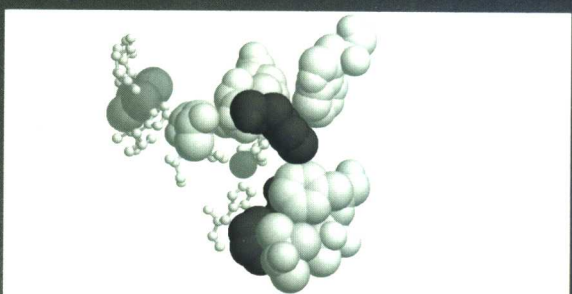
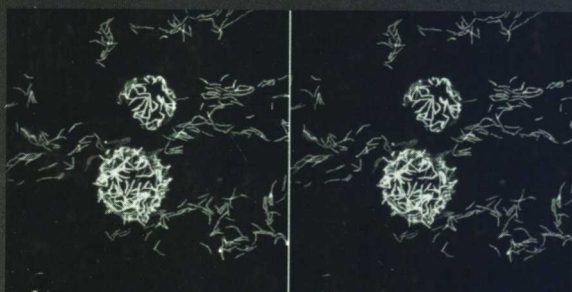


Biocatalysis and Biodegradation

MICROBIAL TRANSFORMATION
OF ORGANIC COMPOUNDS



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Lawrence P. Wackett
C. Douglas Hershberger

Biocatalysis and Biodegradation

MICROBIAL TRANSFORMATION
OF ORGANIC COMPOUNDS

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Foreword

This book was written during one of the most exciting periods in the history of the biological sciences. It is the first of its genre and represents the collective thoughts of its authors on the birth and growth of biodegradation and biocatalysis as related areas in the disciplines of microbiology, biochemistry, and chemistry. Emphasis is placed on the transition period between traditional microbial chemistry and the emerging fields of genomics (structural and functional), proteomics, and bioinformatics. The elements of discovery and prediction pervade each chapter, with emphasis placed on the function and future developments of novel individual and groups of biocatalysts rather than, as in more conventional texts, on descriptions of the pathways used by microorganisms to degrade specific classes of organic compounds.

In this Foreword, I have chosen Sir Frederick Gowland Hopkins as the catalyst and Cambridge University as the reaction vessel to develop the themes of biodegradation and biocatalysis. Hopkins was the most distinguished biochemist at Cambridge University from the early 1900s through the end of World War II. It was Hopkins who encouraged Marjory Stephenson to write her classic book *Bacterial Metabolism*, which was first published in 1930 and followed by second and third editions in 1940 and 1949. Stephenson traced her interest in things microbial to Pasteur's 1857 paper, "Mémoire de la Fermentation dite lactique." This initial work was criticized by Berzelius and Liebig, both leading chemists at the time. Nevertheless, Pasteur held firm to his interpretation of lactic acid fermentation as a process resulting from, and necessary for, the growth and multiplication of living cells. These observations marked the birth of bacterial metabolism, which was defined in 1949 by Stephenson as "the interpretation of the physiological life of the bacterial cell in terms of biochemistry and biophysics both in respect of its own growth and reproduction and also of its action on its chemical and biological environment."

Hopkins also encouraged Ernest Baldwin, a faculty member in the School of Biochemistry, to write *Dynamic Aspects of Biochemistry*, first published in 1947. There is surprisingly little overlap between the two books in spite of Stephenson and Baldwin being in the School of Biochemistry at

the same time. Baldwin's textbook was used to introduce the palpably cold equations of equilibrium thermodynamics to beginning biochemistry students. *Dynamic Aspects of Biochemistry* achieved this objective admirably and, together with Stephenson's *Bacterial Metabolism*, provided substantial evidence to support Hopkins' celebrated aphorism, "Life is a dynamic equilibrium in a polyphasic system."

