Chapter 8

Environmental Applications

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Introduction

Micro-organisms have several uses in the environment, and new biotechnology can potentially be used to improve these micro-organisms. one application is in the control of pollution and treatment of toxic wastes. As discussed in this chapter, micro-organisms are currently used in pollution control, and the potential applications of biotechnology to treat liquid and solid wastes are numerous. Additionally, techniques are beginning to be used to select micro-organisms that can degrade extremely toxic compounds. In the mining industry, microbes are used to leach metals from mine dumps and concentrate metals from dilute solutions, and there are possibilities for using biotechnology to improve the efficiencies of these processes. A third environmental application of biotechnology is in enhanced oil recovery. About 50 percent of the world's subterranean oil is either reserves trapped in rock or is too viscous to pump. It is possible that either micro-organisms themselves or microbially produced compounds could be injected into oil wells to release the trapped oil.

None of the environmental applications Of new' biotechnology are ready to be marketed, and there are still many technological problems to be overcome. Nevertheless, several companies are pursuing research and development (R&D) in these environmental applications, and their development will progress over the next several years.

Pollution control and toxic waste treatment

Waste products and the pollution problems associated with such products have been part of human existence since the dawn of civilization. Troublesome wastes are of three types: those in the atmosphere, those in aqueous systems, and solids, In the treatment of both liquid and solid wastes, there are significant opportunities for the use of biotechnology. Indeed, most liquid and solid wastes have been dealt with for millennia by natural biological processes, Moreover, humans in their initial attempts to control such wastes have generally resorted to contained biological systems, particularly for the treatment of liquid wastes. The possibilities for using biological systems to control atmospheric pollution, in contrast, are rather limited. The discussion here, therefore, focuses on the applications of biotechnology in the treatment of liquid and solid wastes.

Treatment of nontoxic liquid and solid wastes

Of the conventional microbiological systems for the treatment of liquid wastes now in use, the most complex is that found in publicly owned water treatment plants. As shown in figure ***22,*** there are four basic unit operations in a wastewater treatment plant:

- primary processing;
- secondary processing;
- tertiary processing; and
- digestion.

The primary treatment step removes solids from the wastewater. These solids (sludge) are then either disposed of or sent to a sludge digester, and the wastewater is forwarded to second-

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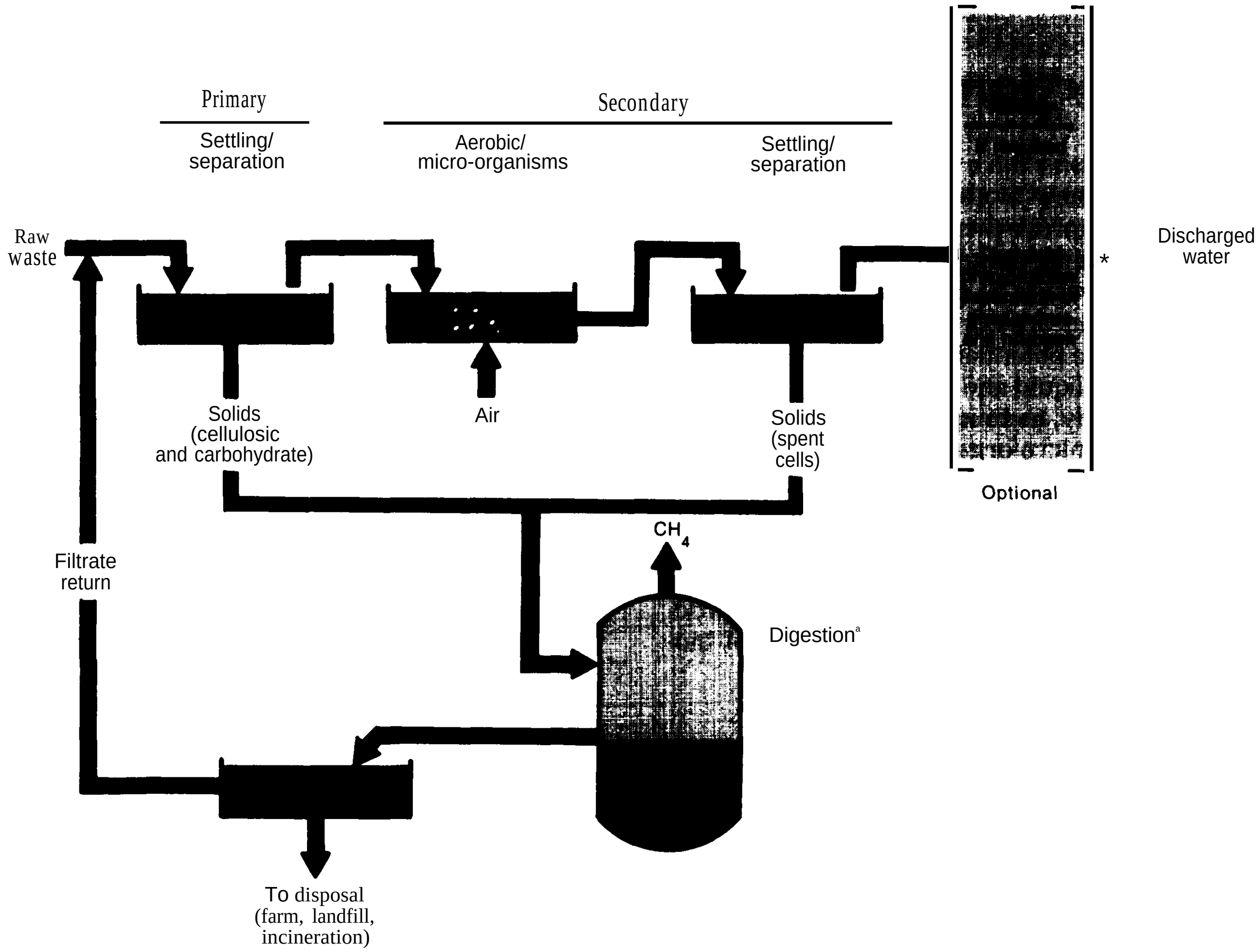
Figure 22.—Steps in Waste Treatment



NOTE: [a]May be aerobic.

