . For heating oil and diesel oil, the initial oven temperature was 50°C and was programmed to reach 205°C at 4°C min^{-1} . Dilutions of the original products in CH_2Cl_2 served as quantitative analytical standards. All fuel residues were expressed as milligrams of hydrocarbon gram of dry soil⁻¹.

For class separation, each fuel sample except gasoline was fractionated on a silica gel column. The silica gel (Aldrich Chemical Co., Inc., Milwaukee, Wis.) was activated at 105°C for 12 h. The glass column (2 by 28 cm) was packed with silica gel suspended in hexane. The 0.5-g hydrocarbon samples were adsorbed on 3 g of silica gel and placed on the column. A 3-g layer of anhydrous sodium sulfate was placed over the sample to absorb any water and to prevent the disturbance of the sample with the solvents. The class fractionations of petroleum products were accomplished by successive elution in a discontinuous solvent gradient of increasing polarity. The saturated, aromatic, and asphaltic classes were eluted with 120 ml of hexane, benzene, and chloroform-methanol (1:1; vol/vol), respectively.

RESULTS AND DISCUSSION

Characteristics of the fuels and soils. Some characteristics of the five fuels used in this study are summarized in Table

TABLE 1. Analysis of the fuel products used in the spill bioremediation study

Fuel product	Class composition (%) ^a			
	Saturates ^b	Aromatics ^c	Polar ^d	Carbon range ^e
Gasoline		Not analyzed		C ₆ -C ₁₁
Jet fuel	83.0	15.7	1.3	C ₉ -C ₁₇
Heating oil	62.5	32.9	1.9	C ₉ -C ₂₂
Diesel oil	53.7	45.0	1.3	C ₉ -C ₂₅
Bunker C	52.6	35.0	12.4	Not analyzed ^f

^a Chromatographic separations done on a silica gel column.

^b Hexane eluate.

^c Benzene eluate.

^d Chloroform-methanol (1:1; vol/vol) eluate.

^e Based on gas chromatographic analysis and comparison with authentic *n*-alkane standards.

^f According to a distillation curve, the bunker C sample contained 15 to 20% of a medium distillate (probably heating oil) added to lower the pouring point of the product.

1. With the exception of bunker C, all products had very low levels of polar compounds. From the three medium distillates, jet fuel had the lowest and diesel fuel had the highest levels of aromatic compounds, with heating oil having intermediate levels. The reciprocal was true for saturated compounds. Diesel oil had the widest carbon number range. The values found in this study showed a good correlation with published product specifications (5). As saturated compounds are generally more easily biodegraded than the corresponding aromatic compounds are (1, 3), the compositional data also helped to interpret some of our biodegradation results.

The textural compositions, organic matter contents, and initial pHs of the three types of soils used in these experiments are summarized in Table 2. Lakewood sand was a particularly low-quality soil with a very acidic pH. A heavy clay soil was not available in the study area, and the selected Penn clay loam was only slightly more heavily textured than the loam soil was.

The analytical approaches for the five fuel products, their mean time zero recoveries, and the standard deviations of these recoveries are summarized in Table 3. As the number of determinations prevented the use of replicate samples in routine analyses, the standard deviations in Table 3 give some general confidence limits for the analytical procedures that were used in terms of recovery and repeatability.

Kinetics of fuel disappearance and data presentation. Our studies generated a four-point depletion curve for each fuel type under a wide variety of incubation conditions (temperatures, soil types, loading rates, bioremediation treatments, poisoned controls). The volume of the data precluded the presentation of all these curves, yet their mathematical

TABLE 2. Characteristics of the three soil types used in the biodegradation experiments

Soil type	Texture (%)			Organic matter (%)	pH
	Sand	Silt	Clay		
Loam, Bayway ^a	36	40	24	2.7	4.3
Lakewood sand ^b	90	4	6	4.9	4.0
Penn clay loam ^c	44	34	22	1.3	4.9

^a From the grounds of the Bayway Refinery (Exxon USA). There was no previous spill exposure.

