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Bioremediation: The Application of Biotechnology for the Clean-up of Oil Spills and Industrial Pollutants

by

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Biotechnology and Environmental Quality

In the face of the increasing severity of environmental damage evidenced during this century, there has been a growing awareness on the part of the public and governments that actions are needed to maintain and restore environmental quality. While waste minimization and recycling programs have been instituted in many industries and by public authorities to help conserve resources and to protect the environment against the release of wastes and pollutants, new technologies are needed for the treatment of wastes and pollutants to aid in sustaining development and environmental quality. The accelerated development of biotechnology during the last decade presents new possibilities for dealing with both the current and emerging problems of environmental contamination by oil spills and releases of industrial chemicals.

Biotechnology is one of several competing technologies for the maintenance of environmental quality and must be viewed within the larger spectrum of scientific and engineering disciplines. However, the importance of biotechnology within this overall context has increased significantly within the past five years and will continue to increase.

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This is, in part, due to the exceptionally rapid advances of knowledge, the low environmental impact and cost effectiveness of using biological as opposed to physical-chemical treatments, and also to problems associated with nonbiological treatments, such as the production of gaseous pollutants by incineration. The advantages of bioremediation over other technologies for environmental clean-up are reduced cost, reduced risk of exposure to harmful chemicals and minimal environmental impact.

Biotechnology has provided solutions to deal with environmental problems. When the disposal of wastes into rivers threatened human health and the well-being of aquatic life, wastewater treatment facilities were developed during the nineteenth century as one of the first applications of biotechnology for the maintenance and restoration of environmental quality, and this treatment has been of great benefit to humankind. Since that time there have been only minor changes in the fundamental designs of the original sewage treatment plans and the way organisms are used, and there are significant numbers of failures of these facilities to meet performance criteria. A particular problem occurs when industrial chemicals enter wastewater treatments that are not specifically designed to cope with those chemicals. Biotechnological processes will continue to play a central role in the treatment of municipal, industrial, and agricultural wastes. In the treatment of aqueous and gaseous effluents, biotechnological processes can be regarded as established technologies based on a history of traditional performance of reducing the biochemical oxygen demand (BOD) of the effluent.

Emerging threats to the environment, such as the atmospheric changes that potentially pose a threat of global climate change, the loss of trees and forests due to atmospheric pollutants, and the formation of deserts, are also appropriate for potential biotechnology treatments. Biopolymers, synthetic fuels and other biological alternatives to chemical processes present opportunities for economic and environmental benefit through biotechnology. Biotechnology has the capacity for contributing to sustainable development.

Bioremediation: An Overview

Bioremediation emerged in the 1990's as an important approach for the clean-up of oil spills and numerous industrial pollutants. Bioremediation uses microorganisms to degrade polluting substances to nontoxic end products, such as carbon dioxide and microbial biomass that can be accommodated by the environment without causing further environmental damage. It is a "green solution" to environmental pollution that relies upon natural processes to remove and to detoxify polluting materials. In essence, bioremediation simply speeds up natural degradation process.

In most cases, bioremediation relies upon naturally occurring microorganisms that are indigenous to the contaminated site. Often, the degradative activities of indigenous microorganisms are limited by environmental factors such as the availability of molecular oxygen, phosphates, and fixed forms of nitrogen that can be used to support microbial growth. By overcoming environmental limitations, such as by tilling soils to improve aeration or adding fertilizers to overcome nitrogen and phosphate limitations, the activities of indigenous microorganisms can be stimulated, and the rates of pollutant degradation accelerated. In cases where there is inadequate genetic diversity within the microbial community to effect the degradation of specific pollutants, seed cultures can be added to initiate the degradative process. Seed cultures may be undefined mixtures of microorganisms, such as occur in manures, or may be very specific cultures of microorganisms with defined metabolic capacities, including genetically modified microorganisms.

