SECOND EDITION

ENVIRONMENTAL BIOTECHNOLOGY

THEORY AND APPLICATION

GARETH M. EVANS AND JUDITH C. FURLONG

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Environmental Biotechnology

Theory and Application

Second Edition

by

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Environmental Biotechnology

This book is dedicated with much love to our respective parents, partners and pooches.

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Foreword

Environmental biotechnology has come of age and many of the technologies have developed at a surprising rate both scientifically and perhaps more importantly as practical techniques too. On both counts then, it's appropriate that there is now to be a second edition of this book. Industry has been struggling, particularly over recent years, with the need for increasing compliance with new laws and a changed public perspective and all of this has made 'green' technologies very attractive. But this can only work in real terms when those technologies are cost effective and the emphasis is on both 'cost' and 'effective'. It is something that, happily, both sides, industry and technology providers, seem increasingly to have woken up to.

Carbon reduction could be a massive force for change. In the water industry alone sludge thickening is a hideously energy-intensive process and accounts for something like a third of the capital costs at a plant and half of its operating costs. If you once start looking at ways to reduce carbon consumption then you're bound to be finding ways to reduce those costs too and so initiatives such as the UK's recent Climate Change Act and the Carbon Reduction Commitment have major implications and are going to drive a major step-change across industry as a whole. It could also be a massive spring-board for sustainable growth too.

Ecosystems are unbelievably complex, but the basic principles that drive them are equally simple and it is precisely this area that is the natural realm of environmental biotechnology and why the second edition of this book is so timely. It provides an excellent framework for understanding the fundamentals and biochemical processes that underpin the practical, and a cogent exposition of those practical applications themselves. It is a complete introduction to the broad church, that is modern environmental biotechnology and as such will be of great value to undergraduates of course, studying the subject, but also to other professionals in the wide and growing range of industries to which the subject is becoming increasingly relevant.

With its logical structure, clear and authoritative explanations and fundamentally readable style, I whole-heartedly recommend it to any students, researchers and environmental managers, in fact anyone who is looking to understand this important science and how it will, I hope, play a big part in shaping our low carbon future.

Dr Dene Clackmann
Principal of Clackmann Associates and Chair of the Carbon Commune Group

Preface

When we began work on the first edition of this book back in 2001, we set out to present a fair reflection of the practical biological approaches that were then currently being employed to address environmental problems, and to provide the reader with a working knowledge of the science which underpinned them. It was a straightforward goal and one which, like the book itself, sprang out of our Environmental Biotechnology modules at the University of Durham, but as we said at the time, this was never intended to be just another 'book of the course'. That thinking remains in this version, but we have been given the rare opportunity in it of being able to revise and update the content to once again, we hope, give a fair and honest overview of the real world applications of this fascinating branch of environmental management, ten years on.

It has been an interesting journey, particularly in respect of the outcomes of technologies and techniques that were innovative and new then – seeing which of our predictions came true and those for which we were wide of the mark. On balance, we feel the former satisfyingly outnumber the latter, but modesty prevents us from banging that particular drum too loudly; more objective minds than ours should make that call, if they so wish.

We received many useful comments from the many reviews and reviewers of the first edition, which was it seems, gratifyingly well received and a good number of their suggestions have helped to shape the changes that we have made. In the process of writing this update, we bore two things in particular, in mind. Firstly, from the comments we have had from successive waves of our own students and some of those who had read the earlier version, we believed that the fundamental approach we have adopted to the subject, works. That in itself is less of a unique insight than the result of the happy accident of our respective backgrounds, which so perfectly mix the academic and the practical, making 'theory and application' a natural focus as much as an appropriate title. Secondly, we were reminded of the words of an editor of our acquaintance: the most powerful drive known to our species is not for survival, nor to procreate, but to alter someone else's copy. We decided that unless it was really justified, we were not going to change our own.

Consequently, although all the case studies are new – as befits the progress that has been made in this field – the familiar shape of the original remains.

We have retained the logical structure we adopted at the outset addressing technologies in as cohesive a manner as possible, which we still feel is the obvious approach, given the intrinsic interrelatedness of so much of our subject matter.