Baldwin was a dedicated teacher and found that students comprehended and remembered reactions if they were presented pictorially. His "whirligigs" were the forerunners of the elegant multicolored cartoons that pervade the pages of microbiology and biochemistry textbooks today. In this regard it is of interest to note that in England soon after the second world war, there were three weekly comic papers, *The Beano*, *The Wizard*, and *The Dandy*. Each contained three or four episodes of ongoing stories designed to attract the attention of children in the 7- to 10-year age group and each episode was presented in full text without a single picture or cartoon. This was at a time when British children yearned for the so-called "American comics" that in the postwar era were finding their way to the shelves of British newsagents. It was the widespread fear of parents that the glorious cartoons in *Superman* would somehow bring down the whole British educational system by stifling the imagination of students in their formative years. Retrospective analysis shows that these apprehensions had no basis in fact. Although Baldwin's whirligigs could not compare with the pictorial presentation of Superman in flight, they have survived in the representation of the structure of ATP as A-P~P~P. Baldwin's squiggle (~) was used to identify a "high-energy" phosphate bond and, as the late Stanley Dagley noted, "in one fell swoop raised ATP to the level of a high explosive." Of course Baldwin did state that the importance of ATP "lies in the ease with which its terminal phosphate radical can be transferred with a part or all of the 11,000 calories of free energy with which it is associated, to other molecules," and he went on to use the A-P~P~P structure to show how ATP plays a central role in energy transactions in living cells. It is remarkable that the squiggle representing a high-energy phosphate bond can be found in almost every biochemistry textbook from 1947 to the present day and that, almost without exception, it is accompanied by an explanation that denies the existence of high-energy phosphate bonds.

The two decades following the second world war saw unprecedented growth in biochemistry and all areas of the biological sciences. The determination of the structure and function of DNA, RNA, and messenger RNA provided the basis for the development of the field of molecular biology. Albert Lehninger at Johns Hopkins University recognized, like Baldwin before him, the need for a textbook that correlated thermodynamic principles with metabolic activities. His book, *Bioenergetics: the Molecular Basis of Biological Energy Transformations*, was written for undergraduates beginning the study of molecular biology. This text, in elegant and simple presentations, showed that thermodynamic principles "comprise a central and unifying theme in biology." In this context, metabolism can be thought of as the total chemical activities of the cell, with the terms "catabolism" and "anabolism" representing the degradation and synthesis of cellular constituents, respectively.

The relationship between catabolism and anabolism can be seen in the carbon cycle, which, at the global level, represents an equilibrium between the processes of photosynthesis and respiration. Light energy from the sun is used by plants, algae, and certain bacteria to drive the synthesis of cel-

lular constituents from carbon dioxide and an appropriate electron donor. The carbon sequestered in these molecules is ultimately released to the atmosphere as carbon dioxide, mainly by the catabolic activities of heterotrophic microorganisms. For example, glucose is catabolized via the Embden-Meyerhoff pathway in a stepwise sequence of reactions to pyruvate. In the process, ATP for biosynthesis is produced by substrate-level phosphorylation, and oxidation reactions are mediated by the removal of hydride ions as NADH. Continuation of glucose degradation is dependent on the regeneration of oxidized NAD^+ . In fermentative microorganisms, this is achieved by the transfer of electrons to organic compounds. For example, yeast regenerates NAD^+ by transferring reducing equivalents to acetaldehyde. The end product of this reaction is ethanol. The same strategy is used by mammals when oxygen concentrations are low. In this case, however, hydrogen and electrons are used to reduce pyruvate to lactate. In contrast, aerobic organisms utilize the tricarboxylic acid cycle to oxidize pyruvate to carbon dioxide and water. Hydrogen and electrons are transferred to oxygen by a sequence of redox proteins, and ATP is generated by oxidative phosphorylation. In anaerobic environments, the acids and alcohols generated by fermentation can serve as growth substrates for other groups of bacteria, which convert them to acetate, hydrogen, and carbon dioxide. These products are converted to methane by methanogenic bacteria. Methane is oxidized to CO_2 by methylotrophic bacteria and thus completes the carbon cycle. Although oxygen is the terminal electron acceptor in aerobic organisms from bacteria to humans, an alternate lifestyle is embraced by iron-, sulfate-, and nitrate-reducing bacteria, and the search for a phosphate-reducing organism continues (R. S. Wolfe, personal communication, 2000).