Treatment of Wastes and Industrial Chemicals in Bioreactors

Liquid wastes are produced by human activities as domestic sewage by various agricultural and industrial operations[2]. In order to maintain water quality, these wastes often are treated in bioreactors before being released to the environment. Contemporary liquid waste treatment facilities aim at reducing the biological oxygen demand (BOD) associated with the organic compounds within the waste. The aim of most liquid waste treatments is a total reduction of degradable compounds that would otherwise cause excessive oxygen consumption in the receiving water bodies. General liquid waste treatment facilities do not target specific classes of compounds.

The introduction of industrial chemicals into sewage treatment facilities and other waste treatment facilities has caused problems because many industrial chemicals are not degraded in traditional waste treatment facilities and escape as pollutants into the environment. Failure of a chemical waste biodegradation system may be due to unfavorable environmental conditions, to the absence of appropriate microorganisms with the necessary catabolic pathways, or to both. Biotechnological processes can, in many cases, overcome the limitations of waste treatment facilities and prevent chemical pollutants from entering the environment.

There are a variety of conventional liquid waste treatment facilities, including activated sludge, trickling filter, and rotating biological contactor units. Each of these waste treatments is aerobic, and oxygen is supplied to foster microbial degradation. The inocula for these facilities come from the microbial communities that naturally develop during the

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[2] For an overview of biotechnology for the treatment of wastes see Levin, Morris A. and Michael A. Gealt, (eds), 1993. *Biotreatment of Industrial and Hazardous Waste*, McGraw-Hill, Inc., New York and Omenn, Gilbert S. (ed),. 1988. *Environmental Biotechnology:Reducing Risks from Environmental Chemicals through Biotechnology*, Plenum Press, New York.

degradation of the waste. For example, an activated sludge portion of the microbial biomass produced during the treatment of a prior batch of waste is used to inoculate an incoming new batch of waste. In the trickling filter and biological rotating contactor systems, a biofilm develops that actively degrades the compound in the waste.

Even commonly used treatment systems such as activated sludge occasionally fail to perform adequately. The development of effective wastewater treatment demonstrate the need for coupling fundamental microbiological studies with engineering principles to develop effective systems. More effective waste treatment systems would contribute to pollution prevention. In the wastewater treatment systems, anaerobic systems are now playing a role alongside traditional aerobic ones. Similar systems for industrial pollutants would make significant contributions to pollution prevention.

Some organic compounds are readily degraded, whereas others are totally undegradable by microorganisms (recalcitrant) at the current time. Many xenobiotic (synthetic - not naturally occurring) pollutants, such as DDT, are halocarbons that have proven recalcitrant to microbial attack. Important groups of halocarbons include halocarbon propellants, solvents and refrigerants, certain organochlorine insecticides, polychlorinated or polybrominated biphenyls and triphenyls, chlorodibenzodioxins, and chlorodivenzofurans. In some cases, compounds once thought to be recalcitrant have later been found to be biodegradable. Until very recently, for example, PCBs (polychlorinated biphenyls) and TCE (trichloroethylene) were thought to be undegradable, but these compounds are now known to be biodegraded.

Because industrial waste often escape degradation by the generalized communities of liquid waste treatment systems, special steps can be taken to design waste treatment facilities for industrial compounds. Sequential batch reactors can be used with each reactor aimed at degrading specific compounds in the industrial waste. Adapted microbial communities are used in each separate reactor so as to accomplish this task. By separating waste streams, microorganisms grow on the specific compounds in each. Compounds such as azo dyes, which are widely used in the textile industry, can be degraded using such bioreactors.

Some compounds are degraded by cometabolism where microorganisms growing on one substrate gratuitously attacks another compound. Supplying the necessary cosubstrate can result in effective degradation of the target compound. Cometabolic tetrachloroethylene degradation, for example, has been demonstrated for methanogenic bacterial consortium growing on acetate in an anaerobic reactor. Extensive aerobic degradation of trichloroethylene, a widely distributed halocarbon pollutant, by a methane-utilizing microbial consortium has been demonstrated. The low specificity of methane monooxygenase allows the conversion of TCE to TCE epoxide, which subsequently spontaneously hydrolyzes to polar (formic acid, glyoxylic acid) products utilizable by microorganisms. *Methylococcus capsulatus* has been reported to convert chloro- and bromomethane to formaldehyde, dichloromethane to CO, and trichloromethane to CO_2 while growing on methane.