SOURCE Office of Technology Assessment.

ary treatment. The secondary treatment system generally consists of natural aerobic microbes in a large open basin with some type of forced aeration. The purpose of this processing step is to degrade the dissolved organic compounds. The sludge resulting from this operation is primarily composed of microbial cells and is either disposed of or sent to a digester. The liquid from the secondary operation is sometimes subjected to tertiary processing, which can involve precipitation and separation of phosphorous and nitrogen, sand filtration, detention ponds, or biological filters. The water from the tertiary unit (or, in the absence of tertiary treatment, from the secondary unit) is returned to the environment.

The sludge digestion process used to treat the sludge resulting from the primary and secondary treatments is conventionally an anaerobic bioprocess. Its purpose is threefold: to reduce the total volume of solids requiring disposal, to reduce the odor, and to reduce the number of pathogenic organisms. Another potential objective of solid waste treatment can be to recover useful methane from the anaerobic bioprocess. Although the effective anaerobic treatment of solid wastes is more a problem of engineering than of biotechnology, there is a possibility that enzymes added to the waste could improve the efficiency of this treatment. Like secondary processing, sludge digestion is a classic bioprocess open to further technological improvements.

The total cost of running publicly owned water treatment systems in the United States has been estimated to be **$5** billion to $6 billion per year (12). The cost of the chemicals used in these systems represents approximately ***20*** percent of the total operating costs (12). The biotechnology-based improvements that could be used by these treatment systems will either:

- increase the capacity of the treatment plants and therefore reduce the need for new capital expenditures,
- replace existing synthetic organic chemical additives, or
- remove newly identified, potentially harmful materials.

Processes similar to those just described for publicly owned water treatment plants are also used in the treatment of industrial wastewater, particularly wastewater from the chemical, petroleum, food processing, and pulp and paper industries. For that reason, biotechnology-based improvements in bioprocessing or solids separation procedures that are applicable to public water treatment systems will very likely be applicable to the industrial sector.

IMPROVEMENT OF CONVENTIONAL WASTEWATER TREATMENT PROCESSES

Both physical and biological processes are utilized in the treatment of wastewater. Improvements in any of these operations would be reflected in reduced capital and operating costs for wastewater treatment. Some specific opportunities for biotechnology-based improvements in wastewater treatment are discussed below.

Solids Separation: Flocculation.-The major physical operation in wastewater treatment is that of solids separation. Suspended solids must be separated during both the primary and secondary treatment steps, Quite frequently, it is also desirable to "thicken" the sludges resulting from these settling operations. The present techniques for accomplishing these separation and thickening operations generally include the use of materiaIs known as flocculants. Because of the increased use and reuse of water, the U.S. market for floe - culants is expanding (8,10).

Examples of classical flocculants are iron or aluminum salts and activated silica. In recent years, synthetic polymers have been used as flocculants, and in some cases, they have produced very promising results (8,10). Unfortunately, most of these synthetic polymers are based on acrylamide, a toxic compound. Moreover, these synthetic polymers are usually subjected to postpoly - merization chemical modification, which adds to their cost. For both safety and economic reasons, therefore, biologically derived flocculants could be very desirable.

A few microbially produced polyelectrolyte polysaccharides that may prove to be effective flocculants have been identified ***(15).*** Before these potential bioflocculants can be commercially applied, microorganisms with the potential for high-Ievel production of effective polysaccharides at low cost will have to be identified. The potential bioflocculants will also have to be tested for their flocculating ability in waste treatment situations. Because the potential bioflocculants are polysac - charides and not proteins, improving their production through recombinant DNA (rDNA) technology may be a complex task (see discussion of polysaccharide biopolymers in ***Chapter 7: Special-t-v Chemicals and Food Additives).*** It should be noted, however, that improvements in microbial polysaccharide production have already been achieved with classical chemical mutagenesis and selection (34).

Sludge Dewatering.–For ease of handling of solid residues from water treatment processes, the water content of such residues must be reduced to a minimum to reduce their total weight. It is particularly important to reduce the water content of these residues to the smallest practical value if the sludge is to be disposed of by incineration.

The sludge dewatering operations with current technology (filtration and centrifugation, for example) result in a solids content of 15 to 40 percent, leaving a water content of 60 to 85 percent.

A significant proportion of the water that is retained is "microscopic" in nature, i.e., it is associated with microbial cells and organic debris present in the sludge. If techniques for releasing this retained water could be developed, they would find a ready and profitable market in the field of residue disposal.

Because much of the water retained in sludge is probably held in polymeric matrixes composed of cellulosics, fats, polysaccharides, and proteins (38), partial degradation of these matrixes by using some combination of cellulases, proteases, amylases, and polysaccharide hydrolyses should release it. Some enzymes potentially useful for sludge dewatering may already be available in sufficient quantities and at economically attractive costs. For other potentially useful enzymes, techniques for economic, high-yield production will have to be developed. In some instances, these developments will simply involve process development using known microbial strains, In other instances, it may be necessary to construct genetically strains of microorganisms for high-level production of specific enzymes and perhaps specifically alter the characteristics of the enzymes through directed protein modification. It may also be desirable to identify new enzymes from nature that have superior characteristics for use in sludge dewatering.

