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Thirty Years and Counting: Bioremediation in Its Prime?

CAROL LITCHFIELD

Bioremediation in its formal sense, meaning any use of living organisms to degrade wastes, has been practiced since humans first populated the world and had to dispose of their trash. Without knowing about the microorganisms in soil and water, people relied on them to destroy waste products from human domestic, agricultural, and industrial activities by converting them to carbon dioxide, water, and additional microbial biomass. With the advent of wastewater treatment plants in the late 19th century (Frankland and Frankland 1894), biotreatment became a more formalized, better-engineered process, although it still was not called bioremediation. Direct land treatment of residues from wastewater treatment plants, refinery sludges, and municipal wastes, as well as composting, has been practiced widely for several decades.

The formal application of this natural technique for treating spills and anthropogenic compounds began 30 years ago with a report entitled *Beneficial Stimulation of Bacterial Activity in Groundwater Containing Petroleum Products,* by R. L. Raymond and coworkers (1975). The authors reported that by adding nutrients to subsurface soil, they could increase the numbers of bacteria that degrade hydrocarbons derived from petroleum and thereby boost the rate of removal of the contaminants. This was the origin of the process that is now called accelerated or enhanced *in situ* bioremediation (ISB). ISB can involve the addition of various nutrients, depending on the soil and groundwater chemistry, but most often includes the addition of an electron acceptor (oxygen in some form or nitrate), phosphates, and perhaps a nitrogen source. The process can be applied to subsurface soils or aquifers as well as to empoundments, lakes, and other bodies of water.

Today, any transformation or removal of contaminants from the environment by organisms is considered to be bioremediation. Several types can be distinguished. Natural bioremediation is the simplest: nothing is added to surficial soils or to the subsurface, but monitoring is performed to ensure that the contaminants are disappearing as a result of microbial action and not because of dilution or migration of the contaminant.

Another type of bioremediation uses aboveground treatment. Excavated soil is treated directly in constructed containers or in a controlled environment, that is, in bioreactors. Bioreactors have the advantage in that they can be used to treat solid, liquid, or gaseous contaminants. And it is worth remembering that not all bioremediation involves microbes; plants have begun to find their way into the field as concentrators or metabolizers (or both) of various compounds. Plants have been found to be especially useful for concentrating heavy metals, which makes for easier collection and disposal of these contaminants.

The biotreatment of petroleum hydrocarbons, one of the earliest modern uses of bioremediation, is less expensive and more effective at cleanup of contaminated sites than the traditional "pump-and-treat" method. Pump-and-treat involved flushing contaminants out of the ground with water and then treating the water—sometimes for decades—and returning it to the ground; moreover, the pump-and-treat method failed to completely remove contaminants with low solubility. This explains why bioremediation became known for treating gas station and refinery spills.

Researchers who had been working on microbial processes for the degradation of various other organic compounds saw wider potential for bioremediation, however. There was particular interest in its use for dealing with chlorinated hydrocarbons, because these compounds have been widely released into the environment, have potential carcinogenic effects, and may contribute to ozone depletion. Soon efforts were under way to use bioremediation, either aerobically or anaerobically, to destroy pentachlorophenol, pesticides, and gaseous products such as styrene.

The introduction of anaerobic biodegradation changed the field dramatically, because anaerobic bacteria can dechlorinate compounds more readily than can aerobic bacteria. Moreover, as oxygen does not need to be added, there is less chance of causing the precipitation of iron compounds, which can plug aquifers. Three classes of materials have traditionally been considered recalcitrant to bioremediation: explosives, metals, and polychlorinated hydrocarbons (PCBs [polychlorinated biphenyls] and their relatives). Nonetheless, progress has been made in all of these areas.

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Doomed to bioremediation

This background on the development of bioremediation shows why most applications of the technology, even today, are for the treatment of petroleum hydrocarbons. Even so, most of the formerly recalcitrant chemicals now seem doomed to bioremediation, thanks to the advances described here. Three recent works (two books and a CD-ROM) offer an overview of the extent of bioremediation's successes and remaining difficulties. A fourth work, a book on bioremediation in general, is described below; it would be useful for anyone teaching a course on bioremediation or environmental science.