The environmental conditions within bioreactors can be altered to favor the degradation of particular compounds. Many modern waste treatment facilities permit varying the oxygen concentration. Oxygen can be substituted for air, as in the UNOX wastewater treatment system, developed by Union Carbide. This system achieves higher biological oxidation rates per unit in volume than are achieved when air is used for aeration. The deep shaft process of ICI uses air injection and pressure to similarly achieve elevated oxidation rates for organic compounds. In other cases, anaerobic zones are included that favor the anaerobic degradation of specific compounds. In this manner, industrial wastes can be treated to prevent the release of organic contaminants into the environment where they can cause environmental harm.

The capability of alternating between aerobic and anaerobic conditions can be very important for the degradation of halogenated compounds such as PCBs. Anaerobic dehalogenation (reductive dehalogenation) removes the halides from such compounds, forming compounds that can then be degraded under aerobic conditions. In the case of PCBs, the higher molecular weight congemers—those PCBs that are highly substituted with chlorine substituents—are attacked under anaerobic conditions to form light congemers—those PCBs that have only a few chloride substituents. The lower weight PCB congemers are attacked under aerobic conditions. Thus, extensive degradation of complex mixtures of PCBs can be achieved by first incubating anaerobically and then aerobically. Similar degradation of other highly chlorinated molecules can likewise be achieved in this way.

Waste treatment systems can also be used to remove inorganic compounds. For example, phosphates can be removed, and thus eutrophication can be prevented. When certain bacteria are grown under anoxic (free of air) conditions, they accumulate poly-β-hydroxybutyrate. If these same bacteria with the accumulated poly-β-hydroxybutyrate are subsequently grown under aerobic conditions, they will take up large amounts of phosphate and incorporate it into polyphosphate, thereby removing it from the wastewater. Several biological phosphorous removal systems have been designed incorporating the removal of phosphate into activated sludge treatment systems. These involve alternating anoxic and aerobic cycles.

While waste treatment systems are best used to degrade organic compounds or to sequester inorganic compounds prior to release into the environment, bioreactors can also be used for the treatment of contaminated soils and waters. Soils, sediments, and waters can be transferred to reactors where environmental conditions and microbial communities can be controlled to optimize the degradation of the contaminating compounds. Some bioreactors can be transported to contaminated sites, minimizing the costs of transferring large amounts of contaminated materials from the site to the bioreactor. The conditions within the reactor can be adjusted to favor the degradation of particular compounds.

In Situ Bioremediation

In situ biodegradation is a natural process which has been going on since the first microbes and excess organic matter were both present in the soil[3]. At its most fundamental, biodegradation is a recycling process essential for the proper maintenance of the carbon and nitrogen cycles in nature. *In situ* biodegradation can be applied to hazardous wastes, and techniques for detecting and enhancing natural *in situ* bioremediation have been developed.

The majority of the novel pollution problems with organic compounds involve chemicals that are xenobiotic, that is compounds synthesized by humans that have no close natural counterparts. Xenobiotic chemicals include pesticides, plastics, and other synthetic compounds that may persist because microorganisms lack the catabolic pathways for degrading them. Given sufficient time, it is assumed that microorganisms will evolve the capacity to degrade such compounds. To short circuit the evolutionary time required for the development of such organisms, it is possible to carry out genetic engineering, or to culture organisms in ways that favor major evolutionary changes.

