CHAPTER 18

TECHNIQUES OF DEALING WITH ENVIRONMENTAL POLLUTION: BIOREMEDIATION

Hillary M Masundire

Biological Science Department
University of Botswana
Private Bag 0022
Gaborone, Botswana

Phone: 267-3552524 Fax: 267-356591
E-mail: masundireh@noka.ub.bw

Techniques of Dealing with Environmental Pollution: *Bioremediation*

Introduction

Pollution may be defined as *the introduction, through human activity, deliberately or accidentally, of substances which harm the biotic and/or abiotic components of the environment* (Suter 11, 1993, Moriarty, 1990). The agent of pollution may reduce or entirely stop some normal processes e.g. the existence and/or growth of some organism(s). From a human perspective, pollution is mostly seen in the light of loss of environmental benefits such as use, productivity, aesthetic appeal or economic value. The substances that cause pollution are called pollutants.

Not all substances released by humans into the environment are necessarily pollutants. Some are mere contaminants. **Contaminants** are *substances which do not have any known direct or indirect biological effects.* For some pollutants, their mere qualitative presence in the environment is potentially harmful while the harmful effects of others can only be felt if they are present in appreciable quantities. Some are lethal in small quantities with their effects being felt after only brief exposure of the victim to the pollutant while some victims may be able to tolerate sub-lethal concentrations of some pollutants.

Pollutants can be categorised into two groups on the basis of the mode of their biological effects.

- *Some pollutants have no apparent direct deleterious biological effects.* However, they affect biological organisms by altering some aspect(s) of the physical and/or chemical environment in a manner that will reduce the ability of some species to survive (Moriarty, 1990). Examples include the effect of increased carbon dioxide concentration in the atmosphere, accumulation of sulphur in the atmosphere and the over supply of nutrients to inland or coastal waters leading to eutrophication. Accumulation of such pollutants alters the environment to an extent such that species compositions and distribution are altered (Lund, 1971).

- *Other pollutants have direct lethal effects based on exposure and dose.* These are described as toxicants or toxins.

The effect of pollutants may be so severe as to cause death of some biota. Such lethal pollutants are then described as being toxic substances. The same substance may be harmless, a pollutant or a toxicant depending on its concentration in the environment.

Managing Pollution

Managing pollution *often involves prevention or reduction of entry of pollutants into the environment.* It may also involve the removal or reduction of pollutants already in the environment. The former is the more desirable while the latter is reactionary and may only achieve short term benefits unless it is attempted in conjunction with prevention or reduction. Pollution clean-up may involve physical, chemical or biological methods.

Biological methods of dealing with pollution include a suite of biological techniques such as *biomanipulation, biological control, biological treatment, biotransformation and bioremediation* which all, in one way or another, use biological organisms to control, reduce or remove pollutants from the environment. Some of these applications have been in use for hundreds of years but using such trendy names. For example, sewage treatment has always used organisms such as bacteria, fungi, algae, protozoa, micro- and macro-invertebrates and higher vertebrates to convert domestic and industrial wastewater non-polluting. Improvements that have been made in biological treatment of sewage have involved all or several of these bioprinciples.

More recently, **bioremediation** has been used to refer to *the process by which biological organisms, chiefly micro-organisms are used to decontaminate soils and aquifers, treatment of oil spills or biodegradation of xenobiotics and metals*. Xenobiotics are compounds of synthetic origin. They are mostly resistant or recalcitrant to biological degradation (Bitton, 1994). **Xenobiotics** may also be of organic origin such as humic substances and lignin. Bioremediation usually involves improving or speeding up normal biological process that would, if left alone, take much longer.

