Can bioremediation bounce back?

As the market for bioremediation using genetically modified microorganisms is eroded by controversy over the technology, transgenic plants may take center stage for environmental cleanup.

Myrna E. Watanabe

Human endeavors—both in peacetime and in war—can result in the pollution of urban and rural environments, making large areas unsafe and potentially uninhabitable. High-profile disasters, such as the Exxon Valdez oil spill and the nuclear accident at Chernobyl, require cleanup that can be expensive, time-consuming, and frequently unsatisfactory. One solution is to harness and improve on nature’s own waste disposers—microbes and plants—using genetic engineering. However, most research in this area has fizzled out long before it reached the market, confounded by the multiple obstacles of cost, complexity, regulatory burden, and lobbyists. Researchers still trying to produce the perfect decontamination system may be doing so for niche markets.

**Costly cleanups**

Remediation is big business: There are about 12,000 contaminated “Superfund” sites listed in the United States, and 400,000 contaminated sites in Western Europe, with potentially tens of thousands of additional unofficial sites. In total, the world market for remediation was $15–$18 billion in 1998 and is growing. Most remediation is of groundwater and soil, and most sites are contaminated with a combination of heavy metals (mercury, lead, cadmium, and arsenic) and organic compounds (petroleum hydrocarbons and trichloroethylene). Several sites owned by the US Department of Energy are also contaminated with radionuclides such as plutonium, uranium, strontium, and technetium. Explosives, pesticides, and plastics make up a smaller, but still important, portion of the market, as does remediation of aquaculture wastes (see box, “Algae too”).

Standard remediation involves removing the contaminant from the site in its unchanged form, and disposing of it elsewhere. For example, contaminated soil is excavated, and landfill soil used as a replacement. Contaminated groundwater is pumped from wells dug in the ground, and the pollutant removed by methods such as filtration. Clearly, standard technologies do not remediate the contamination; they simply move it from one location to another, or transform it from one state into another (e.g., liquid to gas). Furthermore, remediation projects may take decades to complete, can be very costly, and often leave residual contamination. The inadequacy of standard remediation techniques has generated the need for more effective alternatives. Conceivably, bioremediation could provide that answer.

**Nature’s waste disposers**

Bioremediation may be carried out either using microbes or plants, and can be done ex situ or in situ (see Table 1). Bioremediation of organic contaminants may be quicker than standard techniques and, most importantly, may completely eliminate the contaminant, breaking it down into nontoxic molecules. Microbes and plants can be used to clean up heavy metals in soil or water, but they must then be disposed of, usually by burning or, in the case of radioactive materials, vitrification (i.e., the solidification of radioactive wastes into molten soil).

Bioremediation may be cheaper than standard remediation techniques, although each site is different and in some instances a combination of standard methods and bioremediation is needed. Clifford Mark of Micro-Bac International (Round Rock, TX), which specializes in microbial bioremediation, explains that using its microbial products costs $25/cubic yard, compared to standard pump/treat or soil remediation costing $50–$100/cubic yard. Others have estimated that phytoremediation of soil with fine-rooted grasses may cost as little as $10–$35/ton, whereas in situ bioremediation may cost $50–$150/ton, and incineration as much as $200–$1,500/ton. The US Environmental Protection Agency (EPA) estimates that phytoremediation could save up to 50–80% of the cost of conventional treatments.

To date, all commercial microbial remediation uses naturally occurring organisms, generally those identified at contaminated sites. Organisms living in contaminated areas develop the ability to metabolize the contaminant, if even only to a small degree, through a process called adaptation. For example, the bacterium Deinococcus radiodurans, which was identified in the 1950s in food treated by irradiation, is resistant to ionizing radiation. Exposure of microorganisms to hydrocarbons can increase their hydrocarbon-oxidizing potential. Microbes evolve the ability to hyperaccumulate metals and nonmetals, which gives them a selective advantage within a contaminated environ-
ment, allowing them to survive and evolve additional resistance. Adaptation may be the result of enzyme changes, genetic mutations, or the selective enrichment of organisms capable of metabolizing the contaminant. Strains that adapt particularly well to contaminated environments may be those containing plasmids encoding enzymes capable of metabolizing the contaminant.

