

Bioremediation: Techniques for Cleaning up a mess

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Waste products resulting from human life have always been a serious problem. Today these waste products range from raw sewage to nuclear waste. In the past disposal of these wastes meant digging a hole, dumping the waste material in, then filling it all in. Out of sight, out of mind. But lately this method has become insufficient. The toxic materials from these “dig and dump” sites have begun to leak into water sources and into areas that sustain human life. This problem has led to modern-day *bioremediation*. Bioremediation is the transformation or degradation of contaminants into non-hazardous or less hazardous chemicals⁴. Bacteria are generally used for bioremediation, but fungi, algae and plants have also been used. Bioremediation is not a new technology. There has been evidence that compost piles existed as far back as 6000 BC, and in 1891 the first biological sewage treatment plant was created in Sussex, UK. However, the word “bioremediation” did not appear in peer-reviewed scientific literature until 1987¹.

There are three classifications of bioremediation:

- **Biotransformation** - the alteration of contaminant molecules into less or non-hazardous molecules⁴
- **Biodegradation** - the breakdown of organic substances in smaller organic or inorganic molecules⁴
- **Mineralization** - is the complete biodegradation of organic materials into inorganic constituents such as CO₂ or H₂O⁴.

These three classifications of bioremediation can occur either *in situ* (at the site of contamination) or *ex situ* (contaminant taken out of the site of contamination and treated elsewhere).

There are advantages and disadvantages to both *in situ* and *ex situ* strategies. *Ex situ* strategies (aka “pump and treat”) removes the contaminants and

places them in a contained environment². This contained environment allows for easier monitoring and maintaining of conditions and progress, thus making the actual bioremediation process faster. However, the removal of the contaminant from the contaminated site is time consuming, costly and potentially dangerous. By bringing the contaminants to the surface, the workers and the general public have increased exposure to the toxic material. There are several extraction strategies to facilitate *ex situ* bioremediation. The soil can actually be dug up and transported to a bioreactor. Soil washing³ is another method that can be used, where water is flushed through the contaminated region and then transferred to a bioreactor for treatment¹. Similarly, soil venting⁴ can be used, where air is flushed through the contaminated region and the air containing the contaminant is transferred to a bioreactor for treatment. The method of contaminant extraction depends on the nature of the contaminant in question (whether it is gas, liquid or solid phase, its chemical properties, and its toxicity).

In contrast, the *in situ* strategy does not require removal of the contaminant from the contaminated site. Instead either *biostimulation* or *bioaugmentation* is applied¹. *Biostimulation* is the addition of nutrients, oxygen or other electron donors and acceptors to the coordinated site in order to increase the population or activity of naturally occurring microorganisms available for bioremediation. *Bioaugmentation* is the addition of microorganisms that can biotransform or biodegrade contaminants. The microorganisms added can be a completely new species or more members of a species that already exists at the site. The advantage of *in situ* bioremediation is that there is no need to extract the contaminants, so there is less exposure to workers, and it is also less costly. However, there are also disadvantages to this strategy. The site of bioremediation is not contained, therefore, it is harder to control conditions and monitor progress.