Conventional Uses of Biological Processes.—Biological processes are used in two operations of wastewater treatment plants, the secondary treatment step involving an aerobic process and the sludge digestion operation involving an anaerobic process. The performance of these standard aerobic and anaerobic biological treatment processes could conceivably be improved by the addition of specific enzymes that could augment the ability of the natural micro-organisms to degrade, for example, protein, starch, polysaccharides, and cellulosics. Such enzymes could be applied selectively at specific wastewater treatment plants where their particular substrates are present in unusually high concentrations. Enzyme "augmentation" might also help accommodate fluctuating loads on a particular treatment plant. The R&D involved in providing enzymes for this purpose would be similar to that for providing enzymes for sludge dewatering.

One potential byproduct of anaerobic bioprocesses is gas. Solid wastes, when held in sanitary landfill, very often encourage the growth of micro-organisms that produce methane. The generation of methane has become a serious problem in many sanitary landfill sites around the country. Experiments concerning the possibility of tapping this methane as an energy resource are in progress (39). Preliminary results indicate that the costs of the required anaerobic equipment are so high as to make the methane gas thus generated uneconomic as an energy source (39). Research is continuing, however, and it is conceivable that at some point in the future, improved micro-organisms or added enzymes could improve to a limited extent the economics of methane production from solid waste.

CONTROL OF ORGANIC MICROPOLLUTANTS

In recent years, significant pollution problems have arisen with regard to drinking water (27). Analyses of surface waters in the United States and Europe have demonstrated the presence at low concentrations of certain naturally arising soluble organic compounds that, when chlorinated, lead to the formation of trihalomethanes (THMs) (23,24,25,28,35,36). Increasing attention is being focused on these precursors of THM, because THMs are classified as potential carcinogens (23,24,25,28,31,35,36). In addition, there has been a series of toxic compounds discovered in ground water called volatile organic compounds (VOCS). VOCS are apparently leached from a variety of sources in the ground. Both VOCS and the precursors of THMs are potentially amenable to biological treatment methods.

Biotechnology can potentially offer improved techniques for the removal of organic micropollutants (13). It is possible, for example, that their removal could be accomplished by the use of enzymes that are capable of polymerizing aromatic compounds (e.g., fulvic acids and phenolic compounds) that often contaminate drinking water. These low molecular weight aromatic compounds are not precipitated in the traditional flocculation procedures, and they do not adsorb readily to activated carbon (26). These compounds also contribute to the formation of THMs and chlorophenOIS during chlorination procedures (2,23,24,25, 28,35,36).

Enzymatic polymerization should result in the removal of most of these low molecular weight aromatic compounds during flocculation procedures. Horseradish peroxidase is one enzyme that can catalyze polymerization reactions of this type (1, 19,20), but it is not clear that purified or even crude horseradish peroxidase could be employed in a cost-effective manner, Other potentially useful polymerizing enzymes are synthesized by micro-organisms, but the current production levels are much too low for these enzymes to be commercially viable (5,6, 11,33). Development of enzymatic polymerization to remove low molecular weight aromatic compounds will therefore require one or more of the following biotechnological developments (13):

- microbial strain improvement and process development programs using known polymerizing enzyme-producing microbial strains;
- identification of micro-organisms that produce useful polymerizing enzymes in high yield; or
- the genetic manipulation of a microorganism to produce high levels of a polymerizing enzyme.

Another potential approach for using biotechnology to remove organic micropollutants from water is to develop micro-organisms that will better degrade these contaminating compounds. Such micro-organisms could be introduced into the water treatment cycle by seeding them onto activated carbon. When activated carbon is employed in water treatment processes, it accumulates naturally occurring microbes from the water. The goal would be to expand the degradative capacity of that microbial population. Although certain micro-organisms of various genera (*Pseudomonas, Acinetobacter, Arthrobacter, Klebsiella)* will degrade a variety of organic compounds, it will probably be necessary to identify or develop novel micro-organisms for the degradation of specific classes of pollutants. One procedure for accomplishing this, plasmid-assisted molecular breeding, is discussed below in the section on toxic waste treatment. Because micro-organisms of the genera listed above are generally present in natural populations, it should be possible to transfer genes that encode degradative enzymes from strains developed in the laboratory to the naturally occurring micro-organisms to encourage their survival in the environment.

The comments above have been made with respect to the control of organic micropollutants in drinking water. Any technology developed to solve the problems associated with drinking water, however, would most likely be applicable to similar organic contamination problems in industrial wastewater.

CONTROL OF HEAVY METAL CONTAMINATION

Heavy metals in drinking water have long been of concern (3). The concern has focused on lead, zinc, copper, and cadmium, although iron, at relatively high concentrations, can also present health risks (38). In addition to contaminating drinking water supplies, heavy metals can have detrimental effects on the operation and performance of biological processes used in wastewater treatment (3), Moreover, heavy metal contaminants in effluents from wastewater treatment plants can have potentially deleterious effects on downstream flora and fauna (3).

Micro-organisms used in metal accumulation (see section on microbiological mining below) are not useful for concentrating the heavy metals discussed here (except copper), because most metals found in contaminated water are ***toxic*** to micro-organisms. One potential approach to solving the problems of heavy metal contamination ***involves*** the use of metallothioneins (see also section on microbiological mining). These proteins, found principally in higher organisms, have a high affinity for various heavy metals (21). The economics of this process would depend on efficient release of the bound metals and reuse of the metallothionein. In fact, the gene coding for mouse metallothionein has been cloned and expressed (22,46). It is possible, therefore, that this protein could be produced in large amounts by bacteria, immobilized on a solid support, and used to extract metals from any solution passed over the immobilized protein (41). This process would be highly controlled and could be used not only for decontamination of waste streams from any industrial process, but also for concentrating metals by the mining industry.

Toxic waste treatment

The chemical and petroleum industries produce a variety of highly toxic organic wastes that are not initially amenable to conventional microbial treatment. Such wastes can be either liquid or solid. For developing biologically based processes that will degrade or otherwise detoxify them, a variety of techniques can be envisioned. A specific microorganism or enzyme will probably have to be developed for each toxic compound.