While the fundamental arrangement is, of course, still intended to unify the whole work, we have tried to keep each chapter as much of a 'stand-alone' as possible, in an attempt to make this a book which permits the interested reader to just 'dip in'. Ultimately, of course, it still remains for that reader to decide how successful we have been.

The text falls into three main parts. The early chapters again examine issues of the role and market for biotechnology in an environmental context, the essential biochemistry and microbiology which enables them to be met, and the fundamental themes of biological intervention. The technologies and applications themselves make up the central core of the book, both literally and figuratively and, fittingly, this is the largest part. Finally, some of the current aspects of, and future potential for, integration in the wider field of environmental biotechnology are discussed. There is, however, one departure from the original – this time there is no Chapter 11; no final discussion of 'The Way Ahead'. Ten years ago, environmental biotechnology was a much younger field and those predictions had some purpose. Today, it has assumed its rightful place as a realistic alternative to many of the earlier established approaches for manufacturing, land remediation, pollution control and waste management and the pace of change is now just too fast to make useful forecasts that will be meaningful for the next ten years. It would be presumptuous and in any case, on balance, we feel we got most of our forecasts about right last time; we are not about to push our luck!

Despite the passage of time and all the attempts at the rationalisation of global environmental regulation, the whole subject remains inherently context dependent – a point which inevitably recurs throughout the discussion – and local modalities can conspire to shape individual best practice in a way unknown in other branches of biotechnology. What works in one country may not in another, not because the technology is flawed, but often simply because economic, legislative or societal barriers so dictate. The environmental biotechnologist must still sometimes perform the mental equivalent of a circus act in balancing these many and different considerations. It is only to be expected, then, that the choices we have made as to what to include, and the relative importance afforded them, reflect these experiences. Some readers will take issue with those decisions, but that has always been the lot of writers. It would be unrealistic to expect that we should be treated any differently.

As we wrote in the first 'Preface', it has been said that the greatest thing that anyone can do is to make a difference. It remains our hope that with this second edition, we can again in some small way, do just that.

Acknowledgements

Writing any book always involves more people than the authors, or those who work for or on behalf of the publishing company, sometimes very directly, sometimes rather less obviously so. Remembering to say 'thank you' to those who have done something very concrete or obvious is seldom a problem and there are some old friends amongst that group – especially Rob Heap and Bob Talbott – along with Bob Rust, Graham Tebbitt, Vanessa Trescott and Bob Knight, who helped us get everything straight and in time for our first deadline all those years ago! The same thing is true of people who lend you space on their coffee table when you simply have to finish a chapter – so 'thanks' again to Linda Ormiston, OBE. Sadly one of our biggest supporters at Durham – Professor Peter Evans (no relation, by the way) – died shortly before the first edition was completed and it is our great regret that he, who had given us so much encouragement to build up the Environmental Biotechnology course and was so sympathetic to the wider objectives of this book, did not see it published.

Though the personnel has changed – Keily Larkins, Lyn Roberts and Laura Stockton last time, Liz Renwick, Rachael Ballard, Izzy Canning and Fiona Woods this – the good folk at John Wiley & Sons Ltd have been their usual unflappable selves, checking in periodically to make sure that everything is still going to plan and the final manuscript is going to come in pretty much on time.

We are of course, constantly reminded of Newton's words – that we stand on the shoulders of giants – and happily acknowledge the broader debt that we owe not only to the great biologists, biochemists and engineers, but also to all those have travelled this route before us, to our own teachers who inspired us, to our contemporaries who spurred us on and to our parents without whom, in the most literal of senses, none of this would have been possible.

We were and are deeply grateful to all of these people for their help and support – and to anyone we have missed out, we are truly sorry; the slight really was not intentional. Finally, ten years on, our dogs have changed too; Mungo and Megan are, sadly, no more; the burden of missed walks and late meals has fallen on new paws. To Bess and Nell, we can only apologise – such is the lot of the writer's mutt.

