The microbial removal of toxic waste

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The rapid growth of the global chemical industry over the last 35 years has meant that there have been both increased amounts and complexity of toxic waste effluents. Global chemical output increased by 63% in the period from 1996 to 2010[1]; this increase has led to an unprecedented release into the environment of a vast array of chemicals. Bioremediation is now a successful environmental biotechnology used for the remediation of these pollutants, having a number of advantages (for example, cost, environmental friendly means of disposal) over any alternative treatment such as placing in landfill or incineration. Bioremediation offers the opportunity to utilise the natural microbial population to treat the contaminated site, returning the elements making up the contaminants to natural nutrient cycling.

There are around 60,000 chemicals in use in hospitals, households and in industry around the world[2]: Table 1 lists the 2001 production levels of a range of petrogenic-based products. These chemicals alone are being produced at a rate of over 2,2000,000 metric tonnes per year.

Table 1. Chemical production of products from petrogenic sources[1].

Product	Production in metric tonnes
Diesel	1,102,541
Gasoline	771,101
Kerosene	82, 882
Lubricants	39,229
Liquid petroleum gas from natural petroleum refineries	100,100
Naphthas	183,226

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:

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

Once released, these pollutants may either be broken down or may persist until they are detected and quantified and their potential risk assessed. A number of options exist for the disposal (remediation) of pollutants found in the environment[3]. These include:

- **Bioremediation:** involves the application of microorganisms for the removal of contaminants from the environment.
- **Burying:** disposal of a pollutant by placing it in a landfill, which is engineered in a way that makes every effort to protect the environment from the pollutant. This may not result in the complete degradation of the contaminant.
- **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. The ash residue will have to be disposed of through burial.
- **Solidification:** encapsulation of the pollutant in cement which, after hardening, can be disposed of safely in a landfill. The pollutant will rarely be degraded in this form.
- **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.

Cleaning up existing environmental contamination is expensive; for example China, regarded as the world's worst polluter, will need to spend at least 2 per cent of gross domestic product a year (680 billion yuan, equivalent to A$108 billion) to clean up 30 years of pollution by industrial waste[4].

In terms of the bioremediation process, the actual methodology used depends greatly on the quality and quantity of the pollution and is also affected by other factors such as the presence of toxic agents and environmental conditions. Nevertheless bioremediation is an applicable technology for a range of pollutants. Figure 1 shows the range of industries that use bioremediation as a technology.

The origins of bioremediation are rooted in the development of land farming as a technology to treat oil sludges arising from refineries, a process that has been used for at least 40 years. The technique exploited the ability of naturally occurring microorganisms in soil to biodegrade hydrocarbons. Later research showed the microorganisms indigenous to soil, groundwater and marine ecosystems could biodegrade a wide range of compounds released into the natural environment. These include aliphatic and aromatic hydrocarbons, chlorinated solvents and pesticides. Encouraging the rapid and complete biodegradation of these organic pollutants was the aim of the technology[5]. Since then bioremediation has evolved as a method for the removal and destruction of many environmental pollutants.

There are two main techniques in bioremediation for utilising bacteria to degrade toxic waste in aquatic and terrestrial environments. One method, biostimulation uses indigenous bacteria which are stimulated to grow by introducing nutrients into the soil or water environment and thereby enhance the biodegradation process[6,7]. Several laboratory and field experiments using biostimulation of microorganisms have been described where the addition of nutrients has shown positive effects on petroleum hydrocarbon decontamination[6].

Another method, bioaugmentation, involves culturing the bacteria independently and then adding them to the site[8]. For this purpose, bacterial strains can be directly isolated in laboratories using samples taken from contaminated sites or alternatively bacterial strains used in bioremediation exist in various repositories (for example, American Type Culture Collection) and are commercially available, although most are usually covered by patent rights. Each one of these strains may yield spectacular results *in vitro* for specific target compounds[9]. However, in some cases bioaugmentation was not able to show