Living organisms have different tolerance levels to factors of their environment, including pollutants. Concentrations of a pollutant which may be toxic to one species, may not have any observable negative effect on another. *In some cases, a pollutant could be a metabolite for another species.* Such species could then be used to remove those pollutants that they can metabolise. By so doing, the organisms are remedying the environment by ridding it of a pollutant, thus providing bioremediation. *Some species can absorb and accumulate high quantities of a pollutant from the environment without themselves suffering an effect.* They rid the environment of the pollutant by **bioaccumulating** pollutants within their tissues from whence the pollutants may be subsequently excreted. *Some organisms alter the chemical structure of a pollutant into a form that they can metabolise.* In such cases bioremediation is preceded by **biotransformation** or **bioconversion**.

The *concentrations of pollutants may increase per unit of tissue of organisms higher in a food chain involving intake of such a pollutant.* That phenomenon

is known as **biomagnification**. The concentration of DDT has been known to reach near lethal levels in fish-eating birds that are at the top of the food chains that involve the passage of DDT or its residues from algae, crustacean zooplankton and fish, especially piscivorous fishes such as tiger fish (Hydrocinus vitattus).
In order to manage or reduce pollution or effects thereof, the composition of species in a community may be deliberately altered in order to remove or reduce specific pollutants from the environment. This is called **biomanipulation** and has been attempted in reversing the effects human induced eutrophication of inland waters. Particular species of plant, animal, bacteria or fungi may be cultured to be used to clean up specific pollutants from the environment. This is termed biotechnology or sometimes environmental biotechnology. By and large all these different modes of operation of biological systems represent examples of bioremediation.

Sewage Treatment

All forms of sewage or wastewater treatment are based on the ability of some species to selectively reduce or remove specific pollutants from the environment. The symbiotic association between bacteria, algae, protozoa, aquatic invertebrates and sometimes higher life forms in sewage treatment is a well-known example of ***bioremediation.***

In **oxidation ponds**, bacteria, both aerobic and anaerobic, oxidise the organic matter in wastewater. This bacterial metabolism results in the release of carbon dioxide and the mineralisation products from the breakdown of complex organic compounds such as proteins, carbohydrates and lipids. New bacterial cells are formed i.e. bacteria increase in population with a concomitant increase in the breakdown of the incoming organic matter. The products of the bacterial metabolism are utilised by plants, mainly algae, in the ponds. The algae photosynthesise during the day releasing oxygen required by aerobic organisms in the ponds. In the absence of light at night, photosynthesis ceases and anaerobic conditions prevail, favouring the activity of anaerobic bacteria.

In **trickling filters or biological filters**, sewage treatment is effected by the activity of biota forming the biofilm around the filter medium, usually rock. Microbial flora of the biofilm includes **bacteria:** *Zooglea, Pseudomonas, Flavobacterium, Achromobacter, Alcaligenes, Spaerotilus, Nitrosomona and Nitrobacter;* **fungi:** *Fusarium, Penicillium, Aspergilus, Mucor, Geotrichium and several yeasts; and* **algae:** *Ulothrix, Phormidium, Anacystis, Euglena, and Chlorella* (Bitton, 1994). **Animal species** belonging to the groups Protozoa, Rotatoria and several macroinvertebrates constitute the fauna of the biofilm. Their collective action on sewage results in an effluent with low BOD, low nitrate concentrations, low coliform counts ie. a less polluting effluent.

Activated sludge treatment systems are based on the principle of creating optimum conditions for the existence, growth and functioning of micro-organisms that are involved in the wastewater treatment. **Nitrification**, the conversion of ammonium compounds to nitrate, is a two stage process involving conversion of ammonium to nitrite by *Nitrosomonas spp. Nitrosospira, Nitrosococcus and Notrosobilus*. This is followed by the **oxidation** of nitrate to nitrate by *Nitrobacter spp., Nitrospira and Nitrococcus*. This process occurs best at pH between 15°C and 35°C. On the other hand, **denitrification**, the breakdown of nitrate to gaseous nitrogen, occurs optimally under conditions of high nitrate concentrations, anoxia, high amounts of organic matter, pH between 7.0 and 8.5 and temperatures between 35°C and 50°C. These are the conditions that favour the growth of the mediating micro-organisms: *Pseudomonas, Bacillus, Spirillum, Acinctobacter* and other genera. Biologica phosphate removal is effected by bacteria that can accumulate phosphate in excess of their cell requirements. Inorganic phosphate is released under anaerobic conditions and micro-organisms such as *Acinetobacter, Pseudomonas, Mycobacterium* and a few other genera take up this phosphate and accumulate it in polyphosphate granules within their cells. The activated sludge treatment process therefore involves the sequential creation of these conditions.

