Chapter 8

The intentional release of micro-organisms into the environment: Challenges to commercial use

Andrew S. Ball
School of Applied Sciences, RMIT University,
Victoria, Australia

Bioremediation involves the application of micro-organisms for the removal of contaminants from the environment. Bioremediation competes effectively with other remediation approaches, such as thermal desorption and incineration. Further innovation of this technology involves the development of geneticically engineered strains with enhanced biodegradability capabilities. At present, however, there have been very few reported examples where genetically engineered micro-organisms have been released into commercial bioremediations. The main reasons for this include the lack of knowledge of the environmental risks and benefits of releasing genetically modified organisms into a contaminated area. In addition, non-specialist stakeholder support is often overlooked and remains a crucial area for improvement if sustainable remediation is to continue to develop. This chapter focuses on the application and risks associated with bioremediation.

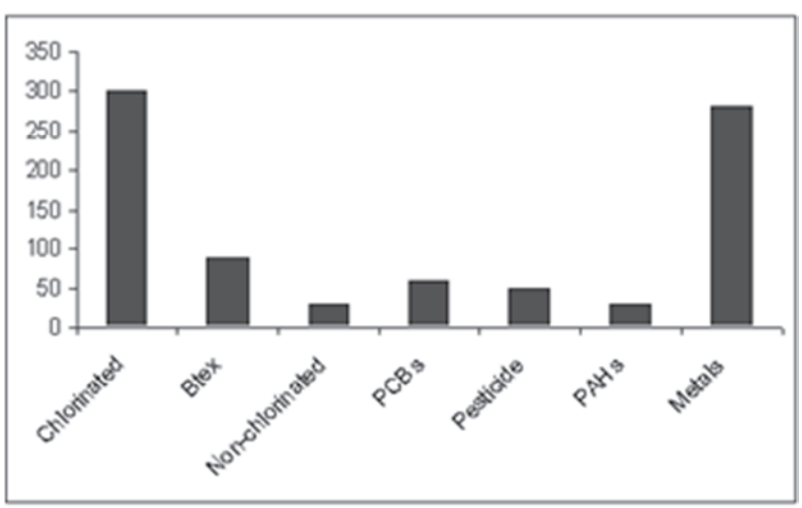
Introduction

The rapid expansion and increasing sophistication of the chemical industries in the past century, and particularly over the last 30 years, has meant that there has been an increasing amount and complexity of toxic waste effluents. It is estimated that there are around 60 000 chemicals in use in hospitals, households and in industry around the world; hundreds more are being introduced annually (Ball and Kadali, 2012). This has led to an unprecedented exposure of the environment to a vast array of chemicals. While most of the chemicals in use are used and subsequently disposed of correctly, it is inevitable that significant quantities of many of these chemicals will be released into the environment, becoming pollutants. This may occur in a number of ways, including (Ball, 2007):

- accidental release of chemicals during production and processing
- release of chemicals during use
- accidental release of chemicals during spillage
- deliberate release of the chemical into the environment.

At the same time, regulatory authorities have been paying more attention to problems of contamination of the environment. Industrial companies are therefore becoming increasingly aware of the political, social, environmental and regulatory pressures to prevent the escape of effluents into the environment. The occurrence of major incidents (such as the Union-Carbide (Dow) Bhopal disaster or the release of radioactive material in the Chernobyl accident, etc.) and the subsequent massive publicity due to the resulting environmental problems have highlighted the potential for imminent and long-term disasters in the public's conscience (Ball, 2007). Even though policies and environmental efforts should continue to be directed towards applying pressure on industry to reduce toxic waste production, bioremediation presents opportunities to detoxify a whole range of industrial effluents, as becomes clear from the example of an overview of toxic compounds and the number of sites where they occur in the United States (Figure 8.1).

Figure 8.1. **Number of sites in the United States that require treatment for pollution**



Note: Btex = benzene, toluene, ethylbenzene, xylene.