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Introduction to Environmental Biotechnology

The Organisation for Economic Co-operation and Development (OECD) defines biotechnology as 'the application of science and technology to living organisms, as well as parts, products and models thereof, to alter living or non-living materials for the production of knowledge, goods and services' (OECD, 2002). Despite the inclusiveness of this definition, there was a time when the biotechnology sector was seen as largely medical or pharmaceutical in nature, particularly amongst the general public. While to some extent the huge research budgets of the drug companies and the widespread familiarity of their products made this viewpoint understandable, it somewhat unfairly distorted the picture. Thus therapeutic instruments were left forming the 'acceptable' face of biotechnology, while elsewhere, the science was all too frequently linked with an uneasy feeling of unnatural interference. The agricultural, industrial and environmental applications of biotechnology are potentially enormous, but the shadow of Frankenstein has often been cast across them. Genetic engineering may be relatively commonplace in pharmaceutical thinking and yet when its wider use is mooted in other spheres, such as agriculture, for example even today much of society views the possibility with suspicion, if not outright hostility.

The history of human achievement has always been episodic. For a while, one particular field of endeavour seems to hold sway as the preserve of genius and development, before the focus shifts and the next wave of progress forges ahead in a dizzy exponential rush in some entirely new direction. So it was with art in the Renaissance, music in the eighteenth century, engineering in the nineteenth and physics in the twentieth. Now it is the age of the biological – in many ways forming a kind of rebirth, following on from the heyday of the great Victorian naturalists, who provided so much input into the developing science.

It is then, perhaps, no surprise that the European Federation of Biotechnology begins its 'Brief History' of the science in the year 1859, with the publication of *On the Origin of Species by Means of Natural Selection* by Charles Darwin. Though his famous voyage aboard *HMS Beagle*, which led directly to the formulation of his (then) revolutionary ideas, took place when he was a young man, he had delayed making them known until 1858, when he made a joint

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presentation before the Linnaean Society with Alfred Russell Wallace, who had, himself, independently come to very similar conclusions. Their contribution was to view evolution as the driving force of life, with successive selective pressures over time endowing living beings with optimised characteristics for survival. Neo-Darwinian thought sees the interplay of mutation and natural selection as fundamental. The irony is that Darwin himself rejected mutation as too deleterious to be of value, seeing such organisms, in the language of the times, as 'sports' – oddities of no species benefit. Indeed, there is considerable evidence to suggest that he seems to have espoused a more Lamarckist view of biological progression, in which physical changes in an organism's lifetime were thought to shape future generations.

Darwin died in 1882. Ninety-nine years later, the first patent for a genetically modified organism was granted to Ananda Chakrabarty of the US General Electric, relating to a strain of *Pseudomonas aeruginosa* engineered to express the genes for certain enzymes in order to metabolise crude oil. Twenty years on from that, the first working draft of the human genome sequence was published and the full genetic blueprint of the fruit fly, *Drosophila melanogaster*, that archetype of eukaryotic genetics research, announced – and developments have continued on what sometimes feels like an almost daily basis since then. Today biotechnology has blossomed into a major growth industry with increasing numbers of companies listed on the world's stock exchanges and environmental biotechnology is coming firmly into its own alongside a raft of 'clean technologies' working towards ensuring the sustainable future of our species and our planet.

Thus, at the other end of the biotech timeline, a century and a half on from *Origin of Species*, the principles it first set out remain of direct relevance, although increasingly in ways that Darwin himself could not possibly have foreseen.

The Role of Environmental Biotechnology

If pharmaceutical biotechnology represents the glamorous end of the market, then environmental applications are decidedly more in the Cinderella mould. The reasons for this are fairly obvious. The prospect of a cure for the many diseases and conditions currently promised by gene therapy and other biotech-oriented medical miracles can potentially touch us all. Our lives may, quite literally, be changed. Environmental biotechnology, by contrast, deals with far less apparently dramatic topics and, though their importance, albeit different, may be every bit as great, their direct relevance is far less readily appreciated by the bulk of the population. Cleaning up contamination and dealing rationally with wastes is, of course, in everybody's best interests, but for most people, this is simply addressing a problem which they would rather had not existed in the first place. Even for industry, though the benefits may be noticeable on the balance sheet, the likes of effluent treatment or pollution control are more of an inevitable obligation than a primary goal in themselves. In general, such activities are typically funded on a distinctly limited budget and have traditionally been viewed as a necessary inconvenience. This is in no way intended to be disparaging to industry; it simply represents commercial reality.