Pollution Control

Not all the contents of wastewater are easily biodegradable. There are a number of synthetic compounds that may be in wastewater which are resistant to biodegradation. Because of their non biological origin, such compounds are described as being foreign to biological systems. They are called xenobiotics (Bitton, 1994). These recalcitrant chemicals include halogenated hydrocarbons, halogenated aromatics, pesticides and polychlorinated biphenyls (PCBs). They may be toxic, mutagenic or carcinogenic. Some of them accumulate through food chains, their lethal effects being felt in high trophic levels. There are micro-organisms with special abilities to breakdown these resistant compounds. The xenobiotics are transformed by micro-organisms resulting in mineralisation, accumulation or polymerisation.

Biodegradation of xenobiotics is effected by micro-organisms that use the xenobiotic as a sole source of carbon and energy. Exposure of wastewater microbes to xenobiotics leads to selection of resistant species that possess the appropriate array of enzymes to enable them to utilise the xenobiotic as a metabolite. TABLE 17.1 summarises some of the microorganisms with the ability to degrade xenobiotics which have have been isolated (Kumaran and Shivaraman, 1988; Bitton, 1994).

Oil Spills

Bragg, *et al.* (1993) unequivocally demonstrate the effectiveness of bioremediation in degradation of polynuclear hydrocarbons in cleaning up an oil spill in Alaska, USA. Naturally occurring micro-organisms were responsible for this both in surface and intertidal sediments. The rate of breakdown of both total resolvable hydrocarbons (TRHC) and polynuclear hydrocarbons was found to increase with the application of nitrogen fertiliser. Bragg *et al.* showed that both total resolvable hydrocarbons (A) and polynuclear, in hydrocarbons would more rapidly degrade fertilised beaches than in the unfertilised controls.

Such forms of bioremediation where the activity of indigenous micro-organisms is enhanced by the addition of nutrients are referred to as in situ bioremediation. The degradation of xenobiotics in soils has been effected by adding micro-organisms such as *Arthrobacter, Rhodococcus chlorophenolicus,* and *Flavobacterium (*Bitton, 1994).

A number of fungus species have also been known to be able to use hydrocarbons as a source of carbon for their energy requirements. Cooney *et al.* (1993) found that each of 13 marine fungi could grow with a single hydrocarbon as the only carbon source. There is therefore potential to use such fungi in cleaning up oil spills in the seas.

Mixed cultures of micro-organisms can be used to clean up contaminated aquifers by enhancing biodegradation of pollutants. Such commercial blends of hydrocarbon degrading bacteria have been used in bioreclamation of soils and aquifers. After several months of operation, this mode of biorestoration achieved 93% removal of hydrocarbons (von Wedel *et al.*, 1988).

How quickly a synthetic chemical will be biodegraded depends on several factors including (i) immediate utilisation for growth or energy, (ii) utilisation and transformation after a lag period, (iii) transformation only by cometabolism with other chemicals utilised for growth and (iv) completely nonbiotranformable due to some unusual structure or presence of a structure that is inaccessible to the micro-organisms. Biotransformation rates are affected by adaptation, nutrients, oxygen, moisture, pH and temperature, often in combination rather than singly. *Biotransformation of chemicals*, characteristically leads to, in a stepwise more oxygenated products that are more rapidly oxidised, ultimately resulting in mineralisation i.e. the conversion of organic carbon to carbon dioxide, which is not common in abiotic processes.