As the number of toxic compounds that are leached or dispersed into the environment increases, the development of technologies for the treatment of toxic wastes becomes more critical. Toxic wastes are often resistant to natural biological degradation and therefore persist in the environment. Because of their toxic character, developing biotechnological approaches for effective treatment of such wastes may be difficult,

Toxic wastes are generally present in the environment in one of two forms. In some cases, they are purposefully concentrated at specific disposal sites in the form of dumps or lagoons. In other instances, the toxic compounds have already been dispersed into the environment, and they are often present at very low concentrations in soil and water over a fairly large geographical area, In general, toxic wastes in dumps or lagoons are likely to be more amenable to biological treatment than those that have been more widely dispersed. Dumps and lagoons have the advantage of presenting a reasonably high concentration of a particular type of compound or family of compounds at a specific site. Thus, the feasibility of developing a very specific treatment process tailored to both the waste to be detoxified and the environment in which it is found is increased. For more widely distributed wastes, even if biological methods for detoxification are developed, it may be impossible to apply them effectively.

It has often been observed in traditional biological waste treatment systems that the microbial population will adjust to the presence of a toxic compound and eventually achieve some degree of efficiency in its decomposition. This phenomenon, traditionally termed acclimatization, probably represents the selection of mutant microorganisms that are able to both tolerate and degrade the toxic compound. In the case of certain toxic wastes, it may be possible to accelerate this natural mutation and selection process in the laboratory by the use of a technique called chemostat selection.

In traditional chemostat selection, the natural microbial populations present in soil or water samples collected from or near the waste disposal sites are grown continuously over several months in the presence of steadily increasing concentrations of the relevant toxic compound. This process provides steadily increasing selective pressure for the growth of mutant micro-organisms able to tolerate and potentially degrade the toxic substrate. The mutation rate in the chemostat can often be increased by the use of chemical or physical agents.

In a more modern version of chemostat selection, plasmid-assisted molecular breeding, laboratory strains of ***Pseudomonas*** that contain plasmids encoding enzymes involved in the degradation of toxic compounds are added to the chemostat (16). This technique is based on the observation that in nature degradative plasmids often evolve by the recruitment of genes from other plasmids in other micro-organisms. Plasmid-assisted molecular breeding has resulted in the generation of both a mixed-culture and a pure ***Pseudomonas*** strain that degrade the normally recalcitrant molecule, 2)4,5-T) which is a component of herbicides and Agent Orange (16,17). It has also been possible to develop microorganisms that degrade novel substrates by introducing into a single bacterial strain plasmids specifying the degradation of different, but analogous, compounds or different portions of a single degradative pathway (32). Because degradation of a toxic compound usually involves a complex and often uncharacterized series of reactions, it has generally been preferable to let nature select for the proper genetic combination rather than to attempt to construct it de novo in the laboratory.

More recently, however, in a joint research project between the University of Geneva (Switzerland) and the University of Gottingen (F.R.G.), researchers have cloned the gene for one of the key enzymes in the degradation of 2,4,5-T. Their hope is to understand better the degradation pathways that have been naturally selected and possibly use this knowledge to develop a more capable micro-organism (4).

one or more of the techniques described above could potentially lead to the isolation of either a mixed culture or a pure strain that degrades a particular toxic compound that might be able to be used at a disposal site or in a contaminated area. The ***Pseudomonas*** strain that degrades 2,4,5-T has been shown to function successfully both in laboratory tests using contaminated soil and in field tests (17). The micro-organisms being investigated now are aerobic. However, if the toxic waste is present in a dump, it may be necessary to develop anaerobic micro-organisms for detoxification.

The development of micro-organisms for the degradation of both organic micropollutants and toxic wastes will require screening of natural microbial populations or chemostat selection for the appropriate degradative abilities. Once micro-organisms with the ability to degrade the offending compound(s) are available, it may be desirable to transfer that ability to a different microbial host by using rDNA technology to increase the efficiency of degradation or to increase the ability of the micro-organisms to survive in the environment in which they are utilized.

For certain toxic wastes, an alternative approach to detoxification might involve the use of specific enzymes. Enzymatic processes would not totally degrade the toxic compound but simply would convert it to a nontoxic derivative that might then be degraded through natural biological processes. Development of such enzymatic processes would probably involve an extensive research effort, and only very hazardous toxic wastes would justify this degree of effort.

Slime control

Slime can be broadly defined as an aggregation of microbial cells held together by the extracellular polysaccharides produced by the micro-organisms. Wherever water moves in significant quantities, slimes proliferate. The proliferation merely requires the presence of a nutrient, even in minute quantities. In the manufacture of paper, slime control is of major concern because slimes have a very deleterious effect on product quality (7,9)29,30). This problem arises because of the high nutrient availability and favorable temperature and pH in the paper processing environment.

The slimicides currently in use are often heavy metal-based poisons that can result in significant pollution and waste treatment problems ***(7,9,29, 30).*** However, the potential for using enzymatic methods for slime control appears quite promising. The formation of slimes is principally due to the extracellular polysaccharides produced by micro-organisms, so it should be possible to use polysaccharide hydrolyses to degrade the slimes rather than toxic agents to destroy the micro-organisms.

Grease decomposition

Facilities processing meats, poultry, and certain other foods have particularly difficult problems with grease. Grease problems also appear throughout the wastewater collection and treatment cycle. Both pipe collection branches and pump stations are susceptible to the problems of grease accumulation, which include plugging of lines, accumulation of debris in wet wells, slippery working surfaces, unsightly conditions, odor, and operational problems at the facility site. Scum layers on sedimentation tanks and scum mats in digesters cause additional problems. The two basic problems are the congealing (solidifying) of the grease and the difficulty, if not an impossibility, of decomposing the grease once it arrives at the wastewater treatment plant.