With regard to pollutants that enter the environment, *in situ* methods are likely to prove most cost effective. However, these *in situ* bioremediation treatments face the problem of identifying limiting factors and delivering appropriately active microorganisms to

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3. For an overview of in situ bioremediation see Hinchee, Robert E. and Robert F. Olfenbuttel (eds),. 1991. *In Situ Bioreclamation: Applications and Investigations for Hydrocarbon and Contaminated Site Remediation*, Butterworth-Heinemann, Stoneham, MA and Hinchee, Robert E. and Robert F. Olfenbuttel (eds),. 1991. *On-Site Bioreclamation: Processes for Xenobiotic and Hydrocarbon Treatment*, Butterworth-Heinemann, Stoneham, MA

the pollutant which, in turn, must be bioavailable. Much work needs to be done on integrated systems that couple engineering and nonbiological aspects of pollutant remediation in the emerging field of bioremediation. In some cases, consortia of microorganisms will be needed and methods for maintaining the appropriate balance of populations within such consortia have yet to be developed.

Site specificity and the inability to predict and to monitor performance have limited the acceptance of biotechnological solutions by engineers and managers charged with the responsibility of deciding on appropriate environmental remediation solutions. Improvements in biotechnological processes for treating environmental contamination should increase the uses of bioremediation in the near future. The majority of current problems of contamination and pollution at specific sites can be treated by microbiological processes based on indigenous organisms.

Currently, several hundred sites are being considered for bioremediation or are actually being treated using this technology. Most of these sites are contaminated with hydrocarbons from creosote or fuel spillages. Some have chlorinated hydrocarbon contaminants such as TCE and PCBs. The degradation of these compounds prevents contamination of neighboring waters and this often is the aim of *in situ* bioremediation. Slow degradation of complex polynuclear aromatics often makes this a long process.

Two major engineering approaches to the design of *in situ* bioremediation have been developed. The first can be applied to shallow contaminated groundwater systems and the saturated zones of soils. Water from a well is used to create a depression in the saturated zone. The water is supplemented with nutrients and an electron acceptor (e.g. oxygen or nitrate) and returned to the aquifer, near the source of the contamination. The groundwater provides nutrients and water to the indigenous bacteria in the contaminated unsaturated soil. The groundwater is drawn into the saturated zone, where it passes over the contaminated regions of the soils, providing nutrients needed for microbial degradation. In such treatment, studies are performed to determine which nutrients are limiting natural *in situ* biodegradation and what concentrations must be supplied for *in situ* bioremediation.

The second approach for *in situ* bioremediation, called *bioventing*, involves treating unsaturated soils. In this approach, air is forced into the vadose zone at a relatively slow rate. Water is returned to the soils along with nutrients via a sprinkler system. Horizontal pipes below the zone of contamination capture the added water and help draw the air into the aquifer.

Biodegradation of non-growth supporting pollutants is a significant process that must be considered in engineering design and the subsequent application of bioremediation technology for specific pollutants. For example, TCE is a widely distributed subsurface contaminant of groundwater that is degraded by monooxygenase and dioxygenase enzymes which are induced by substrates that are structurally unrelated to TCE. There is a wide range of microorganisms which possess the ability to synthesize oxygenases that degrade TCE. These include organisms that can grow with methane, phenol, toluene, and ammonia. By supplying methane, it is possible to stimulate the methanogenic bacteria which degrade chlorinated solvents such as TCE by cometabolism. Thus, methanotrophic bacteria show some promise for bioremediation of halocarbon-contaminated aquifers.

Bioremediation of Oil Spills

Early work on the microbial utilization of petroleum hydrocarbons was ducted in the 1950s and 1960s when petroleum was viewed as an inexpensive carbon source, and single cell protein (microbial biomass) was considered as a possible solution to the perceived impending world food shortage for the predicted global population explosion. Applied studies focused on optimizing microbial growth on low-middle molecular weight hydrocarbons, and basic research studies elucidated the metabolic pathways of alkane, cycloalkane and aromatic hydrocarbon utilization. These studies showed that the microbial degradation of hydrocarbons produced cell biomass and carbon dioxide. They also indicated that the greater the complexity of the hydrocarbon structure, that is, the higher number of methyl branched substituents or condensed aromatic rings, the slower the rates of degradation and the greater the likelihood of accumulating partially oxidized intermediary metabolites.