^b Pine Barrens, N.J., pine-oak forest; state park.

^c Piscataway, N.J., meadow; recreational use.

TABLE 3. Extraction, analysis, and time zero recovery from soil of the five fuels used in this study

Product	Extraction	Analysis	Mean \pm SD recovery (%) ^a
Gasoline	Cold Freon 11	GC ^b	93.7 \pm 1.9
Jet fuel	Soxhlet, CH ₂ Cl ₂	GC	98.5 \pm 2.7
Heating oil	Soxhlet, CH ₂ Cl ₂	GC	98.0 \pm 1.4
Diesel oil	Soxhlet, CH ₂ Cl ₂	GC	97.3 \pm 2.3
Bunker C	Soxhlet, CH ₂ Cl ₂	Residual wt	99.5 \pm 1.5

^a Based on triplicate samples at the low (50-mg g of soil⁻¹) loading rate.

^b GC, Gas chromatography.

reduction to constants was made problematic by the complex nature of the fuels and by the soil incubation system.

The classical pattern of material depletion by a constant force shows first-order (exponential) kinetics. Because of diffusion limitations and increases in the degrading microbial populations, even the depletion of a homogeneous substrate in soil is rarely first order but, rather, is intermediate between first-order (exponential) and zero-order (linear) kinetics. The kinetics are further complicated in the case of fuels by the fact that these consist of numerous individual hydrocarbons, each of which is utilized at a different rate. The slowing tendency of utilization is caused not only by substrate depletion but also by the fact that the remaining hydrocarbons are structurally less degradable than are the ones that already disappeared (1, 3). To some extent, this is compensated for by the increasing numbers (enrichment) of the hydrocarbon-degrading microorganisms in the soil with time. For the reasons described above, there is no precise way to convert the curves obtained in this study to constants. For the purposes of the data in Table 4, half-life is simply the time needed to reduce the total fuel concentration in soil to 50% of the initial amount. If a 50% reduction was not achieved within the time period of the experiment, Table 4 simply indicates this fact (e.g., half-life, >12 weeks). The half-lives used in Table 4 in their restricted sense still give a useful comparison of the relative biodegradability of the five fuels and of the environmental conditions that favor or restrict the process. In addition to Table 4, we included one set of depletion curves (see Fig. 1 through 5) for each fuel type in order to illustrate their typical kinetics of disappearance.

Concerning the data in Table 4, some generalizations apply to all fuel products. Half-lives of fuels were longest in poisoned soils, and only the highly volatile gasoline reached a 50% depletion in such soils. Half-lives of fuels were also longer in untreated than in treated soils and longer at 17°C than at 27°C. A further increase in temperature to 37°C increased rather than shortened the fuel half-lives. This was also observed in a previous study (4) with oil sludges and probably reflects the increased attack of hydrocarbons on microbial membranes at elevated temperatures. Increased fuel concentrations tended to increase half-lives only moderately. As to the effect of soil types, half-lives tended to be longest in the sand, a soil with poor absorbing capacity and low microbial diversity. Half-lives in clay loam were slightly shorter than those in loam.

Unleaded gasoline. The disappearance kinetics (Fig. 1) and half-lives (Table 4) of unleaded gasoline differed only very slightly in untreated, treated, and poisoned soils. Compared with the medium distillates, biodegradation played a smaller relative role in the overall removal of gasoline hydrocarbons from soil. The C₆ to C₉ components of gasoline (Table 1), under the conditions of these experiments, were lost more

TABLE 4. Half-lives of five fuels in three soil types under various incubation conditions