Techniques that result in the emulsification and decomposition of grease would significantly improve the operation of all waste treatment facilities. Bacterial formulations have been used in the past for grease decomposition (18). Improvement of these cultures might be possible. Additionally, an enzymatic approach, such as the use of lipases, could improve the operation of waste facilities. * However, because grease contamination generally is in the form of nonaqueous, congealed deposits, substrate availability may be a significant prob-

*See ***Chapter 7: Special%\,*** Chemicals and Food Additives

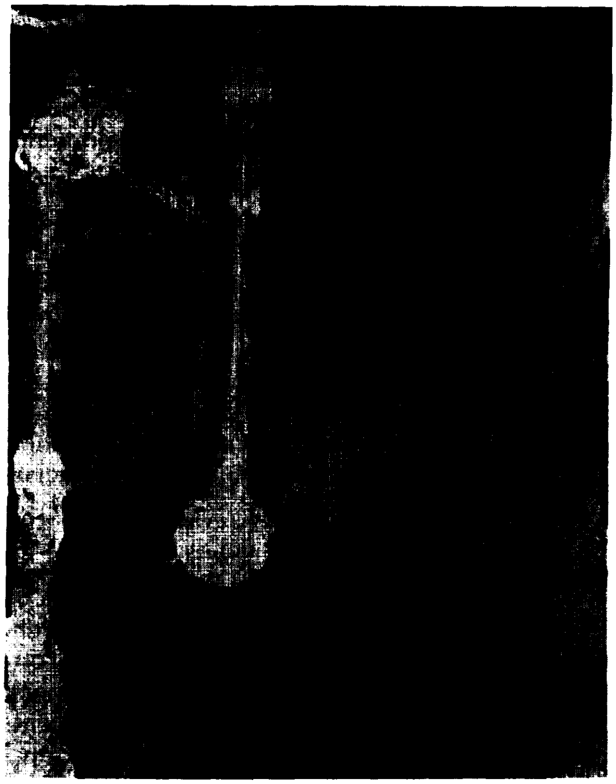


Photo credit, David W Taylor, Naval Ship Research and Development Center

Grease buildup in a holding tank on a U.S. Navy ship after 5 months of normal operation

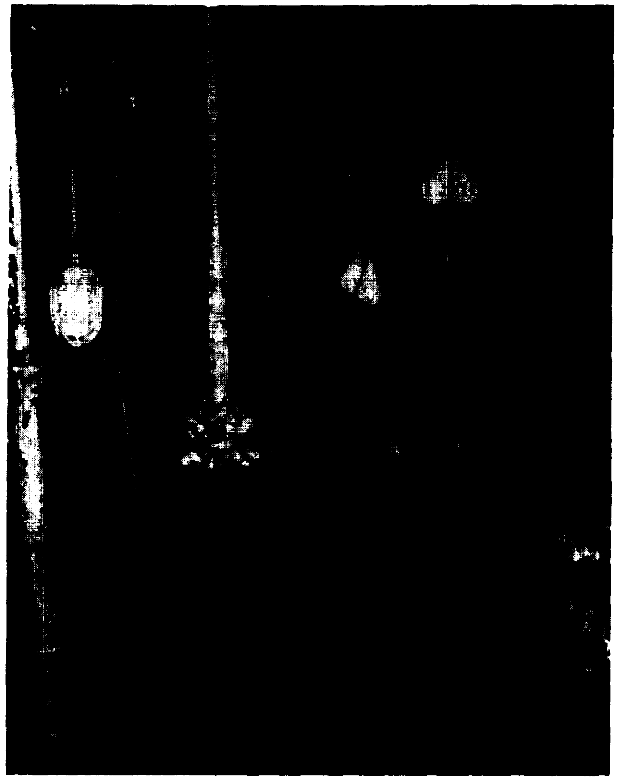


Photo credit: David W. Taylor, Naval Ship Research and Development Center

Grease buildup in the same tank after 4% months of operation with daily addition of decreasing bacteria produced through classical genetic selection techniques

lem. A mechanism for delivering the enzyme to the substrate might solve the problem, but no approaches for accomplishing this have been postulated.

Commercial aspects of biotechnology in pollution control and toxic waste treatment

In contemporary times, basic developments and improvements in water treatment have originated primarily in Western Europe and spread through the Western Hemisphere. Higher population and industrial densities coupled with fewer water resources have forced Western European countries to advance the technology at a much faster pace than required in the United States. In a sense, Western Europe has been the proving ground for new technologies used for water and wastewater treatment. This historical pattern suggests that Western Europe has probably been making initial assessments of the impact of advanced biotechnology in this area. Japan is also conducting a small amount of R&D in this area.

In the United States, there probably is more activity oriented to biotechnology, much of it financed by the U.S. Government, in the municipal solid waste treatment sector than in either the air or liquid waste treatment sectors. Additionally, R&D efforts aimed at improving the technology of wastewater treatment are concentrated in a handful of small bioprocess-oriented companies and certain academic microbiology laboratories. Only recently did interactions begin between these research groups and the plant operators involved in purifying wastewater (14). In the past, industry has relied primarily on engineering consultants, not technology-based companies, to address pollution problems; these consultants have used the most basic existing technologies for treatment of organic wastes.

Two potential barriers to the commercial application of novel approaches to the problems of pollution control and waste treatment are the performance of the products that are developed and scientific uncertainty regarding their application. For example, although the technology for high-level production of enzymes and metallothioneins certainly exists or can be developed, the performance of these products in the desired application is as yet untested. If their performance turns out to be poor, then the R&D effort for commercialization would be much more extensive and might not be worth pursuing. Furthermore, although reasonable approaches can be designed to identify or develop microa-ganisms for the degradation of organic micropollutants and toxic wastes, the success of these approaches is uncertain. It is also unclear whether genetically manipulated micro-organisms or micro-organisms that have been otherwise selected in the laboratory will be able to survive in a nonlaboratory environment. Their ability to survive and function in the field will probably be greatest if the desired degradative activities can be introduced through minimal alteration of a naturally occurring micro-organism.