Once released into the environment, depending on the nature of the pollutant, the chemical can be found in air, soil and water. For example, if the pollutant (e.g. benzene) is in a gaseous state under atmospheric temperature and pressure, it will largely be found in the gaseous phase, while a solid contaminant (e.g. lead) will be found largely in soils or sediments (Ball, 2007). These pollutants can be found in air, water or soil and can be metals or organic compounds not normally found in nature. Once released into the environment, these pollutants may either be broken down or may persist until they are detected and quantified and their potential risk assessed. It may be that the pollutant(s) have to be removed and degraded, or degraded *in situ* (Figure 8.2) (Ball and Kadali, 2012).

Figure 8.2. **Sampling of groundwater for determination of hydrocarbon contamination**

Source: Andrew S. Ball, RMIT University, Australia

Remediation technologies

A number of options exist for the disposal (remediation) of pollutants found in the environment. These include (Ball, 2007):

- Incineration: the process of the destruction of a pollutant through conversion to carbon dioxide and water through combustion with the residue of incombustible material forming an ash residue.
- Burying: disposal of a pollutant by placing it in a sanitary landfill, which is engineered in a manner that protects the environment from the pollutant.
- Solidification: encapsulation of the pollutant in cement which after hardening can be disposed of safely in a landfill.
- Thermal desorption: this is an environmental remediation technology that utilises heat to increase the volatility of contaminants such that they can be removed from the soil. The volatilised pollutants are then collected or thermally destroyed.
- Bioremediation: the application of biological treatment to the cleanup of hazardous chemicals by metabolic conversion into non-toxic substances (Cookson, 1995).

This chapter focuses on the application and risks associated with bioremediation and consequently will focus on this sustainable remediation technology.

Bioremediation

The advantage of using bioremediation rather than digging up the contaminated soil and placing it elsewhere is that only moderate capital investment is required as the process is low in energy input. In addition, the processes are environmentally safe, do not generate waste and are self-sustaining. In many cases, bioremediation not only offers a permanent solution to the problem, but is also cost effective. Cleaning up existing terrestrial environmental contamination in the United States alone can cost as much as USD 1 trillion. Bioremediation can help reduce the costs of treatment as follows (Ball and Kadali, 2012):

- Treating contamination in place: most of the cost associated with traditional cleanup technologies is associated with physically removing and disposing of contaminated soils. Because engineered bioremediation can be carried out in place by delivering nutrients to contaminated soils, it does not incur removal-disposal costs.
- Harnessing natural processes: at some sites, natural microbial processes can remove or contain contaminants without human intervention. In these cases where intrinsic bioremediation (natural attenuation) is appropriate, substantial cost savings can be realised.
- Reducing environmental stress: because bioremediation methods minimise site disturbance compared with conventional cleanup technologies, post-cleanup costs can be substantially reduced.

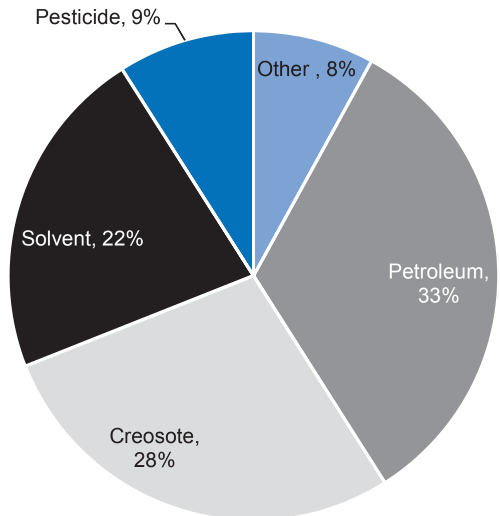
As a technology, bioremediation has a global application. In the United Kingdom alone it has been estimated that there are some 100 000 sites, which will take between GBP 10 000 million and GBP 20 000 million to clean up. In terms of the nature of the bioremediation process used, this depends greatly on the nature and quantity of the pollution. Nevertheless, bioremediation is an applicable technology for a range of pollutants. Figure 8.3 shows the range of industries that use bioremediation as a technology (Ball, 2007).

Technologies involved in bioremediation

In terms of technologies utilised within the wider remit of biotechnology, a number of specific terms are used to describe the activity of micro-organisms and the way they are used (Ball, 2007). This section discusses the main ones.