Other microbes can be harnessed to clean up recalcitrant and dangerous heavy metals. For example, several bacteria, which normally reduce Fe(III), can also remediate radionuclides such as uranium, plutonium or technetium. These bacteria use their own or another organism’s siderophores—natural metal-chelating agents—to transport metal ions into the cell.

Because of limitations of modern techniques, only a tiny percentage of naturally occurring microorganisms can be grown in the laboratory. However, these unculturable organisms offer unexplored avenues for bioremediation, and research has concentrated on finding ways to culture such microbes. One example is the isolation of strain CBDB1 of Dehalococcoides ethenogenes, which was found to dechlorinate ubiquitous environmental contaminants, chlorobenzenes. The organism was cultured in a synthetic medium with supplements.

However, there are limits to the use of microbes in remediation. Although the EPA encourages use of unique technologies for remediation, adding naturally occurring microbes to sites is regulated on a state-by-state basis in the United States. The European Union (EU) also requires approval for genetically modified organisms (GMOs), although the procedures that must be adhered to differ for organisms that will be used in a contained manner versus those that will be released into the environment. Some researchers suspect that adding extrinsic microbes may not be a viable answer: Foreign microbes may not survive or may not remediate contaminants any better than indigenous microbes.

An alternative option is to enhance the natural microbial environment by adding substances (so-called amendments) that make the microbes work more effectively. Amendments include oxygen, nutrients, co-metabolites, and organic substances. Amending naturally occurring microbes may be preferable to adding extrinsic microbes to a site.

Table 1. Common bioremediation technologies

<table>
<thead>
<tr>
<th>Methods</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tbody>
<tr>
<td>Biostimulation (stimulating native microbial activity by introducing nutrients, oxygen, or other electron donors or acceptors)</td>
<td>Inexpensive</td>
<td>Effective at limited number of sites; uncertain results</td>
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<tr>
<td>Bioaugmentation (stimulating bioremediation with additional microorganisms)</td>
<td>Increased rate of remediation</td>
<td>Can be expensive; some products not effective</td>
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<tr>
<td>Ex situ applications</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Land-farming (mixing surface soil with waste and aerating the mix by tilling)</td>
<td>Easy to implement; rapid cleanup</td>
<td>Requires large surface area</td>
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<tr>
<td>Biopile (self-contained treatment in elevated beds)</td>
<td>Can be implemented in small site area</td>
<td>Difficult to control and monitor process; may be slow</td>
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<tr>
<td>In situ applications</td>
<td></td>
<td></td>
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<tr>
<td>Dissolved oxygen in situ treatment</td>
<td>Very rapid remediation; works in both saturated and unsaturated zones; can be retrofitted to existing pump-and-treat and air sparging/vapor extraction sites; good process for introducing biological enhancements; excellent operating flexibility</td>
<td>May require more complicated design</td>
</tr>
<tr>
<td>Bioslurping (vacuum-enhanced drainage to treat hydrocarbon contamination)</td>
<td>Can be effective in both saturated and unsaturated zones; removes contaminated groundwater in conjunction with biological enhancement</td>
<td>Limited operating flexibility; unpredictable results; may be high maintenance; requires water treatment/disposal</td>
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<tr>
<td>Chemical oxygen enhancement (addition of a chemical in water that releases oxygen)</td>
<td>Relatively easy to implement; may serve to temporarily appease regulators</td>
<td>Not good for sites with moderate to high contamination; expensive on unit cost basis; no operating flexibility</td>
</tr>
<tr>
<td>Bioventing (circulation of air through subsurface to encourage microbial degradation)</td>
<td>Works in unsaturated soils; relatively simple design</td>
<td>Limited effectiveness in saturated soils; difficult to deliver biological enhancements</td>
</tr>
<tr>
<td>Natural attenuation (bioremediation using native microbes and chemicals)</td>
<td>Inexpensive; good for sites with proper chemical/physical and regulatory conditions</td>
<td>Slow cleanup prolongs site closure; usually requires long-term monitoring; increased site characterization requirements</td>
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*Used with permission, Brian L. Clark, Enzyme Technologies (Portland, OR).*
Algae too!

