Chapter 7

The Use of Genetically Engineered Micro-Organisms in the Environment

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Figure

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Although most genetically engineered micro-organisms are being designed for contained facilities like fermenters, some are being examined for their usefulness in the open environment for such purposes as mineral leaching and recovery, oil recovery, and pollution control.

All three applications are characterized by:

- the use of large volumes of micro-organisms;
- less control over the behavior and fate of the micro-organisms;
- a possibility of ecological disruption; and
- less basic research and development (R&D) —and a higher degree of speculation—than the industries previously discussed.

Mineral leaching and recovery

All micro-organisms interact with metals, Two interactions that are of potential economic and industrial interest are leaching metals from their ores, and concentrating metals from wastes or dilute mixtures. The first would allow the extraction of metals from large quantities of low-grade ores; the second would provide methods for recycling precious metals and controlling pollution caused by toxic metals.

Microbial leaching

In microbial or bacterial leaching, metals in ores are made soluble by bacterial action. Even before bacterial leaching systems became accepted industrial practice, it was known that dissolved metals could be recovered from mine and coal wastes. Active mining operations currently based on this process (such as those in Rio Tinto, Spain) date back to the 18th century. Presently, large-scale operations in the United States use bacterial leaching to recover copper "rem waste material. Estimates for the contribution of copper leaching to the total annual **J.S.** production range from 11.5 to 15 percent.

Leaching begins with the circulation of water through large quantities–often hundreds of ins—of ore. Bacteria, which are naturally associated with the rocks, then cause the metals to be leached by one of two general mechanisms: either the bacteria act directly on the ore to extract the metal or they produce substances, such as ferric iron and sulfuric acid, which then extract the metal. It appears that simply adding acid is not as efficient as using live bacteria. Although acid certainly plays a role in metal extraction, it is possible that direct bacterial attack on some ores is also involved. In fact, some of the bacteria that are known to be involved in mineral leaching have been shown to bind tenaciously to those minerals.

The application of the leaching process to uranium mining is of particular interest because of the possibility of in situ mining. Instead of using conventional techniques to haul uranium ore to the surface, microbial suspensions can extract the metal from its geological setting. Water is percolated through underground shafts where the bacteria dissolve the metals. The solution is then pumped to the surface where the metal is recovered. This approach, also called "underground solution mining, " **is** already used in Canadian uranium **mines,** where it began almost by chance. In 1960, after only 2 years of operation, researchers at the anium M Uranium Mine found that the natural underground water contained large amounts of leached uranium. In 1962, over 13,000 kilo-

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grams (kg) of uranium oxide were obtained from the water. Thereafter, water was circulated through the mines as part of the mining operation. It has been suggested that extending this practice to most mines would have significant environmental benefits because of the minimal disruption of the land surface. Although the process is slower than the technology currently employed, the operating costs might be lower because of the simplicity of the system, since no grinding machinery is needed. Furthermore, deeper and lower grade deposits could be mined more readily.

Bacterial leaching can also extract sulfur-containing compounds, such as pyrite, from coal, producing coal with a lower sulfur content. Sulfur-containing coals from such areas as Ohio and the Appalachian Mountains are now less desirable than other coals because of the sulfur dioxide they release during burning. They often contain up to 6 percent sulfur, of which 70 percent can be in the form of pyrite. According to recent data, mixed populations of different bacteria, rather than a single species, are responsible for the most effective removal of sulfur—a finding that may lead to the genetic engineering of a single sulfur-removing bacterium in the future.

Applied genetics in strain improvement

The bacterium most studied for its leaching properties has been ***Thiobacillus ferrooxidians (which*** leaches copper), but others have also been identified in natural leaching systems. Although leaching ability is probably under genetic control in these organisms, practically nothing is known about the precise mechanisms. This is largely because little information exists in two critical areas: the chemistry of interaction between the bacteria and rock surfaces; and the genetic structure of the micro-organisms. The finding that mixed populations of bacteria interact to increase leaching efficiency complicates the investigation.

Because of the lack of genetic and biochemical information about these bacteria, the application of genetic technologies to mineral leaching remains speculative. Progress in obtaining more information is slow because less than a dozen laboratories in the Nation are actively performing research.

But even when the scientific knowledge is gathered, two obstacles to the use of genetically engineered micro-organisms will remain. The first is the need to develop engineered systems on a scale large enough to exploit their biological activities. A constant interchange must take place between microbial geneticists, geologists, chemists, and engineers. E.g., the geneticists must understand the needs identified by the geologists as well as the problems faced by the engineers, who must scale-up laboratory-scale processes. The complex nature of the problem can be approached most successfully by an interdisciplinary group that recognizes the needs and limitations of each discipline.

The second obstacle is environmental. Introducing large numbers of genetically engineered micro-organisms into the environment raises questions of possible ecological disruption, and liability if damage occurs to the environment or human health.

In summary, the present lack of sufficient scientific knowledge, scientists, and interdisciplinary teams, and the concerns for ecological safety present the major obstacles to the use of genetic engineering in microbial leaching.

Metal recovery

The use of micro-organisms to concentrate metals from dilute solutions such as individual waste streams has two goals: to recover metals as part of a recycling process; and to eliminate any metal that may be a pollutant. The process makes use of the ability of micro-organisms to bind metals to their surfaces and then concentrate them internally.

Studies at the Oak Ridge National Laboratory in Tennessee have shown that micro-organisms can be used to remove heavy metals from industrial effluents. Metals such as cobalt, nickel, silver, gold, uranium, and plutonium in concen trations of less than 1 part per million (ppm) car. be recovered. The process is particularly useful for recovering metals from dilute solutions **01**

10 to 100 ppm, where nonbiological methods may be uneconomical. Organisms such as the common yeast *Saccharomyces cerevisiae* can accumulate uranium up to 20 percent of their total weight.

The economic competitiveness of biological methods has not yet been proven, but genetic improvements have been attempted only recently. The cost of producing the micro-organisms has been a major consideration. If it can be reduced, however, the approach might be useful.

As with other biological systems, genetic engineering may increase the efficiency of the extraction process. In the *Saccharomyces* system, differences in the ability to recover the metals have been demonstrated within populations of cells. Selection for cells with the genetic ability to accumulate large amounts of specific, desired metals would be an important step in designing a practical system.

