2 BIOLOGICAL DEGRADATION OF HAZARDOUS WASTES

Industrial waste problems, especially those involving hazardous waste, are an ongoing problem. They have a high profile, and as such are reported regularly in the media. Examples of past mishaps are Love Canal, New York; Times Beach, Missouri; and Serveso, Italy. In our modern day world, with our use of plastics, cars, leather, and pesticides, it is now accepted that they have "side effects." Within the last number of years, there has been a move to clean these problems. Presented here is a review of current studies concerning biological treatment of hazardous waste, along with non-biological strategies.

Biological treatment is an expanding field which has shown success in remediation of hazardous waste problems. It is not the only treatment technology available and does not have to be used in isolation, nor is it without limitations. The chapter begins with an introduction, explaining some of the problems with hazardous waste, ways to reduce it, some past legislation, and facts about hazardous waste. Then there is a brief section reviewing current abiotic treatment methods available, including some of their advantages and limitations. Discussed are some of the types of biological treatment techniques, (e.g., land composting, activated sludge processes, filters), before assessing what we know about the microbiological basis for biological degradation. Also addressed is how we test if a compound is susceptible or not, the genetical basis of degradation, and the terms "mineralization" and "recalcitrance." There follows work done in degradation methods using aerobic bacteria in pilot plant demonstrations or in-situ studies. These are the success stories that have originated in laboratory research and bench scale demonstrations. A review of current laboratory work follows. The work discussed to date has been aerobic but there are anaerobic bacteria that degrade many compounds with certain advantages over aerobic degradation.
They have a different type of metabolism. This will be discussed prior to the laboratory work. Included in this section and not in the pilot studies/in-situ section is work on 1,1,1-trichloroethane, which is an in-situ study. It is placed here because it is an example of anaerobic degradation. The work with fungi has so far been ignored. Although there has been a great deal of information about fungal degradation in the past years, the majority of published works dealt with bacteria.

INTRODUCTION

Hazardous waste does not consist of one chemical or attribute; the definition of it is a wide one. The Environmental Protection Agency (EPA) stated in its report to Congress that:¹ The term "hazardous waste" means any waste or combination of wastes which pose a substantial present or potential hazard to human health or living organisms because such wastes are lethal, non-degradable, persistent in nature, biologically magnified, or otherwise cause or tend to cause detrimental cumulative effects. General categories of hazardous waste are toxic chemical, flammable, radioactive, explosive, and biological. These wastes can take the form of solids, sludges, liquids, or gases. So, the range of hazardous wastes, and their effects is large and diverse.

Attributes of hazardous wastes are: irritation, corrosivity, and bioconcentration. The observable effects are a function of exposure, the longer a person is exposed, the more serious the effects.

Nearly all industry produces some waste and since no one is likely to independently shut down an industry that makes the waste, we need some guidelines and regulations. On the federal level, industrial waste was originally regulated by the Clean Water Act of 1977 and the Clean Air Act of 1963 and subsequent amendments; hazardous wastes received special emphasis in the Resource Conservation and Recovery Act of 1976 (RCRA), the Toxic Substances Control Act of 1976 (TOSCA), and the Comprehensive Environmental Response, Compensation and Liability Act of 1980 (CERCLA) and subsequent amendments.

Through CERCLA, the Superfund aid was initiated and it was seen as an assault on growing hazardous waste problems, but to date, only 12-15 sites have been detoxified and restored. Most other sites have been limited to containment. The EPA estimates that there are some 2000 hazardous waste sites, but more alarmingly the Office of Technical
Assessment believes that 10,000 sites will eventually need detoxification. Meanwhile the U.S. industry continues to produce new wastes at the rate of 260 million metric tons per year.²

The EPA states that the preference for waste management priorities should first be via waste minimization techniques and then only after this is to focus on treatment.³ There has been emphasis on the treatment abandoned hazardous waste dumps, preventing the illegal dumping of wastes, and regulating industrial discharges and dumping. But there has been a lack of strategies to deal with both the toxicity and volume of industrial waste at the source. Valentino and Walmet⁴ discuss this process problem and discuss strategies to deal with it. They list as five most common strategies from generally least costly to most costly:

- Reduction in the overall amount of waste that is produced. The materials used in the front end of the process can be reduced by good housekeeping policies. There are numerous examples of this strategy. For example, an electronics firm has reduced the overall amount of solvent needed to degrease metal parts, and an electroplating shop has, instead of rinsing all parts with fresh water, made minor changes that allow the plater to "cascade" rinse water i.e., use water from the third rinse to perform the second rinse and so forth, thus cutting the total quantity of water requiring treatment by up to 90 percent.

- Reuse of the processing chemicals or solvents with little or no treatment, sometimes within the same company. Often, however a waste that cannot be used by one company can be reused somewhere else. For example, a waste alkali can be used to neutralize an acid stream at another facility. Cement dust captured in the pollution control equipment of a cement kiln is very useful in final drying and in stabilizing metallic sludges to prevent leaching after land disposal.

- Recycling the processing chemicals through a treatment process. The characteristics of the useful end product may be altered, but recycling may produce useful materials and energy. An example is the reclamation of titanium from utility-plant precipitator ash or precious metal from plating wastes.
Research, development, and demonstration (RD&D) of new waste-reduction technologies. RD&D can range from basic research, to demonstrating the feasibility of tailoring an existing technology to the process of a particular business. An example is the development of a system for the anaerobic digestion of cheese-whey, a by-product of the cheese manufacturing process. While it is not a hazardous waste, cheese whey has an oxygen demand 350 times stronger than normal sanitary domestic sewage, and it creates serious disposal problems. In the past, many cheese-manufacturing plants have either spread the whey on farmland (which results in odor and run-off into surface water) or sent the whey down the sewers into a municipal sewage treatment works, which overloaded the treatment capacity of the system. The development of an anaerobic treatment system for cheese whey in which certain bacteria are used to convert waste material into a mixture of harmless gases, including methane is reducing the load of the systems and producing sizable quantities of biogas, which can be used as fuel.

Replacement of the entire industrial process. This is the most costly strategy and usually beyond the capabilities of all but the most prosperous and willing industries. An example of replacement, which has applied to industrial as well as residential practices, is the wholesale reduction of oil-based paints in favor for water-based paints. Another example that illustrates a costly alternative is heat-treating certain metal parts to provide suitable hardness. Small items were previously hardened in molten cyanide baths. Because cyanide is toxic, many cyanide baths have been replaced by controlled atmosphere furnaces that are much more complex and costly to operate.

Legislation for the process problem would be difficult since each industry is different, with different starting chemicals, techniques, and products. Another problem with legislation is that waste problems vary on a regional basis and with locale. The waste problem in central New Jersey will not have the same characteristics as one in California.
Weather patterns differ greatly across the United States. Leaching of landfills is much less of a problem in arid regions than it is in the rainy areas. Solar evaporation may be an effective waste-concentration option in New Mexico but not in New York. Similarly, certain industries that are regional in nature have specialized waste treatment problems. For example, the petrochemical industry is concentrated on the Gulf Coast. Mining and ore refining tend to occur in only a few major locations. Steel has historically been heavily concentrated in Pennsylvania and Ohio. New York has unusually large concentrations of many small electroplaters in the New York City area, leather tanneries in two Mohawk River Valley counties, and a large number of diverse, old manufacturing operations in the Buffalo area and Elmira areas.