Examples of In-Situ Bioremediation

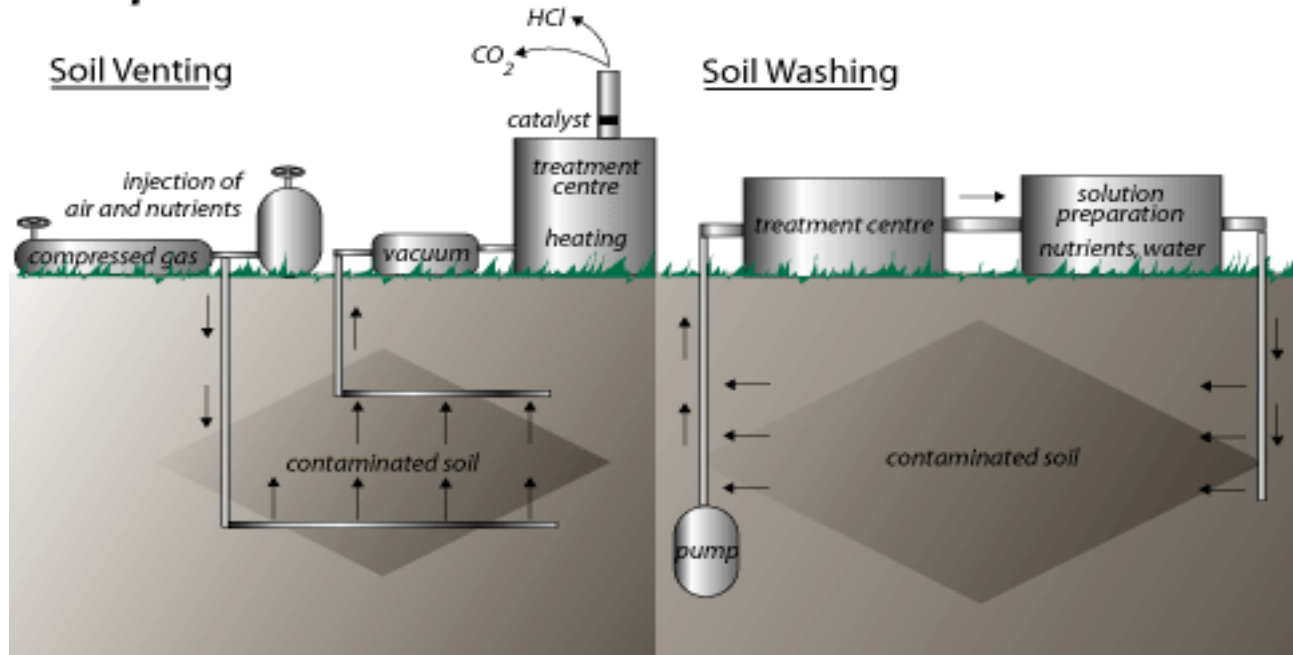


Figure 1. In-situ Bioremediation.

One example of this is the attempt to biostimulate microbes at an oil spill site. Nutrients added to the site end up diffusing through the water, and the result is a solute concentration the same as before the supplementary nutrients were never added⁵. Another disadvantage of in situ bioremediation is that if the soil (or other media that contains contamination) is heterogeneous, there will be uneven flow of liquid or gas containing the nutrients or microbes, so different areas will undergo different rates of remediation¹. Despite the complications with bioremediation, it is still being used or studied for use in the remediation of crude oil spills, sewage effluents, chlorinated solvents, pesticides, agricultural chemicals, gasoline contaminants, contaminants from wood processing, radioactive metals and toxic metals⁵.

For the last 50 years, the United States have used nuclear energy for many purposes. However, the research, development and production of nuclear material has left the United States with a lot of nuclear waste to deal with¹. Normally this waste is just contained and buried. This has resulted in more than 120 sites in 36 states and territories that contain hazardous and radioactive wastes, including 475 billion gallons of contaminated groundwater, 75 million cubic metres of contaminated sediment, and 3 million cubic metres of leaking waste buried in landfills, trenches

and spill areas¹. In the early 1990's the threat of the Cold War ended and the US Department of Energy (DOE) shifted their focus towards cleaning up these sites. The first few years consisted mainly of cataloging and preliminary characterization, which cost more than \$23 billion (the next ten years are budgeted to exceed \$60 billion¹). Such a large and costly project must have priorities and the focus of the DOE centres around radionuclides and toxic metals found in the nuclear waste sites. Some radionuclides found at DOE facilities are uranium (U), strontium (Sr), plutonium (Pu), cesium (Cs) and technetium (Tc). Some toxic metals present at DOE facilities include lead (Pb), chromium (Cr) and mercury (Hg).