Table 17.1: Some micro-organisms with the ability to degrade xenobiotics

Organic Pollutant	Bioremedial Organism
Phenolic compounds	*Achromobacter, Alcaligenes, Acinetobacter, Arthrobacter, Azotobacter, Bacillus cereus, Flavobacterium, Pseudomonas putida, P. aeruginosa, Nocardia, Candida tropicalis, Debaromyces subglobusus, Trichosporon cutaneoum, Aspergillus, Penicillium, Neurospora.*
Benzoates and relateds	*Arthrobacter, Bacillus spp. Micrococcus, Moraxella, Mycobacterium, P. putida, P. fluorescens.*
Hydrocarbons	*Escherichia coli, P. Putida, R Aeruginosa, Candida.*
Surfacants	*Achromobacter, Alcaligenes, Bacillus, Citrobacter, Clostridium resinae, Flavobacterium Pseudomonas, Nocardia, Candida, Cladosporium.*
Polychlorinated biphenynls (PCBs)	*Achromobacter, Pseudomonas, Aspergillus, Phanerochaete*
Pentachlorophenol (PCP)	*Flavobacterium, Pseudomonas, Phanerochaete*
Pesticides	
DDT	*P. aeruginosa*
Linurin	*Bacillus sphaericus*
2,4-Dichlorophenol	*Arthrobacter, Pseudomonas cepacia*
2,4,5-Tricholorobenzene	*Pseudomonas cepacia*
Parathion	*Pseudomonas spp., E. coli, P. stutzeri, P. aeruginosa*

Heavy Metals

Some metals such as copper, cadmium, mercury and chromium are highly toxic at certain concentrations. These heavy metals are very important and significant pollutants in environments that receive industrial wastes. A number of micro-organisms which can transform some of these metals have been identified. *Pseudomonas fluorescens, E. Coli, clostridium sp., Asp[ergillus niger* and *Saccharomyces cerivisiae* can transform mercury to methyl mercury by methylation. Methyl mercury is biodegradable.

Biotransformation and ultimately **bioremediation** of metals are effected in one or more of several including production of micro-organisms. These include production of strong acids, production of organic acids, production of ammonia, production of hydrogen sulphide, production of extracellular polysaccharides able to chelate (bind metal ions and maintain them in solution) heavy metals. TABLE 17.2 summarises some micro-organisms and can be that are known to biotransform metals used in bioremediation.

One major current concern is the contamination of soils and aquifers by some of these pollutants. Bioremediation lends itself to ridding oils and aquifers of these toxicants.

Table 17.2: Micro-organisms with ability to biotransform metals (Bitton, 1994)

Metal Pollutant	Metal Biotransforming Micro-organisms
Mercury (Hg)	*Pseudomonas flourescens, E. coli, Clostridium, Aspergillus niger, Saccharomyces cerevisiae.*
Cadmium (Cd)	*E. coli, Bacillus cereus, Aspergillus niger.*
Lead (Pb)	*Pseudomonas, Alcaligenes, Flavobacterium.*
Arsenic	*Aspergillus, Fusarium.*
Selenium	*Aeromonas, Flavobacterium, Penicillium, Aspergillus.*
Chromium	*Enterobacter cloacae.*

Bioremediation of Soils and Aquifers

Bioremediation could be applied *in situ*. This is a technique that enhances the ability of natural micro-organisms to biodegrade pollutants by adding nutrients and an increasing supply of oxygen. This involves addition of nutrients, nitrogen and phosphorus followed by physical turbation of the soil e.g. by ploughing to increase aeration (oxygen supply). This technique can be effectively used to biodegrade diesel oil and grease in soil. It has been recorded to reduce hydrocarbons by 95% within 5 months (Want *et al*, 1982; Vance, 1991). Piotrowsir (1989) demonstrated that bioremediation of aquifers contaminated with pentachlorophenol (PCp) and creosote could be significantly improved by injection of hydrogen peroxide and nutrients (nitrogen and Phosphorus).