Most of the production of waste in the U.S. is from industry which is responsible for 69%. Municipal output accounts for 35%.5

The bulk of potentially hazardous waste is generated from four industries: primary metals; organic chemicals and explosives; electroplating and metal finishing; and inorganic chemicals. The hazardous waste management industry often deals with input materials in the five groupings of: metals/metal finishing; paint/solvent/coatings; organics; petroleum; and inorganics.

Some estimates show that 71% of the waste is produced from the chemical and petroleum industries, 22% from metal and related industries, and 7% from other industries.5

As stated before, waste production is regional in nature. Of the hazardous waste produced in the U.S. some 60% of it is from the following states: New Jersey, Ohio, Illinois, California, Pennsylvania, Texas, New York, Michigan, Tennessee, and Indiana.

Legislation as a tool in itself is not enough to deal with the problem; economically viable alternatives are necessary. Different states show different types of waste management, but land treatment is ubiquitous in treatment technologies. In the past the land took much of the brunt with landfills and surface impoundments, with deep injection into the groundwater system being the next biggest category, and other forms being responsible for only a small percentage. With the rising cost of land treatment, depletion of space, and concern over contamination of our groundwater, other technologies will have to take on a more significant role in treating hazardous wastes.
ABIOTIC TREATMENT TECHNIQUES

Wastewater Treatment

Selection of a treatment process is dependent on the nature of the wastewater and the quality of the effluent desired. Hazardous components of the wastewater may be either separated or converted to non-hazardous forms in order to permit the disposal of the wastewater effluent by conventional methods. Conversion processes can be done in one step or in multiple steps. The hazardous components which are separated from the wastewater must be disposed of. This may take additional steps, e.g., sludge dewatering.

Liquids-Solids Separation

Separation of suspended matter from wastewater can be accomplished by a number of different processes. Large heavy solids are easier to remove than finely divided light solids.

Screening devices are used to remove large pieces of solid matter that would interfere with subsequent processing operations or would cause damage to equipment such as pumps. Coarse screening devices may consist of parallel bars, rods or wires, perforated plates, gratings, or wire mesh.

Gravity sedimentation--This process involves the containment of wastewater for a sufficient period of time to allow some or all of the suspended materials to either settle out or float to the surface of the wastewater. In its simplest form as a batch process, a given volume of wastewater is transferred to a vessel and held there until nearly all the settleable and floatable matter separates. The floating matter can be skimmed off and the wastewater decanted for discharge or further treatment. Sludge may be allowed to collect until several batches of wastewater have been processed. Then it is removed. The vessel may have a conical bottom so that the sludge can be removed via a valve. Large settling ponds may be constructed which are drained periodically to permit sludge removal. Solids-contact or sludge-blanket clarifiers are useful where sludges are flocculent and of low density. They are designed with large mixing and reaction zones that coupled with the sludge blanket account for greater efficiency in solids removal. Gravity
sedimentation works like the clarifiers but more time is taken for the settling.

**Dissolved-Air Flotation** is useful for suspended matter that does not sink or float in a reasonable period of time. Separation is brought about by the introduction of finely divided gas bubbles which become attached to the particulate matter, causing it to float to the surface where it is removed by skimming. Introduction of the gas bubbles is usually accomplished by reducing the pressure of the wastewater causing dissolved gases to be released. This is commonly used to separate greasy or oily matter from industrial wastes. Granular-Media Filters or deep bed filtration is a polishing step that removes small amounts of suspended solids and produces a highly clarified water. Chemical coagulation and sedimentation typically precede this stage. Graded sand and pulverized coal are commonly used in the filter beds. Conventional operation is usually by downflow. The ability of the granular media filter beds to produce a clear effluent results from the straining action and adhesion, which removes particles finer than the pore space.

**Surface Filters** make use of a fine medium such as a cloth or close mesh screen. In the rotary vacuum filter, the medium is in the form of a continuous belt and it rotates over a perforated drum that is partially submerged in the slurry to be filtered. Water is pulled through the filter cake that forms on the belt to the inside of the drum, where it is transferred to the vacuum system.

**Centrifugation** is a useful alternative to filtration for sticky sludges that do not dewater rapidly on a filter. They operate by a rapid rotation of a liquid suspension, which induces a much greater force than gravity to hasten the separation of the suspended matter.

**Chemical Treatment**

Chemical treatment is a widely used process for the destruction or separation of hazardous constituents in wastewater. This can be done by neutralization of acidic or alkaline wastewater until a suitable pH is obtained. Precipitation/Coagulation/Flocculation is used for the removal of heavy metals. Precipitation refers to the formation of a solid phase, coagulation is where the containment is trapped by the formation of a precipitate, and flocculation is the agglomeration of a coagulating chemical.
Oxidation-Reduction or the redox processes are used for converting toxic pollutants to harmless or less toxic materials that are more easily removed. These processes involve the addition of chemical reagents to wastewaters, causing changes in the oxidation states of substances both in the reagents and in the wastewaters. In order for one substance to be oxidized, another must be reduced. Ozone is a powerful oxidizing agent that is usually oxygen at temperatures and pressures up to 350°C and 180 atmospheres, respectively, to treat organic wastes.

Ion exchange involves a change in the chemical form of a compound; the exchange of ions in solution with other ions held by mixed anionic or cationic groups or charges. Typically, a waste solution is percolated through a granular bed of the ion exchanger, where certain ions in solution are replaced by ions contained in the ion exchanger. If the exchange involves cations, the exchanger is called a cation exchanger and correspondingly anion exchanger is one that involves anion exchange.

Physical Methods

There are several methods used for separating pollutants from wastewater: activated carbon, steam stripping, evaporation, reverse osmosis, and solvent extraction. The chemical and physical characteristics of the pollutant are important in the selection of the physical removal method. Steam stripping is effective for substances that have an appreciable vapor pressure at the boiling point of water, whereas evaporation is effective for those chemicals that will not volatilize. Soluble, small organic molecules are adsorbed by activated carbon; large ions are separated by reverse osmosis.

Activated Carbon Adsorption--Here the inorganic and organic chemicals are adsorbed onto activated carbon. Usually hydrophobic chemicals are more likely to be removed. The degree of adsorption is linked to the molecular weight, methanol-water coefficient, or solubility (these are also linked to the recalcitrance and/or toxicity). The smaller the size of the grain, the more surface area is available and so equilibrium is reached quicker with powdered activated carbon compared to the granular form. But then the powdered form needs more pumping to get the wastewater through and hence the costs are increased. There are two principle systems, one is downward flow through the bed (pressure or gravity flow) and the other is upflow through a packed or
expanded bed. Activated carbon adsorption is applicable to the treatment of dilute aqueous wastes, but they should be treated to remove suspended solids, oil, and grease. Temperature and pH are also important for the different compounds to be treated. The carbon is either disposed of or regenerated. Carbon has also been added directly to biological treatment effluent in a contacting basin. The advantages of this are that the sludge toxicity is reduced by selectively removing the toxic organics from solution and that the carbon adsorption capacity is extended by bioregeneration of the "biocompatible" species adsorbed on the surface. For aqueous solvent waste containing contaminants in concentrations up to 10,000 mg/l, the activated sludge process has been proposed as a potential applicable treatment. However these concentrations may be toxic to the sludge or they may be easily stripped to the atmosphere, thereby creating another hazard. The sludge may also contain recalcitrant waste, due to adsorption of the contaminants and be difficult to dispose of.

Evaporation is the process that heats the liquid, venting the vapors to the atmosphere and concentrating the pollutants into a slurry.