In many respects, there is a logical fit between this thinking and the aims of environmental biotechnology. For all the media circus surrounding the grand questions of our age, it is easy to forget that not all forms of biotechnology involve xenotransplantation, genetic modification, the use of stem cells or cloning. Some of the potentially most beneficial uses of biological engineering, and which may touch the lives of the majority of people, however indirectly, involve much simpler approaches. Less radical and showy, certainly, but powerful tools, just the same. Environmental biotechnology is fundamentally rooted in waste, in its various guises, typically being concerned with the remediation of contamination caused by previous use, the impact reduction of current activity or the control of pollution. Thus, the principal aims of this field are the manufacture of products in environmentally harmonious ways, which allow for the minimisation of harmful solids, liquids or gaseous outputs or the clean-up of the residual effects of earlier human occupation.

The means by which this may be achieved are essentially twofold. Environmental biotechnologists may enhance or optimise conditions for existing biological systems to make their activities happen faster or more efficiently, or they resort to some form of alteration to bring about the desired outcome. The variety of organisms which may play a part in environmental applications of biotechnology is huge, ranging from microbes through to trees and all are utilised on one of the same three fundamental bases – accept, acclimatise or alter. For the vast majority of cases, it is the former approach, accepting and making use of existing species in their natural, unmodified form, which predominates.

The Scope for Use

There are three key points for environmental biotechnology interventions, namely in the manufacturing process, waste management or pollution control, as shown in Figure 1.1.

Accordingly, the range of businesses to which environmental biotechnology has potential relevance is almost limitless. One area where this is most apparent

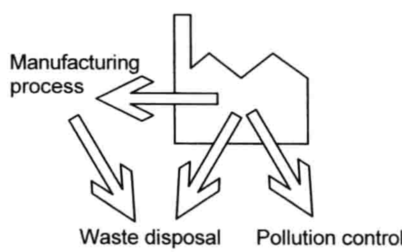

Figure 1.1 *The three intervention points*

is with regard to waste. All commercial operations generate waste of one form or another and for many, a proportion of what is produced is biodegradable. With disposal costs rising steadily across the world, dealing with refuse constitutes an increasingly high contribution to overheads. Thus, there is a clear incentive for all businesses to identify potentially cost-cutting approaches to waste and employ them where possible. Changes in legislation throughout Europe, the US and elsewhere, combined with growing environmental awareness and a burgeoning demand for reduced carbon footprints have inevitably driven these issues higher up the political agenda and biological methods of waste treatment have gained far greater acceptance as a result. For those industries with particularly high biowaste production, the various available treatment biotechnologies can offer considerable savings.

Manufacturing industries can benefit from the applications of whole organisms or isolated bio-components. Compared with conventional chemical processes, microbes and enzymes typically function at lower temperatures and pressures. The lower energy demands this makes leads to reduced costs, but also has clear benefits in terms of both the environment and work-place safety. Additionally, biotechnology can be of further commercial significance by converting low cost organic feedstocks into high value products or, since enzymatic reactions are more highly specific than their chemical counterparts, by deriving final substances of high relative purity. Almost inevitably, manufacturing companies produce wastewaters or effluents, many of which contain biodegradable contaminants, in varying degrees. Though traditional permitted discharges to sewer or watercourses may be adequate for some, other industries, particularly those with recalcitrant or highly concentrated effluents, have found significant benefits to be gained from using biological treatment methods themselves on site. Though careful monitoring and process control are essential, biotechnology stands as a particularly cost-effective means of reducing the pollution potential of wastewater, leading to enhanced public relations, compliance with environmental legislation and quantifiable cost-savings to the business.