For centuries, algae have been used in Asia for bioremediation of aquaculture wastes; the algae grown with the fish help to keep the water clean. However, industrial aquaculture is now an economic necessity, and a solution is needed for the nitrogen and phosphorus waste products of commercial finfish and invertebrate farming. Working with the red alga, Porphyra (called "nori" by Japanese food aficionados), Charles Yarish and Thomas Chen and colleagues of University of Connecticut (Stamford and Storrs, respectively) plan to genetically engineer a strain resistant to bacterial and fungal infections, which destroy 30–40% of oceanic nori culture each year. The genetically engineered nori will be maintained in a closed system. To prove that this can be done, Chen and colleagues produced transgenic nori containing a marker gene. They now plan to insert an antimicrobial Cecropia moth gene into the algae by electroporation.

Great hopes dashed

In theory at least, genetically modifying microbes to be more effective scavengers for toxic contaminants could enhance and extend the value of microbes in bioremediation. However, the climate for use of such microbes has changed radically during the last five years. In 1994, biotechnology consultant David Glass, now chief executive officer of Applied PhytoGenetics (Athens, GA), wrote: "As EPA gains more experience with uses of GMOs in bioreactors and the environment, many existing barriers are beginning to disappear." Glass further extolled the "predictability and safety of the technology" and the "growing public acceptance". Most of the $500 million market for bioremediation in the United States is for augmentation by naturally occurring microbes. Glass and other industry insiders say that there is no need to engineer microorganisms for this purpose, because naturally occurring microbes are effective enough. Moreover, the cost of producing GMOs is high, and with the exception of academic research, a permit from the EPA is needed for field use—an expensive proposition. Furthermore, the switch in public perception—now more skeptical of the safety of GMOs and opposed to their release into the environment—has confounded efforts in this area.

Today, the field of GMOs for bioremediation is limited to academic institutions that are able to carry out research under an EPA exemption; the researcher does not require a full permit, but the GMO must be contained and field release is prohibited. However, the first field trial of a GMO—a strain of Pseudomonas fluorescens engineered to degrade naphthalene—for bioremediation was carried out four years ago by the University of Tennessee (Knoxville, TN) and Oak Ridge National Laboratory (Oak Ridge, TN). The GMO contained a naphthalene catabolic plasmid along with a reporter gene that caused the organism to bioluminesce in the presence of the contaminant.

There may be other unique uses for GMOs in the remediation of sites suffering from a combination of metal and/or organic and radionuclide contamination. Researchers are looking to identify the genes that allow microbes to take up heavy metals or organic contaminants, using these to transform the radioactivity-tolerant species D. radiodurans. For example, Lawrence Wackett, Mcknight Professor of Biochemistry at University of Minnesota (St. Paul, MN), helped to engineer a radiation-resistant strain that could also treat mercury contamination. However, the approval process would be difficult and the sites would need a combination of bioremediation and standard remediation.

Wackett also modified Escherichia coli to overproduce the enzyme atrazine chlorohydrolase to degrade the herbicide atrazine. To satisfy the EPA regulatory approval process for field testing, Wackett and colleagues treated the engineered E. coli with a crosslinking agent that killed the cells but left their enzymes intact and functional. This would permit the shipment of bags of dead cells, which could then be added to the soil. Wackett says that this product was successfully field tested and the site was closed last year.

A group in Spain has engineered a bacterium Ralstonia eutropha to contain a gene for mouse metallothionein, a small protein that binds heavy metals. Such a protein is expressed on the bacteria's outer membrane, enabling it to immobilize heavy metals—in this case, cadmium. Laboratory experiments showed that genetically engineered R. eutropha could, indeed, hyperaccumulate cadmium.

However, the commercial potential of GMOs in remediation seems limited. James Tiedje of the Center for Microbial Ecology at Michigan State University (East Lansing, MI)—who is pursuing a pilot-scale, closed-system bioremediation of polychloro- biphenyls (PCBs) using GMOs—explains that it is already difficult enough to convince engineers to use nonengineered strains of microbes, let alone GMOs. Gary Sayler, director of The Center for Environmental Biotechnology at the University of Tennessee, adds that such manipulated organisms may be used in contained reactors, but using GMOs for bioremediation in the field is basically "dead in the water". In the face of such an unwelcoming climate, researchers are beginning to turn their attentions to plants.