Reverse osmosis--Osmosis is the process where a solvent (e.g., water) moves from an area of low concentration to high across a semipermeable membrane which does not allow the dissolved solids to pass. In reverse osmosis, a pressure greater than the osmotic pressure is applied so the flow is reversed. Pure water will then flow through the membrane from the concentrated solution.

Solvent extraction is a process whereby a dissolved or adsorbed substance is transferred from a liquid or solid phase to a solvent that preferentially dissolves that substance. For the process to be effective, the extracting solvent must be immiscible in the liquid and differ in density so that gravity separation is possible and there is minimal contamination of the raffinate with the solvent. The hydrophobic solutes are more likely to be extracted. Solvent extraction can be performed as a batch process, or by the contact of the solvent with the feed in staged or continuous equipment.

Steam stripping is where water vapor at elevated temperatures is used to remove volatile components of a liquid. Countercurrent flow is generally used to promote gas-liquid contact, thus allowing soluble gaseous organics from the liquid waste to be continuously exchanged with molecules within the stripping gas. Again, this is useful only for waste with low water solubilities.
Incineration

Incineration is a high temperature oxidation process that converts the principal elements (carbon, hydrogen, and oxygen) in most organic compounds to carbon dioxide and water. Given with the problems of disposing on land, incineration may take on a lead role in waste treatment. However, it is not without its problems. There is fear among the general public about the nature of the stack emissions but it is an efficient method. The destruction of the molecular structure usually eliminates the toxicity of the chemical. But the existence of other elements in a waste may result in the production of particulate pollutants that require removal in off-gas treatment systems. There are several types of incinerators available.

The liquid injection incinerators operate by spraying the combustible waste mix with air into a chamber where flame oxidation takes place. The purpose of spraying is to atomize the waste into small droplets which present a large surface area for rapid heat transfer, thereby increasing the rate of vaporization and mixing with air to promote combustion. Air is supplied to provide the necessary mixing and turbulence. These incinerators are widely used for destruction of liquid organic wastes.

Rotary-kiln incinerators are designed to process solids and tars that cannot be processed in the liquid incinerator. The rotary-kiln is a cylindrical shell lined with refractory material that is horizontally mounted at a slight incline. It is rotated from 5 to 25 times at high temperatures, 1500 to 3000°F with excess air, the residence time varied depending on the nature of the waste. The rotation causes a tumbling action that mixes the waste with air. The primary function is to convert, through partial burning and volatization, solid wastes to gases and ash/residue. If the ash is free of dangerous levels of hazardous wastes, it is put in a landfill.

Fume incineration--Large quantities of organic vapor fumes are produced by many industries, including fat rendering, metal painting and varnishing, and various types of printing. These vapors are generally mixes of hydrocarbons, alcohols, and acetates. The mixes may not be acutely toxic but they do cause odor problems. An integrated heat-recovery system demonstration at Case-Hoyt Company, near Rochester, captures waste heat and simultaneously reduces plant air emissions by oxidizing solvents into harmless gases as the heat is recovered.
The multiple hearth incinerator is used for wastes that are difficult to burn or that contain valuable metals that can be recovered. It consists of a refractory-lined circular steel shell, with refractory hearths located one above the other. Solid waste or partially dewatered sludge is fed to the top of the unit where a rotating plow rake plows it across the hearth to dropholes. The uncombusted material falls to the next hearth and the process is repeated until the combustion is complete.

Fluidized-bed incinerators (FBI) are applicable to the destruction of halogenated organic waste streams. This type of incinerator consists of a vessel in which inert granular particles are fluidized by a low velocity air stream which is passed through a distributor plate below the bed. An FBI consists of a windbox (through which combustion air is introduced to the reactor), and a reactor zone (containing a bed of sand, waste injection, and removal ports). Temperatures are in the range of 1300 to 2100°F, gas residence times usually a few seconds. They have been used to treat municipal sewage sludge, low quality fuels, pulp and paper effluents, food processing waste, refinery waste, radioactive waste, and miscellaneous chemical waste.9

A molten salt incinerator uses a molten salt such as a sodium carbonate as a heat transfer and reaction medium. In the process, waste material along with air is added below the surface of the bed so that any gases formed during combustion are forced to pass through the melt. Reaction temperatures in the bed range from 1500 to 2000°F and residence times are less than a second. Any acidic gases formed are neutralized by the alkalinity of the bed. This can change the fluidity of the bed so it needs replacement frequently.

Plasma arc incineration is based on the concept of reducing or pyrolyzing waste molecules to the atomic state using a thermal plasma field. They system uses very high energy at temperatures near to 10,000°C to break bonds of hazardous waste chemical molecules down to the atomic state. An electrode assembly ionizes air molecules which create a plasma field. Hazardous waste mixtures interact with the field, forming simple molecules such as carbon dioxide, hydrogen, hydrogen chloride, and other minor matrix compounds such as acetylene and ethene. Westinghouse Electric has a mobile plasma arc unit called Pyroplasma that reportedly treats liquid wastes at the rate of a 3 gal/min.10 The high temperatures decomposes PCB and other wastes in an oxygen-deficient atmosphere. Hydrogen chloride is treated with sodium to form water and salt.
Lime or cement kiln incineration—A cement kiln is basically a large rotary kiln in which raw materials are fed countercurrent to combustion gas flow. The wet process kilns uses a 30% water slurry feed and are the most suitable for hazardous waste destruction. The products formed are alkaline and so act as a scrubber, removing acid gases formed during combustion. This system operates at 2800°F resulting in very efficient removal of wastes. A project is in operation at Blue Circle Atlantic Inc., a cement company in Ravena where it is evaluating the incorporation of hazardous wastes into an existing cement-kiln operation. The facility is not yet in operation but should be able to handle large amounts of waste and alleviate some problems locally.

Wet Air Oxidation

Wet air oxidation involves the aqueous phase oxidation of organic materials at high temperature and pressure. A major advantage over other incineration methods is that the water in the waste stream is kept in the liquid state. Water is pumped into the reactor along with oxygen which is heated by the hot effluent. Two types of reactors are used, a bubble tower reactor and a stirred tank cascade reactor. This process is good for wastes that are too dilute to incinerate but too toxic for biological methods. The products are usually acetic acid and carbon dioxide.

Solidification Techniques

There are several innovative non-thermal processes that have been developed under the SITE program that immobilize wastes by vitrification or other types of solidification. The SITE program is the Superfund Innovative Technology Evaluation, a $20 million/year program which has been developed to encourage the private development and demonstration of new technologies for cleaning up hazardous wastes. For example, the researchers at Battelle Pacific Northwest Laboratories have developed an in-situ vitrification process (which was originally designed for the containment of nuclear wastes) in which electrodes are sunk into a contaminated area and attached to a diesel powered generator. The current produced temperatures of about 3600°F which is much higher than the fusion temperature of soil. An exhaust hood is placed over the site to collect and treat any combustion products. The
result is a massive glass-like product consisting of completely immobilized organics, inorganics, steel drums, and other components that are essentially locked up and inert. The time taken to complete the process depends on the electrode depth and frequency. Another solidification process uses a reagent called Urrichem that immobilizes slurried hazardous components. The contaminated soil is excavated and intimately mixed with the Urrichem off-site. After blending, the slurry is pumped out of the mixer and hardens into a concrete like mass within 24 hours. Chemfix is a process developed by Chemfix Technologies (Metairie, LA). In this technique, a proprietary blend of soluble silicates and additives is used to convert high molecular weight organic and inorganic slurries into a cross-linked, clay like matrix.

