

Biofilms in Bioremediation

Current Research and Emerging Technologies

Edited by

Gavin Lear

School of Biological Sciences
The University of Auckland
Auckland
New Zealand



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Front cover image: A dead sea bird following the Rena oil spill, New Zealand. Photo by Rambo Estrada, 2011.

Back cover image: Scanning electron micrograph image of a mixed freshwater microbial biofilm community. Designed by Andrew Dopheide, 2011.

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Preface

The continued release of contaminants into the environment, predominantly by industrial and agricultural activities, means that there is an ongoing need to remediate sites exposed to elevated concentrations of a wide range of pollutants. A number of chemical and physical processes are currently used to encourage the degradation of, or altered mobility of pollutants in field, laboratory and bioreactor settings. However, the microbial bioremediation of troublesome contaminants is increasingly seen as being both cost-effective and reliable, and a number of approaches are in widespread commercial use. Microbial bioremediation largely capitalizes on the metabolic activities of biofilm-dwelling microorganisms which are widely accepted as being responsible for the majority of pollutant degradation in natural environments. Common methods for the *in situ* biological treatment of contaminated sites include the use of permeable reactive biobarriers in which the natural movement of water is used to direct subsurface contaminants through engineered 'walls' of enhanced biofilm microbial activity. Alternatively, contaminated media can be extracted and manipulated within biofilm bioreactors. In this way, a greater level of experimental control is provided and the fate of pollutants, as well as their degradation products may be more easily monitored. Where the biomass and activity of potential degraders in the natural community is too low, new microbial strains can also be introduced. Regardless of the approach used, it is evident that biofilm-dwelling microbial communities rather than planktonic organisms dominate the bioremediation of most pollutants. Thus, an increased understanding of the complex structure of microbial biofilms and the communication and

cooperation among individual microbial cells will inevitably aid their successful use in bioremediation applications. Fortunately, recent advances in both molecular and microscopy-based methods have revolutionized our understanding of the microbial biofilm 'mode-of-life'. The three-dimensional structure of biofilms and their coating by extracellular polymeric substances have been interrogated using a broad range of microscopy-based techniques to reveal how this mode of community organization provides microbial cells with substantial additional protection from toxic substances and mechanical stresses. Gradients in the availability of nutrients, toxicants and gases across biofilm structures have also been observed and characterized. The complex spatial organization of biofilms provides a variety of microniches to support an increased diversity of microbial life and their associated metabolic potential. Evidence of this is provided by the outputs of DNA sequencing studies, while advanced isotope labelling methodologies have permitted the accurate identification of even low-abundance taxa involved in key metabolic processes. As advances in biofilm research continue, the scientific community is finding ever more applications and ways to manipulate the degradation of pollutants by biofilms present in soils, natural waters, and on the surfaces of other organisms frequenting polluted environments, including plants and even fish!

In the first part of this book we provide an up-to-date review of the latest scientific research that has contributed to our understanding of the vital importance of microbial biofilms for the biological remediation of contaminated environments. In part two, the results of a variety of key case

studies are presented to highlight the broad range of treatment approaches and applications at our disposal. Finally, as the application of biofilms in

bioremediation continues to increase, we seek to predict the future trends and likely growth areas in biofilm-related research.

Gavin Lear

Biofilms are structured communities of microorganisms that attach to surfaces and exert a pervasive impact across a range of sectors. Detrimental biofilms can incur significant costs to industry in terms of biofouling and corrosion, and in healthcare as a cause of antimicrobial resistance, persistent infections, and the failure of medical interventions. However, opportunities to exploit beneficial biofilms also exist, for example in microbial fuel cells, bioconversion processes such as waste-water treatment and bioremediation. This book treats the roles of biofilms in bioremediation. Biofilms, here taken to include flocs, comprise physical structures that support ecosystems of microorganisms. In turn, the microorganisms construct and engineer these biofilms, forming microbial communities (Flemming and Wingender, 2010). Biofilms are organized as stable communities of mono- or multi-specific cells of bacteria with or without eukaryotes, attached to each other and surfaces in different environments (Costerton, 1995). Biofilms: implications in bioremediation. *Trends Microbiol.* 2006 Sep;14(9):389-97. doi: 10.1016/j.tim.2006.07.001. Biofilms are assemblages of single or multiple populations that are attached to abiotic or biotic surfaces through extracellular polymeric substances. Gene expression in biofilm cells differs from planktonic stage expression and these differentially expressed genes regulate biofilm formation and development. Microorganisms in biofilms cooperate through quorum sensing. Biofilm Formation. A biofilm is a cooperative aggregate of microorganisms associated with a surface and enclosed in a polymeric matrix (‘‘slime’’). Biofilms may form on plant surfaces to form symbiotic relationships with the plant (e.g. nitrogen-fixing *Rhizobium* on roots). Alternatively, biofilms may form on plant surfaces to cause crop diseases (e.g. citrus canker is associated with biofilms). Aggregate of microorganisms in which cells that are frequently embedded within a self-produced matrix of extracellular polymeric substances (EPSs) adhere to each other and/or to a surface. Note 1: A biofilm is a system that can be adapted internally to environmental conditions by its inhabitants. Note 2: The self-produced matrix of extracellular polymeric substances, which is also referred to as slime, is a polymeric conglomeration generally composed of extracellular biopolymers in various structural