The wreck of the tanker Torrey Canyon in 1969 focused environmental concern on the fate of hydrocarbon pollutants in the oceans, and research interest quickly shifted to examining the biodegradation of oil under real environmental conditions. These studies revealed that hydrocarbon-degrading microorganisms are ubiquitously distributed in the environment, and that the rates of hydrocarbon biodegradation are limited by abiotic environmental factors; low levels of phosphate and fixed forms of nitrogen in marine environments limit rates of hydrocarbon degradation, and molecular oxygen is required for rapid hydrocarbon biodegradation. The persistence of petroleum pollutants depends on the quantity and quality of the hydrocarbon mixture and on the properties of the affected ecosystem. In one environment, petroleum hydrocarbons can persist almost indefinitely, whereas under another set of conditions, the same hydrocarbons can be completely biodegraded within a few hours or days.

Studies on the natural fate of hydrocarbons in the environment formed the basis for bioremediation, the biotechnological process in which the rates of hydrocarbon biodegradation are accelerated by overcoming the rate limiting factors in order to remove contaminating pollutants[4]. Bioremediation most often uses microorganisms and their biodegradative capacity to remove pollutants. The end-products of effective bioremediation, such as water and carbon dioxide, are nontoxic and can be accommodated without harm to the environment and living organisms. Using bioremediation to remove pollutants is inexpensive as compared to physical methods for decontaminating the environment that are extraordinarily expensive. While many current technologies call for moving large quantities of toxic waste-contaminated soil to incinerators, bioremediation can be performed on site and requires simple equipment that is readily available. Bioremediation, though, is not the solution for all environmental pollution problems. Like other technologies, bioremediation has limitations as to the materials that can be treated, conditions at the treatment site, and the time that is available for the treatment.

The two approaches taken for the bioremediation of petroleum pollutants are the addition of microorganisms (seeding) that are able to degrade hydrocarbons and the modification of the environment, for example, by adding fertilizers or by aerating the contaminated site.

Because hydrocarbon-degrading bacteria and fungi are widely distributed in marine, freshwater, and soil habitats, adding seed cultures has proven less promising for treating oil spills than adding fertilizers and ensuring adequate aeration. Nevertheless, many companies are developing and/or marketing hydrocarbon-degrading seed cultures. Most microorganisms considered for seeding are obtained by enrichment cultures from previously contaminated sites. Some of these seed cultures may be useful for treating heavy oils that contain hydrocarbons that are relatively resistant to degradation, but seed cultures are likely to be of little benefit for the treatment of the bulk of petroleum contaminants.

The initial steps in the biodegradation of hydrocarbons by bacteria and fungi involve the oxidation of the substrate by oxygenases for which molecular oxygen is required. Conditions of oxygen limitation normally do not exist in the upper levels of the water col-

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4. For an overview of petroleum hydrocarbon biodegradation and bioremediation see Atlas, R. M. 1984. *Petroleum Microbiology.* McGraw Hill, NY and Atlas, R. M. and R. Bartha. 1992. Hydrocarbon biodegradation and oil spill bioremediation. *Advances in Microbial Ecology* 12: 287-338.

umn in marine and freshwater environments. Low concentrations of oxygen, however, is often a critical rate limiting factor for the biodegradation of hydrocarbons in soils and aquifers. In surface soils, oxygenation is best assured by providing adequate drainage and by tilling the soils. This can be accomplished with simple farm equipment. When hydrocarbons have migrated into subsurface soils and if they have contaminated aquifers, oxygen can be provided by forced aeration or through the addition of stabilized peroxides that slowly decompose and release molecular oxygen.