Alternative methods have also included landfills, deep well injection disposal, and ocean dumping. Landfills were developed because it was believed that by placing waste in designated ground areas, there would be a natural decomposition over time. Unfortunately the water table rises in a landfill and this mounding effect means that we get a leaching of the water containing toxics since the water flow is always from areas of high to low head, i.e., there is a gradient set up that favors water movement originating in the landfill and away from it thus contaminating our groundwater supplies. This is a special problem for an area like Long Island, New York, that relies on groundwater and not surface water for their domestic/industrial supplies. Deep injection wells have similar problems. When the aquifers in which the deep well sits are pumped, the contaminants are drawn into the well. Ocean dumping has upset the delicate ecosystem balance in some areas. Thus there is a need for effective techniques that industry can safely use to try and combat the nation's hazardous waste problems. Biological treatment may be such.

BIOLOGICAL CONTROL METHODS

Biological treatment of hazardous wastes is becoming a realistic option in the treatment strategies for hazardous wastes. As we research more about specific bacteria and fungi that do the work and know more about what conditions favor degradation, we are finding more uses for them. A major advantage is that the cost is much less than incineration. A limitation is the length of time involved, since biological treatment can take months. There are different mechanisms that we can use to degrade
a compound. Selection is based on the type of pollutant, type of degradation (i.e., aerobic or anaerobic), and various other parameters.

There are suspended growth systems such as activated sludge, aerated lagoons, and anaerobic digesters where the microorganisms are suspended; and fixed film processes, which include filters and rotating biological contactors where the microorganisms grow on a fixed surface. Since toxin degradation is a slow process, the fixed film processes may be best since the microbial retention is very high. In aerobic degradation, the major portion of the cost is the aeration. Wastes in any form—solid, aqueous, and gaseous—can be treated biologically. The treatment can be done several ways: by applying the waste to the soil, by composting, using a hybrid liquid/solid treatment technique, treating the wastes in-situ, using soil filter gases, and with wastewater treatment systems. Care must be taken in assessing biological treatment since non-degradable pesticides and other organics may be unaffected by the treatment or toxic intermediates may be formed and therefore a finishing process such as activated carbon may be needed.

Land Treatment

Land treatment is the biological option most widely used to treat hazardous wastes. Many of the industrial waste treatment sites using biological treatment are at petroleum refineries.

The steps in the process typically are: spread the waste on the land, let it dry, till the soil to mix the waste in, control moisture, and if necessary add nutrients so that the waste-destroying bacteria will grow.

In a land treatment system, bottom liners under the treatment zone are generally not needed. Physical, chemical, and biological processes in the soil are relied on to degrade, immobilize, or transform the wastes to the environmentally acceptable forms. Most pollutants are captured and transformed in the top 6-12 in. in the soil, though the treatment zone may extend down 5 ft.

Land treatment is suitable with wastes such as petroleum refinery sludges, creosote sludges and wastewaters, and processing sludges from wood, paper, and textile manufacture. It is also widely used for municipal sludges and wastewaters, and food processing sludges and wastewaters. Oil, metals, and other constituents of environmental concern are successfully controlled by land treatment.
While most land treatment sites are at operating facilities, at least one is at an abandoned wood preserving facility now being remediated under Superfund or CERCLA. At this Minnesota site a treatment pilot test was run for a year proving that the creosote constituents could be degraded by acclimated soil microorganisms.

To scale up to full operation, about 12,000 cu. yd. of contaminated soils and sludges were removed from the impoundment and temporarily stored. A lined treatment area was then constructed over the impoundment, and the stored wastes were put back in place.

The process has been encouragingly effective, as shown by polycyclic aromatic hydrocarbon removals of 90% after 193 days of treatment. Total cost, including excavation of the waste and construction and operation of the facility, was approximately $80/cu. yd. This cost is much less than incineration, the only other in-situ treatment technique that completely eliminates the hazardous compound.

Costs for unlined land treatment systems are a little cheaper but since we need to protect the groundwater system, extensive site testing is necessary to obtain federal RCRA permits.

As a result of the Hazardous and Solid Waste Amendments of 1984 and the current land disposal restrictions rule, EPA is in the process of demonstrating achievable treatment techniques to be used as an alternative to the land disposal of hazardous wastes. Research has been done by waste generators that also treat the waste, commercial facilities, and EPA in-house research.

### Composting

Composting uses less space than land treatment and it controls gases and leachate. In composting, piles of the waste 3-6 ft. high are treated. Aeration is provided either by turning the piles mechanically or through a forced aeration system. Bulking agents such as wood chips are sometimes added to facilitate mixing and oxygen transfer. These bulking agents are typically screened out at completion of composting, and mixed in with the next batch.

Composting has been used mostly in treating municipal sewage sludge, but has also been used to treat several industrial solid wastes. These include industrial wastewater treatment sludge, food processing wastes, and some industrial wastes containing low levels of pesticides.
A flow diagram for a biochemical compost bed system is shown in Figure 1.

A pilot scale bacteria-based composting system has successfully been used for treating diesel-fuel contaminated soils. Aeration is provided by an air compressor that draws a vacuum at the bottom of the pile and returns the air at the top. Additional air is added as necessary. Nutrients and water are provided through an irrigation system located at the top of the pile. The system is a fully enclosed vessel that is batch loaded. Studies to date have shown that the diesel contaminants have half-lives in the range of 30-60 days. Costs of a full-scale system are estimated at under $100/cu. yd. of soil, again significantly less than incineration.

**Liquids/Solids Treatment Systems (LSTS)**

These are hybrids, intermediate between land treatment and conventional water-suspended biological systems. The waste is not in a solid form as in land treatment, nor in water as in conventional municipal wastewater treatment, but halfway between. The wastes are in a suspended solid, slurry or sludge form.

LSTS systems reduce the level of contaminants in a waste primarily by 1) dissolving the organics, 2) biodegrading the dissolved organics into less toxic and less environmentally significant forms, and 3) releasing the products as gases to the atmosphere. The key step, dissolving the wastes from solid to liquid, can result from microbial action and/or physical-chemical action.

There are two basic LSTS process types: single-reactor and two-stage. In either case it is vital to have sufficient mixing of the liquid/solid mixture to achieve effective mass transfer of both organics in the liquid phase, adequate oxygen transfer to the microorganisms, and to keep the suspension of solids in the solution.

The single-batch reactor is the simpler process and the most widely used. Here both the dissolving and biodegradation steps occur together in a single tank, pit, or lagoon. Air is introduced to provide mixing for the extraction process and oxygen for biological reasons. Aeration and mixing may be provided by submerged aerators, floating aerators, or compressor/sparger (lance) systems.

At completion of treatment, the aerators and mixing are stopped and the solids allowed to settle. The treated liquid is decanted off the top,
Figure 1. Biochemical compost bed system flow diagram.
while the treated solids are treated further, by conventional land treatment or by disposal on-site. If the process is done in a lagoon or surface impoundment, it may be possible to decant the liquid and leave the solids in place.

LSTS have been used in coal tar waste sites in Florida and Illinois. In Florida, about 600,000 gal. of the wastes were found in a gas holder and in Illinois 2000 cu. yd. of material in an in-ground concrete structure. After 9 months, significant reduction of tar volume, phenol concentration and flash point were reported.