The *Proceedings of the 2002 National Conference on Environmental Science and Technology* (Uzochukwu et al. 2003) is based on a conference held in Greensboro, North Carolina, and sponsored by Battelle, a multinational company specializing in science and technology. The objectives of this meeting were to address pollution prevention and solutions to pollution, and to improve communication on these topics. As is typical at Battelle-sponsored conferences, accepted papers were limited to six pages, and thus they were limited in scope and in the amount of data presented. A good companion for this book is *In Situ and On-site Bioremediation, 2003: Proceedings of the Seventh International In Situ and On-site Bioremediation Symposium,* edited by Victor S. Magar and Mark E. Kelley (2004). This CD-ROM is also based on a Battelle-sponsored symposium, but the authors have been given more leeway in the length of their presentations. Together, these two volumes provide a detailed overview of the state of the art in bioremediation. The third work for discussion is *Bioremediation,* volume 8 in the series *Recent Advances in Marine Biotechnology,* edited by Milton Fingerman and Rachakonda Nagabhushanam (2003).

These collections usefully demonstrate several areas of continuing research into biodegradation pathways and their application to the remediation of pollution, such as that caused by methyl tertiary-butyl ether (MTBE). MTBE is the compound that was added to gasoline as the replacement for lead to reduce engine knocking. It was initially considered among the compounds recalcitrant to bioremediation, but more recent research has shown that it is indeed biodegradable. MTBE degradation was the subject of two papers at the 2002 conference, one describing a field study on biostimulation and bioaugmentation for aerobic *in situ* biodegradation (Bagga and Rifai 2003) and the other showing, in an analysis of MTBE-contaminated sites in Texas, that natural attenuation is a viable option (Shore and Rifai 2003). Further information on natural and enhanced bioremediation of MTBE can be found in the Magar and Kelley collection (2004), where details of both laboratory and field studies are described. Thus, recent research has laid to rest the earlier concerns of the Environmental Protection Agency (EPA) and some environmental groups that MTBE would persist in the environment.

Landfarming is a form of bioremediation that involves applying wastes to fields (much as a farmer plows fertilizer into the fields) and allowing the indigenous soil microorganisms to consume the pollutants. It has been widely practiced in the United States and other countries to destroy petroleum refinery wastes. But can these fields that have been contaminated with oily sludges be used again for other purposes? Some research suggests that the answer may be yes. Anoliefo and colleagues (2003) report the results of pretreating soil contaminated by up to 10 percent crude oil with white rot fungus. They found that the growth of the common bean was not seriously affected after the oil-contaminated soil had been pretreated. This finding has important implications for the future utilization of highly polluted but bioremediated soils. To be sure, other questions remain to be answered: Was the nutritional and general chemical composition of beans grown in these soils any different as a result of the oil and the pretreatment? Was there any incorporation of residual pollutants in the plant parts? Regardless, if the central finding is confirmed at other sites, it will represent a promising step forward that could expand the reuse of urban as well as suburban polluted sites.

Better models, please

One oft-recurring theme in bioremediation, and especially in subsurface treatment technologies, is the question of the fate and transport of pollutants. This is especially important for enhanced bioremediation, because it is essential to know where the pollutants, the microbes, and any added nutrients are and how they move over time. Several models have been used to try to provide answers, in particular the generally available model known as MODFLOW, along with Bioplume and its subsequent improvements (Borden and Bedient 1986).

Environmental Science and Technology has three papers on this subject, and I wish that the authors could have shared data and models. If they had, I think they could have made a major push forward in subsurface modeling. That opinion is prompted by the paper by Aburime and colleagues (2003), which stresses the importance of initial field data on model predictions and describes the various results that emerge from different models. This leads the geologist and the regulator to wonder which model is correct. Numerous models have also been developed to describe processes in the vadose zone (the upper layers of the subsurface region, above the water-bearing layer) such as bioventing, in which a vacuum is used to oxygenate the area and allow for microbial degradation of the pollutants. A paper by Rucker (2003) describes a multilayer model for the migration of contamination from the vadose zone, while another contribution, by Chang and colleagues (2003), describes results obtained through sequential estimation theory. All three of these papers offer important information, but that information needs to be integrated into a single modeling effort before we can expect better prediction of the fate and transport of contaminants in the vadose zone. This is critical for the success of *in situ* treatment, yet it appears that for the time being each large site will still have to have a special model developed to reflect local conditions.

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Indeed, modeling is a recurring theme in many sections of Magar and Kelley's (2004) collection. For a model to be useful, it should be field validated. An especially timely paper by DeMarco and colleagues (2004) attempts this with a three-dimensional semianalytical model that allows for uncertainties in biodegradation rates, formation of metabolic by-products, sorption of the contaminant, groundwater velocity, and source concentrations. The model then calculates the probability that EPA groundwater standards will be exceeded and applies the model to a case study in Texas. Applications of this type of model to more sites may result in a model more generally applicable than the current ones. The other major factor that feeds into modeling of fates and transport is the bioavailability of the pollutant. If the compound is too tightly sorbed onto the surface of soil particles, or is bound in the microscopic pores of rocks and shales, then it becomes available to beneficial organisms only when surfactants release it or the geological structure is altered through fracturing or removal to the surface. Bioavailability is the subject of an entire section in the Magar and Kelley CD-ROM but is not directly considered in the collection by Uzochukwu and colleagues (2003).

