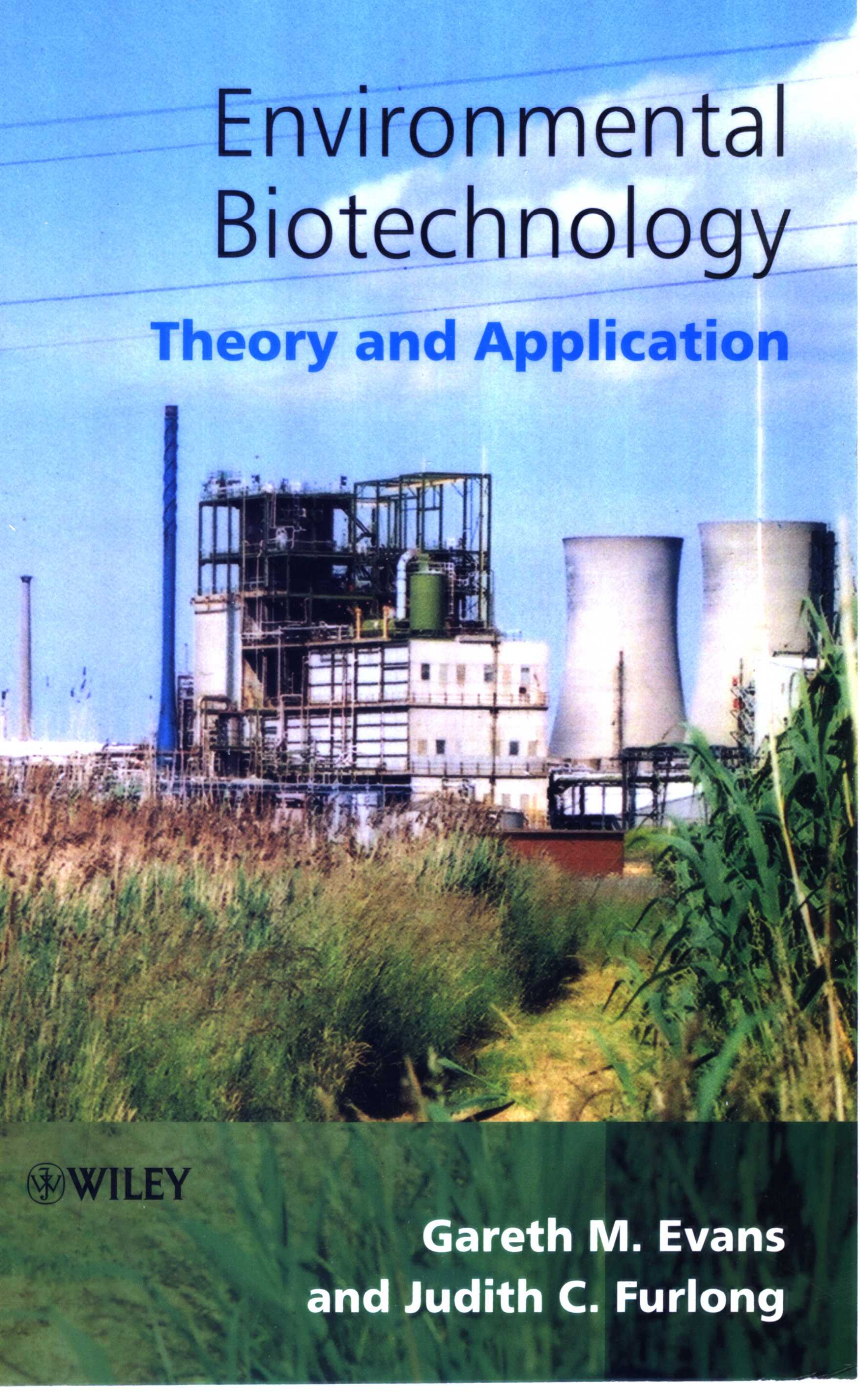
Environmental Biotechnology

Theory and Application



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Foreword

by Professor Bjørn Jensen

Chairman of the European Federation of Biotechnology, Environmental Biotechnology Section and Research and Innovation Director, DHI Water and Environment.

Environmental biotechnology has an exciting future. Just the thought of having microorganisms work for you – simply by feeding them with natural substrates and having hazards turned into minerals and nature's own basic constituents – is really intriguing. Of course, we all know that it is not that simple, but nevertheless it is both the fundamental premise, and the ultimate goal, which we must bear in mind in all developments in the field.