Currently, there is no effective way to deactivate radioactive materials, except to allow them to decay in a site that is far away from human life. Unfortunately, many of radionuclides have very long half-lives:

Radionuclide	Half-life
Sr-90	28 years
Cs-137	30 years
Pu-239	24,100 years
Tc-97	2.6 million years
U-238	4.5 billion years
U-235	7.13 million years

Exposure to this radiation can cause radiation burns and cancer. In addition, strontium (Sr) can replace calcium in bones and become concentrated enough to cause radiation injury from within the body. The problem with containment sites of these radioactive materials is that they can leak. Most of the leaking is caused by solubilization of the metals into water. This allows the radioactive metals to travel with the water, which may flow through or around the site. One solution against this problem is to make the metals insoluble. This essentially makes the radionuclides immobile and prevents leaking from the contaminated sites. One way of doing this is to introduce metal-reducing bacteria to the containment site and allowing the bacteria to precipitate the metals¹ out of the solution. The bacteria can directly reduce the radioactive metals from an oxidized, soluble form to a reduced, insoluble form. The bacterium does this by taking electrons from organic compounds, and using the radioactive metal as the final electron acceptor in the process of energy production¹. For example, a bacterium can reduce U (VI), Cr (VI) and Tc (VII) to U (IV), Cr (III) and Tc (IV).

The bacteria can also indirectly reduce the radioactive metals by using an intermediate electron donor¹. For example, the bacterium can use either Fe³⁺ or SO₄²⁻ as a final electron acceptor. Fe³⁺ becomes Fe²⁺ and SO₄²⁻ becomes H₂S⁻. Fe²⁺ and H₂S⁻ can then reduce the radioactive metals to an insoluble state. The result of these redox reactions can be seen as the precipitant within the metal-reducing bacteria. Radioactive metals are not the only contaminants on which this redox bioremediation technique can be used. It can also be used to immobilize toxic metals or change them into less toxic forms. The negative effects of a few toxic metals include:

- Cr (IV)- Throat and lung cancer, shortened lifespan, reproductive problems and lower fertility
- Pb - plumbism (lead poisoning), anaemia, effects on the intestines and central nervous system. Also in children (generally having a less well-developed blood brain barrier than adults): behavioural

changes; decreased intelligence; brain damage and even death have been observed.

CH₃Hg- neurotoxicity.

Although using bioremediation is a great idea, quite often the contaminants or the contents of the contaminated site are also toxic to the active microbe involved in the bioremediation process. This problem can make it very difficult to keep the rate of bioremediation high. A solution to this problem is genetically engineered microbes (GEM) that are resistant to the extreme conditions of the contaminated site and also have bioremediary properties. For example, radiation is damaging to most life forms including most bacteria. However, there is one species of bacterium, *Deinococcus radiodurans*¹, that is resistant to radiation damage (it can withstand up to 1.5 million rads of radiation. If the genes for metal reduction can be transferred into this radiation-resistant species, or the radiation-resistant genes transferred into the metal-reducing bacteria, a GEM could be made that would be more efficient at bioremediation of sites leaking radioactive metals. This is only one example of bacterial species being studied for their ability to resist extreme conditions. Others include *bacillus infernos*¹, which can resist high temperatures, and *Methanococcus jannaschii*¹, which can resist high pressures (up to 230 atm) in addition to high temperatures.

Dehalococcoides have the natural ability to dechlorinate tri-, tetra- and penta-chloro dioxins⁶. The genes responsible for this dechlorination have been identified and modified to become overexpressed. This allows the dechlorination abilities of *Dehalococcoides* to be augmented. Further studies into placing these overexpressed genes into more robust organisms for bioremediation purposes are being done. The value of a GEM with the ability to bioremediate sites with dioxins is obvious when considering the toxicity of dioxins. Acute dioxin toxicity causes symptoms such as disturbance of hair arrangement, atrophy of the thymus gland, and hypertrophy of the liver (according to animal tests) and a skin disease named chloracne in humans⁷. Chronic poisoning includes carcinogenicity, liver disorders, teratogenicity, and

heterotopic endometriosis⁷. Dioxins are also suspected of causing endocrine disruption. Intake of dioxins over the tolerable daily limit can result in degradation of immunity, malignant tumours and morphological deformations of reproductive organs⁷. Sources of dioxins included waste incinerators, impurities in agricultural chemicals and products containing PCBs. Dioxins can be found in air, water, soil, food and even breast milk⁷.