If the technological barriers to commercial application can be surmounted, the other areas of importance will be markets, Government policy, and regulation. Biotechnological improvements in the area of conventional wastewater treatment processes and slime control would provide economic benefits. If the performance is satisfactory, markets for these products should develop. The primary limitation to commercialization will be the rate of acceptance by the treatment plant operators.

In the case of pollution control, whether it be control of organic micropollutants, heavy metals, or toxic wastes, the primary nontechnological barrier will be Federal Government policy. Biotechnological solutions to these problems are likely to be vigorously pursued only if the Government sets goals and criteria for reducing these contaminants that must be met by both the public and private sectors, The effort for developing these biotechnological solutions will probably initially require Federal funding, However, the requirements could eventually create a demand for a commercial product, and funding might then shift partially to the private sector. At the present time, most industries will not fund biotechnological research on waste treatment problems. They are only interested in licensing or purchasing such technology if it has already been developed.

Another potential barrier to commercialization of products for pollution control is Government regulation of the products themselves. In the case of enzymes and other proteins, few significant safety problems requiring regulation are anticipated, although care must be taken in handling these products. The application of micro-organisms, in contrast, could involve significant regulatory implications. Since the micro-organisms proposed here will have the potential for being released into the environment, it will probably be necessary to establish their safety or to develop methods for their containment at the site of treatment. U.S. policy with regard to the regulation of micro-organisms, particularly genetically manipulated ones, is dynamic. The regulatory constraints that will be placed on the use of micro-organisms in the future, therefore, cannot be accurately predicted. The benefits of using micro-organisms in the area of pollution control to protect human health will have to be carefully balanced against any perceived dangers associated with their use.

Photo credit" G E. Pterce and M K Mulks

Pseudomonas putida, a bacterium capable of degrading hydrocarbons

Microbiological mining

Micro-organisms have been used to some extent in mineral leaching and metal concentration processes for many years. For the most part, these processes have been fortuitous, relying on micro-organisms found associated with mine dumps. With the recent advent of novel biological techniques, people in the mining industry and biologists have begun to think about ways to manipulate genetically some of the micro-organisms important in metal recovery processes to increase their efficiency and allow them to function on a larger variety of substrates.

Mineral *leaching*

More than 10 percent of the copper produced by the United States is leached from ores by micro-organisms (41,48). The micro-organisms used are found naturally associated with ores; the ores are not inoculated with selected strains. Until recently, the use of micro-organisms in the mining industry received little research attention because of the ease of mining high-grade ores and the relatively low energy cost for conventional mineral processing. The use of micro-organisms is gaining new attention, not only because of the depletion of high-grade ore and the soaring cost of energy, but also because of the possibility for genetic manipulation to increase the efficiency and broaden the application of microbial leaching.

There are many advantages to the use of micro-organisms. Besides having a low energy requirement due to their growth at ambient temperature and pressure, micro-organisms work efficiently and are less polluting than smelting techniques. It is possible they could be used for leaching in deep underground sites that are inaccessible to more traditional mining equipment. Mining with microorganisms requires relatively low capital and operating costs, making it feasible for small-scale mining operations. The major drawback to the use of micro-organisms is that the biological processes are slow compared to the equivalent chemical ones (4 I).

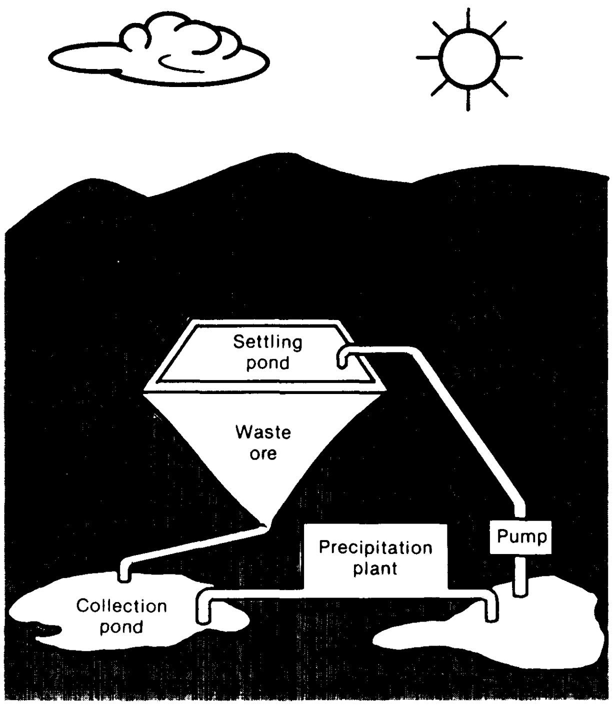
Microorganisms have been used mostly to leach copper and uranium (40). The organism that is most often used in these operations, and consequently the best studied, is ***Thiobacillus ferrooxidans. T. ferrooxidans*** has also been shown to effect solubilization of cobalt, nickel, zinc, and lead (43). This organism, and most of the other bacteria found in mine dump sites, are autotrophic: they use carbon dioxide from the air for their carbon source, and they generate their energy from the oxidation of inorganic matter. In other words, they need no raw material input from miners who wish to exploit them.

The solubilization (leaching) of metals from ore by bacteria occurs in two ways: indirect and direct. The ***indirect method*** involves the transformation of ferrous iron to ferric iron by ***T. ferrooxidans.*** The ferric iron is a very powerful oxidizing agent that consequently converts metal sulfide minerals into acid soluble metal sulfate compounds. For example, ferric iron reacts with copper sulfide to form soluble copper sulfate. The ***direct method*** involves an enzymatic attack by the bacteria on sulfide minerals to give soluble sulfates and, in the process, also oxidizes the ferrous iron to ferric iron. The result is the same, i.e., the metal is soluble in an acid solution. The metal-laden solutions are collected and the metals are removed from solution by chemical and physical processes (see fig. 23). Additionally, since the use of coal as an energy source will increase, bacteria may be used to extract the sulfur from coal, making it less polluting.