Environmental biotechnology is not an easy subject to cover, but therefore so much the more important that it should be. For years, the environmental technologies had a little too much tail wind because of the overwhelming sympathy for green solutions. This often led to misuse and discredit of the technologies among some end users. In those years, too many studies underestimated the complexity of the task. The inevitable outcome was poor documentation, not giving enough credit to the processes involved and the degree of process control required. The reputations of these technologies were also hampered by the fact that some of those who were in favour of them were too ambitious as to when these technologies should be applied, and gave too little emphasis to other competing approaches which might have been more useful.

Environmental biotechnology has now fully regained its reputation, due to the hard work of skilled and dedicated scientists. Reliable documentation within a number of areas is rapidly accumulating, and new emerging approaches and tools are distinguishing the field. For these reasons, this book is extremely well timed.

The book covers both the basic fundamentals and biochemical processes involved, as well as the technologies themselves within different areas of application. As part of the framework, it also provides a thorough description of the character of pollution and pollution control, and there are chapters on more modern approaches to the subject, such as integrated environmental biotechnology and genetic tools – all in all a complete introduction to the study of environmental biotechnology.

There is no doubt that this book will be one of inspiration for all professionals in the field. It is a very good framework for understanding the complex nature of processes and technology, and as such it will be useful for researchers, practitioners and other parties who need a working knowledge of this fascinating subject.

Preface

This work inevitably sprang out of our environmental biotechnology modules at the University of Durham, but it is not intended to be just another 'book of the course'. Though it is clearly rooted in these origins, it reflects our wider, and rather varied, experiences of the field. In many respects, we have been fortunate; teaching has undoubtedly drawn on the 'theory', while our own consultancy has tended to focus us on the 'application'. Indeed, our own particular backgrounds mean that our partnership is based in both the academic and the practical. Like many before us, we came to the subject largely by accident and via other original disciplines, in the days before educational institutions offered anything other than traditional programmes of study and, please remember, this was not so long ago. The rise of environmental studies, which must surely be amongst the most inherently applicable of applied sciences, and the growing importance of biotechnology usage in this respect, remain two of the most encouraging developments for the future of our planet.

Within a very short time, biotechnology has come to play an increasingly important role in many aspects of everyday life. The upsurge of the 'polluter pays' principle, increasing pressure to revitalise the likes of former industrial sites and recent developments within the waste industry itself have combined to alter the viability of environmental biotechnology radically in the last five years. Once an expensive and largely unfamiliar option, it has now become a realistic alternative to many established approaches for manufacturing, land remediation, pollution control and waste management. Against a background of burgeoning disposal costs and ever more stringent legislation and liabilities, the application of biologically engineered solutions seems certain to continue its growth.

The purpose of this book is a straightforward one: to present a fair reflection of the practical biological approaches currently employed to address environmental problems, and to provide the reader with a working knowledge of the science that underpins them. In this respect, it differs very little from the ethos of our course at Durham and we are grateful to each successive wave of students for constantly reminding us of the importance of these two goals. In other ways, this work represents a major departure. Freed from the constraints of time and the inevitable demands of exams, we have been afforded the luxury in this book of being able to include far more in each section than could reasonably be covered in a traditional series of lectures on the topic. In some places, this has allowed us to delve in deeper detail, while in others it has permitted some of the lesser well-known aspects of this fascinating discipline to be aired anew.

We have adopted what we feel is a logical structure, addressing technologies in as cohesive a manner as possible, given the intrinsic interrelatedness of so much of our subject matter. While the fundamental structure is, of course, intended to unify the whole work, we have tried to make each chapter as much of a 'standalone' as possible, in an attempt to make this a book which also encourages 'dipping in'. Ultimately, of course, the reader will decide how successful we have been.

The text falls into three main parts. The early chapters 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, aspects of integration and the future development of environmental biotechnology are addressed.

This subject is inherently context-dependent – a point which 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 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. It is equally inevitable that some readers will take issue with these decisions, but that has always been the lot of writers. As an editor of our acquaintance once confided, the most powerful drive known to our species is not for survival, nor to procreate, but to alter someone else's copy.

It has been said that the greatest thing that anyone can achieve is to make a difference. We hope that, in writing this book, we will, in some small way, do just that.

Acknowledgements

The authors of any book always owe a debt of thanks to many people. Not in the slightly sycophantic way of the film awards, but in a very real sense, there truly are those without whom it would not have been possible to get the job done. The writers of this book are no exception and would like to say a public thank you to everyone who helped us along the way. To single anyone out always runs the risk of being divisive, but to omit a few particular individuals would be churlish in the extreme. We are particularly grateful to Lynne and David Lewis-Saunders for the use of our compact and bijou residence in the Dales, where so much of this book was written and to Linda Ormiston, OBE, for the loan of her coffee table, where most of the rest of it took shape.