Oil recovery

Since 1970, oil production in the United States has declined steadily. The supply can be increased by: accelerating explorations for new oilfields; by mining oil shale and coal and converting them to liquids; and by developing new methods for recovering oil from existing reservoirs.

In primary methods of oil recovery, natural expulsive forces (such as physical expansion) drive the oil out of the formation. In secondary methods of recovery, a fluid such as water or natural gas is injected into the reservoir to force the oil to the well. Approximately 50 percent of domestic crude in recent years has been obtained through secondary recovery.

Recently, new methods of oil recovery have been added to primary and secondary methods, which are called tertiary, improved, or enhanced oil recovery (EOR) techniques. They employ chemical and physical methods that increase the mobility of oil, making it easier for other forces to drive it out of the ground. The major target for EOR is the oil found in sandstone and limestone formations. It is here that applied genetics may play a major role, engineering micro-organisms to aid in recovery.

Oil susceptible to these processes is localized in reservoirs and pools at depths ranging from 100 ft to more than 17,000 ft. In these areas, the oil is adsorbed on grains of rock, almost always accompanied by water and natural gas. The physical association of the trapped oil and the surrounding geological formations varies significantly from site to site. The unknown characteristics of these variations are largely responsible for the economic risk in an attempted EOR.

Enhanced oil recovery

Of the original estimated volume of more than 450 billion barrels (bbl) of U.S. oil reserves, about 120 billion bbl have been recovered by primary and secondary techniques, and another 30 billion bbl are still accessible by these methods. The remaining 300 billion bbl however, are probably recoverable only by EOR methods. These figures include the oil remaining in known sandstone and limestone reservoirs and exclude tar sands and oil shale.

Four EOR processes are currently used. All are designed to dislodge the crude oil from its natural geological setting:

- In *thermal processes,* the oil reservoir is heated, which causes the viscosity of the oil to decrease, and with the aid of the pressure of the air introduced, supports the combustion that forces the petroleum to the producing well. Thermal processes will not be improved by genetic technologies.
- Various crude oils differ in their viscosity—ability to flow. Primary and secondary methods can easily remove those that flow

as readily as **water, but many of the reservoirs contain oil as viscous as road tar.** ***Miscible processes*** use injected chemicals that blend with the crude oil to form **mixtures that flow more readily. The chemicals used include alcohols, carbon dioxide, petroleum hydrocarbons such as propane and butane-propane mixtures, and petroleum gases.** A fluid such as water is generally used to push a "slug" of these chemicals through the reservoir to mix with the crude oil and move it to the surface.

- Chemicals are also used in alkaline flooding, polymer flooding, and combined surfactant/polymer flooding.

In ***alkaline flooding,*** sodium hydroxide, sodium carbonate, or other alkaline materials are used to enhance the flow of oil. Neither natural nor genetically engineered micro-organisms are considered useful in this process.

Polymer flooding is a recent apparently successful method of recovery. It depends on the ability of certain chains of long molecules, known as polymers, to increase the viscosity of water. Instead of altering the characteristics of the crude oil, the aim is to make the injected water more capable of displacing it.

In the ***combined surfactant/polymer flooding*** technique, a detergent-like material (surfactant) is used to loosen the oil from its surrounding rock, while water that contains a polymer to increase its viscosity is used to drive the oil from the reservoir. (See figure 27.)

- ***Other EOR methods*** include many novel possibilities, such as the injection of live micro-organisms into a reservoir. These may produce any of the chemicals used in miscible and chemical processes, from surfactants and polymers to carbon dioxide. One target for EOR is the half million stripper wells (producing less than 10 barrels per day (bbl/d) in the United States.

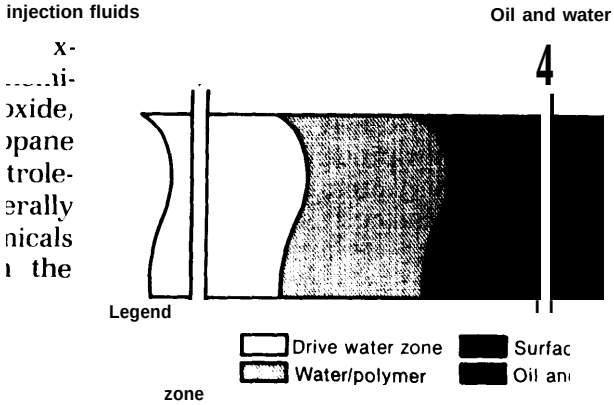
MICROBIAL PRODUCTION OF CHEMICALS USED IN EOR

EOR methods that use chemicals tend to be expensive because of the cost of the chemicals. Nevertheless, potentially useful polymers were found in the early **1960's** and have since been responsible for the recovery of more than 2 million bbl. Polymers such as polyacrylamide and xanthan gum can increase the viscosity of water in concentrations as low as one part in a thousand. Xanthan gum is readily made in large quantities by micro-organisms. Different strains of ***Enterobacler aerogenes*** produce a wide variety of other polymers. A useful biopolymer—one formed by a biological process—might be designed specifically to improve oil recovery.

Xanthan gum, produced by ***Xanthomonas campestris*** and currently marketed by the Kelco division of Merck & Co., Inc., is useful but far from ideal for oil recovery. While it has excellent viscous properties, it is also very expensive. Furthermore, unless it is exceptionally pure, it can plug reservoir pores, since the fluid often has to travel through hundreds of meters of fine pores. To avoid such plugging, the fluid must be filtered to remove bacterial debris before it is injected.

Nevertheless, micro-organisms can be selected or genetically engineered to overcome many obvious difficulties. * With improved properties, polysaccharides (polymeric sugars)

Figure 27.-Chemical Flooding Process



SOURCE: Office of Technology Assessment, *Enhanced Oil Recovery Potential in the United States* (Washington, D. C.: U.S. Government Printing Office, January 1978).

• **A good organism, for example, might have the following desired properties: nonpathogenic to humans, plants, or animals; rapid growth on simple, cheap raw materials; ease of separation from its products; limited detrimental effect on reservoirs, e.g., plugging; easy disposal of cells, e.g., byproduct credits; ability to**

obtained by microbial fermentation could compete with those obtained from alternative sources, especially seaweed. Controlled fermentation is not affected by marine pollution and weather, and production could be geared to market demand.