Fuels, soil type, and incubation temp (°C) ^a	Fuel added (mg g (dry wt) of soil ⁻¹)	Half-life ^b		
		Untreated ^c	Treated ^d	Poisoned ^e
Unleaded gasoline				
Sand				
27	50	2.1	1.5	2.3
Loam				
17	50	1.9	ND ^f	2.2
27	50	1.7	1.5	2.0
37	50	1.2	ND	1.8
27	100	1.7	1.5	2.0
27	135	1.7	1.5	2.0
Clay				
27	50	2.7	2.3	4.5
Jet fuel				
Sand				
27	50	>12.0	6.0	>12.0
Loam				
17	50	>12.0	ND	>12.0
27	50	>12.0	4.4	>12.0
37	50	>12.0	6.0	>12.0
27	100	ND	ND	>12.0
27	135	>12.0	7.5	8.0
Clay				
27	50	3.5	1.7	>18.0
Heating oil				
Sand				
27	50	>18.0	>18.0	>18.0
Loam				
17	50	>18.0	18.0	>18.0
27	50	12.0	5.5	>18.0
37	50	12.0	6.6	>18.0
27	100	>18.0	9.2	>18.0
27	135	>18.0	12.5	>18.0
Clay				
27	50	9.0	5.5	>18.0
Diesel oil				
Loam				
27	50	>18.0 ^g	7.0 ^h	>18.0
27	100	>18.0 ^g	7.0 ^h	>48.0
Bunker C				
Sand				
27	50	>48.0	>48.0	>48.0
Loam				
17	50	>48.0	>48.0	>48.0
27	50	>48.0	>48.0	>48.0
37	50	>48.0	>48.0	>48.0
27	100	>48.0	>48.0	>48.0
27	135	>48.0	>48.0	>48.0
Clay				
27	50	>48.0	>48.0	>48.0

^a Analytical data on the fuels are summarized in Table 1, and analytical data on the soils are summarized in Table 2.

^b Half-lives are in days for unleaded gasoline and are in weeks for all other fuels.

^c Untreated describes samples that received the petroleum product only.

^d Treated soil samples were treated with lime to an approximate pH of 7.5, received N and P fertilizer, and were tilled weekly.

^e Poisoned (2% HgCl₂) samples served as evaporation controls.

^f ND, Not determined.

^g When tilled only (no lime and fertilizer), half-lives decreased at 50 mg g of soil⁻¹ to 10 weeks and at 100 mg g of soil⁻¹ to 11.3 weeks.

^h In this case the treated samples received either the normal (60 μ mol of N and 5 μ mol of P g of soil⁻¹) or only 25% of this amount. The effects of fertilizer levels on half-lives were marginal and were averaged.

rapidly by evaporation than by biodegradation, while biodegradation primarily removed the C₁₀ to C₁₁ components. This became clear when the gas chromatograms from untreated and treated soil samples were compared (data not

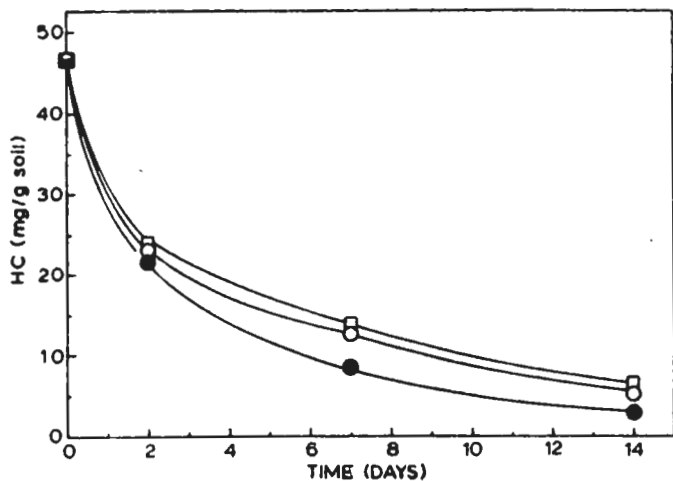


FIG. 1. Disappearance of gasoline hydrocarbon (HC; 50 mg g of soil⁻¹) from columns of loam soil. Symbols: O, untreated soil; ●, bioremediation-treated soil; □, poisoned soil.

shown). There was little or no difference in the loss of short-retention-time materials, but treatment that promoted biodegradation visibly increased the loss of the components with longer retention times.