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Some of the most interesting and exciting chapters in the volume by Uzochukwu and colleagues (2003) are in the section called "Innovative Environmental Technologies." It begins with an overview paper by Bonito (2003) that describes various advancements of sensor technology and includes many links to relevant manufacturers' Web sites. Bonito briefly describes new instrumentation—a chlorophyll detector, a handheld electronic nose that can detect aerosolized chemicals, and an electronic tongue that can provide chemical analyses in real time, for example—then discusses developments more applicable to remediation, namely, webs of sensor pods that can communicate with each other and send data wirelessly. Trips to the field to recover information from data loggers may soon be a thing of the past. These advances could provide great savings of time and money during remediation efforts. Dalton and colleagues describe another technological advancement in their paper on atmospheric sampling using glow discharge ionization (Dalton et al. 2003). Although the technology for aerosol sampling has not yet been miniaturized for field use, it seems likely that it will be soon, given the rapid progress in this area.

Not to be outdone by the volume from the 2002 conference, the Magar and Kelley CD (2004) contains a section on "Environmental Forensics and Novel Methods in Support of Site Remediation." The forensics here is concerned with identifying sources of pollutants and their age. The Nordtest methodology for oil spill identification, an internationally recognized standard test that identifies the sources of a spill by using gas chromatography to obtain molecular "fingerprints" of heavy oils, has been modified. The test relies on the diagnostic ratios of polyaromatic hydrocarbons (PAHs) and 17 diagnostic biomarkers to differentiate similar oils. Results from the application of the modified test to two well-known oil spills are described (Daling et al. 2004). The other technology that has been applied to the forensics of pollution and the estimation of the biodegradation of a contaminant is the use of stable isotopes of carbon or hydrogen (Crowley and Kalin 2004, Kuder et al. 2004, O'Sullivan et al. 2004, Philip et al. 2004, Saber et al. 2004). While these applications are not new, they do reflect more widespread acceptance and use of the technologies.

A different and interesting approach to determining the likelihood of microbial metabolism of a pollutant was reported by Sublette and colleagues (2004) in the Magar and Kelley CD. In this paper, Sublette and his coworkers used beads containing nutrients and placed these "bug traps" in monitoring wells. After 30 days the beads were recovered and the amount of microbial growth determined. Those beads containing nutrients had considerably more biofilm than those without. These results suggest that when monitoring wells are already at a site, they could be used to estimate rates of degradation and the preferred nutrient additions without resorting to the artificial laboratory microcosms commonly used.

Despite the earlier prevalent view that chlorinated hydrocarbons are recalcitrant to bioremediation, research on microbial physiology over the past 18 years has repeatedly shown that this is not so. Indications that the dry-cleaning solvent trichloroethylene (TCE) might be amenable to biotreatment were noted in an early patent in this field (Lawes and Litchfield 1988). Previously, McCarty and coworkers had demonstrated anaerobic dechlorination of TCE and the cometabolism of TCE (Bouwer et al. 1981, Bouwer and McCarty 1983a, 1983b). Even pentachlorophenol is readily degradable aerobically and anaerobically (reviewed by Litchfield and Rao 1998). The largest section in the Magar and Kelley CD-ROM is on the reductive dechlorination and halorespiration of various chlorinated aliphatics, aromatics, and pesticides, and it proves that they should be considered recalcitrant no longer. The progress is exemplified by the full-scale bioremediation of a carbon tetrachloride spill at Schoolcraft, Michigan (Lisiecki et al. 2004). Other extensive field pilot studies have been reported by Granade and colleagues (2004), Finn and colleagues (2004), and French and colleagues (2004), to name only a few.

Another interesting development in the arena of anaerobic degradation has been the production of HRC (Hydrogen Release Compound), a tripolyacetate and glycerol mixture that hydrolyzes in groundwater to provide lactate, from which anaerobic microorganisms produce hydrogen. This follows the successful introduction of an oxygen-releasing compound for aerobic situations. Either of these compounds may be added

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to the contaminated soils, and thus provide an electron acceptor in a nonhazardous, usable form (see *www.regenesis.com/products*).