Biological processes have disadvantages primarily in the costs of appropriate raw materials and in the need for large quantities of solvent. Current efforts to find cheaper raw materials, such as sugar beet pulp and starch, show promise. The need for solvents to precipitate and concentrate the polymers before shipment from plant to field can be circumvented by producing them onsite.

Micro-organisms can also produce substances like butyl and propyl alcohols that can be used as cosurfactants in EOR. It has been calculated that if n-butanol were used to produce crude oil at a level of 5 percent of U.S. consumption, 2 billion to 4 billion lb per year—or four to eight times the current butanol production—would be required. Micro-organisms capable of producing such surfactants have been identified, and genetically superior strains were isolated several decades ago at the Northern Regional Research Laboratories in Illinois. Other chemicals, such as alcohols that increase the rate of formation and stability of chemical/crude oil mixtures and the agents that help prevent precipitation of the surfactants, have also been produced by microbial systems.

The uncertainties of the technical and economic parameters are compounded by the lack of sufficient field experiments. Laboratory tests cannot be equated with conditions in actual oil wells. Each oil field has its own set of characteristics—salinity, pH (acidity and alkalinity), temperature, porosity of the rock, and of the crude oil itself—and an injected chemical behaves differently in each setting. In most cases, not enough is known about a well's characteristics to predict the nature of the chemical/crude oil interaction and to forecast the efficiency of oil recovery.

use water available at site; growth under conditions that discourage the growth of unwanted micro-organisms; no major problems in culturing the bacterium; and genetic stability.

IN SITU USE OF MICRO-ORGANISMS

One alternative to growing micro-organisms in large fermenters then extracting their chemical products and injecting them into wells, is to inject the micro-organisms directly into the wells. They could then produce their chemicals in situ.

Unfortunately, the geophysical and geochemical conditions in a reservoir seldom favor the growth of micro-organisms. High temperature, the presence of sulfur and salt, low oxygen and water, extremes of pH, and significant engineering hurdles make it difficult to overcome these limitations. The micro-organisms must be fed and the microenvironment must be carefully adjusted to their needs at distances of hundreds to thousands of feet. The oil industry has already had discouraging experiences with micro-organisms in the past. In the late 1940's, for instance, the injection of sulfite-reducing micro-organisms, along with an inadvertently high-iron molasses as a carbon source, resulted in the formation of iron sulfide, which clogged the rock pores. One oil company developed a yeast to break down petroleum, but the size of the yeast cells (5 to 10 micrometers, um) was enough to clog the 1-um pores.

Nevertheless, information from geomicrobiology suggests that this approach is worth pursuing. Preliminary field tests have also been encouraging. The injection of 1 to 10 gal of ***Bacillus*** or ***Clostridium*** species, along with a water-suspended mixture of fermentable raw materials such as cattle feed molasses and mineral nutrients, has resulted in copious amounts of carbon dioxide, methane, and some nitrogen in reservoirs. The carbon dioxide made the crude less viscous, and the other gases helped to repressurize the reservoir. In addition, large amounts of organic acids formed additional carbon dioxide through reactions with carbonate minerals. The production of microbial surfactants further aided the process.

Although previous assessments have argued that reservoir pressure is a significant hindrance to the growth of micro-organisms, more recent studies indicate the contrary. The micro-organisms must, however, be selected for increased salt and pH tolerance.

EOR AND GENETIC ENGINEERING

The current research approach, funded by the Department of Energy (DOE) and, independently, by various oil companies, is a two-phase process. The first phase is to find a micro-organism that can function in an oil reservoir environment with as many of the necessary characteristics as possible. The second is to alter it genetically to enhance its overall capability.

The genetic alteration of micro-organisms to produce chemicals used in EOR has been more successful than the alteration of those that may be used in situ. * However, recombinant DNA (rDNA) technology has not been applied in either category. All efforts have employed artificially induced or naturally occurring mutations.

CONSTRAINTS TO APPLYING GENETIC ENGINEERING TECHNOLOGIES IN EOR

The genetic data base for micro-organisms that produce useful polysaccharides is weak. Few genetic studies have been done. Hence, theoretically plausible approaches such as transferring enzyme-coding plasmids (see ch. 2) for polysaccharide synthesis, cannot be seriously contemplated at present. Only the crudest methods of genetic selection for desirable properties have been used thus far. They remain the only avenue for improvement until more is learned about the micro-organism's genetic mechanisms.

The biochemical data base for the characteristics of both the micro-organisms and their products is also lacking. The wide potential for chemical reactions carried out by microbes remains to be explored. At the same time, a system must be devised to allow easy characterization, classification, and comparison of products derived from a variety of micro-organisms.

The physical data base for oil reservoirs is limited. The uniqueness of each reservoir suggests that no universal micro-organism or method of oil recovery will be found. Compounding this problem is the lack of sufficient physical, chemical, and biological information about the reservoirs, without which it is difficult to see how a rational genetic scheme can be constructed for strains. Clearly, the activities of micro-organisms under specified field conditions cannot be studied unless researchers know what the appropriate conditions are.

Three ***institutional obstacles*** exist. First, publication in this field is limited because most research is carried out in the commercial world and remains largely confidential. Second, neither the private nor the public sector has been enthusiastic about the potential role of micro-organisms in EOR, The biological approach has only recently been given consideration as a way to advance the state of the art of the technology, and most oil companies still have limited staffs in microbiology. To date, DOE's Division of Fossil Fuel Extraction has conducted the main Federal effort. Third, any effort to use micro-organisms must be multidisciplinary in nature. Geologists, microbiologists (including microbial physiologists and geneticists), chemists, and engineers must interact to evolve successful schemes of oil recovery. Thus far, such teams do not exist.

Environmental and legal concerns have also inhibited progress. Microbial EOR methods usually require significant quantities of fresh water and thus may compete with municipal and agricultural uses. Furthermore, the use of micro-organisms introduces concerns for safety. All strains of ***Xanthomonas,*** which produce xanthan gum polymer, are plant pathogens. Other micro-organisms with potential, such as ***Sclerotium rolfii*** and various species of ***Aureobasidium*** have been associated with lung disease and wound infections, respectively.