LSTS have the potential to achieve the treatment goals in a shorter time using less land area than other on-site biological management approaches, such as land treatment and composting. The process uses tank reactors, and thus may eliminate regulatory obstacles or concerns related to the possible land-treatment ban or to contaminant migration in the groundwater.

Soil Biofilters

Hazardous wastes in gaseous form can be biologically treated too. On-site biofilters can reduce nuisance odors, volatile organic compounds, and particulates in waste gases. The soil biofilter involves two mechanisms; mechanical filtration and treatment by bacteria on the soil particles.

A biofilter consists of a distributing pipe network underneath soil or gravel. The gas to be treated is pumped through the media and escapes through holes in the pipe wall. It rises through the media bed which is usually 3-10 ft. deep. Pollutants captured on the biofilter can be removed by filtration, physical and chemical absorption, chemical oxidation, and structural treatment.

The technique is not a new one, filters have been used for many years to remove odors from agricultural, food, and wastewater processing operations, and emissions from paper and chemical manufacturing plants. The soil filters have successfully removed low concentrations of H₂S, SO₂, NH₃, and NO₂ as well as particulates in these gases. Other potential applications include treatment of exhaust gases from air stripping units for wastewater and solvents, from mechanical technologies such as belt presses for oily sludges, from aerated waste treatment systems, and from in-situ soil stripping of
volatile organic compounds (VOCs) using vacuum and forced aeration methods.

The S.C. Johnson Wax Co., in Racine, Wisconsin\textsuperscript{14} has demonstrated removal of VOCs in a soil bed. This, in turn, has led to research on the biodegradation of chlorinated ethenes, such as those likely to be in exhaust gases under anaerobic conditions. The research demonstrated the aerobic degradation of TCE and other chemicals.

A soil biofilter is being planned for the treatment of VOCs being removed from contaminated soil and groundwater in New Jersey.\textsuperscript{14} Natural gas will be introduced with the contaminated air stream to stimulate growth of the needed methane-oxidizing bacteria.

Wastewater Treatment

Biological treatment has been very successful in the removal of organic pollutants and colloidal organics from wastewater. Activated sludge, biological filters, aerated lagoons, oxidation ponds, and aerobic fermentation are some of the methods available for wastewater biodegradation. In removal of toxic waste, more care is needed since the bacteria are prone to destruction from shock loading or increases of toxic material fed in without allowing time for the population to grow large enough to deal with it.

Biodegradation occurs because bacteria are able to metabolize the organic matter via enzyme systems to yield carbon dioxide, water, and energy. The energy is used for synthesis, motility, and respiration.

With simple dissolved matter, it is taken into the cell and oxidized, but with more complex inorganics, enzymes are secreted extracellularly to hydrolyze the proteins and fats into a soluble form which can then be taken into the cell and oxidized. Hence the more complex matter takes longer to process.

Some organic compounds are "refractory," they cannot be oxidized while others are toxic to the bacteria at high concentrations.

The purpose of biodegradation is to convert the waste into the end products and material that will settle and can be removed as sediment. Again, biodegradation may not be one hundred percent, or toxic by-products may be formed. Further treatment by chemical methods or dilution may be needed to get the contaminant to a concentration prescribed as safe.
Nitrogen and phosphorus are essential in the oxidation process for the synthesis of new cells, and trace amounts of potassium and calcium are also required. The former are sometimes deficient so nitrogen is added in the form of ammoniacal nitrogen (nitrite and nitrate are not readily used by bacteria).

BOD or biochemical oxygen demand, measures the strength of the organics present and is defined as the amount of oxygen needed by the bacteria for oxidation. The more concentrated the organic material the higher the BOD. A BOD:N:P ratio of 100:5:1 is thought to be the optimum ratio of nutrients needed by bacteria.

Activated Sludge Process

This involves the generation of a suspended mass of bacteria in a reactor to degrade soluble and finely suspended organic compounds.

In this method the wastewater with its organic compounds is fed into the aeration tank. This is supplied with air and is vigorously mixed to allow maximum contact of bacteria and waste. The contents, referred to as MLSS (mixed liquor suspended solids) are then fed to a sedimentation tank where the treated solids settle to the bottom and the top liquid layer is treated and discharged. Part of the biological solids are recycled back to the aeration tank to maintain the correct mix; the remainder is waste.

This method is flexible and can be used on almost any type of biological waste. An industrial application has been demonstrated for phenol degradation using a petroleum refinery wastewater: there was an 85-90% removal of phenol and cyanide in the steel industry.¹⁵

Trickling Over Process

Here the wastewater is distributed by a flow distributor over a fixed bed of medium on which the bacteria grow forming a slime layer to which oxygen is supplied. The wastewater flows down over the slime layer which absorb organic materials and nutrients, releasing the oxidized end products to the drainage system underneath. Eventually some of the layer will detach with the wastewater, and then some additional separation is necessary.
Stabilization

This is a procedure where wastewater is stabilized by the actions of bacteria in shallow ponds. There are basically two types of ponds, ones where there is a natural supply of oxygen from algal photosynthesis (oxidation ponds) and mechanically supplied oxygen (aerated lagoons). The bacteria metabolize the wastes and the solids settle at the bottom as a sludge. Also there is anaerobic decomposition where the bacteria at the bottom will degrade the waste without oxygen's presence. Or there can be a lagoon that has both aerobic and anaerobic decomposition, with an interchange of products between the two layers of bacteria in a symbiotic relationship.

DETERMINATION OF BIOLOGICAL DEGRADABILITY

Some terms are important in understanding the fate of hazardous wastes in biological systems. They are biodegradable, persistent, recalcitrant, and mineralization. A compound that is biodegradable can be changed by the action of microorganisms to another compound. This does not necessarily mean that the product is less toxic than the parent compound, or that intermediate toxic compounds are formed that may inhibit the degraders, or that the product will not be toxic to the next degraders or to man himself. Park et al. demonstrated this possibility in their study of bacterial degradation of 7,12-dimethylbenzanthracene in non-acclimated soil. When this compound was degraded aerobically, the parent C DMBA had a half life of 17 days. There was a decrease in the parent compound accompanied by an increase in metabolites: 4-hydroxy, 5-hydroxy, and 10-hydroxy DMBA and 7,12 dihydro-12-methyl 7-methylene benz (a)anthracene. But there was some associated mutagenicity that was thought to be due to the polarity of the compounds, with those that are low and moderately polar having more mutagenic potential than the highly polar compounds. The pH of the soil also plays a role since at low pH fungi dominate the ecosystem and they are more likely to form epoxides (associated with mutagenicity) than bacteria which are dominant at higher pH. So there are some possible
types of reaction that we do not want. We need information so that we can select favorable degradation reactions so as to reduce some types of toxic waste. Recalcitrant means that the compound cannot be biodegraded under any circumstances, i.e., it's the compound itself that is inherently resistant and not the treatment system that has failed to account for some vital fact. Persistence is a "conditional" property of the biodegradable compound in that it may be biodegraded if the correct circumstances favoring biodegradation are present.

Mineralization is the complete conversion of an organic compound to the end products of carbon dioxide and water. Primary biodegradation is the single transformation of a compound and partial biodegradation is somewhere between primary degradation and mineralization.