Victories have also been won over supposedly recalcitrant explosives and highly reactive compounds. During the manufacture of explosives, perchlorate ($HClO_4$) is formed, and this has become a serious groundwater contaminant. Owsianiak and colleagues (2004) described an *in situ* field test showing that induced anaerobic conditions resulted in an average 94 percent reduction of the perchlorate concentration in the groundwater. This impressive feat has prompted these authors to press ahead with a full-scale bioremediation. Similarly, Weeks and colleagues (2004) showed that, in the laboratory, both perchlorate and the explosive RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) could be treated in an aboveground fluidized bed bioreactor dosed with acetic acid as the carbon and energy source and using the perchlorate as the electron acceptor. The success in the lab has led the way to a field test. Meanwhile, Raymond and colleagues (2004) reported the successful degradation of TNT (2,4,6-trinitrotoluene), RDX, and HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-triazine, another explosive) by using a sequential aerobic–anaerobic system at the Iowa Army Ammunition Plant. They had previously used this approach at several other field-scale and full-scale ammunition sites, both for landfarming, as described here, and in aboveground bioreactors. Reductions of the contaminants ranged from 99 percent for RDX to 92 percent for HMX. All of these studies reinforce the view that explosives and other highly reactive compounds cannot duck microbial attack, provided the treatment system is properly designed.

A place for plants, too

Fifteen years ago, phytoremediation was considered something of an oddity. That plants could significantly participate in the active uptake and concentration of chemicals seemed unlikely. TCE has since been found to be amenable to phytoremediation by Wilde and colleagues (2004), who used contaminated groundwater to drip-irrigate cottonwood trees, while Spriggs and colleagues (2004) have planted poplar, cottonwood, and willow trees at the Naval Training Center in Florida, which is contaminated with a range of aliphatic chlorinated compounds. Their report is a preliminary description of the field test and of their laboratory studies. In addition, Dubois and colleagues (2004) reported that native grasses in Canada could remove 11 percent of HMX in 11 weeks. Allen and colleagues (2004), however, were unable to demonstrate any reduction of PAHs in a greenhouse test using alfalfa and ryegrass, so, with our current understanding of the rhizosphere and of plant metabolism, it appears that phytoremediation may not be applicable in all cases.

Still, we know that plants are able to concentrate in their tissues toxic metals present at dilute concentrations in soil or groundwater. A review of this field has been published by Kamnev (2003), which also has an extensive bibliography of the literature through 2002. A comprehensive bibliography up to 2000 is at the Web site of the EPA Phytoremediation Handbook Team (*http://clu-in.org/products/phytobib/biba-b.html*), where more than 1400 citations are listed. Kansas State University also maintains an excellent site at *www.engg.ksu.edu/HSRC/phytorem,* and the *International Journal of Phytoremediation* (*www.aehs.com/journals/phytoremediation/)* is in its sixth volume. Phytoremediation has certainly come of age and should be considered for wetland or surficial soil restoration.

Managing metals

The other area that has seen major advances in the last 10 to 15 years is the bioremediation of metals by microorganisms, specifically through reduction of the metals to less toxic or nontoxic valence states. Microorganisms have been found that can reduce chromium (VI) to the less hazardous chromium (III), selenate to selenite, and even uranium (VI) to uranium (IV). In *Bioremediation* (Fingerman and Nagabhushanam 2003), the chapter by Ruml and Kotrba (2003) deals with microbial control of heavy-metal pollution. After an introduction on metals, a survey of which metals are essential and which are not, and a description of their binding, the authors discuss metallothioneins and metallothionein-like proteins from plants and the use of biomass as a sorbant for metals in solution. They include an informative table showing what is known about the sources and the efficacy of biosorbants. There is also a brief mention of bioleaching and extensive descriptions of metal precipitation by species of *Ralstonia* and *Citrobacter,* sulfide precipitation due to sulfate-reducing bacteria, microbial methylation, and mobilization. Applications include biosorption and entrapment of precipitates in flocs, both briefly described. Interestingly, the authors speculate about using bacteria to generate transgenic plants that could accumulate heavy metals from soils and wetlands. Finally, there are 25 pages of references that alone would have proved the chapter's worth. For anyone not involved in heavy metal research, this excellent chapter is most usefully read before proceeding to the chapters dealing with the bioremediation of specific metals.

Mercury remediation using natural and recombinant microbes has been the subject of much attention. Wagner-Döbler (2003) reviews this field and her own research. After a brief review of microbe–metal interactions and the biotransformation process, the author suggests immobilization of mercury by microbes to improve the growth of plants. She cites as a parallel cadmium immobilization, which improved tobacco growth in laboratory microcosms and may represent a new application for *ex situ* slurry reactors. She has also been involved in the design of a bioreactor to produce elemental mercury without volatilization. The reactor was scaled up and has been tested in Germany; it is currently in use at a chloralkali factory in the Czech Republic. Wagner-Döbler briefly describes the system and makes the point that mercury transformation is a one-step process and therefore amenable to pure culture systems. The use of biofilms to cause mercury reduction is also described. All in all, it appears that after years of research on this dangerous but valuable element, practi-

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cal techniques for treating contamination are on the horizon.