Immediate environmental and legal concerns, therefore, arise from the potential risks associated with the release of micro-organisms into the environment. When they naturally cause disease or environmental disruption, their use is clearly limited. And when they do not, genetic engineering raises the possibility that they might. Such concerns have reduced the private sector's enthusiasm for attempting genetic

* Some of the goals have been to: improve polymer properties to enhance their commercial applicability; improve polymer production (a major mistake has been to reject a micro-organism in the initial screening because its level of production was too low); improve culture characteristics, e.g., resistance to phage, rapid growth, ability to use cheaper raw materials; and eliminate enzymes that naturally degrade the polymers.

engineering. (See ch. 10 for a more detailed discussion of risk.)

GENETIC ENGINEERING OF MICRO-ORGANISMS FOR USE IN OTHER ASPECTS OF OIL RECOVERY AND TREATMENT

Two other aspects of microbial physiology deserve attention: the microbial production of oil muds or drill lubricants, and the treatment of oil once it has been recovered. Drilling muds are suspensions of clays and other materials that serve both to lubricate the drill and to counterbalance the upward pressure of oil. Microbially produced polysaccharides have been developed for this use. Exxon holds a patent on a formulation based on the production of xanthan gum, from ***Xanthomonas campestris,*** while the Pillsbury Co. has developed a polysaccharide (glucan) from various species of ***Sclerotium.*** **At least two of** the small genetic engineering firms have begun research programs to develop biologically produced polysaccharides with the desired lubricant qualities.

Interest in the postrecovery microbial treatment of oil after its extraction centers around the ability of micro-organisms to remove undesirable constituents from the crude oil itself. As an indication of recent progress, three distinct microbial systems have been developed to help remove aromatic sulfur-containing material, a major impurity.

Overview of genetic engineering in mining and oil recovery

The underlying technical problem with the use of genetically engineered organisms in either mining or oil recovery is the magnitude of the effort. In both cases, large areas of land and large volumes of materials (chemicals, fluids, micro-organisms) must be used. The results of testing any new micro-organism in a laboratory cannot automatically be extrapolated to large-scale applications. The change in magnitude is further complicated by the lack of rigid controls. Unlike a large fermenter whose temperature, pH, and other characteristics can be carefully regulated, the natural environment cannot be controlled. Nevertheless, despite the formidable obstacles, the potential value of the products in these areas assures continuing efforts.

Pollution control

Life is a cycle of synthesis and degradation—synthesis of complex molecules from atoms and simple molecules and degradation by bacteria yeast, and fungi, back to simpler molecules and atoms when organisms die. The degradation of complex molecules is an essential part of life. Without it, ". . . we'd be knee-deep in dinosaurs. "[1] A more quantitative statement is equally thought provoking. Livestock in the united States produce 1.7 billion tons of manure annually. Almost all of it is degraded by soil microorganisms.

For a long time people have exploited microbial life forms to degrade and detoxify human sewage. Now, on a smaller scale, science is beginning to use micro-organisms to deal with the pollution problems presented by industrial toxic wastes. Chemicals in their place can be useful and beneficial; out of place, they can be polluting.

Pollution problems can be divided into two categories: those that have been present for a long time in the biosphere-e. g., most hydrocarbons encountered in the petroleum industry and human and animal wastes—and those that owe their origin to human inventiveness-e. g., certain pesticides. Chemicals of both sorts, through mishap, poor planning, or lack of knowledge at the time of their application sometimes appear in places where they are potentially or actually hazardous to human health or the environment.

Pollution can be controlled by microbes in two ways: by enhancing the growth and activity

[1] R. B. Brial Sup "Bacterial Supplementation, What It Can and Cannot Do. " oral presentation to the Ninth Engineering Foundation on Environmental Engineering in the Food Processing Industry, 1979 (Available from Flow Laboratories, Inc., Md.)

of microbes already present at or near the site of the pollution problem, and by adding more (sometimes new) microbes to the pollution site. The first approach does not provide an opportunity for applying genetics, but an example will indicate how it functions.

Enhancing existing microbial degradation activity

Sun Oil successfully exploited indigenous microbes to clean up a 6,000 gal underground gasoline spill that threatened the water supply of a town in Pennsylvania. z [3]First, engineers drilled wells to the top of the water table and used pumps to skim gasoline from the water surface. About half the gasoline was removed in this fashion, but company calculations showed that dissipating the remaining gasoline would require about 100 years. To speedup the process, it was decided to encourage the growth of indigenous bacteria that could degrade the gasoline.

Pollution-control microbes, like all organisms, require a number of different elements and compounds for growth. If the amount of any nutrient is limited, the microbe will not be able to metabolize the pollutant at the fastest rate. The cleanup depended on increasing the growth rate of the bacteria by supplying them with additional nutrients. In the case of the gasoline-degrading bacteria, the gasoline already supplied the hydrocarbon, but the water-gasoline environment was deficient in nitrogen, phosphate, and oxygen. Those three nutrients were pumped down to the water table, bacterial growth increased, and the gasoline was metabolized into innocuous chemicals by the bacteria. As a result, it was degraded in a single year.

Adding microbes to clean up pollution

Genetics may have important applications in approaches to pollution control that depend on adding microbes to the pollution site. Three firms–Flow Laboratories, Polybac Corp., and Sybron / Biochemicals Corp.—sell microbes for such use. Two companies select bacteria for enhanced degradation activity and two mutate bacteria to the same end, but none of the three firms currently uses genetic engineering techniques.

Some "formulations" (mixtures) of bacteria are designed to degrade particular pollutants, such as one that was used to digest the 800,000 gal of oily water that lay in the bilges of the Queen Mary. After a 6-week treatment with the formulation, the water from the bilges was judged safe for disposal into the Long Beach, Calif., harbor. It was discharged without causing an oil slick or harming marine life. q Flow Laboratories markets its services to companies with industrial pollution problems. It investigates the problem, develops a formulation to degrade the pollutants, and sells it.

In addition to industrial pollution problems, Flow markets its products and services for use in sewerage systems, which collect and hold human wastes to facilitate degradation and detoxification. Sludge bacteria in sewerage plants degrade the waste, but they are not present in the lines that carry wastes to the treatment plant. As a result, greases and oils from fat discarded through garbage disposals and from cosmetic oils and creams coat the inside of sewerage lines and reduce their carrying capacity.[5]

Cities have resisted using added microbes in sewerage systems. Standard textbooks simply state that the ideal bacteria will establish themselves in a well-planned and well-managed system. The idea that "better" bacteria can be added to improve the plant operation is not readily accepted.