Biotechnology could be used by the mining industry to create more efficient micro-organisms. Recombinant DNA technology could be used to effect the following improvements in selected bacteria:

- an enhancement in the rate at which the bacteria regenerate the ferric iron;
- greater tolerance to acidic conditions;
- greater tolerance to saline conditions;
- a decrease in the bacteria's sensitivity to some metals, especially thorium, silver, mercury, and cadmium; and
- an increase in the bacteria's ability to withstand high temperatures for deep mine operations.

Figure 23.—One Possible Configuration for a Leaching Process



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It is likely that rDNA technology will be able to address some of these problems in the near future (41)43,47)!

Microorganisms used in a metal-leaching operation are subjected to very different stresses than those used in a laboratory setting. These differences must be kept in mind when considering the use of rDNA technology, especially since most of the experience with the new technology has focused on well-defined laboratory strains in controlled environments. The bacteria used in leaching endure variable weather conditions, some quite inhospitable for most organisms. When a micro-organism is placed in the environment, it will most likely have to interact with other organisms, and this fact has to be taken into account when researching organisms of interest. Additionally, the mineralogy at each mine site is unique, so microorganisms either will have to be modified for each site or will have to be able to act on varied feedstocks. It is unlikely that feedstocks will be prepared to suit the micro-organism. It seems that the most likely application for genetically manipulated micro-organisms in mineral leaching will be in the same area in which microorganisms are used now, for the treatment of large quantities of discarded waste rock that have small quantities of valuable metals (41,43).

Because the leaching process takes place in the environment, the biological process cannot be completely controlled. Nevertheless, there are ways to optimize the reaction conditions for the microorganisms of interest. The particle size and particle-to-solution ratio of the mine dumps can be manipulated. It is also possible to some extent to control the pH, temperature, and oxygen and carbon dioxide levels. By optimizing these conditions, the leaching organisms can be given an advantage over naturally occurring organisms.

In recent years, the search for new microorganisms in such primeval environments as hot acid springs, volcanic regions, and deep ocean thermal vents has revealed many micro-organisms capable of metal transformations under harsh conditions. Not only are these organisms likely to have application in commercial metal recovery, but they also represent an enormous gene pool for improving existing leaching bacteria through rDNA technology.

Concentration of metals

Another area where micro-organisms could be useful to the mining industry is the concentration of metals from aqueous solutions. The R&D of this kind of process is somewhat easier than R&D of leaching because it can occur in more controlled laboratory situations, making manipulation of the organism's environment possible. There are two biological methods for concentrating metals. In one case, the metals are nonspecifically adsorbed to the surface of the organism. In the other, the metals are specifically bound and taken up by the organism. In the latter mechanism, metals can be "concentrated up to 10)000 times. There is a great diversity of organisms that have been shown to concentrate metals, including bacteria, fungi, and algae. The metals they concentrate are primarily copper, uranium, silver, and the lanthanides. Recombinant DNA technology could be useful in developing organisms to expand the range of metals concentrated.

Another approach to concentrating metals involves the use of specific metal-binding proteins produced in higher organisms. One of the best studied metal-binding proteins is metallothionein, which binds cadmium, zinc, mercury, and copper. The use of these proteins is discussed earlier in the section on pollution control and toxic waste treatment.

Commercial aspects of biotechnology in microbiological mining

In the United States, there is no Federal R&D funding specifically earmarked for mining microbiology. The National Science Foundation and the U.S. Department of Energy (DOE) have funds under various programs that can be used for basic research studies on microorganisms important in mining. In fiscal year 1984, neither agency anticipates funding at levels more than $300,000. The Bureau of Mines of the U.S. Department of the Interior did not fund any microbiology in fiscal years 1981 and 1982. In fiscal year 1983, it funded the Idaho National Engineering Laboratory at about $300,000 to study the leaching and concentrating of cobalt. The Bureau intends to continue the funding of this project at the same level in fiscal year 1984 (45).

Much of the R&D funding in this field comes from both large and small firms in the mining industry. Atlantic Richfield Co. is doing a substantial amount of research in this area. Other large companies investing in microbiological mining include General Electric, Koppers, Eastman Kodak, International Nickel Co., Chevron, W. R. Grace, and Standard Oil of California. Additionally, at least four small U.S. companies, Advanced Mineral Technologies, Inc. (Socorro, N. Mex.), Poly - bac (Allentown, Pa.), Genex, and Biogen S. A.* are researching mining and metal microbiology.

Two spinoff applications could derive from the work in the area of microbiological mining. one application is the recovery of expensive metals such as silver from processes such as photograph developing. In the past, the developing solutions containing the silver were disposed, but with the increased price of silver over the past few years, there has been increasing interest in silver recovery. Another application is using microa-ganisms to reactivate metal catalysts, recovering metals that have been deposited on the catalyst. Both the catalyst is regenerated and the metal is recovered (48).

Several other countries, notably the United Kingdom, Australia, South Africa, and Canada, are interested in the applications of biotechnology in the mining industry. The majority of the R&D, however, is being done by private industry. Very little is funded by the Governments of these countries.

As of mid-1983, there were no genetically manipulated micro-organisms on the market (4.4). Yet it is possible that research efforts could yield useful, new bacteria for leaching and concentration of metals in a few years. If scale-ups and field trials (for leaching) were carried out expediently, marketable products for leaching and concentration could be available in less than 10 years (42). This research is proceeding slowly, however, because of the currently depressed state of the minerals market. Most industry experts hesitate to speculate when micro-oganisms used for mining might reach the marketplace, because the worldwide availability and price of these metals will determine how fast the research will proceed. There will have to be a scarcity of the metal before much microbiological research will be done.