Greener is cleaner

Phytoremediation, the remediation of contaminated ground using plants, has a much lighter regulatory burden and is advancing at a faster pace. The use of transgenic plants in field trials requires just 30 days' advance notification of the US Department of Agriculture (USDA), which regulates these plants under the Plant Pest Act. (Noxious weeds require a field-testing permit.) Environmental release of any GMO in the EU must be approved under Directive 2001/18/EC, and the European Commission has further suggested that risk assessment studies be carried out for all GMOs planned for release.

At least for now, the main focus is on the use of wild-type plants in bioremediation. M. Cristina Negri of Argonne National Laboratory (Argonne, IL) notes that plants are the pump and treatment system—functioning like a straw to suck up contaminated groundwater. Indeed, the physical and biochemical characteristics of plants make them ideal for bioremediation. Research in phytoremediation has therefore focused on species that can pick up large quantities of water (e.g., hybrid poplars and eastern cot...
Plants can accumulate and metabolize organic contaminants, reducing them to carbon dioxide and water. The extensive root systems of plants allow them to absorb large amounts of water and nutrients, and they can hyperaccumulate various contaminants. Plants sequester heavy metals in their roots—where they are stabilized, shoots, stems, or leaves. Within the root system—the so-called rhizosphere—live bacteria and fungi may produce substances that chelate metals or otherwise render metals more amenable to absorption through the roots. Roots themselves produce metal chelators and other substances that aid in uptake. Alternatively, the plant can volatilize the metal into a form that is less bioavailable than the original toxic contaminant and release it into the atmosphere. Plants also can be used to form a barrier, protecting an area from further incursion of contaminants, or as a cap or cover for an area that has not, or has only partially, been remediated. Research has shown that nickel and zinc can be hyperaccumulated in plants such as members of the mustard family (Brassicaceae), including Thlaspi and Alyssum, along with grasses and hybrid poplars. Recently, three teams independently discovered the presence of genes for phytochelatin synthases—a small, cysteine-rich protein that binds metals, rendering them nontoxic—in plants and in the worm Caenorhabditis elegans. Phytochelatins can bind to lead, cadmium, arsenic, and mercury, and the chelated metal is then packed into cell vacuoles, rendering the metals inaccessible to the plant’s metabolism.

Plants, like microbes, can adapt to survive within contaminated environments. Researchers therefore study plants growing in polluted sites to determine if they could be used as hyperaccumulators. Recently, a group led by Lena Ma from the University of Florida, Gainesville, identified the brake fern (Pteris vittata), which naturally accumulates arsenic, growing on a site contaminated with chromated copper arsenate. The phyto remediation firm Edenspace (Dulles, VA) is now considering commercializing the fern.

Plants that do not conserve water, such as hybrid poplars and willows, are the best choices for phyto remediation of contaminated ground water, as they steadily take up water and nutrients from the soil. Grasses are also used to remediate organic contamination in soil. Although phyto remediation has only been considered effective on large plots where the contaminated water is only 2–3 meters below ground level, plants can be forced to root much deeper—perhaps as deep as 10–12 meters—by using engineering props. At Argonne National Laboratory, trees are planted in a hole lined with thick plastic liner and backfilled with topsoil. The roots are isolated from superficial groundwater and will grow down until they reach deeper water.

Although phyto remediation is one of the technologies recommended by the EPA for cleanup of brownfields—for industrial or commercial sites that may be contaminated—phyto remediation is still a young technology and additional proof that it is effective is needed.

### Transgenic troubles
A small but growing number of companies are applying phyto remediation (see Table 2). However, although many field trials are underway, few employ genetically engineered plants. Nevertheless the future looks green: Applied PhytoGenetics’ Glass is optimistic that transgenic plants could be used commercially for phytoremediation within two to four years.

However, Applied PhytoGenetics is currently the only company studying the potential applications of genetically engineered plants for remediation. Richard Meagher of the University of Georgia (Athens, GA), and one of the company’s founders, has been examining plants that effectively remove mercury from the environment. The team first transferred a bacterial mercuric reductase gene into Arabidopsis, resulting in a plant that can convert mercury into a gas. They then transformed yellow poplar, Liriodendron tulipifera, with the same gene, conferring mercury resistance. In an unpublished study, they have further transformed plants with three of five genes resulting in increase of arsenic uptake.