Monitored natural attenuation (intrinsic bioremediation) is one method of applying *in situ* bioremediation. One component of natural attenuation is the use of indigenous micro-organisms to degrade the contaminants of concern without human intervention (such as supplementing the available nutrients). Site characterisation and long-term monitoring comprise the activities required to implement natural attenuation. Long-term monitoring is used to assess the fate and transport of the contaminants compared against the predictions. The reactive transport model can then be refined to obtain better predictions. Natural attenuation processes typically occur at all sites, but to varying degrees of effectiveness depending on the types and concentrations of contaminants

Figure 8.3. **Range and weighting of industries that utilise bioremediation**



present and the physical, chemical and biological characteristics of the soil and groundwater. As they rely on naturally available micro-organisms in each site in combination with abiotic processes, natural attenuation processes may reduce the potential risk posed by site contaminants in three ways:

- the contaminant may be converted to a less toxic form through destructive processes, such as biodegradation or abiotic transformations
- potential exposure levels may be reduced by lowering of concentration levels (through destructive processes or by dilution or dispersion)
- contaminant mobility and bioavailability may be reduced by sorption to the soil or rock matrix.

In situ bioremediation (ISB) is the use of micro-organisms to degrade contaminants in place with the goal of obtaining harmless chemicals as end products. Most often, *in situ* bioremediation is applied to the degradation of contaminants in saturated soils, although bioremediation in the unsaturated zone can occur. ISB has the potential to provide advantages such as complete destruction of the contaminant(s), lower risk to site workers and lower equipment/operating costs. ISB can be categorised by metabolism or by the degree of human intervention. At a high level, the two categories of metabolism are aerobic and anaerobic. The target metabolism for an ISB system will depend on the contaminants of concern. Some contaminants (e.g. fuel hydrocarbons) are degraded via an aerobic pathway, some anaerobically (e.g. carbon tetrachloride) and some contaminants can be biodegraded under either aerobic or anaerobic conditions (e.g. trichloroethene).

Accelerated *in situ* bioremediation is where substrate or nutrients (termed biostimulation) are added to an aquifer to stimulate the growth of a target consortium of bacteria. Usually the target bacteria are indigenous; however, enriched cultures of bacteria (from other sites) that are highly efficient at degrading a particular contaminant can be introduced into the aquifer (termed bioaugmentation). Accelerated ISB is used where it is desired to increase the rate of contaminant biotransformation, which may be limited by lack of required nutrients, electron donor or electron acceptor. The type of amendment required depends on the target metabolism for the contaminant of interest. Aerobic ISB may only require the addition of oxygen, while anaerobic ISB often requires the addition of both an electron donor (e.g. lactate, benzoate) as well as an electron acceptor (e.g. nitrate, sulfate). Chlorinated solvents, in particular, often require the addition of a carbon substrate to stimulate reductive dechlorination. The goal of accelerated ISB is to increase the biomass throughout the contaminated volume of aquifer, thereby achieving effective biodegradation of dissolved and sorbed contaminant.

The addition of either nutrients or micro-organisms generally bring about an increase in the rate of bioremediation, but the increased cost of utilising this approach ensures that their application is based around the particular requirements of the remediation. For example, if the site is to be built upon shortly, enhancing the natural rate of remediation through the addition of biostimulation and/or bioaugmentation may be necessary and cost effective. In contrast, if the site is to be left for some time (i.e. years) then monitored, natural attenuation will generally be employed as it is the most cost-effective bioremediation.

Environmental risks of bioremediation

In terms of deleterious effects of bioremediation on the environment, there are several potential problems which may arise:

- Firstly, there is the scenario that the bioremediation fails and the contaminant remains in the environment. This may be a result of the low bioavailability of the compound or perhaps pollutant toxicity.
- Secondly, there is the possibility that the bioremediation has resulted in only a partial breakdown of the pollutant. If the intermediate product is more toxic than the original compound, then this will lead to greater environmental damage. This has been observed during the degradation of polychlorinated ethene in groundwater where a more toxic intermediate, vinyl chloride, has been the main product of bioremediation rather than ethene.
- Thirdly, if biostimulation has been employed, then there is the possibility that the treatment itself (e.g. addition of nutrients such as nitrogen and phosphorus) may cause deleterious effects on the environment through increased nutrient availability, which in soils would mean the release of nutrients into surface water resulting in increased eutrophication leading to algal blooms.
- Finally, if bioaugmentation is employed, the addition of an organisms not native to that environment is added, there is an inherent risk that these organisms may significantly affect the functionality of the natural microbial community, causing deleterious effects on the environment.