The value of adding bacteria to large sewerage sytems has not been adequately tested. Because of the size of municipal systems (which already contain tons of sludge bacteria), some have argued that adding a few additional

[2]R. L. Raymond, V. W Jamison, J. O. Hudson, "Beneficial Stimulation of Bacterial Activity in Groundwaters Containing Petroleum Products," AIChE symposium series 73:390-404, 1976.

[3]V. W. Jamison, R. L. Raymond, J. O. Hudson, "Biodegradation of High-Octane Gasoline, " *Proceedings of the Third International Biodegradation Symposium*, J. M. Sharpley and A. M. Kaplan (eds.) (City????: Applied Science Publishers, 1976).

[4]Anon., *Environmental Science and Technology* 13:1180, 1979.

[5]R. E. Kirkup and L. R. Nelson, "City Fights Grease and Odor problems in Sewer Systems," *Public Works Magazine*, October 1977.

pounds of bacteria is unlikely to have any effect. Thus far, the Environmental Protection Agency (EPA) has not recommended adding bacteria to municipal systems; however, EPA suggests that they might be useful in smaller installations and for specific problems in large systems.

Dry formulations are available for use in cleaning drains and pipes in smaller installations, such as restaurants and other food processing facilities. In restaurants, the bacteria are added to the drain at the end of the workday. Bacteria have been selected for their inability to produce hydrogen sulfide, which means that the degrading process does not produce the unpleasant odors frequently encountered in the digestion of oils and fats.[6]

As of November 1979, the pollution control industry had few plans for the genetic manipulation of bacteria, except for the selection of naturally occurring better performers. Consumer resistance to "mutants" is a factor that discourages the move to microbial genetics. Probably even more important is the high cost of establishing and maintaining microbial genetics laboratories. It has been estimated that the cost of carrying a single Ph. D. microbial geneticist is over $100,000 annually.[7] This expense is quite high relative to the $2 million to $4 million sales of all biological pollution control companies in 1978.[8]

Resistance to the use of genetically manipulated bacteria is not universal. Many industrial wastes are oxidized to nontoxic chemicals by biological treatment in aerated lagoons. The process depends on the presence of microbes in the lagoons; over time, those that grow best on the wastes come to dominate the microbial populations. Three companies now sell bacteria that they claim outperform the indigenous strains found in the lagoons. E.g., the Polybac Corp. has sold its products to all seven Exxon biological waste treatment plants to treat chemical wastes. One of its formulations has been used to degrade toxic dioxins from an herbicide spill. One month's treatment with the bacterial formulation reduced the orthochlorophenol concentration from 600 to 25 ppm in a 20,000 -gal lagoon.[9]

Sybron/Biochemical, a division of Sybron Corp., sells cultures of bacteria that are intended to aid in the biological oxidation of industrial wastewater; this company also lists 20 different cultures for application to specific wastes. Patent number 4,199,444 was granted on April 22, 1980, for a process involving the use of a mutant bacterial culture to decolor waste water produced in Kraft paper processing.[10] Other patents are pending on a mixture of two strains that degrade grease and a strain that degrades "nonbiodegradable" detergents.

There is disagreement about the value of adding microbes to decontaminate soils or waters. One point of view argues that serious spills frequently sterilize soils, and that adding microbes is necessary for any biodegradation. The other contends that encouraging indigenous microbes is more likely to succeed because they are acclimated to the spill environment. Added bacteria have a difficult time competing with the already-present microbial flora. In the case of marine spills, bacteria, yeast, and fungi already present in the water participate in degradation, no one has been able to demonstrate the usefulness of added microbes.

Commercial applications—market size and prospects

The estimated market size of pollution-control biological products in 1978 was $2 million to $4 million, divided among some 20 companies,

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'Anon., ewage That Sewage System With ment *Environmental Science and* :1198-1199 197 198-1199, 1979.

[7] Anon., "Biotechnology DNA Research Expenditures in U.S. May Reach $500 Million in 1980, With About $150-200 Million for Commercial Products, " told. *Drug Research* Reports, "The Blue Sheet, " May 28, 1980, p. 22.

ess W *Business Week,* July 5, 1976, p. 280; *Chemical* Week 121:47, 1977; and *Food Engineering 49: 138, 1977,* cited in T. Davi ner, "Microorganisms for Waste Treatment, " *Microbial* 'Non-Bi II, (2 cd., vol. II, (London: Academic Press, 1979), pp. *211-222.*

footnote 6.

Davis, J. E. Blair, and W. Randall, "Communicant ion: Development of Color Removal Potential in Organisms Treating Pulp and Paper Wastewater," *J. Water Pollution Control Fed.,* 382 1978, pp. 382-385.

Tekeo N. oam Contr "Foam Control of Detergent," *Industrial Wastes,* January/February 1980; L. David, J. E. Blair, and C. Randall, '(Mixed Bacterial Cultures Leak 'Non-Biodegradable' Detergent," *industrial Wastes,* May/June 1979.

and the potential market was estimated to be as much as $200 million.[12] These estimates can be compared to Polybac's own sales records. In 1976, its first year, its sales totaled $0.5 million and in 1977, $1.0 million. It expects to reach $5 million in 1981.

To date genetically engineered strains have not been applied to pollution problems. At least one prominent genetic engineering company has decided not to enter the pollution control field, concluding that it was improbable that added microbes could compete with indigenous organisms. More specifically, the possibility of liability problems make the approach even less attractive. Pollution control requires that "new" life forms be released into the environment, which is already seen as precariously balanced. Such new forms might cause health, economic, or environmental problems. The problems of liability that might arise from such applications are enough to deter entrepreneurs from contemplating work in the field at this time.

An additional reason for the reluctance of some companies to engage in this activity is that the opportunities for making money are limited. Selling microbes, rather than their products, may well be a one-shot opportunity. The microbes, once purchased, might be propagated by the buyer. Nevertheless, at least two small companies have announced that they are pursuing efforts to use genetic engineering.

The low-key efforts in this field might accelerate quickly if a significant breakthrough occurred, To date, no "new" organism has appeared that will degrade previously intractable chemicals. The effect of such a development might be enormous.