Microbes are not the only species that can be enhanced by genetic modification for bioremediatory purposes. Plants have also been studied and used. Bioremediation by plants is called *phytoremediation*. Arsenic is one target of phytoremediation⁸. The health effects of arsenic include liver, lung, kidney and bladder cancers. One plant, *Arabidopsis thaliana* has been genetically modified to overexpress two bacterial genes, *arsC* and *g-ECS*¹. The gene *arsC* codes for arsenate reductase, which allows the plant to modify arsenate into arsenite, and *g-ECS* codes for γ -glutamylcysteine synthase, which makes a thiol that can detoxify arsenite by forming arsenic-protein thiates that are then stored in vacuoles¹. Essentially this genetically modified plant can take up arsenate, detoxify it and store it. Phytoremediation can also be used to destroy high-energy compounds such as TNT, GTN, RDX, TETRYL and HMX⁹. Tobacco plants have been genetically modified to express bacterial pentaerythritol tetranite (PETN) reductase allowing these plants to take up high-energy compounds and reduce them to non-explosive substances⁹. Another genetically modified plant possesses a bacterial mercuric reductase gene allowing it to take up mercury (Hg) out of the soil and store it safely¹⁰.

Although genetically modified plants and GEMs have great applications in bioremediation, creating them has serious ethical implications. For this reason, the DOE has formed a group called BASIC (Bioremediation And its Societal Implication and Concerns) Program¹. This group consists of community leaders, engineers, representatives, scientists and lawyers. Together they discuss the ethical and societal implications of bioremediation, foster respect and collaboration between scientist and stockholders, and enhance research communication and develop educational materials¹. One of the largest concerns with GEMs is that they are not naturally

occurring and therefore they could potentially upset the natural environment. This concern can be extended to bioremediation that does not even use GEMs: in bioaugmentation, the addition of more microbes to a naturally occurring population in the contaminated site can upset its natural balance. Furthermore, the addition of a new species of microbe to the site can also upset the balance of co-existing microbe populations present. In biostimulation, the preexisting microbe population responsible for bioremediation is being expanded beyond its normal size and this could result in a disturbance to the balance of surrounding microbe populations. Also, the nutrients added for biostimulation could stimulate microbe species other than that intended for bioremediation. The consequences of this are unknown. Although ideally the microbes added for bioaugmentation or expanded by biostimulation would die off when the addition of nutrients ceased, there is the possibility that these expanded populations would continue to thrive, resulting in severe consequences to the environment. Especially with in situ bioremediation, where the contamination site is not remediated within a contained bioreactor, these environmental balance issues must be considered and addressed. The balance between the damage to the environment caused by toxic materials and the potential damage that may be caused by bioremediating microbes must be determined.

Bioethics concerning GEMs and ecological damage control are not the only factors outside of science that need to be considered in bioremediation. Cost is also an important factor. How much is it worth in dollars to clean up a contaminated site? It has been predicted that by 2010, the global environmental technology industry could be worth more than \$1.5 trillion, of which biotechnological processes could account for 15-20%⁵. This large quantity of money has not been overlooked by businesses. Many bioremediation and related technologies companies have formed: Battelle Environmental Systems & Technology, Bioremediation Service, Inc., BioSolutions International, Inc., Chemical Specialities International, International Biochemicals (InterBio), Micro-Bac International, Inc., Oppenheimer Biotechnology, Inc. (OBI), Phytotech, Inc., Regenesis Bioremediation Products, Inc¹¹. Even though bioremediation reduces the cost of decontaminating

sites significantly, it still costs money and time. But ultimately, the question is not whether decontamination is worth the effort and expense. The question is whether or not we can afford to neglect these environmental problems that have accumulated over time.

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