In general, bioremediation utilises the natural ability of mixed populations of micro-organisms. The dynamics of such populations are complex and the potential for use of a released organism to enhance the bioremediation process therefore depends both on the environment and the nature of the pollutant (Aleer et al., 2011). However, with the release of any organism in the environment, the risk of utilising such a strategy must be fully considered. In some countries (e.g. Australia) the likelihood of being able to obtain permission from a body such as the Environmental Protection Agency to release a micro-organism is very small. However, in other countries (e.g. the United States), it is more likely to be permitted if a case is made. Nonetheless, this obstacle remains a significant challenge to the commercial use of micro-organisms in many countries.

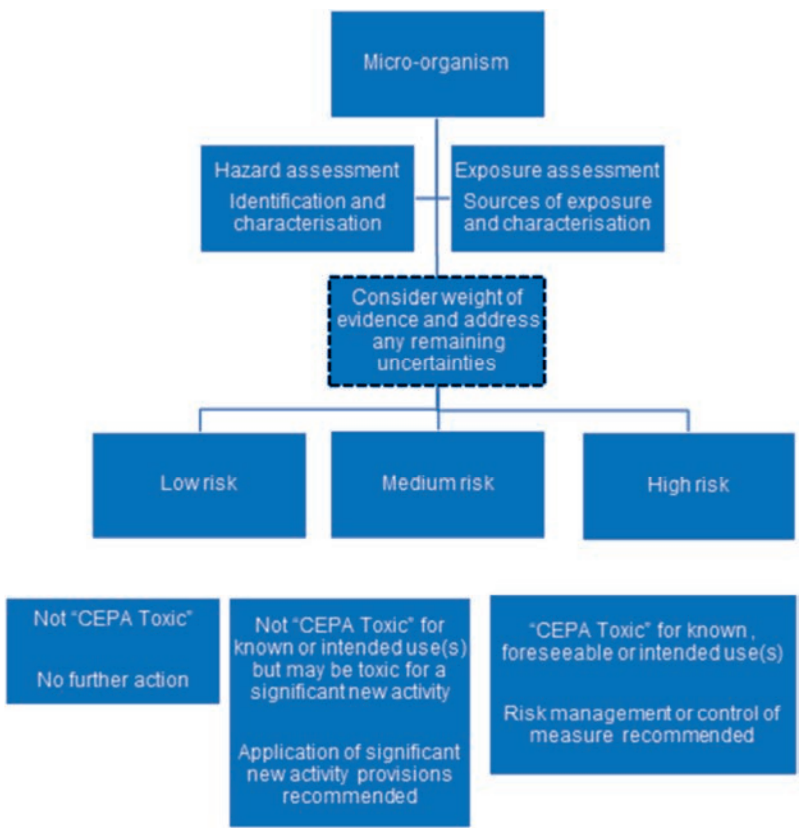
Nature protection and the introduction into the environment of micro-organisms

Given the potential risks associated with the release of micro-organisms into the environment, many countries have developed strategies and protocols for risk assessment. The approaches taken generally use the paradigm that risk is proportional to the product of hazard and exposure:

$$\text{Risk} \propto \text{Hazard} \times \text{Exposure}$$

For example, the Canadian EPA has established guidelines for risk assessment and a safety mechanism (Figure 8.4).

Figure 8.4. **Canadian draft guidelines for risk assessment for the release of micro-organisms into the environment**



Source: Department of Health and Ageing and enHealth Council (2002), "Environmental health risk assessment: Guidelines for assessing human health risks from environmental hazards", Commonwealth of Australia, Canberra, www.nphp.gov.au/enhealth/council/pubs/pdf/envhazards.pdf.

In terms of hazard assessment, this involves characterisation of the micro-organism and identifies the potential adverse effects on the environment and/or human health and predicts the extent and duration of these effects. This characterisation involves:

- taxonomic identification, for risk assessment purposes (OECD, 2003)

- assessment of strain history in terms of any known pathogenicity
- record of any genetic modifications related to the proposed strain
- the potential of the organism in terms of its potential for horizontal gene transfer
- consideration of the biological and ecological properties of the organism
- examination of any information relating to previous release.