**Basis for Biodegradation**

Why are some compounds degraded by microorganisms? The degradation is done not as a favor to us but because the organisms gain energy needed for growth, repair, reproduction, and other biological functions needed for survival. Food sources contain oxygen in hydroxyl (OH) or carboxylic acid (COOH) groups. Oxidation reactions take place where electrons are transferred along an electron chain with compounds accepting and passing on electrons to a terminal electron acceptor. The coenzymes may be NADH and NAD which get reduced and then coupled with the electron transport chain to produce high energy bonds in ATP. Many compounds are biochemically inert such as alkanes, saturated ring structures, and unsubstituted benzene. They are devoid of oxygen and not subject to dehydrogenation reactions. The ability of bacteria to utilize these compounds lies in the fact that they can catalyze oxidation using oxygen. Other bacteria have enzymes which work without oxygen and coenzymes are needed.

Studies have been done that attempt to relate the physical and/or chemical properties of a chemical to its recalcitrance; however experience has shown that many chemicals which are thought not to be removed by abiotic mechanisms actually undergo transformations in model treatment systems.
Genetics

Bacteria can only do things for which they have a genetic capability. They must be able to produce the right enzymes to do the job, and the right environment must exist for them to be able to produce the right enzymes. If a chemical is present in concentrations either too low or too high, then it may not be biodegraded: too low and the enzymes will not be induced, too high and the compound may be toxic. One reason why bacteria are robust at biodegradation is that they may still be able to degrade a different compound to the one that they normally utilize if the active site of the molecule has not been altered. The xenobiotic still needs to be able to induce the production enzymes or the reaction will stop. The bacteria will not be able to metabolize it and will die. This use of a different substrate is fortunate for us and has been termed "gratuitous" metabolism. Complicating biodegradation further is "cometabolism" where two substrates are needed i.e., one compound cannot fulfill all the bacterial needs and act as the sole carbon and energy source. A second compound is needed as growth substrate. Biological degradation treatment systems have to be designed to take care of another problem. Bacteria need a continuous carbon source for growth and yet if too much substrate is added, initially the bacteria are unable to metabolize it. They need a period of "acclimation" where they are growing and strengthening and perhaps even undergoing genetic changes. If the intermediate is toxic then the bacteria may be killed or the formation of the next strain that metabolizes it may be stopped so that it accumulates and destroys the system. Bacteria often work with a succession of strains so that complete mineralization needs more than one organism which does not alone have the required genetic capability. As will be discussed in the anaerobic section, mixed cultures can degrade better both qualitatively and quantitatively than single cultures. This may involve mutation. Vandenbergh et al.\textsuperscript{18} took different strains of bacteria, found out what they could grow on and not; and then did transformation and conjunction experiments with them. They found that after plasmids had been introduced, strains that couldn't previously metabolize toluene,
could now degrade it. They also isolated the plasmids necessary for activity. Their findings support the idea of evolution since the bacteria specific for the biodegradation of halaromatic compounds could not utilize toluene. There has been an evolution pathway involving mutations and transfer of genetic material between bacteria so that although at one time they could degrade toluene, they could now only degrade the products of it and not the parent compound.

A less desirable aspect of the ability of bacteria to degrade compounds has been reported by Reed et al.\textsuperscript{19} who noted that some pesticides are degraded quickly by microorganisms before they have eliminated the pests. This phenomenon is called accelerated pesticide degradation. The study examined the response of microbial populations in soils after incorporation of the herbicides, alachlor, butylate, EPTC, carbofuran, cloethocarb, and isophenos. Enzyme assays for alkaline phosphomonoesterase, phosphodiesterase, and rhodanese were also studied. To test the sensitivity of selected microorganisms to the pesticides, formulations of the pesticides were applied to cultures on agar plates and zones of inhibition noted. Those bacteria that grew on pesticides were examined for specific enzymes. The activities varied but were greater for those in the field than control strains indicating that there had been a selection for increased activity in the presence of pesticides. Several isolates capable of growing on the carbamate insecticide, carbofuran, and the thiocarbamate herbicides, butylate and EPTC, also exhibited high rhodanese activity suggesting that this enzyme may be involved in the metabolism of these pesticides. These results suggest that the bacteria capable of developing high rhodanese activity in response to applications of thiocarbamate herbicides (e.g., butylate) may also be involved in the degradation of the chemically similar carbamate insecticides (e.g., carbofuran) without prior exposure to them. Again evolution appears to be the factor here; individual bacterium may acquire the ability to utilize a pesticide by the evolution of specific enzymes. Several organisms may have to coexist in a community to metabolize a pesticide. Assays for specific enzymatic activities of microorganisms shown to metabolize certain pesticide substrates indicate that cross-adaption of the microorganisms for degradation of chemically similar pesticides in the soil environment may exist. The growth pattern of microorganisms is conceptually illustrated in Figure 2. This is a classical viewpoint.
Testing for Recalcitrance

There have been several endeavors to find out which chemicals are recalcitrant and which are not and under what conditions. Obviously if
we have a list of possibilities, then designing hazardous waste treatment becomes all the easier. However, there are problems and these will be discussed in this section. First we need an experimental technique that lets us test for recalcitrance. A "tiered" approach has been advocated by Grady. The philosophy behind tiered testing is that tests are started that isolate those compounds which are readily biodegraded under less than optimum conditions and then give more and more favorable conditions up through the tiers until a compound can be classified as recalcitrant. This is a cost saving approach. Recalcitrance can only be deduced from the failure of biodegradation under the most favorable conditions. It cannot be proved.

**Aerobic Tiered Testing**

*Tier 1* is a screening test where the test compound is the sole carbon and energy source to organisms that have not had prior exposure to it. Biodegradation is measured by dissolved organic carbon (DOC) removal, oxygen uptake, or carbon dioxide evolution. Cometabolism is precluded and so the genetic capability of the bacteria must be wide. The use of an unacclimated culture and the short time of the test means that induction must be the only mechanism responsible for the degradation. Only compounds readily degradable will give positive results. If biodegradation does take place, then we need to know if inhibition is important. Therefore growth of bacteria are tested with the substrate over a range of concentrations. For a non-biodegradable compound inhibition can be tested on another group of bacteria that are growing on a different substrate (i.e., are able to utilize that substrate unless they are inhibited by the test compound). *Tier 2* testing is acclimation and enrichment. Here a culture of bacteria is grown on more than one carbon source. The test compound is applied first in low concentrations and increased if there is evidence of biodegradation. Since there is more than one carbon source, DOC removal, oxygen uptake, and carbon dioxide emission cannot be used. Here chemical analysis is needed specific for the test compound. *Tier 3* is the assessment of the degree of biodegradation. If we get a positive result from Tier 2, then we can go back and do a Tier 1 test with a mixed culture. If the results are positive, the test compound can be degraded by a culture as the sole carbon and energy source. If the results are negative at this point, it doesn't mean that the compound is recalcitrant, but that cometabolism is
needed or that complete biodegradation is impossible. Following the fate of carbon can be done through radioactive labelling and seeing if the carbon ends up in new cells or metabolic intermediates which may then be mineralized. Tier 4 is the actual kinetic studies done on a multicomponent media.

**Anaerobic Tiered Testing**

The philosophy of anaerobic tiered testing is the same as the aerobic. Tier 1 is the use of a single carbon and energy source on an unacclimated, mixed anaerobic culture with the production of carbon dioxide and methane. Since it is possible to predict more or less the amount of gas produced if a substrate is completely mineralized, then mineralization can be deduced easily. Due to the slow growth of anaerobic bacteria, this stage can take a long time. Tier 2 is the acclimation and enrichment stage. The test compound is increased in a multicomponent setting. Complete mineralization needs several interacting populations and furthermore, usually requires changes in the composition of the populations.

