1 Biotechnology and Bioremediation – An Overview

Ajay Singh[1] and Owen P. Ward[2]

1 Introduction

The large-scale manufacturing, processing and handling of chemicals have led to serious surface and subsurface soil contamination with a wide variety of hazardous and toxic hydrocarbons. Many of the chemicals, which have been synthesized in great volume, including polychlorinated biphenyls (PCBs), trichloroethylene (TCE) and others, differ substantially in chemical structure from natural organic compounds and are designated as xenobiotics because of their relative recalcitrance to biodegradation. Other compounds, for example the polycyclic aromatic hydrocarbons (PAHs), are also toxic and the high molecular weight PAHs (having four or more fused rings) are typically recalcitrant to biodegradation. The latter compounds, the products of incomplete combustion of natural organic materials and hydrocarbons, occur in the soil environment as a result of naturally ignited forest fires. However, intensification of energy-related and other industrial processes with associated production of wastes and by-products, rich in PAHs, has led to serious soil contamination of many industrial sites. The resultant accumulations of the various organic chemicals in the environment, particularly in soil, are of significant concern because of their toxicity, including their carcinogenicity, and also because of their potential to bioaccumulate in living systems. A wide variety of nitrogen-containing industrial chemicals are produced for use in petroleum products, dyes, polymers, pesticides, explosives and pharmaceuticals. Major chemical groups involved include different nitroaromatics, nitrate esters and nitrogen-containing heterocycles. Many of these chemicals are toxic and threaten human health and are classified as hazardous by the United States Environmental Protection Agency.

[1] Petrozyme Technologies Inc., 7496 Wellington Road 34, R.R. #3, Guelph, Ontario N1H 6H9, Canada, e-mail: asingh@petrozyme.com, Tel.: 519-767-2299, Fax: 519-767-9435
[2] Department of Biology, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada

Soil Biology, Volume 2
Biodegradation and Bioremediation
(ed. by A. Singh and O. P. Ward)

Interest in bioremediation of polluted soil and water has increased in the last two decades primarily because it was recognized that microbes were able to degrade toxic xenobiotic compounds which were earlier believed to be resistant to the natural biological processes occurring in the soil. Although microbial activity in soil accounts for most of the degradation of organic contaminants, chemical and physical mechanisms can also provide significant transformation pathways for these compounds (Rogers 1998). Bioremediation is generally considered a safe and less expensive method for the removal of hazardous contaminants and production of non-toxic by-products (Providenti et al. 1993; Ward et al. 2003).

There have been many experimental successes with the more difficult to degrade contaminants, but there have also been many notable failures. However, it has been suggested that, although microorganisms have the primary catalytic role in bioremediation, our knowledge of the alterations occurring in the microbial communities remains limited and the microbial community is still treated as a "black box" (Iwamoto and Nasu 2001; Dua et al. 2002). Put in a more positive light, bioremediation remains a developing field, largely because it has traditionally been carried out in a natural environment where many of the organisms are uncharacterized and because no two environmental projects are identical (Watanabe 2001; Verstraete 2002).

Biotechnology has the potential to play an immense role in the development of treatment processes for contaminated soil. As with any microbial process, optimizing the environmental conditions in bioremediation processes is a central goal in order that the microbial, physiological and biochemical activities are directed towards biodegradation of the target contaminants. Environmental factors influencing microbial growth and bioactivity will include moisture content, temperature, pH, soil type, contaminant concentrations and oxygen for aerobic degradation and redox potential for anaerobic degradation. Deviations of these parameters away from optimal conditions will reduce rates of microbial growth and transformation of target substrates and perhaps cause premature cessation and failure of the bioremediation process. Biodegradation potential may also be limited by the toxicity of the pollutants to the degrading microbes. Some species have developed cellular defenses enabling them to tolerate high concentrations of toxic contaminants.

Understanding the biochemical and physiological aspects of bioremediation processes will provide us with the requisite knowledge and tools to optimize these processes, to control key parameters and to make the processes more reliable. Since the majority of bioremediation processes rely on the activities of complex microbial communities, we have much to learn about the interactive and interdependent roles played by individual species in these communities. We need to develop strategies for improving the bioavailability of the many hydrophobic contaminants which have an extremely low water solubility and tend to be adsorbed by soil particles and persist there. We need to continue to elucidate the complex aerobic and anaerobic metabolic pathways which microbes have evolved to degrade organic contaminants and to understand the nature of rate-limiting steps, bottlenecks and underlying genetic and biochemical regulatory mechanisms. We need to continue to characterize many of the key enzymatic reactions that participate in contaminant transformation and to relate contrasting reaction rates, substrate specificities and enzyme mechanisms to differences in protein structures. Such new knowledge can provide us with the requisite information to test, design and engineer biocatalysts with improved substrate specificities, reaction rates or other desired catabolic properties and ultimately to engineer improved catabolic pathways for bioremediation. We must recognize that some chemical species are inherently intractable to enzyme transformation and we should be open to the possibility of combining chemical or physical strategies with biological systems to achieve overall effective remediation. We must also continue to devise better methods for monitoring and assessing the progress and effectiveness of microbial biodegradation processes at both the research and process implementation level. Clearly, the availability of advanced molecular techniques provides a new impetus and enhances our abilities to address many of these issues.