As a result of the hazard assessment and together with the other assessments listed in Figure 8.3, the application is categorised into one of three risk estimates (Slovic, 1987; 1997):

- High risk: a determination of high risk implies that severe, enduring or widespread adverse effects are probable for exposure scenarios predicted from known, foreseeable or intended uses; control measures or risk management would be recommended.
- Medium risk: a determination of medium risk implies that adverse effects predicted for probable exposure scenarios may be moderate and self-resolving. In this case, use may be recommended with monitoring.
- Low risk: a determination of low risk implies that any adverse effects predicted for probable exposure scenarios are rare, or mild and self-resolving.

Examples of use of released micro-organisms in bioremediation

Over the last decade, a number of companies have been established to develop and commercialise biodegradation technologies. For example, one bioremediation company, Envirogen (New Jersey), has developed recombinant PCB (polychlorinated biphenyl) degrading micro-organisms with improved stability and survivability in mixed populations of soil organisms. The same company has also developed a naturally occurring bacterium that degrades trichloroethylene (TCE) in the presence of toluene, a toxic organic solvent killing many other micro-organisms. However, the use of microbes for bioremediation is not limited to detoxification of organic compounds. In many cases, selected microbes can also reduce the toxic cations of heavy metals (such as selenium) to the much less toxic and much less soluble elemental form. Other commercially available products include BioWorld Augmentation, which represents “a group of specific micro-organisms selected for each type of contaminant”. Recently RemActiv™ has been introduced into the market; this is a liquid additive that contains selected micro-organisms and a specially formulated nutrient mix.

Feedback on the use of these organisms as a bioaugmentation treatment is mixed. This is not unexpected as the environmental conditions under which these additives operate effectively are limited and as every commercial bioremediation represents a unique set of pollutants and environmental conditions, it is not surprising that under some conditions, treatment is effective while under other conditions, treatment can be less effective or even ineffective. However, throughout the deployment of augmented organisms in the environment for remediation of a range of contaminants, to the best of the author’s knowledge, there have been no reports of any detrimental environmental effects caused by the released micro-organism. This is an important observation, confirming that bioremediation represents a sustainable and environmentally safe technology, provided that due testing and analysis in both the laboratory and the field have been completed prior to full-scale treatment.

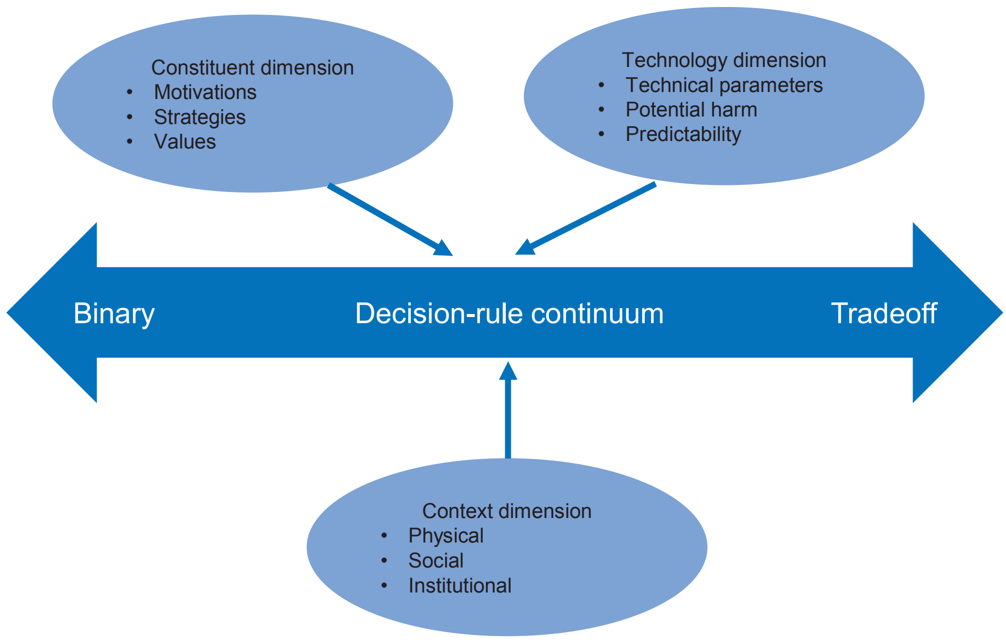
Challenges to commercial use of bioremediation technologies