Those involved in processing organic matter, for example or with drying, printing, painting or coating processes, may give rise to the release of volatile organic compounds (VOCs) or odours, both of which represent environmental nuisances, though the former is more damaging than the latter. For many, it is not possible to avoid producing these emissions altogether, which leaves treating them to remove the offending contaminants the only practical solution. Especially for relatively low concentrations of readily water soluble VOCs or odorous chemicals, biological technologies can offer an economic and effective alternative to conventional methods.

The use of biological cleaning agents is another area of potential benefit, especially where there is a need to remove oils and fats from process equipment, work surfaces or drains. Aside from typically reducing energy costs, this may also obviate the need for toxic or dangerous chemical agents. The pharmaceutical and brewing industries, for example both have a long history of employing enzyme-based cleaners to remove organic residues from their process equipment.

In addition, the development of effective biosensors, powerful tools which rely on biochemical reactions to detect specific substances, has brought benefits to a wide range of sectors, including the manufacturing, engineering, chemical, water, food and beverage industries. With their ability to detect even small amounts of their particular target chemicals, quickly, easily and accurately, they have been enthusiastically adopted for a variety of process monitoring applications, particularly in respect of pollution assessment and control.

Contaminated land is a growing concern for the construction industry, as it seeks to balance the need for more houses and offices with wider social and environmental goals. The re-use of former industrial sites, many of which occupy prime locations, may typically have associated planning conditions attached which demand that the land be cleaned-up as part of the development process. With urban regeneration and the reclamation of 'brown-field' sites increasingly favoured in many countries over the use of virgin land, remediation has come to play a significant role and the industry has an on-going interest in identifying cost-effective methods of achieving it. Historically, much of this has involved simply digging up the contaminated soil and removing it to landfill elsewhere. Bioremediation technologies provide a competitive and sustainable alternative and in many cases, the lower disturbance allows the overall scheme to make faster progress.

As the previous brief examples show, the range of those which may benefit from the application of biotechnology is lengthy and includes the chemical, pharmaceutical, water, waste management and leisure industries, as well as manufacturing, the military, energy generation, agriculture and horticulture. Clearly, then, this may have relevance to the viability of these ventures and, as was mentioned at the outset, biotechnology is an essentially commercial activity. Environmental biotechnology must compete in a world governed by the Best Practicable Environmental Option (BPEO) and the Best Available Techniques Not Entailing Excessive Cost (BATNEEC). Consequently, the economic aspect will always have a large influence on the uptake of all initiatives in environmental biotechnology and, most particularly, in the selection of methods to be used in any given situation. It is impossible to divorce this context from the decision-making process. By the same token, the sector itself has its own implications for the wider economy.

The Global Environmental Market

The global environmental market is undergoing a period of massive growth. In 2001, the UK's Department of Trade and Industry estimated its value at around 1500 billion US dollars, of which some 15–20% was biotech-based. Although the passage of time has now shown some of the growth forecasts then made for the following years to have been somewhat optimistic, a recent study predicts that the market will have grown to 7400 billion US dollars by 2025 (Helmut Kaiser Consultancy, 2009). There are several major factors acting as drivers for this growth, including a greater general awareness of environmental issues, the widespread adoption of sustainable best practice by industry and geo-political changes that open new territories for technology transfer. In addition, biotechnology has increasingly gained acceptance for clean manufacturing applications, with the use of biomimetics in particular showing marked expansion over recent years, while energy production, waste management and land remediation have all benefited from the ongoing trend stimulating the sales of biotechnology-based environmental processing methods. Water treatment in its broadest sense has been perhaps the biggest winner in all this, the sector now accounting for some 25% of the total global environmental market (Helmut Kaiser Consultancy, 2009).

The export of environmental technologies is now a significant contributor to the global market, which will continue to expand in the burgeoning worldwide trend towards driving economic development alongside strong ecological awareness. Although such technology transfer is likely to continue to play a major role on the global scene, it is also probable that many countries will increasingly build their own comprehensive indigenous environmental industry over the coming years, thus circumventing their dependence on innovation imports.