**Testing for Recalcitrance**

It is important that we can differentiate whether biodegradation has occurred or not. Intrinsically biodegradable compounds may be removed by abiotic mechanisms (e.g., sorption onto the biological floc or stripping due to aeration). These are not desirable; stripping may be venting the toxin to another media (i.e., the air) and sorption onto the floc may result in a sludge that is unacceptable for disposal. Bearing these abiotic processes in mind, we should not necessarily believe long lists reporting biodegradability for various chemicals. Ghisalba noted that the EPA’s report "Water Related Environmental Fate of 129 Priority Pollutants" (1979) contains many contradictory statements and is suspect. Since this may have resulted from sorption and stripping and not biodegradation, it is important for us to be able to accurately measure biodegradation and furthermore, to be able to design the engineering system so that biodegradation can be maximized. Pitter did experiments on many chemicals in order to assess their biodegradability. He used the substrate as a sole source of carbon for bacteria in activated sludge and calculated the percent chemical oxygen demand (COD)
removal based on the initial concentration. He lists many chemicals and notes that biodegradation can be partial, acceptable, or total. This is an early study. While it does not directly address problems in the design system, it does conclude that there are many parameters that effect the reaction outcome. The physico-chemical aspects (e.g., temperature, solubility, the degree of dispersion of the compound in the media, pH, dissolved oxygen as well as the molecule size, chain length, kind and number of substituents, and stereochemistry) are as important as the biological factors (e.g., the microbiological culture, adaptation, age of the culture, toxicity of the compound, or other substrates). In order to reach a persistent, repeatable degradation of toxics, we must understand how these factors interact. The generation of intermediates, or end products and their effect on the treatment system has been an area of research. Chudoba reported the adverse effects of the accumulation of waste product on bacterial activity. First there was a severe deterioration of the flocculating and settling properties of the mixed culture. Also there was a decrease in the COD removal. The waste product, proportional to the amount of substrate degraded, consisted of high molecular saccharidic polymers. There are still many questions that need to be answered. Can changing reactor configurations lead to a more stable culture? How do the dynamics of the different populations in a culture effect the system? What is the role of intermediates/end products? How can we best acclimate a culture? What about the toxicity of substances to be biodegraded?

Harman et al. expressed dissatisfaction with past screening techniques that did not always include toxicity testing as a parameter. Such investigations failed to establish a primary cause of persistence. Since a large number of xenobiotics are persistent, this may be due to a lack of necessary enzymes by the microorganisms or inhibition by the chemical. Knowing the cause of persistence is important; if it is because of a lack of the appropriate enzymes we can choose either acclimation or bioaugmentation with microorganisms that do possess the enzymes. The authors were interested in what tests we can use to assess persistence and what operating conditions are favorable for the biotransformation of specific compounds. They outline testing procedures for orthochlorophenol (OCP) (highly inhibitory and moderately persistent) and 2,4-dichlorophenoxyacetic acid (DCP) (moderately inhibitory and slightly persistent) using bacteria from activated sludge for SBR's fed with potato starch wastewater. Biodegradation, toxicity, and metabolic activity tests
were undertaken. The biodegradation tests were done by agar plating and shaker flasks. There was more growth of DCP than OCP. Acute toxicity tests were done with Daphnia and the Microtox tests, where OCP was more inhibitory. Metabolic activity tests were carried out by adding the compounds to bacteria and measuring the decreased oxygen uptake compared to a control. OCP showed the greatest decrease in oxygen uptake. Also cellular ATP levels were measured with OCP being found to have smaller levels. The study found discrepancies between individual tests and concluded that no one technique is useful for establishing persistence. To narrow the cause, acute toxicity tests were done and found OCP to be more toxic and inhibitory. To find the rate and degree of biodegradation a simulation test is needed (e.g., the batch shaker test). Use of the three tests will determine if a xenobiotic will persist in the reactor, the cause of persistence, and the estimated rate and degree of biodegradation that can be achieved. The metabolic testing is useful as a complimentary analysis.

Another point in question is if the technique we use to assess biodegradability really show this. In some instances where a high concentration of a chemical is required in order to achieve analytical precision, a toxic but biodegradable substance may be falsely labelled non-biodegradable due to its toxicity to the test organisms. Blok et al. tested the sensitivity of different tests to a variety of chemicals. They suggest a method to minimize this false labelling of chemicals and provided insight into the testing procedures. Various chemicals were selected that were known to be biodegradable, had given variable results, but could be toxic. These chemicals were then tested for degradability via different techniques.

The BOD₅ and closed bottle inhibition tests establish toxicity by measuring the inhibitory effect of chemicals on the oxygen uptake resulting from the degradation of a readily degradable substrate (glucose/glutamic acid or a fatty acid ethoxylate). If a chemical is non-toxic and biodegradable, a higher oxygen uptake than the controls is expected.

The activated sludge - inhibition of respiration test measures the inhibitory effect of the test chemical on oxygen uptake of a respiring sludge while it is degrading a standard substrate at high concentration, thus giving a high respiration rate.

The growth inhibition test is a measure of a decrease in the turbidity of microorganisms in solution as their growth is stopped.
because of the addition of a toxin. The Microtox measures a light reduction by the luminescent bacterial species *Phytobacterium phosphoreum* due to a toxic presence. In the repetitive die away test two cultures are incubated, one with a growth substrate alone and one with the test chemical. Toxicity is a measure of the reduced oxygen uptake.

The tests were compared by an EC50, that is the concentration of the substrate that causes a 50% reduction in the specific test parameter. Microtox is the most sensitive of the tests and may overestimate the toxicity of a chemical. The inhibition of respiration test proved to be consistent and may be good for testing where there are high inoculum concentrations. The growth inhibition, RDA, and BOD tests seemed to have similar sensitivities. From the chemicals tested, no single test gave EC50 values that were consistently related to toxicity. It was found that in most cases, the EC50 concentration were inhibitory. Therefore, the authors suggest that the test substance concentrations used in biodegradability testing should be less than one-tenth that of the EC50 values obtained in toxicity testing.

PILOT STUDIES

**PCB Biodegradation**

Polychlorinate biphenols (PCB) are very toxic chemicals and suspected carcinogens. They include polychlorinated dibenzodioxins (PCDD), polychlorinated dibenzofurans (PCDF), and chlorinated benzenes. They have been found in sediments where they are a threat to the drinking water. A consortium of bacteria has been isolated that appears able to degrade PCBs. Some strains of bacteria were isolated that had varying degrees of competence in degrading PCBs. The bacteria metabolized the PCBs by using enzymes specific to the strain. In many cases a particular isomer may not be completely degraded, and a second species is needed to catalyze the degradation of the intermediate (although it cannot degrade the parent compound). Some 25 strains of indigenous, and chemically mutated (grown naturally in diverse PCB sediments) have been isolated and tested. Of these *Alcaligenes eutrophs* and *Pseudomonal putida* degraded 13 different isomers to the extent of between 80 and 100%. Also *Arthrobacteria* were found to degrade most isomers. The
Biological Degradation of Hazardous Wastes

degradation pathway may occur via oxidation of PCBs to chlorobenzoic acid. *Alcaligenes* are believed to have a dioxygenase that attacks the carbon at position 3,4. *Corynebacterium* sp MBI is believed to have a 2,3 dioxygenase. Effective strains usually contain plasmids. A mutant strain that has lost its plasmid can no longer metabolize PCBs. A few strains mineralize some chlorinated biphenols but in most cases a strain can degrade one ring of the PCB, but are unable to degrade the resulting chlorobenzoates which accumulate. *Pseudomonas* sp B13 is a hybrid that has the relatively non-specific oxygenase of *P.putida* and can completely mineralize the chlorobenzoates. In general, the more heavily chlorinated structures resist degradation. The mechanism of hydroxylation of PCBs has not been elucidated, nor an enzyme isolated. However, two pathways have been proposed.