In the Battelle-sponsored book and CD (Uzochukwu et al. 2003, Magar and Kelley 2004), there are more specific examples of the application of bioremediation to metal-contaminated soils and groundwater. Zhang and colleagues (2003) summarize the factors that affect uranium reduction as those that result in anaerobic conditions, conditions that seem to be an almost universal requirement. Arsenic, chromium, and copper concentrations were all found to reach essentially zero milligrams per liter abiologically within 22 days of the addition of organosulfur compounds, provided microbes were active and maintaining a reducing environment (Farone et al. 2004). Horst and colleagues (2004) raised a warning, however, in showing that if aerobic conditions were restored to an aquifer following treatment, the anaerobically removed metals could return to previous levels. Such a situation could also result if the pH of an aquifer became acidic, mobilizing heavy metals precipitated as sulfides. The likelihood of such processes has not been seriously considered in most scenarios for *in situ* heavy-metal precipitation, and this is a potential problem with the technology.

Will sulfur fall next?

The three most up-to-date overviews of bioremediation in the book by Fingerman and Nagabhushanam (2003)—the chapters by Kamnev, Wagner-Döbler, and Ruml and Kotrba—have already been discussed. Although the volume is supposedly on bioremediation, there is actually little else here on the subject. These three chapters and another one, by Vallero and colleagues (2003), do, however, make the book a worthwhile offering. Vallero and colleagues provide a valuable review of the biotechnological treatment of sulfur-containing wastes. The chapter begins with an overview of the sulfur cycle, and then proceeds to a discussion of methanogens versus sulfate reducers and the stoichiometry of organic metabolism. It also discusses the disadvantages—which outweigh the advantages—of using sulfate reduction in bioremediation. The chapter goes on to consider bioreactor design and technology for methanogenesis, citing methods to reduce sulfide formation. The authors next describe sulfidogenic bioreactors and list 24 examples of bioreactors containing different electron donors and exhibiting different efficacies. Sulfide oxidation is considered, along with flue-gas desulfurization and coal desulfurization. The latter is a long-dreamed-of possibility, and the authors believe that the most promising technology for achieving it is the removal of sulfur via sulfide, although that remains unproven. They also describe recent work on desulfurization of diesel and oils. One laboratory study achieved up to 48 percent removal, although comparable results have not yet been seen in pilot-scale studies. This chapter certainly opens up possibilities for future studies and hints at future technologies for the disposal of sulfidic wastes, as well as the clean use of high-sulfur fuels.

Finally, for those who are just beginning to become involved in bioremediation or who are teaching an introductory course in this field, the book by Catherine N. Mulligan, *Environmental Biotreatment: Technologies for Air, Water, Soil, and Wastes* (2002), is the most recent entry in the field. There are six chapters, each with numerous illustrations of the technologies and equipment used. Equations are presented in an easy-to-understand format. On the downside, a more extensive discussion of microbial metabolism and the carbon, nitrogen, and sulfur cycles in the chapter entitled "Principles of Biological Treatment" would have made the bioremediation processes that are discussed later more understandable. My other quibble is that the case studies are not presented in sufficient detail to enable the reader to understand the exact processes that were used, but perhaps the author felt that this lack could be remedied by going to the literature. There is an excellent glossary, although it would have been improved by the inclusion of addresses of some of the valuable Web sites now available (such as *http://members.tripod.com/~bioremediation/*). Still, if I were teaching advanced undergraduate or entry-level graduate students, this would be an up-to-date textbook worth considering.

I hope this article has shown something of what the past 30 years of bioremediation have brought to the treatment of polluted sites. And what should we expect in the next 5 to 10 years? For starters, the field has advanced to the point where bioremediation will be among the standard technologies considered for new cleanup efforts. We should also see an improvement in the models used to predict the fate, transport, and degradation of contaminants so that they will become more universal in scope and easier to use. This will result from a better understanding of the long-term fate of metals, of precipitated metals, and of the bioavailability of organic contaminants. Two areas where much greater understanding seems likely are (1) plant–rhizosphere interactions with regard to contaminants and (2) better control and design of processes that include both aerobic and anaerobic treatment trains. Finally, it seems likely that simpler, cheaper, and more accurate methods to monitor bioremediation on site or *in situ* will be brought from the lab to the field.

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