We are, of course, terribly aware of the loss of the late Professor Peter Evans and enormously grateful to him for encouraging us to build up the environmental biotechnology course. He was very supportive of the wider objectives of this present work and it is a cause of much sadness that he will not see its publication. Our thoughts are with Di: both she and the University of Durham lost a thoroughly good man.

Thanks must also go to old friends – John Eccles, Rob Heap and Bob Talbott – for their assistance and to David Swan, Bob Rust, Graham Tebbitt, Vanessa Trescott and Bob Knight, for helping to get various facts and figures straight and in time for our deadline. Keily Larkins and Lyn Roberts of John Wiley & Sons Ltd have played a great game throughout. Always helpful and supportive, between them they have made contact often enough to reassure themselves that things really were progressing, but not so often as to intrude. This must be an awfully difficult balancing act and they have managed it very well.

We also know, to use the oft-quoted statement of Newton, that we stand on the shoulders of giants; that whatever knowledge we may possess and hopefully impart in this book, was gained thanks to those who have travelled this route before us. The debt to the great biologists, biochemists and engineers is clear, but it exists just as much to our own teachers who inspired us, to our contemporaries who spurred us on and to our parents without whom, quite literally, none of this would have been possible.

To all of these people we are deeply grateful for their help and support, as well as to our dogs, Mungo and Megan, for being quite so forgiving when the need to finish another chapter meant that their walks had to be curtailed.

Contents

1

Introduction to Biotechnology

The *Chambers Science and Technology Dictionary* defines biotechnology as 'the use of organisms or their components in industrial or commercial processes, which can be aided by the techniques of genetic manipulation in developing e.g. novel plants for agriculture or industry.' Despite the inclusiveness of this definition, the biotechnology sector is still often 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 makes this understandable, it does distort the full picture and somewhat unfairly so. However, while therapeutic instruments form, in many respects, the 'acceptable' face of biotechnology, elsewhere the science is all too frequently linked with unnatural interference. While the agricultural, industrial and environmental applications of biotechnology are potentially very great, the shadow of Frankenstein has often been cast across them. Genetic engineering may be relatively commonplace in pharmaceutical thinking and yet in other spheres, like agriculture for example, society can so readily and thoroughly demonise it.

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 development forges ahead in dizzy exponential rush in an entirely new direction. So it was with art in the renaissance, music in the 18th century, engineering in the 19th and physics in the 20th. Now it is the age of the biological, possibly best viewed almost as a rebirth, after the great heyday of the 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 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 after his death, 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 later still, in the year that saw the first working draft of the human genome sequence published and the announcement of the full genetic blueprint of the fruit fly, *Drosophila melanogaster*, that archetype of eukaryotic genetics research, biotechnology has become a major growth industry with increasing numbers of companies listed on the world's stock exchanges. 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 for what has been termed the 'chemical evolution' of biologically active substances and are commonly used in laboratories for *in vitro* production of desired qualities in biomolecules.

The Role of Environmental Biotechnology

While pharmaceutical biotechnology represents the glamorous end of the market, 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 two-fold. 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 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

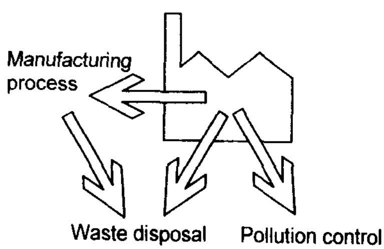

Figure 1.1 *The three intervention points*

employ them where possible. Changes in legislation throughout Europe, the US and elsewhere, have combined to drive 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 biocomponents. 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 workplace 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 reuse 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 ongoing 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 Market for Environmental Biotechnology

The UK's Department of Trade and Industry estimated that 15–20% of the global environmental market in 2001 was biotech-based, which amounted to about 250–300 billion US dollars and the industry is projected to grow by as much as ten-fold over the following five years. This expected growth is due to greater acceptance of biotechnology for clean manufacturing applications and energy production, together with increased landfill charges and legislative changes in waste management which also alter the UK financial base favourably with respect to bioremediation. Biotechnology-based methods are seen as essential to help meet European Union (EU) targets for biowaste diversion from landfill and reductions in pollutants. Across the world the existing regulations on environmental pollution are predicted to be more rigorously enforced, with more stringent compliance standards implemented. All of this is expected to stimulate the sales of biotechnology-based environmental processing methods significantly and, in particular, the global market share is projected to grow faster than the general biotech sector trend, in part due to the anticipated large-scale EU aid for environmental clean-up in the new accession countries of Eastern Europe.