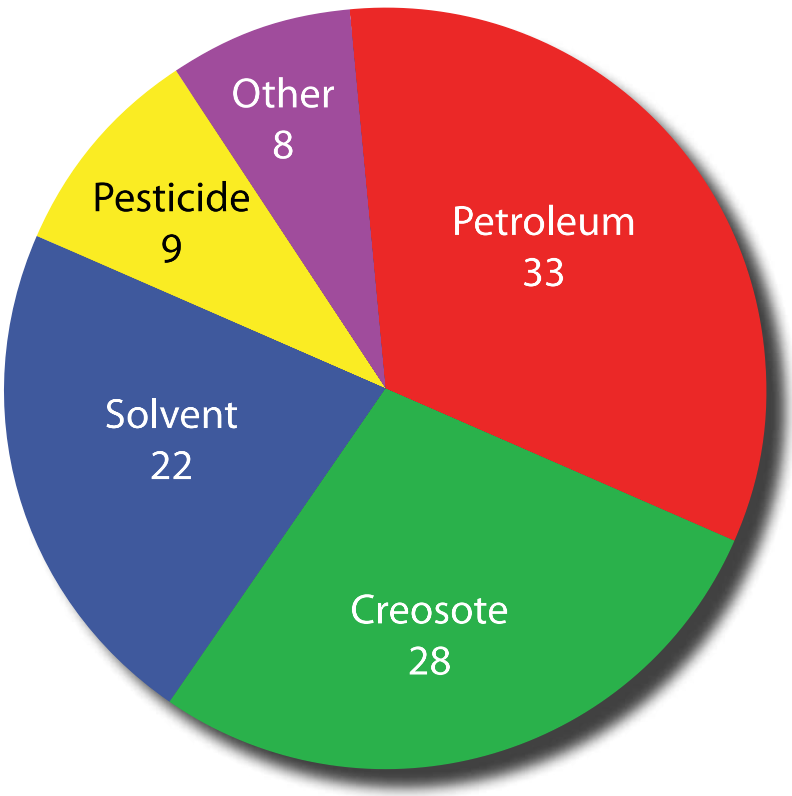

Figure 1. The range and weighting (as expressed as a percentage) of industries that utilise bioremediation[2].

higher remediation efficiencies compared with biostimulation[10] because inoculated microorganisms are not acclimatised to the target site and they could lose their intrinsic degradation activity[6]. Nevertheless, a number of previous studies have reported that bioaugmentation showed superior treatment efficiency[11,12].

Bioremediation is of interest worldwide as a potential clean-up option, especially for inaccessible or sensitive environments[13]. However, for some environments the promise of bioremediation has yet to be realised. This is because bioremediation strategies that are successful in one location might not work in another and microbial processes that remediate contaminants in laboratory incubations might not function well in the field. This is due to lack of adaptability of microbes to the field conditions[14]. In our recent study[15], we used a mesocosm system to assess the potential of a microbial consortia under environmental conditions and then demonstrated their efficacy in the field.

Another factor limiting the implementation of bioremediation is that unlike the concepts of excavation and disposal, which are easy to grasp, the mechanism controlling the growth and activity of microorganisms in contaminated environments are not well understood[16]. To assist in our understanding, in another recent study[17], we assessed the activity of a number of hydrocarbonoclastic bacteria to utilise a range of hydrocarbons, leading to the isolation of a range of hydrocarbonoclastic organisms with application in environments with multiple contaminants.

In conclusion, there exists 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 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 microorganisms. For chlorinated hydrocarbons, the addition of non-genetically modified halorespiring organisms into an environment has proved successful in both North America and Europe.

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Biographies

Professor Andy Ball has more than 25 years' research experience in environmental microbiology with extensive environmental project experience. In January 2012, Professor Andrew Ball was appointed Professor of Environmental Biotechnology at RMIT University. Previously in 2005 he was appointed Foundation Chair of Environmental Biotechnology at Flinders University. He started his career as a Research Fellow in Liverpool University and, more recently, held the position of Reader at the University of Essex, UK, where he was a member of staff for 16 years.

Krishna Kadali is a research scientist at RMIT University. He just started his career as a research scientist recently at RMIT University in January 2012. He has done his PhD and Masters at Flinders University in Environmental Biotechnology. His research interest is in molecular biology and its applications in bioremediation.