Bunker C The disappearance of bunker C from soil was very slow and incomplete (Fig. 2). None of the samples reached a 50% reduction during 48 weeks of incubation (Table 4). Bioremediation initially accelerated bunker C disappearance, but no further stimulation was evident after 8 weeks of incubation. We interpret these results as signifying that most bunker C components were structurally resistant to biodegradation. The maximal weight losses from bunker C matched closely the amount of medium distillate used to lower the pour point of this product (Table 1). We conclude that bioremediation has only very limited beneficial effects on gasoline and on bunker C elimination from soil, although for quite different reasons.

Jet fuel. Jet fuel disappeared from soil quite rapidly (Fig. 3 and Table 4). As evident from the poisoned samples, volatility losses of jet fuel were potentially quite high; but for the reasons discussed earlier, volatility losses from biologically active samples were actually much lower than those indicated by the poisoned controls. Bioremediation substantially

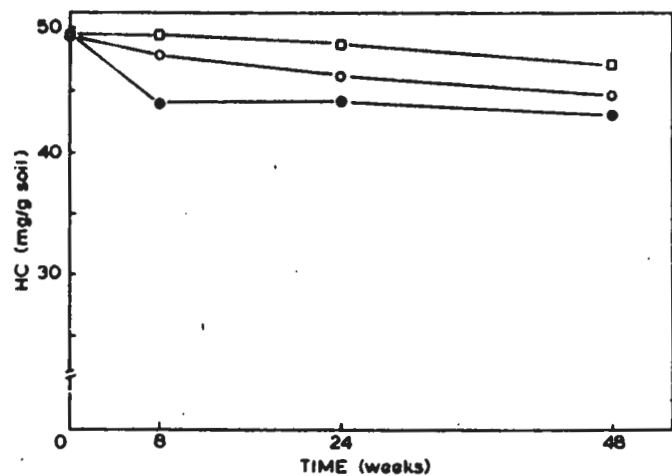


FIG. 2. Disappearance of bunker C hydrocarbon (HC; 50 mg g of soil⁻¹) from columns of Lakewood sand. Symbols: O, untreated sand; ●, bioremediation-treated sand; □, poisoned sand.

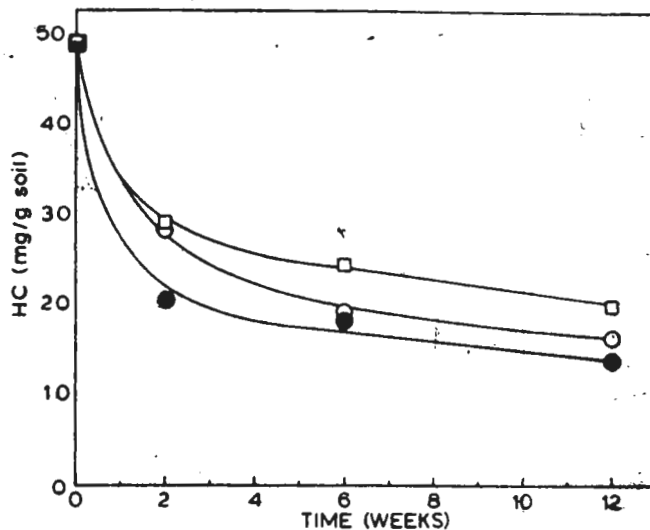


FIG. 3. Disappearance of jet fuel hydrocarbon (HC; 50 mg g of soil⁻¹) from columns of Penn clay loam. Symbols: O, untreated loam; ●, bioremediation-treated loam; □, poisoned loam.

accelerated jet fuel disappearance in the first weeks after the spill. In untreated but biologically active soil samples, disappearance tended to catch up to that in the bioremediation-treated samples in the later phases of incubation. Although the redox potential profile of the soil columns was not measured, from experiments conducted in thin surface soil layers (9), we concluded that this is largely due to oxygen limitation in the soil columns. The oxygen limitation did not allow bioremediation to manifest its full beneficial effect in this incubation system. In poisoned controls, volatilization failed to reduce jet fuel concentrations to 50% in 12 weeks, except in one case (clay loam) that we consider to be an experimental artifact. We believe that the clay loam, with its considerable cation exchange capacity, immobilized most of the Hg²⁺ ions from the solution in the upper portions of the soil columns and so allowed some biodegradation to occur in the lower portions of the columns.