Other sources paint a broadly similar picture. The BioIndustry Association (BIA) survey, *Industrial Markets for UK Biotechnology – Trends and Issues*, published in 1999 does not quote any monetary sector values per year, but gives the size of the UK sector as employing 40 000 people in 1998 with an average yearly growth over 1995–98 of 20%. Environmental biotech is reported as representing around 10% of this sector. An Arthur Anderson report of 1997 gives the turnover of the UK biotech sector as 702 million pounds sterling in 1995/96, with a 50% growth over three years. A 1998 Ernst and Young report on the European Life Sciences Sector says that the market for biotechnology products has the potential to reach 100 billion pounds sterling worldwide by 2005. The Organisation for Economic Cooperation and Development (OECD) estimates that the global market for environmental biotechnology products and services alone will rise to some US$75 billion by the year 2000, accounting for some 15 to 25% of the overall environmental technology market, which has a growth rate estimated at 5.5% per annum. The UK potential market for environmental biotechnology products and services is estimated at between 1.65 and 2.75 billion US dollars and the growth of the sector stands at 25% per annum, according to the *Bio-Commerce Data European Biotechnology Handbook*. An unsourced quote found on a Korean University website says that the world market size of biotechnology products and services was estimated to be approximately 390 billion US dollars in the year 2000.

The benefits are not, however, confined to the balance sheet. The Organisation for Economic Cooperation and Development (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. The OECD investigation provides what is 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 concludes 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 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, the relatively low market penetration to date 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. Moreover, there has been a discernible tendency in recent years towards niche specificity, with companies operating in more specialised subarenas 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. 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 and the market is, as a consequence, inevitably fragmented. Often the complexities of individual projects make the application of 'standard' off-the-shelf approaches very difficult, the upshot being 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. A sizeable proportion of companies active in this sphere, have no 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. The fact remains that one of the major barriers to the wider uptake of biological approaches is the high perceived cost of these applications. Part of the reason for this lies in historical experience. For many years, the solutions to all environmental problems were seen as expensive and for many, particularly those unfamiliar with the multiplicity of varied technologies available, this has remained the prevalent view. 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. Greater awareness of the benefits of biotechnology, both as a means to boost existing markets and for the opening up of new ones, is an important area to be addressed. Many providers, particularly in the UK, have cited a lack of marketing expertise as one of the principal barriers to their exploitation of novel opportunities. In addition, a lack of technical understanding of biotech approaches amongst target industries and, in some cases, downright scepticism regarding their efficacy, can also prove problematic. Good education, in the widest sense, of customers and potential users of biological solutions will be one major factor in any future upswing in the acceptance and utilisation of these technologies.

Modalities and local influences

Another of the key factors affecting the practical uptake of environmental biotechnology is the effect of local circumstances. Contextual sensitivity is almost certainly the single most important factor in technology selection and represents a major influence on the likely penetration of biotech processes into the marketplace. Neither the nature of the biological system, nor of the application method itself, play anything like so relevant a role. This may seem somewhat unexpected at first sight, but the reasons for it are obvious on further inspection. While the character of both the specific organisms and the engineering remain essentially the same irrespective of location, external modalities of economics, legislation and custom vary on exactly this basis. Accordingly, what may make abundant sense as a biotech intervention in one region or country, may be totally unsuited to use in another. In as much as it is impossible to discount the wider global economic aspects in the discussion, disassociating political, fiscal and social conditions equally cannot be done, as the following example illustrates. In 1994, the expense of bioremediating contaminated soil in the United Kingdom greatly exceeded the cost of removing it to landfill. Six years later, with successive changes of legislation and the imposition of a landfill tax, the situation has almost completely reversed. In those other countries where landfill has always been an expensive option, remediation has been embraced far more readily.

While environmental biotechnology must, inevitably, be viewed as contextually dependent, as the previous example shows, contexts can change. In the final analysis, it is often fiscal instruments, rather than the technologies, which provide the driving force and sometimes seemingly minor modifications in apparently unrelated sectors can have major ramifications for the application of biotechnology. Again as has been discussed, the legal framework is another aspect of undeniable importance in this respect. Increasingly tough environmental law makes a significant contribution to the sector and changes in regulatory legislation are often enormously influential in boosting existing markets or creating new ones. When legislation and economic pressure combine, as, for example, they have begun to do in the European Landfill Directive, the impetus towards a fundamental paradigm shift becomes overwhelming and the implications for relevant biological applications can be immense.

There is a natural tendency to delineate, seeking to characterise technologies into particular categories or divisions. However, the essence of environmental biotechnology is such that there are many more similarities than differences. Though it is, of course, often helpful to view individual technology uses as distinct, particularly when considering treatment options for a given environmental problem, there are inevitably recurrent themes which feature throughout the whole topic. Moreover, this is a truly applied science. While the importance of the laboratory bench cannot be denied, the controlled world of research translates imperfectly into the harsh realities of commercial implementation. Thus, there can often be a dichotomy between theory and application and it is precisely