As this chapter has shown, bioremediation offers the possibility of technically effective and relatively less expensive remediation. Assuming that the promise of bioremediation strategies are realised, why would anyone object to using these natural treatments? A failure to anticipate issues that can derail plans to deploy any technology, including bioremediation, can be problematic (Axlerod, 1994). While some issues may revolve around the technical aspects of bioremediation, others may derive from non-technical, social concerns. Site-specific bioremediation decision making can be viewed as a social process that is informed by scientific and technical data, rather than as a physical process. While it is not asserted that bioremediation represents a controversial technology, the use of a simple clean-up option may become controversial (Priest, 1994). Bioremediation encompasses a suite of potential remediation options whose remediation targets, mechanisms and capabilities differ. Therefore, generic questions about the suitability of bioremediation have limited applicability to the particular situations in which it might be considered for deployment. Yet, neither is every possible permutation of contaminant, site, remediation mechanism and remediation goal likely to produce a unique social response. The approach probably lies somewhere in the middle – an exploration of the generic factors that may influence patterns of social responses to specific bioremediation applications (Hagedorn and Allender-Hagedorn, 1997).

To date, there have been relatively few systematic studies of social responses to bioremediation. However, a recent study (Conroy and Ball, unpublished data) suggests that a lack of education in terms of understanding the biological basis of the technology remains a barrier. Therefore, despite increasing applications of bioremediation, social issues related to its deployment have not been documented. While bioremediation may prove to be socially acceptable for cleaning up contamination, it may not be fully acceptable either across the suite of approaches it encompasses or across the range of sites at which it is proposed for deployment (Stern and Dietz, 1994; Davison et al., 1997). Further, the acceptability of this technology should be viewed as multidimensional instead of one-dimensional (e.g. as only as a matter of risk, or risk communication, or education). Acceptability evolves over time through interactions with individuals and organisations, and in response to new technical and non-technical information (Eagly and Kulsea, 1997). Without systematic data, complete analysis of the social dimensions of bioremediation cannot be undertaken. Instead, a systematic approach to identifying and analysing the social determinants of the acceptability of bioremediation can be made. This approach relies on a conceptual framework and draws from published literature to illustrate the attributes of bioremediation and its use.

Although the technology is based on natural processes and does not involve the use of genetically modified organisms, public concerns are centred on the apparent "lack of activity on site" which leads to a public perception that no real "effective treatment" is being applied to the site. To gain a better understanding of social acceptability issues and to improve the ability to predict outcomes in deliberations over the social acceptability of controversial technologies, Wolfe and Bjornstad (2002) developed a conceptual framework for organising what was perceived to be the most important issues. The resulting framework, PACT (Public Acceptability of Controversial Technologies), provides a common logic through which to view site-specific decision making about remediation technologies (Figure 8.5). The PACT is built around dimensions that operate to influence decision-oriented dialogs over controversial remediation technologies in any location.

Figure 8.5. **Overview of public acceptability of controversial technologies (PACT), used to assist in site-specific decision making about remediation technologies**



Source: Wolfe, A.K. and D.J. Bjornstad (2002), "Why would anyone object? An exploration of social aspects of phytoremediation acceptability", *Critical Reviews in Plant Sciences*, Vol. 21, No. 5, pp. 429-438.

The factors relevant to specific decision settings and technologies varies from situation to situation. This PACT-based analysis focuses on an array of attributes that could strongly influence acceptability. In this context, acceptability refers to participants' willingness to consider the technology in question as a viable alternative, rather than to whether the technology ultimately is deployed. The PACT provides a framework through which to see how participants' position changes over time, from absolute positions of support or opposition at one extreme to completely negotiable positions at the other. Changes in positions may be related to any of the PACT's dimensions – from decisions about who should or should not participate in decision making to the kinds of technologies worth considering.