The first involves initial hydroxylation in the 2,3 position of the less substituted ring, followed by meta cleavage and subsequent degradation of the alphatic portion to form unsubstituted benzoic acids. The chlorines on the alphatic carbon are lost in this process. This may not be the mechanism for the degradation of PCB's substitutes in all the orthopositions. A second pathway may be the preferential 3,4 dioxygenase of *A.eutrophus* H850 or the 2,3 dioxygenase of *Corynebacterium* sp MBI.

The U.S. EPA has written about a biological process developed by Bioclean Inc. called the Bioclean Naturally Adapted Microbial Process. Here the microbes can be bacteria or fungi; the method being aerobic, anaerobic, or facultative. This has been successful with cleaning pentachlorophenol bearing sediments. The process was evaluated along with abiotic mechanisms for the decontamination of PCB bearing sediments and was found to be quite useful. The bioclean process does not need pretreatment of the sediments and does not appear to generate any RCRA wastes or emissions. It involves two steps:

1. The extraction, sterilization, and solubilization of the contaminants using high pH and temperature.

2. The bacterial destruction of the contaminant.

These take place over a three day period and have the capacity for treating 22-28 m³/day. The processing cycle has the following stages:
1. The contaminated sediment is fed to a digester in a slurry if that is how it is received. The final digester charge is 2/3 water and 1/3 solids. It is made alkaline by the addition of sodium hydroxide and then the temperature raised to 82°C for one hour. The mix is agitated throughout.

2. It is extracted, cooled to 30°C, and neutralized. Bacteria is inoculated and left for 48-72 hours. The process is aerobic so sterile filtered air is fed through.

3. The treated batch is discharged to a dewatering pit where the sediment is separated for redeposition.

4. A decontamination period follows after which Stage 1 can begin again.

Under SITE, the "superfund innovative technology evaluation" scheme developed to accelerate new hazardous waste treatment technologies, Detox Industries (Texas) reported a way to treat PCE, creosote, oil, phenolics, and pentachlorophenol in-situ. They culture mutant microorganisms that feed on the target contaminant and then apply them to the site in an aqueous solution. The compounds are slowly metabolized over a period of several months. Ultimately the bacteria die from lack of nutrients. Problems with the process are that heavy metals can inhibit the growth of the bacteria, but Detox states that 14,000 cu. yd. of PCB contaminated soil was reduced from levels of 2900 ppm to 1 ppm at 20% of the cost of excavation.

Kampbell et al. investigated the use of a soil bioreactor for the degradation of isobutane, butane, and propane and found that the mineralization was rapid and extensive. At higher concentrations the biodegradation rate was limited by the microbial capacity to metabolize the organic compound. At 90 cm depth they found less biodegradation and they linked this to the liner which was used to protect the groundwater from being contaminated, but kept the soil wetter. So better drainage is needed. The probable intermediates are alcohols and ketones which are produced through the hydroxylation of the alkenes by monoxygenase bacterial enzymes which start alkene metabolism. These are readily degraded in oxygenated soil. The hydrocarbons were completely degraded without any accumulation of the propellant
hydrocarbons or intermediates in the leachate. The overall efficiency of the bioreactor is 95-99% at 24°C although the acclimation time was slow. The ability to degrade volatile organic compounds decreased at low temperatures. But for soil temperatures between 12°C and 24°C, the bioreactor functioned well. The study showed an average transformation of greater than 90% for the volatile organic compounds between 1984-1985. If the temperature is sustained, then there could be good biodegradation all year. The bioreactor removed TCE as rapidly as the most refractory propellant hydrocarbon. The study showed that organic compounds can be removed from the air by permeation through soil bioreactors. Further work is needed to determine the most appropriate texture and type of soil, to see if the addition of mineral nutrients will enhance biodegradation, and to assess the effect of flow rate, bed depth, organic vapor concentration, and soil moisture content on the system. The predicted behavior of the bioreactor based on laboratory studies agreed closely with the actual behavior of the field system.

Methyl Ethyl Ketone

Skladany et al.²⁹ found in-situ treatment of methyl ethyl ketone (MEK) to be successful. Detox industries supplied two submerged bioreactors to a plant that had MEK as a waste product. The first was to act as the biodegradation unit and the second as the polishing unit. Bacteria were to be grown on the fixed plastic film and supplied oxygen, organic carbon, the inorganic nutrients nitrogen and phosphorous, and a pH range of 7-8. The reactors were put in place and given four weeks pretreatment before MEK was fed in batch mode. They found that the MEK was degraded to such an extent that the bacteria would no longer have a growth substance and so additional waste producers were fed to the reactor. At this point a heat exchanger was installed in the reactor in readiness. The compounds were cyclohexanone, miscellaneous organics, and miscellaneous solvents. The cyclohexanone was readily biodegraded, the MEK continued to be removed to >99% and the others degraded slightly less. The system was sensitive to shock loadings and care had to be taken in loading the compounds. However the in-situ overall biodegradation of MEK and other compounds proved to be successful. The reactors were in operation between May 1986 to May 1987 and 3,092,919 gallons of wastewater were treated.
A patent was granted to Research Co., San Francisco, CA for an in-situ process for the biodegradation of hydrocarbon soil where oxygen is pumped into the soil using a borehole in the earth's surface having hydrocarbon-degrading microbes in place.

**Landfill Leachate**

Ying et al. investigated the feasibility of adding powdered activated carbon (PAC) to sequencing batch bioreactors already in place for the treatment of landfill leachate from the Hyde Park landfill, Niagara, NY instead of a subsequent PAC treatment. Since many organic compounds are readily absorbed on to PAC, the authors proposed that biodegradation would be enhanced because of the long residence time of the compounds on PAC. This was a successful pilot scale study where improvements were found in the effluent quality, with more organic removal and better sludge settling with better dewaterability, and the bacteria being more resistant to shock loading of the wastewater. The effluent needed treatment with only 4% of the carbon which would have been needed for treating the raw leachate alone. Nitrification and denitrification were observed in all bioreactors with no aeration during the last two hours of treatment so the PAC-SBR treatment can accomplish nitrogen removals as well. So a high quality effluent was produced compared to the two stage biodegradation followed by activated-carbon treatment. Additional work by Weber et al. studied the effect that an intermittent treatment of a SBR-PAC reactor would have on degradation rates. A pilot study was initiated to test the feasibility of the process. A former hazardous waste site was found to be contaminated by a wide variety of hazardous organic substances. Since there was a projected low groundwater pumping rate at this site, the on-site treatment was expected to be most cost effective if carried out on an intermittent basis. They found that the rate of COD removal was adversely affected by decreases in operation frequency and decreasing operation temperatures. But all the biological BOD was removed within a reaction period of 24 hours despite periods as long as three weeks between operations. The addition of PAC was advantageous for decreasing the aeration times, for conditions of low temperature and extended lag periods. This pilot study showed that intermittent biological treatment can achieve significant reduction of waste pollutants. COD removal was decreased by decreasing operation frequency but if the reaction periods are extended, the biodegradable