Since microorganisms require nitrogen, phosphorus and other mineral nutrients for incorporation into biomass, the availability of these within the area of hydrocarbons degradation is critical. Various types of fertilizers can be applied, including oleophilic fertilizers, such as Inipol EAP22 produced by Elf Equitaine, that are designed to concentrate the nutrients at the oil-water interface where hydrocarbon biodegradation occurs. *In situ* bioremediation of aquifers is a relatively new and promising technique that is limited by finding engineering solutions for distributing mineral nutrients and oxidants in aquifers to permit the full potential of microorganisms for the biodegradation of polluting hydrocarbons to be realized.

Because there is no definition of how clean is clean following an oil spill, regulatory uncertainty necessarily occurs regarding acceptable performance criteria for bioremediation. For bioremediation to become an effective technology, there must be agreement on performance criteria. Surrogate test organisms for risk based ecological effects testing are needed and standardized tests are necessary to verify claims about commercial cultures for oil spill bioremediation. Contingency plans must be made prior to a spill that include consideration of regional differences. While the use of genetically engineered microorganisms for oil spill bioremediation is blocked by regulation, this is not a major problem since the use of such organisms is not scientifically supported as necessary. Bioremediation of oil pollutants can be achieved for the most part by environmental modification—nutrient and oxygen supplementation—and through the actions of naturally occurring microorganisms.

The Exxon Valdez spill in Prince William Sound, Alaska formed the basis for a major study on bioremediation and the largest application of this emerging technology. Oleophilic and slow release fertilizers were tested and subsequently used to treat hundreds of miles of contaminated shorelines. Results from the use of fertilizer solutions unequivocally demonstrate that oil biodegradation rates in Prince William Sound were limited by the availability of nitrogen and phosphorous, and that the clean appearance of rock surfaces following fertilizer bioremediation treatment was directly caused by biodegradation. Rates of stimulation by bioremediation with fertilizers typically was about 3-5 times natural rates of oil biodegradation. Greater stimulation might be achieved by higher levels of nutrient addition, but this could risk ecological side effects such as toxicity to marine life and eutrophication with associated algal blooms. The addition of fertilizers caused no eutrophication, no acute toxicity to sensitive marine test species, and did not cause the release of undegraded oil residues from the beaches. The success of bioremediation program in Prince William Sound has set the stage for the consideration of bioremediation as a key component in any cleanup strategy developed for future oil spills.

Role of Genetically Engineered Microorganisms in Bioremediation

The potential application of recombinant organisms to the environment raises questions relative to risk and regulation[5]. In this regard, methods are clearly needed for monitoring the survival and spread of such organisms; additional research is necessary

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[5] For a review of the potential uses and concerns about deliberate introductions of genetically engineered microorganisms into the environment Stewart-Tull, Duncan E. S. and Max Sussman (eds),. 1992. *The Release of Genetically Modified Microorganisms–Regem 2*, Plenum Press, New York and Sussman, M., C. H. Collins, F. A. Skinner, and D. E. Stewart-Tull (eds),. 1988. *The Release of Genetically-Engineered Micro-Organisms*, Academic Press, San Diego, CA

concerning how to insure the survival of introduced organisms in communities in ways that preclude their untoward effects in nontarget systems.

The absence of a catabolic pathway for xenobiotic compound is no longer an absolute obstacle to finding or engineering microorganisms that can degrade a specific compound. Searching for mutant strains can be extended by using recombinant DNA technology to more rapidly evolve organisms with greater catabolic capacities. Recent advances in molecular biology allow the regulation of gene expression and the substrate specificity of enzymes to be altered. The expression of catabolic genes—genes that code for the enzymes that degrade organic compounds—is often closely regulated, and degradative activity in the environment can be adversely affected by presence of repressors or the absence of inducers. This can be overcome by replacement or modification of the endogenous promotor(s) so that gene expression is not dependent on the presence of specific compounds or environmental factors. Most often genetic engineering of environmental applications relies upon altering the expression of genes already present among the indigenous microbial populations. Depending upon circumstances, constitutive expression or activation of degradative genes in response to temperature, chemicals or specific environmental factors can be obtained. Under the appropriate conditions, these alterations can significantly enhance the degradation potential of microorganisms resulting in a more effective process at lower cost.