Over the last decade, as many predicted, the regulatory framework across the world has experienced a radical tightening, with existing legislation on environmental pollution being more rigorously enforced and more stringent compliance standards implemented. It is hard to imagine that this trend will stop in the coming years, which once again feeds the expectation that it will act as a significant stimulus for the sales of biotechnology-based environmental processing methods. This would seem particularly likely in the current global main markets for environmental technologies, namely Asia in general, China, Japan, Europe and the USA (Helmut Kaiser Consultancy, 2009).

The benefits are not, however, confined to the balance sheet. The OECD (2001) concluded that the industrial use of biotechnology commonly leads to increasingly environmentally harmonious processes and additionally results in lowered operating and/or capital costs. For years, industry has appeared locked into a seemingly unbreakable cycle of growth achieved at the cost of environmental damage. This OECD investigation provided probably the first hard evidence to support the reality of biotechnology's long heralded promise of alternative production methods which are ecologically sound and economically efficient. A variety of industrial sectors, including pharmaceuticals, chemicals, textiles, food and energy were examined, with a particular emphasis on biomass renewable resources, enzymes and bio-catalysis. While such approaches may have to be used in tandem with other processes for maximum effectiveness, it seems that their use invariably leads to reduction in operating or capital costs, or both. Moreover, the research also concluded that it is clearly in the interests of governments of the developed and developing worlds alike to promote the use of biotechnology for the substantial reductions in resource and energy consumption, emissions, pollution and waste production it offers. The potential contribution to be made by the appropriate use of biotechnology to both environmental and economic sustainability would seem to be clear.

The upshot of this is that few biotech companies in the environmental sector perceive problems for their own business development models, principally as a result of the wide range of businesses for which their services are applicable and the large potential for growth. Competition within the sector is not seen as a major issue either, since the field is still largely open and unsaturated, and from the employment perspective, the biotech industry seems a robust one. Although the economic downturn saw the UK science labour market in general shed both permanent and contract staff throughout 2009, the biotech sector increased its demand for skilled scientists and predictions suggest that it will continue to buck the trend in the future (SRG, 2010). Moreover, there has been an established tendency towards niche specificity, with companies operating in more specialised sub-arenas within the environmental biotechnology umbrella. Given the number and diversity of such possible slots, coupled with the fact that new opportunities, and the technologies to capitalise on them, are developing apace, this trend seems likely to continue, though the business landscape is beginning to change. In some sectors, aggressive rivalry for market penetration has begun to produce bigger, multi-disciplinary environmental companies, largely through partnerships, acquisitions and direct competition. It is not without some irony that companies basing their commercial activities on biological organisms should themselves come to behave in such a Darwinian fashion. However, the picture is not entirely rosy.

Typically the sector comprises a number of relatively small, specialist companies. According to the *OECD Biotechnology Statistics 2009*, based on government survey data for 22 OECD countries and an additional four non-member countries, the majority of both biotechnology and biotechnology R&D companies have fewer than 50 employees – the average by country being 67 and 63% respectively (van Beuzekom and Arundel, 2009). As a consequence, the market has tended to be somewhat fragmented. Often the complexities of individual projects make the application of 'standard' off-the-shelf approaches very difficult, inevitably meaning that much of what is done must be significantly customised. While this, of course, is a strength and of great potential environmental benefit, it also has hard commercial implications which must be taken into account. Although the situation has begun to be addressed over recent years, historically a sizeable proportion of companies active in this sphere have had few products or services which might reasonably be termed suitable for generalised use, though they may have enough expertise, experience or sufficiently perfected techniques to deal with a large number of possible scenarios.

Historically, one of the major barriers to the wider uptake of biological approaches has been the high perceived cost of these applications. For many years, the solutions to all environmental problems were seen as expensive and for some, particularly those unfamiliar with the multiplicity of varied technologies available, this view has been slow to fade. Generally, there is often a lack of financial resource allocation available for this kind of work and biotech providers have sometimes come under pressure to reduce the prices for their services as a result. Awareness of the benefits of biotechnology, both as a means

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