These topics, introduced below, represent the main focus of this book.

2 Microbial Communities and Bioremediation

Since microbial communities play a significant role in biogeochemical cycles, it is important to analyze the community structure and its changes during bioremediation processes. The challenge of characterizing the roles of a range of hydrocarbon-metabolizing organisms in degrading the myriad of petroleum substrates present in hydrocarbon-contaminated soils is clearly substantial. However, such studies can provide major insights into important biochemical and physiological aspects of bioremediation and microbial catabolism. Culture-dependent and -independent methods are being applied to microbial community characterization. The temporal and spatial changes in bacterial populations and the diversity of the microbial community during bioremediation can be determined using sophisticated molecular methods (Stapleton et al. 1998; van Elsas et al. 1998; Widada et al. 2002). Recent advances in molecular techniques, combined with genomic information, are greatly assisting microbiologists in unraveling some of the mysteries related to the diverse roles of microbes in these communities. One of the exciting outcomes of these studies is an advance in our understanding of the degree and importance of lateral gene transfer in complex microbial communities. Catabolic genes have the ability to spread through a microbial community at high frequencies (Top and Springael 2003; Van der Meer and Senchilo 2003). An assessment of microbial community dynamics during hydrocarbon bioremediation is presented in Chapter 2.

3 Contaminant Bioavailability

For efficient microbial degradation of chemical contaminants to occur, the contaminants must be bioavailable to the degrading cells. The biodegradation rate of a contaminant depends on the rate of contaminant uptake and mass transfer. Bioavailability of a contaminant in soil is influenced by a number of factors such as desorption, diffusion and dissolution. The decrease in bioavailability due to long-term contamination of soil, often referred to as aging or weathering, is a result of chemical oxidation reactions and slow chemical diffusion of the contaminant into small pores incorporating contaminants into the organic matter. Use of chemical or bio-surfactants during the biodegradation process helps overcome bioavailability problems (Van Hamme et al. 2003). The molecular structure of the contaminant and hydrophobicity may also affect the pollutant uptake by the microorganisms. Indeed, the cells may also have active or selective systems for transporting the contaminants into the cell. Given that many of these contaminants have low solubility in aqueous media, understanding mechanisms of their uptake by the degrading microbes and developing strategies to promote or accelerate their accession represent important aspects of effective bioremediation processes. Chapter 3 provides a critical analysis of the bioavailability of organic pollutants. The hydrophobicity/low water solubility properties of PAHs cause them to associate with hydrophobic components in soil, thereby limiting their accession to microorganisms. This topic is discussed in detail in Chapter 5.

4 Microbial Catabolism of Organic Pollutants

Biodegradation involves the breakdown of organic compounds either through biotransformation into less complex metabolites or through mineralization into inorganic minerals, H_2O, CO_2 (aerobic) or CH_4 (anaerobic). Both bacteria and fungi have been extensively studied for their ability to degrade a range of environmental pollutants including recalcitrant polycyclic aromatic hydrocarbons, halogenated hydrocarbons and nitroaromatic compounds. The biochemical pathways/enzymes required for the initial transformation stages are often specific for particular target environmental contaminants, converting them to metabolites which can be assimilated into more ubiquitous central bacterial pathways. An overview of some of the biodegradation systems used by microorganisms in the catabolism of key organic contaminants in soil is presented in Table 1. The extent and rate of biodegradation depend on many factors including pH, temperature, oxygen, microbial population, degree of acclimation, accessibility of nutrients, chemical structure of the compound, cellular transport properties, and chemical partitioning in growth medium. Figure 1 provides a schematic of aspects of the biodegradation system involved in bioremediation.

Some recalcitrant chemicals contain novel structural elements that seldom occur in nature and which may be incompletely transformed as microbes lack the degradative pathway for complete degradation of these xenobiotics. While microbes may not have the metabolic pathways for mineralization of certain newly introduced synthetic chemicals, there is evidence that microorganisms have the capacity to evolve such catabolic systems over time. In bioremediation processes, it is generally an objective to exploit microbial technology to accelerate the rate of pollutant removal.

Many contaminants in soil exist in anaerobic environments. A couple of decades ago, by observing the anaerobic dechlorination of PCBs over time in Hudson River sediments, it became clear that microbes could transform contaminants under anaerobic conditions. By the late 1980s, there was conclusive evidence that hydrocarbons could be degraded in the absence of oxygen. These anaerobic degradation systems required terminal electron acceptors such as iron (III), manganese oxide or nitrate to replace that function of oxygen in aerobic systems. We have now entered a period of intensive research and discovery focused on the catalytic mechanisms which facilitate the anaerobic catabolism of pollutants. Anaerobic aspects of hydrocarbon bioremediation are discussed in detail in Chapter 4. As is mentioned above, anaerobic processes are

http://www.springer.com/978-3-540-21101-3

Biodegradation and Bioremediation
Singh, A.; Ward, O.P. (Eds.)
2004, XVII, 310 p., Hardcover
ISBN: 978-3-540-21101-3