A hydrocarbon-degrading pseudomonad was engineered for its ability to degrade petroleum hydrocarbons. It was the organism that the Supreme Court of the United States in a landmark decision ruled could be patented. This engineered microorganism has not been used in the bioremediation of oil spills. It degrades low molecular weight hydrocarbons, but does not degrade the higher molecular weight hydrocarbons that occur as persistent contaminants following oil spills. It has not been used in the bioremediation of oil spills.

Given the current regulatory framework for the deliberate release of genetically engineered microorganisms, it is unlikely that any such organism would gain the necessary regulatory approval in time to be of much use in treating an oil spill. Such organisms, however, could be useful in enclosed oily waste treatment systems, perhaps replacing current disposal methods in which oily wastes are spread over surface soils and allowed to degrade in a process called "land farming" or "landtreatment."

Future Research Needs

One of the commonly cited problems for bioremediation, especially when introducing microorganisms is considered, is the lack of knowledge about microbial community interactions. Research is needed on the factors that control the survival and functioning of microorganisms within complex communities. At present it is not possible to know whether or not an introduced organism will survive, persist, or function. This lack of knowledge limits the ability to predict the outcome of biological waste and pollutant treatment systems. It also raises concerns about the potential long term impacts of introducing organisms. Some researchers feel it is almost impossible to introduce organisms with novel traits, particularly those that overexpress degradative capacities, and to have those microorganisms survive long enough to be of environmental benefit. Others feel that steps must be taken to ensure that introduced microorganisms can be recalled, particularly if they are genetically modified; it is possible to engineer suicide functions into genetically modified microorganisms and this has been proposed as a safety measure.

There is a clear need for performance standards against which the success of bioremediation can be measured. The development of biosensor detection systems could contribute to monitoring system performance as well as serving for other pollutant monitoring. Currently, there is no definition of how low a level of a pollutant can safely remain in the environment, nor how quickly specific pollutants must be eliminated as environmental contaminants. This makes it difficult to evaluate when bioremediation should be employed and when alternate treatments are appropriate.

The Organization for Economic Cooperation and Development (OECD) is in the process of developing a report on the state of the art of biotechnology for a clean environment that covers a very broad range of topics. Both long-standing traditional applications of biological systems for waste treatment and modern approaches for pollution, remediation, and minimization are to be considered in this report. The report should serve as a broad guidance document for Governments. Research areas will be identified, particularly where there are bottlenecks to employing biotechnological solutions to environmental problems.

The American Academy of Microbiology examined the scientific foundations of bioremediation and issued a report in 1992 on the current status and future needs. The report states that a major problem in the development of bioremediation technology is the lack of field sites that are well-characterized with respect to contaminants, geohydrology, and geochemistry; such sites are urgently needed for understanding the natural events that are taking place and also for the transfer technology developed in the laboratory to field conditions. An integrated interdisciplinary approach is essential for the application and verification of bioremediation, and this can only be achieved under environmental conditions. Although some sites may already exist, their openness and flexibility of use is unlikely to support more than a few efforts in the bioremediation community. Predictability of process performance cannot be made with a high level of confidence. In some cases, predictability is limited by the lack of biological information, in other cases by lack of accurate parameter estimation and availability of appropriate models. There is a need to orient aspects of bioremediation research to modern biotechnically integrated science and engineering effort using defined field demonstration sites as vehicles for integration. There is a need for realistic economic analyses of costs and cost saving in the use of bioremediation, as compared to other systems for hazardous waste management, and to promote research, development and demonstration of the next generation technology. Given the magnitude of the cost of hazardous waste management and the potential savings bioremediation may create, it can be anticipated that there will be greater investment in environmental biotechnology and the use of bioremediation.