Several academic groups continue to explore means of improving the remediation properties of plants. Julian Schroeder’s group at University of California (San Diego, CA) has cloned and transferred the gene for phytochelatin synthase into the yeast Saccharomyces cerevisiae, as proof of principle. Once the gene is added, the yeast, which normally cannot tolerate cadmium, can survive in its presence. Norman Terry’s group at University of California (Berkeley, CA), is taking this approach into plants, genetically engineering Indian mustard plants with phytochelatin genes to enhance their ability to accumulate cadmium.

A group at University of Washington (Seattle, WA) introduced a human cytochrome P450 gene into tobacco to increase the plant’s ability to metabolize the chlorinated solvent trichloroethylene, and has also introduced a cytochrome P450 gene from Arabidopsis into poplars. Neil Bruce at

### Table 2. Selected bioremediation companies

<table>
<thead>
<tr>
<th>Company</th>
<th>Areas of specialization</th>
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<tbody>
<tr>
<td><strong>Microbial remediation</strong></td>
<td></td>
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<tr>
<td>Envirogen (Lawrenceville, NJ)</td>
<td>Microbial degradation of hydrocarbons</td>
</tr>
<tr>
<td>Micro-Bac International (Round Rock, TX)</td>
<td>Microbial product development in water/wastewater, oil production enhancement and bioremediation; bacterial strains for hydrocarbon remediation</td>
</tr>
<tr>
<td>Oppenheimer Biotechnology (Austin, TX)</td>
<td>Bioremediation for hydrocarbon remediation</td>
</tr>
<tr>
<td>US Microbics (Carlsbad, CA)</td>
<td>Naturally occurring microbes for water and soil remediation. (Has six subsidiaries.)</td>
</tr>
<tr>
<td><strong>Phytoremediation</strong></td>
<td></td>
</tr>
<tr>
<td>Applied PhytoGenetics (Athens, GA)</td>
<td>Startup company; heavy-metal remediation with transgenic plants.</td>
</tr>
<tr>
<td>Edenspace Systems (Dulles, VA)</td>
<td>Remediation from soil and water.</td>
</tr>
<tr>
<td>Phytokinetics (North Logan, UT)</td>
<td>Remediation of groundwater and surface soil with plants; remediation of hydrocarbons, chlorinated hydrocarbons, solvents, and heavy metals</td>
</tr>
<tr>
<td>Wolverton Environmental Services (Picayune, MS)</td>
<td>Remediating indoor air pollution using plants</td>
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the Institute of Biotechnology, University of Cambridge (UK), has introduced a gene encoding the enzyme pentaerythritol tetranitrate (PETN) reductase into tobacco, producing plants that can metabolize the explosive PETN (ref. 20). In this issue, his group reports that they have successfully inserted a gene for the enzyme nitroreductase into tobacco plants to degrade trinitrotoluene (TNT), another troublesome explosive residue (see page 1168).

Although the future is promising for transgenic plants, there are also concerns about their use, not dissimilar to those surrounding genetically modified food plants. Will they spread uncontrollably? Will they decrease genetic variability by interbreeding with wild plants? Will they rob the soil of its nutrients as well as the toxin? Will they somehow find their way into the food chain and harm human and animal health?

Another problem is knowing what to do with the heavy metals accumulated by the plants. Some have suggested that they could be extracted and sold, but there may be safer ways to destroy them or render them non-bioavailable. Although phytoremediation is estimated to cost significantly less than standard remediation, it still will take many years to completely clean up a site, requiring that wells be monitored until it is clear that the method is working. Trees and plants also require care and/or harvesting.

The future

Clearly bioremediation with naturally occurring organisms is becoming a significant part of the remediation business. Although the market for genetically engineered bioremediation is not as promising as it appeared a decade ago, pioneering work continues. However, it seems certain that significant research will still be needed to bring it to a stage of development when local regulatory agencies can be convinced that these methods are safer, cheaper, and more effective than the alternatives. The future role of biotechnology within bioremediation is much less clear: There may be niche markets for GMOs, and genetically modified plants for recalcitrant contaminants, but the market may well be small and public acceptance is not guaranteed.

8. Road map to understanding innovative technology options for brownfields investigation and cleanup CD-ROM. (EPA 542-B-99-009).