Genetic research in pollution control

The Oil and Hazardous Materials Spills Branch of EPA currently supports research aimed at isolating organisms to degrade three specific chemical compounds. The work is being carried out on contract; as of November 1979, no field trials of the organisms had been undertaken. Two of the toxic chemicals, pentachorophenol and hexachlorocyclopentadiene, are relatively long-lived compounds and present long-term problems. A fungus and a bacterium that can degrade the first compound have been isolated,[13] and Sybron/Biochemical already sells a culture specifically for pentachlorophenol degradation. The third toxic compound is methyl parathion. Its inclusion is more difficult to understand, since it is degraded within a few days after its application as a pesticide.

Efforts have been made to isolate bacteria that can degrade (2,4-dichlorophenoxy) acetic acid (2,4-D) and (2,4,5-trichlorophenoxy) acetic acid (2,4,5-T), the components of Agent Orange.[14] Strains of the bacterium ***Alcaligenes paradoxus*** rapidly degrade 2,4-D, and the genetic information for the degradation activity has been located on a plasmid. The investigator who found that strain, while optimistic about the opportunities for isolating and transferring other resistance genes, has been unable to find a bacterium that degrades 2,4,5-T or its very toxic contaminant, 2,3,7,8-tetrachlorodibenzo-para-dioxin (TCDD or dioxin).

By far the best known research in this area is that of Dr. Ananda M. Chakrabarty who engineered two strains of ***Pseudomonas,*** each of which has the ability to degrade the four classes of chemicals found in oil spills. Chakrabarty began with four different strains of ***Pseudomonas.*** None of them presented a threat to human health, and each could degrade one of the four classes of chemicals. His research showed that the genes controlling the degrading activities were located on plasmids. Taking advantage of the relative ease of moving such genes among bacteria, he produced two recombinant bacteria.

Chakrabarty has presented evidence that his bacterium degrades complex petroleum mixtures such as crude oil or "Bunker C" oil, and he

[12] See footnote 8.

[13] P. E. Thuma S. G. Brownlee, and R. S. Valentine, "Laboratory Feasibility and Pilot Plant Studies: Novel Biodegradation Processes for the Ultimate Disposal of Spilled Hazardous Materials," National Environment Research Center, U.S. Environmental Protection Agency, Cincinnati, Ohio, 1978.

[14] "pesticide Degrading A Biological Answer to Environmental Pollution by Pollution by Phenox 1979.

has proposed a method for using it to clean up oil spills. The bacteria are to be grown in the laboratory, mixed with straw, and dried. The bacteria-coated straw can be stored until needed, then dropped from a ship or aircraft onto oil spills. The straw absorbs the oil and the bacteria degrades it. [15] To completely cleanup a spill will probably require mechanical efforts in addition to the biological attack. It was the production of one of Chakrabarty's strains that led to the Supreme Court decision on "the patenting of life. " (See ch. 12 for further details.)

The essential difference between the well-publicized Chakrabarty approach and a less well-known one is that all the desired activities in Chakrabarty's approach are combined in a single organism; while in the other method, bacteria bearing single activities are mixed together to yield a desired "formulation." In yet another approach, Sybron/Biochemical uses mutation and selection to produce specialized degradation activities. It also sells mixed cultures for some applications.

The single-organism, multiple-enzyme system has the advantage that every bacterium can attack a number of compounds. The mixed formulations allow the preferential proliferation of bacteria that feed on the most abundant chemical; then, as that chemical is exhausted, other bacteria, which flourish on the next most abundant chemical, become dominant. The preferential survival of only one or a few strains in a mixed formulation might result in no bacteria being available to degrade some compounds. The multienzyme bacteria, on the other hand, can degrade one chemical after another, or alternatively, more than one at the same time.

Federal research support for engineering microbes to detoxify hazardous substances

EPA currently limits its support to research aimed at selecting indigenous microbes, an area that has already attracted some commercial research support. Commercial firms are looking for large-scale markets, such as sewerage systems, or commonly occurring smaller markets, such as gasoline spills and common industrial wastes.

Whatever potential exists in identifying, growing, and using naturally occurring microbes for pollution control pales beside the opportunities offered by engineering new ones, Unfortunately, the potential risks increase as well. EPA has taken a preliminary step toward assessing the risks by soliciting studies to determine what environmental risks may exist from accidentally or deliberately released engineered microbes.

Summary

While some unreported efforts may be underway, genetics has apparently been little applied to pollution abatement. Nevertheless, the production of "new" life forms that offer a significant improvement in pollution control is a possibility. The constraints are questions of liability in the event of health, economic, or environmental damage; the contention that added organisms are not likely to be a significant improvement; and the assumption that selling microbes rather than products or processes is not likely to be profitable.

The factors that have discouraged developments in this area would probably become less deterring if convincing evidence were found that microbes could remove or degrade an intractable pollutant. In the meantime, the research necessary to produce marked improvements has been inhibited. Overcoming this inhibition may require a governmental commitment to support the research, to buy the microbes, and to provide for protection against liability suits. Such a governmental role would be in keeping with its commitment to protecting health and the environment from the toxic effects of pollutants.

- 436 573, 19, 1976, Patent hea
London, England.

Issue and Options—Biotechnology

ISSUE: How can the Federal Government promote advances in biotechnology and genetic engineering?

The United States is a leader in applying genetic engineering and biotechnology to industry. One reason is the long-standing commitment by the Federal Government to the funding of basic biological research; several decades of support for some of the most esoteric basic research has unexpectedly provided the foundation for a highly useful technology. A second is the availability of venture capital, which has allowed the formation of small innovative companies that can build on the basic research.

The argument for Government promotion of biotechnology and genetic engineering is that Federal help is needed in those high priority areas not being developed by industry.

The argument against such assistance is that industry will develop everything of commercial value without Federal help.

A look at what industry is now attempting indicates that sufficient investment capital is available to pursue specific manufacturing objectives, such as for interferon and ethanol, but that some high-risk areas that might be of interest to society, such as pollution control, may need promotion by the Government. Other areas, such as continued basic biological research, might not be profitable soon enough to attract industry's investment, Specialized education and training are areas in which the Government has already played a major role, although industry has both supported university training and conducted its own inhouse training.