Another technique often used is that of addition of micro-organisms and/or enzymes to contaminated soils or aquifers. The micro-organisms may be added as single species or as consortia of several species. *Arthrobacter, Rhodococcus chlorophenolicus, and Flavobacterium* grown in large fermenters from where they are harvested and inoculated into contaminated soils or aquifers (Bitton, 1994, Thonlas and Ward, 1989).

Biomanipulation

This involves the *deliberate altering of species composition of communities with the intention of selectively removing specific pollutants* or *to manage specific environmental problems.* It requires knowledge of the community's functional groups as well as trophic linkages. Biological control of pests and/or weeds may be cited as examples of biomanipulation, in such cases an organism, bacterium, fungus, invertebrate etc. is deliberately introduced in order to selectively kill an undesirable organism in the environment.

In managing **eutrophic waters**, biomanipulation may be applied to reduce the concentration of target nutrients which ultimately, hopefully lead to recovery of such eutrophic waters. Eutrophic waters are usually characterised by high concentrations of phosphorus and nitrates which lead to overproduction of plants, algae or macrophytes and general loss of biodiversity. The plant species that thrive best under such conditions are usually unpalatable to most herbivores and, in the case of some blue-green, may produce toxic products. Removal of zoo-planktivorous fishes has been shown to lead to improvement in the quality of eutrophic waters particularly with reference to species diversity (Ryding & Rast, 1989).

Bioremediation – A Way Forward

Current trends in the field of **bioremediation** are the *identification and isolation of genes or portions thereof in the form DNA in those micro-organisms that can biodegrade pollutants followed by subsequent transfer of those genes or DNA into naturally occurring but non biodegrading micro-organisms so as to confer the ability to biodegrade.* Other efforts concentrate on ways of improving those microorganisms known to have the ability to biodegrade. There is a need for more illustrative success case studies in order to convince environmental managers about the viability of applying the technique of bioremediation.

Caution is necessary against taking bioremediation at face value especially with regards to potential dangers such as may arise from introduction of "foreign" microbes into new environments. This calls for comprehensive research on potential impacts of such alien species of microbes are used in bioremediation.

The brief discussion above attempted to highlight the fact bioremediation has been known and used to control and/or manage pollution for a long time. In a number of cases bioremediation has been applied without necessarily using the term bioremediation. As a concept, bioremediation is not at all new. However, some of its applications may be new. In other cases, bioremediation is being used to solve new problems. New discoveries are being made of species that can be used in bioremediation of special problems. It is these last two phenomena that may lead recent converts to bioremediation to consider it to be a new concept. It certainly is not.

As a way forward, it can only be advocated that more research into finding those species of organisms, micro and macro, that can be used to clean up the myriad of pollution problems facing us today be strengthened.

References

Bitton, G. (1994). *Wastewater microbiology.* Wiley-Liss, Inc. 478 pp.

Bragg, J. R., R. C. Prince, E. J. Hamer and R.M. Atlas. (1993). "Monitoring effectiveness of bioremediation of Alaskan oil spill" in Guerroro R. & C. Pedros-Alio (eds) *Trends in Microbial Ecology.* Spanish Society for Microbiology p643-646.

Cooney, J. J., S. Wuertz, M. M. Doolittle, M. E. Miller, K. Henry & D. Ricca. (1993). "Marine fungi: Novel catalysts for bioremediation of oil spills", in Guerrero R. & C. Pedros-Alio (eds). *Trends in microbial ecology. Spanish Society for Microbiology.* 639-641.

Moriarty, F. (1990). *Ecotoxicology: The study of Pollutants in Ecosystems.* Academic Press. 289 pp.

Ryding, Sp. & W. Rast. (1989). *The control of eutrophication in Lakes and Reservoirs.* Man and Biosphere Series. Parthenon Publishing Company. 314 pp.

Suter 11, G. W. (1993). *Ecological Risk Management.* Lewis Publishers. 538 pp.

PART 4