Heating oil. Heating oil was less volatile and more persistent than jet fuel was (Fig. 4 and Table 4). Heating oil in soil responded well to bioremediation treatment that typically shortened its half-life to 50% or less as compared with that of untreated soil.

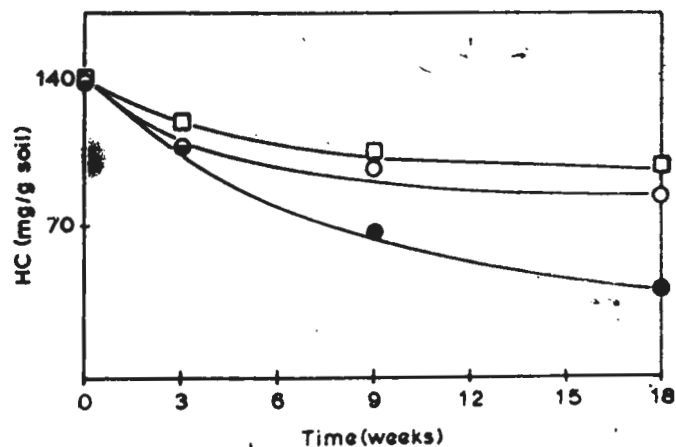


FIG. 4. Disappearance of heating oil hydrocarbon (HC; 135 mg g of soil⁻¹) from columns of loam soil. Symbols: O, untreated loam soil; ●, bioremediation-treated loam soil; □, poisoned loam soil.

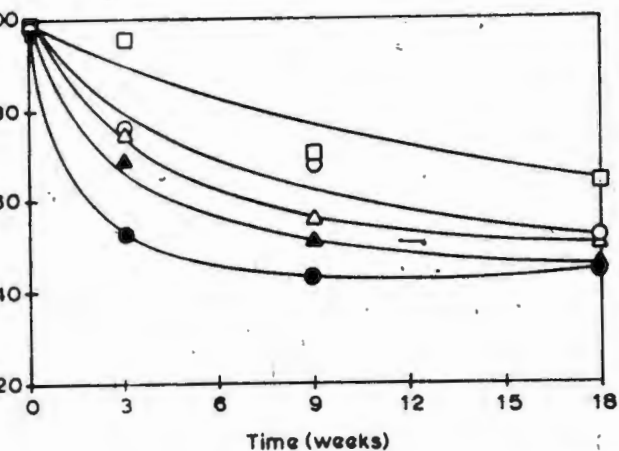


Fig. 5. Disappearance of diesel oil hydrocarbon (HC; 100 mg g soil⁻¹) from columns of loam soil. Symbols: ○, untreated loam soil; ●, bioremediation-treated loam soil; □, poisoned loam soil; △, untreated loam soil only; ▲, bioremediation-treated (25% of normal fertilizer) loam soil.

Diesel oil. Diesel oil behaved quite similarly to heating oil (Fig. 5 and Table 4), with a comparable or slightly longer half-life. As in the case of heating oil, bioremediation reduced diesel oil half-lives by 50% or more. Fertilization at normal and at 25% of the normal level had only a slight and probably not significant effect on the half-lives of diesel oil, indicating that fertilizer can be applied at more conservative rates without reducing the biodegradation efficiency. Tilling increased the oxygen availability and considerably shortened the half-lives compared with the oxygen availability in the undisturbed soil columns, but it did not have the same half-life reduction as it did in combination with pH control and fertilization.