OPTIONS:

A. Congress could allocate funds specifically for genetic engineering and biotechnology R&D in the budget of appropriate agencies, such as the National Science Foundation (NSF), the U.S. Department of Agriculture (USDA), the Department of Health and Human Services (DHHS), the Department of Energy (DOE), the Department of Commerce (DOC), and the Department of Defense (DOD).

Congress has a long history of recognizing areas of R&D that need priority treatment in the allocation of funds. Biotechnology has not been one of these. Even though agencies like NSF receive congressional funding, its Alternative Biological Sources of Materials program is one of the few applied programs that is not congressionally mandated. As a result, the fiscal year 1980 budget saw a ***reduction*** in the allocation of funds, from $4.1 million in 1979 to $2.9 million. A congressionally mandated program, analogous to the successful NSF Earthquake Hazard Mitigation program, could be written into law. other programs, such as the competitive grants program at USDA (or the Office of Basic Biological Research at DOE), are also modestly funded.

Increasing the amount of money in an agency's biotechnology program could bring criticism from other programs within each agency if their levels of funding are not increased commensurately. The Competitive Grants Program at USDA has similar problems; those who are most critical of it argue that it should not take funds from traditional programs. Nevertheless, Congress could promote two types of programs: those with long-range payoffs (basic research), and those which industry is not willing to undertake but that might be in the national interest.

B. Congress could establish a separate Institute of Biotechnology as a funding agency.

The merits of a separate institution lie in the possibility of coordinating a wide range of efforts, all related to biotechnology. Among present organizations, biotechnology and applied genetics cut across several institutes and divisions within them. Medically oriented research falls primarily under the domain of the National Institutes of Health (NIH). EPA is concerned with the prevention of pollution; while NSF's effort in biotechnology has been restricted to modest support scattered through several divisions.

The creation of an organization such as the National Technology Foundation (H.R. 6910) would represent the kind of commitment to engineering, in general, that currently does not exist.

Competition for funds within other agencies would be avoided, since funding would now occur at the level of congressional appropriations. A separate institute, carrying the stamp of Government recognition, would make it clear to the public that this is a major new area with great potential. This might foster greater academic and commercial interest in biotechnology and genetic engineering.

On the other hand, biotechnology and genetic engineering cover such a broad range of disciplines that a single agency would overlap the mandates of existing agencies. Furthermore, the creation of yet another agency carries with it all the disadvantages of increased bureaucracy and competition for funds at the agency level.

C. ***Congress could establish research centers in universities to foster interdisciplinary approaches to biotechnology. In addition, a program of training grants could be offered to train scientists in biological engineering.***

The successful use of biological techniques in industry depends on a multidisciplinary approach involving biochemists, geneticists, microbiologists, process engineers, and chemists. Little is now being done, publicly or privately, to develop expertise in this interdisciplinary area.

In 1979, President Carter proposed the creation of generic technology centers (useful to a broad range of industries) as one way to stimulate innovation. The centers would conduct the kind of research that an individual company might not consider cost effective, but that might ultimately benefit several companies. Each center would be jointly funded by Government and industry, with Government providing the seed money and industry carrying most of the costs within 5 years. If the centers were established at universities, startup costs could be minimized.

Several congressional bills contain provisions for centers similar to these. For example, on October 21, 1980, President Carter signed into law a bill (S. 1250) that would establish Centers for Industrial Technology to foster research links between industry and universities. They would be affiliated with a university or nonprofit institution.

One or more of these centers could be specifically designated to specialize in biotechnology. In addition, training grants could be used to support the education of biotechnologists at the centers or elsewhere. Currently, there is no nationwide training program to train students in this discipline. Education programs, especially for the postgraduate and graduate training of engineers, could further the idea of using biological techniques to solve engineering problems.

D. Congress could use tax incentives to stimulate biotechnology.

The tax laws could be used to stimulate biotechnology in several ways. First, they could expand the supply of capital for small high-risk firms, which are generally considered more innovative than established firms, because of their willingness to undertake the risks of innovation. Much of the pioneering work in the industrial application of genetic techniques has been done by such firms. By nature, they are speculative, high-risk investments. Second, the tax law could provide special subsidies to new high-technology firms, which cannot use the standard investment incentives, such as the investment tax credit, because they usually have no taxable profits for the first several years against which to apply the tax credit. Third, tax incentives could be provided for both established and new firms to make the investment of money for R&D more attractive.

There are a number of ways to expand the supply of venture capital. One is to decrease the tax rate on capital gains or the period an asset must be held for it to be considered a capital gain rather than ordinary income. This change could be limited to stocks in high-technology firms in order to focus its impact and minimize revenue loss. Other options involving the stock of high-technology companies are: a tax credit to the investor who purchases the stock; defer-

ment of capital gains taxes on the sales of these stocks if the proceeds are reinvested into similarly qualifying stock; and more liberal capital loss provisions.

In addition to focusing on the supply of capital, tax policy could attempt to directly increase the profitability of potential growth companies. Since most are not profitable for several years, they cannot take full advantage of the investment tax credit—or even the provision for carrying net operating losses back 3 years and forward 7 years to offset otherwise taxable profits. Two proposals may remedy this situation. First, the investment tax credit could be refundable to the extent it exceeded any tax liability of the firm. A preliminary estimate of the revenue loss for this proposal was $1 billion for 1979. Second, new companies could be permitted to carry net operating losses forward for 10 years. This change would give new firms the same number of years over which to deduct losses as established firms.

The final type of tax incentive is directed at increasing R&D expenditures. Two major proposals would permit companies to take tax credits on a certain percentage of their R&D expenses, and on contributions to universities for research.

The R&D credit has been advocated for several reasons. First, it would increase the after-tax return on R&D investments, making them more attractive. Second, it would reduce the degree of risk on such investments; with a 10-percent credit, the real after-tax expense of a $1 million investment is $900,000. Finally, it would give firms maximum flexibility in selecting projects for investment.

Questions have been raised about the cost effectiveness of the credit. For calendar year 1980, the Treasury Department estimated the cost of a lo-percent R&D credit to be $1.9 million. Since R&D costs average only 10 to 20 percent of the total cost of bringing a new product or process to the market, the net reduction in the cost of commercializing an invention would be 1 to 2 percent. Moreover, the commercial stage of innovation is thought to be riskier and costlier than the technical stage. Another problem is that the credit maybe a windfall for firms that would be investing in R&D anyway. Finally, the credit would subsidize R&D devoted to minor product changes or incremental improvements in addition to R&D directed to more fundamental breakthroughs.