Conclusion

Bioremediation is now a successful environmental biotechnology, having a number of advantages (e.g. cost, environmentally friendly means of disposal) over any alternative treatment of contaminated land such as landfilling or incineration. There exist large areas of the world where contaminated land can be found, constituting an environmental and health hazard. Bioremediation offers the opportunity to utilise the natural microbial population to treat the contaminated site, returning the elements making up the contaminants to the natural nutrient cycling. However, each application varies with contaminants and environmental conditions and therefore there is no single "off the shelf" solution for effective treatment. For petrogenic hydrocarbons, the natural microbial community often performs better than any introduced micro-organisms. For chlorinated hydrocarbons, the addition of non-genetically modified halorespiring organisms into an environment has proved successful in both North America and Europe. Whilst there exists a market for microbial inocula, the potential application and use of genetically modified organisms has yet to be realised. One of the main limitations to the use of this technology is social acceptance (Hoban et al., 1992). Applying the PACT to bioremediation reveals flaws in the typical one-dimensional method often used for gaining technology acceptance (e.g. educating the public about the technology and its benefits or communicating effectively the attributes of the technology in question).

References

Aleer, S., et al. (2011), "Harnessing the hydrocarbon degrading potential of contaminated soils for the bioremediation of waste engine oil", *Water, Air, & Soil Pollution*, No. 218, pp. 121-130.

Axelrod, L. (1994), "Balancing personal needs with environmental preservation: Identifying the values that guide decisions in ecological dilemmas", *Journal of Social Issues*, Vol. 50, No. 3, pp. 85-104.

Ball, A.S. (2007), "Terrestrial environments, soils and bioremediation", in: *The SAGE Handbook of Environment and Society*, Sage Publications, London, pp. 385-394.

Ball, A.S. and K.K. Kadali (2012), "The removal of toxic waste", *Microbiology*, Vol. 2 112, No. 9, pp. 97-99.

Cookson, J.T. (1995), *Bioremediation Engineering: Design and Application*, McGraw Hill, New York.

Davison, A., I. Barns and R. Schibeci (1997), "Problematic publics: A critical review of surveys of public attitudes to biotechnology", *Science Technology and Human Values*, Vol. 22, No. 3, pp. 317-348.

Department of Health and Ageing and enHealth Council (2002), "Environmental health risk assessment: Guidelines for assessing human health risks from environmental hazards", Commonwealth of Australia, Canberra, www.nphp.gov.au/enhealth/council/pubs/pdf/envhazards.pdf.

Eagly, A. and P. Kulsea (1997), "Attitudes, attitude structure and resistance to change", in: *Environment, Ethics, and Behavior: The Psychology of Environmental Valuation and Degradation*, pp. 122-153.

Hagedorn, C. and S. Allender-Hagedorn (1997), "Issues in agricultural and environmental biotechnology: Identifying and comparing biotechnology issues from public opinion surveys, the popular press and technical/ regulatory sources", *Public Understanding of Science*, No. 6, pp. 233-245.

Hoban, T., E. Woodrum and R. Czaja (1992), "Public opposition to genetic engineering", *Rural Sociology*, Vol. 57, No. 4, pp. 476-493.

OECD (Organisation for Economic Co-operation and Development) (2003), "Guidance document on the use of taxonomy in risk assessment of micro-organisms: Bacteria", *Series on Harmonisation of Regulatory Oversight in Biotechnology*, No. 29, ENV/JM/MONO(2003)13, OECD, Paris, www.oecd.org/officialdocuments/displaydocumentpdf?cote=env/jm/mono(2003)13&doclanguage=en.

Priest, S.H. (1994), "Structuring public debate on biotechnology: Media frames and public response", *Science Communication*, Vol. 16, No. 2, pp. 166-179.

Slovic, P. (1987), "Perception of risk", *Science*, No. 236, pp. 280-285.

Slovic, P. (1997), "Public perception of risk", *Journal of Environmental Health*, No. 59, pp. 22-23.

Stern, P.C. and T. Dietz (1994), "The value basis of environmental concern", *Journal of Social Issues*, Vol. 50, No. 3, pp. 65-84.

Wolfe, A.K. and D.J. Bjornstad (2002), "Why would anyone object? An exploration of social aspects of phytoremediation acceptability", *Critical Reviews in Plant Sciences*, Vol. 21, No. 5, pp. 429-438.


Biosafety and the
Environmental Uses
of Micro-Organisms
CONFERENCE PROCEEDINGS
OECD