Our results show that the environmental persistence of petroleum distillate fuels increases in the following order: jet fuel > heating oil > diesel oil. Bioremediation treatment substantially reduced the persistence of all three of these fuels. The tested incubation temperatures had largely predictable effects on disappearance rates and indicated that in temperate regions, medium distillate contaminants of soil can be reduced to essentially insignificant concentrations

within one growing season. Inferior soil types and contamination levels up to the maximum holding capacities of the soils reduced disappearance rates only moderately and did not appear to preclude bioremediation in any of the tested situations.

The laboratory screening described in this report identified spills of medium fuel distillates as promising candidates for bioremediation. These fuels were subsequently selected for larger-scale outdoor bioremediation studies (X. Wang and R. Bartha, *Soil Biol. Biochem.*, in press). The potential problem of polycyclic aromatic residues from diesel oil has been addressed separately (X. Wang, X. Yu, and R. Bartha, *Environ. Sci. Technol.*, in press), and the changes in the soil microbial community caused by fuel spills are the subject of an accompanying paper (9).

ACKNOWLEDGMENT

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What's news in environmental health

Scientists dig deep for site cleanup answers

Micro-organisms living hundreds to thousands of feet beneath the eastern Washington desert may hold the secret to cleaning part of the contamination at the U.S. Department of Energy's Hanford Site and other areas polluted with hazardous and radioactive wastes. Microbes in the subsurface environment may be able to break down some of the wastes produced during the last 50 years of nuclear materials production at Hanford.

Scientists recently began drilling a deep hole on the Hanford Site to search for microscopic organisms. They expect to reach a depth of 350 feet and hope to find "versatile" micro-organisms which can survive in a low-oxygen environment and consume other materials, including hazardous wastes.

The drilling is part of the U.S. Department of Energy's Subsurface Science Program, which researchers at the Pacific Northwest Laboratory, other DOE laboratories and eight universities are searching for answers about subsurface organisms. Battelle operates PNL for DOE. Battelle researchers already have shown that some micro-organisms can render

certain chemical compounds harmless. One can neutralize potentially toxic nitrates in groundwater while simultaneously destroying carbon tetrachloride, a carcinogenic solvent used extensively over the years at many industrial facilities.

Subsurface micro-organisms also may play a part in the effort to clean up nuclear waste by creating biological barriers and altering the mobility or solubility of radioactive contaminants within the ground.

Core samples taken from the Hanford Site will be analyzed to help develop future strategies for waste cleanup. "We may be able to manipulate the microbial populations we expect to find to make them work faster or consume specific types of contaminants," says Jim Frederickson, project co-manager.

FDA issues guidelines for safe egg-handling

Foodborne illness due to *Salmonella enteritidis* (*S.e.*) contaminated eggs has been increasing and spreading to many states as evidenced by recent Centers for Disease Control reports. From January 1985 through October 1989, 189 *S.e.* outbreaks in the United States caused 6,604 illnesses

and 43 deaths. More than 30 outbreaks were reported in the first seven months of 1990.

CDC's studies implicated eggs as the likely source in 73% of the outbreaks where a food was identified. Time/temperature management of eggs repeatedly has been identified as an important component of food safety control efforts, the U.S. Food and Drug Administration notes.

Most eggs do not contain *S.e.* For those that do, it is not yet known exactly how or when the bacteria reproduce when they are present inside an intact egg.

In April 1990 the Conference for Food Protection recommended that concerns about *S.e.* be communicated to all health and food protection agencies and to the food industry. In response, the FDA Model Food Codes Interpretation has redesignated shell eggs as a potentially hazardous food and recommended that food establishment operators be required to refrigerate and properly cook eggs.

The concept of "potentially hazardous food" has been widely used and understood by food regulators for decades. Under FDA model codes and most state and local statutes, regulators can require proper cooking and refrigeration only for foods so designated. Consumers concerned with the *S.e.* problem may be unfamiliar with the terminology, however, and become unnecessarily concerned about the general safety of eggs as food.