*Biogen is about 80-percent U.S. owned, but most of its work in microbiological mining is done by Biogen S.A. in Switzerland.

Microbial enhanced oil recovery

Conventional oil extraction technologies can recover only about 50 percent of the world's subterranean oil reserves. The balance either is trapped in rock or is too viscous to pump. The application of micro-organisms or their products possibly could be used to aid in the recovery of trapped oil. The use of microbial processes for this purpose is called microbial enhanced oil recovery (MEOR).

The interest in MEOR has increased substantially since 1975. Several conferences on the subject have brought together petroleum engineers and microbiologists to begin to analyze the roles that micro-organisms could play in the recovery of trapped oil. TO date, several field tests have been done, but none have yet revealed a micro-organism that is broadly applicable in MEOR (51).

There are three general experimental approaches to MEOR (.51):

- the stimulation of endogeneous micro-organisms by injection of nutrients into the well,
- the injection of laboratory-selected micro-organisms into the well, and
- the production by micro-organisms of specific biological compounds and the subsequent use of these compounds in wells.

As discussed further below, new biotechnology offers possibilities in the latter two approaches.

Uses of micro-organisms in oil wells

Various microorganisms are now being isolated and examined for properties useful for oil extraction. Micro-organisms evolve gases, notably carbon dioxide, that could aid in repressurizing an oil well. An ideal microbe would use the less valuable parts of oil as a carbon source to produce surfactants or emulsifiers to lower the viscosity of the oil allowing it to be pumped to the surface. Several problems complicate this senario. No micro-organism has yet been found that degrades only the less useful components of oil; micro-organisms usually also degrade the compounds important to the petroleum industry. Some micro-organisms will not degrade the oil at all, but these micro-organisms need to have a carbon source, usually molasses, pumped into the well, and this increases the cost of production.

Microbes currently being studied survive only under conditions of moderate heat, salinity, and pressure (55,56). Given the wide variability in geological deposits, these micro-organisms have limited usefulness. However, there is substantial evidence that the oil reservoir is not as an untenable, restrictive environment for micro-organisms as some laboratory studies would indicate. Micro-organisms can, in fact, be isolated from deep reservoirs, and they may have developed specialized mechanisms to cope with low amounts of oxygen. Other micro-organisms have been isolated that do not need oxygen for growth. Further study of these organisms may lead to the development of micro-organisms useful to the petroleum industry (52).

Use of *microbially produced compounds in oil wells*

Another approach to MEOR, the use of microbially produced compounds in oil wells, could be a relatively near-term application of biotechnology. Biological compounds that could be injected into wells include surfactants and viscosity enhancers and decreases. The search has begun for these compounds, but it is becoming increasingly obvious that little is known about these compounds and the micro-organisms that produce them.

Even with the lack of knowledge, however, two promising compounds have been isolated and studied. One substance, characterized at the University of Georgia, is a glycolipid from a bacteria named H-13. This substance reduces the viscosity of various heavy crude oils (51). Another substance, originally isolated in Israel but now studied in the United States, is called emulsan and has the property of emulsifying oil, allowing better flow and dispersal (54). * Field trials have included the cleaning of an oil tanker hold and an aircraft carrier runway (57). Emulsan proved effective at these jobs and holds promise for use in oil wells. Emulsan is being developed by Petroferm, USA (Amelia Island, Florida), and produced and marketed by Pfizer (50).

● Emulsan is discussed further in ***Chapter 7: Specialty Chemicals and Food Additives***.

Commercial aspects of biotechnology in microbial enhanced oil recovery

Many of the major oil companies are thought to be investing in MEOR (49). The U.S. leader in this field appears to be Phillips Petroleum. Small U.S. firms doing R&D in MEOR include Petroferm, Genetics International (Boston), and Worne Biotechnology (Medford, N.J.). Only one company, Shell Oil Co., has stated that MEOR is too speculative for its R&D laboratories (55). Additionally, the LJ.S. Government, through DOE, is investigating MEOR.

Foreign companies and countries are also investigating MEOR, notably the Swiss firm Petrogenetic AG, the British Government and British Petroleum, the U. S. S. R., and the Peoples Republic of China (49,53),

The status of potential markets for MEOR is very questionable because of the lack of knowledge about MEOR's real potential. However, MEOR could potentially increase the production of oil and decrease the costs of recovery significantly.

Priorities for future research

The applications of new biotechnology in the environment are at a rudimentary stage, primarily because of the lack of knowledge about the genetics and biochemistry of the potentially useful microaganisms and the environment in which they operate. Currently, most basic research is done with pure cultures that do not represent the real world situation. There is certainly no guarantee that a species of bacterium will perform in an outdoor environment as it does in the laboratory. Additionally, scale-up problems will be great because of the large size of the operations. Studies in all of these research areas are interdisciplinary. Unless there is close collaboration between biologists and engineers, it is unlikely that the research will be very productive,

Specific challenges for pollution control and toxic waste treatment include:

- the isolation and characterization of enzymes to polymerize low molecular weight organic compounds,
- better characterization of metallothioneins from various species,
- the identification of polysaccharides to serve as bioflocculants,
- the development of enzymes for sludge dewatering,
- the development of microbial strains or enzymes that degrade toxic compounds, and
- the development of improved polysaccharide hydrolyses to degrade slimes.

Specific challenges for microbiological mining include:

- the development of micro-organisms that could leach valuable metals such as thorium, silver, mercury, gold, platinum, and cadmium;
- a better understanding of the interactions between the micro-organisms and the mineral substances; and
- the development of DNA transfer technologies for use at low pHs.

Specific challenges for MEOR include:

- better biochemical and physiological understanding of microorganisms already present in oil reservoirs,
- the development of a microaganism that degrades only the less useful components of oil, and
- screening of microorganisms for the production of surfactants and viscosity enhancers and decreases.

Chapter 8 references

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