One of the provisions of a pending congressional bill (H.R. 5829) provides for a credit of 25 percent for incremental research expenditures above those for a base period. By limiting the credit to incremental expenditures, the bill would create a more cost-effective credit, if passed.

The final type of tax credit would be for corporate contributions to university research. The Treasury Department estimated that a 25 percent credit for research in all fields would cost $40 million in 1980. This credit would be targeted to more fundamental research and not to the subsidy of short-term, incremental projects that are usually a significant part of corporate R&D budgets.

E. Congress could improve the conditions under which U.S. companies can collaborate with academic scientists and make use of the technology developed in universities in whole or in part at the taxpayer expense.

Developments in genetic engineering have kindled interest in this option. Nevertheless, the Government's role in fostering university-academic interaction is far from accepted. Such a role may limit the flexibility of a cooperative effort. At the very least, disincentives such as patent restrictions could be removed.

The controversy has been summed up as follows.

> At the next level of involvement, the Government could identify potential partners, and facilitate negotiations. A more active role would involve the Government's providing startup funds. Finally, the Government could be a third partner, sharing costs with industry and the university. In this case, too large a Government role could lead to Federal intervention in activities that should be the responsibility of business and industry.

'Dennis [illegible] G. S. [illegible]; *Science* 207: 379-384, 1980,

Certainly the Government can facilitate communication; in the health field, NIH, for instance, is an effective stimulus for contacts among scientists.

The possible advantages and disadvantages of university-industry interaction is illustrated by a recent case involving a plan by Harvard University to collaborate with a genetic engineering company. The plan had called for the establishment of a corporation to commercialize the results of research being done in the laboratory of a Harvard molecular biologist, who would have been a principal in the firm. The University would not have been involved in financing or managing the firm, which would also have been housed separately from the campus. However, Harvard would have derived substantial income if the company proved successful through a gift of 10 to 15 percent of the equity and a royalty on sales. After much debate among the Harvard faculty and educators nationwide, the administration decided not to implement the plan because of concerns about possible adverse impacts on academic values.

proponents of such arrangements argue that the universities should reap some return from the commercialization of research conducted by their staff. In addition, many universities are pressed for money, and joint ventures or research funding arrangements with industry provide an attractive source of funds for research programs, especially when Federal support may decline. In return, industry would gain access to the kind of fundamental research that is the foundation for innovation and appears to be especially crucial in the field of genetic engineering, where the gap between basic research and product development is smaller than for other fields.

Opponents of these arrangements, especially ones involving significant interaction as in the Harvard plan, fear that the profit-seeking goals of industry may be incompatible with academic values. The following possible adverse impacts, among others, have been articulated: **1) in**crease in secrecy, to the detriment of the free exchange of ideas so important in academia; 2) discrimination by the university in its hiring and promotion policies in favor of those doing the revenue-producing research; and 3) distortion in the direction of research and in the training of graduate students.

F. Congress could mandate support for specific research tasks, such as pollution control using microbes.

Investment in creating microbes to degrade pollutants is slow because the potential market is thought to be small and because of the severe liability problems that might arise from intentional release of commercially supplied microbes.

But microbes may be useful in degrading intractable waste and pollutants. Genetic determinants for desired degradation activities may be present in naturally occurring organisms, or scientists may have to combine genes from different sources into a single organism. Current research, however, is limited to isolating organisms from natural sources or from mutated cultures. More elaborate efforts, involving recombinant DNA (rDNA) techniques or other forms of microbial genetic exchange, will require additional effort.

A decision by the Federal Government to support research and to reduce liability concerns is probably needed before the potential of microbial control of pollution can be realized. Federal activity might depend on the results of an evaluation **of the** technical feasibility **of** microbial pollution control, which could be made by either an interagency task force or a special commission. If the evaluation is negative, Congress might elect to do nothing to encourage the technology. If the evaluation is positive, Congress might select from the following suboptions:

1. Initiate no research support nor any Federal relief from or limit on potential liability claims. This option would not foreclose private commercial efforts, but it would limit them because of restricted research funds and large liability questions. If sufficiently large markets were anticipated or found, the limitations would be overcome.
2. Initiate research support programs. Research might be directed at problems posed by particular pollutants (contract re-

search). Federal support of biological research is managed by several agencies, and this course would create few, if any, major administrative problems.

3. Guarantee markets **for particular products. In addition to patent protection, which would be of little value in the case of an organism purposefully disseminated into the environment, the Government could offer to buy desirable microbes. This public sector market might provide enough incentive to research to make Federal funding unnecessary, or the market incentive and research support might be used jointly.**
4. **Fix a limit on liability and set up liability insurance, funded partly or wholly by the Government. This option would reduce the financial risk for entrepreneurs who venture to clean up pollutants with microbes, Such an insurance scheme would require that a Federal agency (EPA, for instance) be satisfied that little risk was attendent in the use of the microbe.**
5. **Arrange a scheme to test micro-organisms for known and anticipated risks before they are released. The Federal Government might have to bear these costs as part of a research program.**
6. **Leave most efforts to industry and allow each Government agency to develop programs in the fields of genetic engineering and biotechnology as it sees fit.**

This option, currently the status quo, seems to be favored by some industry officials. If it is worth doing, they argue, industry will do it. To a large extent, the availability of venture capital in the United States has allowed many companies to pursue projects that are deemed practical and economically important. The production of interferon, insulin, ethanol, ethylene glycol, and fructose are cited as examples of successful applications that were motivated by industry.

Generic research, or research that is fundamentally useful to a broad range of companies, will probably not be undertaken by any one company. When the payoff does not come soon enough, the Government has traditionally taken the responsibility for funding the work. E.g., NIH supported 717 basic research projects involving rDNA in fiscal year 1980 at a cost of $91.5 million. Similarly, high-risk research with high capital costs would be likely targets for Government support.

Leaving all R&D in industry's hands would still produce major commercial successes, but would not ensure the development of generic knowledge or the undertaking of high-risk projects.