The FDA, therefore, has asked local officials to help the public understand that the designation "potentially hazardous food" simply identifies foods for which time/temperature management is indicated. These include a wide variety of perfectly wholesome nutritious foods commonly found in home refrigerators, such as meat, poultry, fish, dairy products and cooked vegetables.

International garbage trashing U.S. beaches

While Americans are demonstrating a growing concern about keeping beaches clean, trash from other countries continues to foul the United States coastline.

A recent survey, conducted by Keep America Beautiful, Inc., in conjunction with a nationwide beach cleanup project, reported that some 424 tons of trash was collected from 700 miles of beaches, much

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¹⁸Stephan Budiansky and Russell Clemings, "Toxic Wastes? A Little Fungus May Help," U.S. News and World Report, 09 November 1987, 85.

¹⁹Ford, 69.

²⁰Walter H. Corson, ed., The Global Ecology Handbook (Boston: Beacon Press, 1990), 267.

²¹Ibid., 5.

²²Ibid., 265.

²³Richard A. Denison and John Raston, eds., Recycling and Incineration - Evaluating the Choices (Washington DC: Island Press, 1990), 236.

²⁴Ibid., 237.

²⁵Ibid., 238.

²⁶Louis Blumberg and Robert Gottlieb, War on Waste (Island Press, 1989), 28.

²⁷Jon R. Luoma, "Trash Can Realities," Audubon, March 1990, 86.

²⁸McGraw - Hill, eds. Encyclopedia of Science and Technology (New York: McGraw - Hill, Inc., 1987), s.v. "Incineration," by Robert J. Bryan.

²⁹Jon Luoma, "Burn Garbage for Electricity - Yes, No, Maybe...", Audubon, March 1990, 96.

³⁰Ibid., 97.

³¹McGraw - Hill, 33.

³²Ibid., 33.

³³Newsday, Rush to Burn: Solving America's Garbage Crisis? (Washington, D.C.: Island Press, 1989), 10.

³⁴Ibid.

³⁵Gregory Matzuk, "Energy Resource Recovery: A Technical Overview," American City and County, 106, January 1991, SW/RR 11.

³⁶Ibid.

³⁷Newsday, 11.

³⁸Ibid.

³⁹Ibid.

- ⁴⁰Matzuk, 14.
- ⁴¹Ibid.
- ⁴²Ibid., 39.
- ⁴³Ibid., 1.
- ⁴⁴Ibid., Chapter 5.
- ⁴⁵Ibid., 105.
- ⁴⁶Ibid., 1.
- ⁴⁷Ibid., 61.
- ⁴⁸Luoma, "Trash Can Realities," 87.
- ⁴⁹Newsday, 214.
- ⁵⁰Ibid., 212.
- ⁵¹Ibid., 205.
- ⁵²Ibid., 212.
- ⁵³Mario Gialanella and Louis Luedtke, "Air Pollution Control and Waste Management," American City and County, 106, January 1991, SW/RR 18.
- ⁵⁴Newsday, 208.
- ⁵⁵Ibid., 221.
- ⁵⁶Ibid., 218.
- ⁵⁷Ford, 70.
- ⁵⁸Luoma, "Trash Can Realities," 89.
- ⁵⁹Ibid., 95.
- ⁶⁰Ibid., 96.
- ⁶¹Ibid., 98.
- ⁶²Fletcher, notes.
- ⁶³Luoma, "Trash Can Realities," 87.
- ⁶⁴Ibid., 88.

⁶⁵Melinda Beck, "Buried Alive," Newsweek, 27 November 1989, 70.

⁶⁶Sharon Begley, Hideto Takayama, and Mary Hager, "Teeing Off on Japans Garbage," Newsweek, 27 November 1989, 70.

⁶⁷Ibid.

⁶⁸Ibid.

⁶⁹Neil Seldman, "Waste Management: Mass Burn is Dying," Environment, 31 (September 1989): 43.

⁷⁰Ibid.

⁷¹Ibid., 44.

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