The catabolic and anabolic processes used by microorganisms for the degradation and synthesis of carbohydrates, lipids, proteins, and other cellular constituents are reasonably well known. Together, they represent the field of central or intermediary metabolism. Thus it seems appropriate to ask how the pathways of central metabolism relate to biodegradation and biocatalysis. In this context, it is important to recognize that catabolism and anabolism are powerful terms that directly relate to the fundamental activities of living cells, whereas biodegradation and biocatalysis are used to identify individual specific objectives within the area encompassed by metabolism. In its broadest sense, biodegradation refers to the microbial catabolism of a compound into molecules that can enter the central metabolic pathways used by aerobic and anaerobic microorganisms. For example, lignocellulose is the main structural component of woody plants, and lignocellulose biodegradation is the most important process in the recycling of terrestrial biosynthetic compounds. This is accomplished by a cascade of thermodynamically favorable reactions mediated by microorganisms. Certain fungi use extracellular hydrogen peroxide-dependent enzymes to depolymerize lignocellulose. The aromatic alcohols and ethers liberated by ligninases, together with free cellulose, serve as carbon and energy sources for a variety of microorganisms, and ultimately the carbon sequestered in lignocellulose is returned to the atmosphere as carbon dioxide. Biocatalysis, in contrast to biodegradation, is a term that is usually used to identify catabolic or anabolic reactions that lead to the accumulation of specific metabolic products. The reduction of acetaldehyde to ethanol by alcohol dehydrogenase and the generation of high-fructose corn syrup by glucose-6-phosphate isomerase are classic examples of biocatalysis.

Although examples of biodegradation and biocatalysis abound in nature, it is only in the 38 years since the publication of Rachel Carson's *The Silent Spring* that biodegradation has achieved serious recognition as a research area that focuses on the pathways and reactions used by microorganisms to degrade environmental pollutants. In this context, it is desirable to have an understanding of the nature of environmental pollutants. There are, however, no unifying concepts to satisfy all situations. In simple terms, a compound can be considered a pollutant if its concentration reaches a level that has deleterious effects on life forms at any stage from the bottom to the top of the food chain. At the turn of the century, London hatters used mercury to provide a sheen for top hats, and the frequency of dementia in this group of individuals was very high. The Mad Hatter in Lewis Carroll's *Alice in Wonderland* provides anecdotal evidence for this condition, which is due to the formation of neurogenic dimethyl mercury by intestinal bacteria. A more recent example is seen in the high incidence of dimethyl mercury poisoning of the inhabitants of Minamata, a small fishing village in Japan. Mercury from a chlorine plant was released into the estuary near the village. Methanogens in anaerobic sediments produced dimethyl mercury, which moved through the food chain to fish and finally to the fishermen and their families.

The cycle of carbon in nature operates on the assumption that all biosynthetic organic compounds are biodegradable. There is, however, a second major source of organic compounds that has to be considered in terms of carbon recycling and biodegradation. Every year since the Precambrian era, a small amount of the plankton in ancient oceans has been buried before it can be completely converted to carbon dioxide by microorganisms. This material undergoes thermodynamic stabilization by diagenesis, and the end product is graphite. The early stages of diagenesis occur in shallow sediments where organic material is subjected to extremes in pH and redox conditions. During this time, carbon-carbon bonds are broken and oxygen and other elements are displaced, leading to the temporary formation of new and often more complex compounds (humic acids) even though overall stabilization of the system takes place. Eventually, most of this material is converted to kerogen, a complex polymer of unknown structure. As sediments sink to lower levels, kerogen is subjected to increases in temperature and pressure. These conditions, in conjunction with catalytic changes due to the composition of the rock, can eventually lead to the formation of crude oil. It is important to realize that crude oil represents an intermediate stage on the geological time scale between kerogen and graphite. Thus no two oils have the same chemical composition.

Today, petroleum derived from crude oil drives the economies of industrial nations, and not surprisingly, crude oil and products formed from it find their way into environments where their presence is not anticipated or appreciated. At the beginning of the new millennium, the public is still enamored of stories relating to the use of bacteria to clean up oil spills. However, work supported by the American Petroleum Institute reveals the chemical complexity of oil and the realization that the timeframes required for complete biodegradation make it unlikely that significant bioremediation technology for catastrophic events such as the *Exxon Valdez* spill will ever be developed. Most crude oils contain significant quantities of linear alkanes. Bacteria that degrade these hydrocarbons are ubiquitous in the environment. The introduction of nitrogen and phosphorus sources to environments polluted by oil to accelerate biodegradation by indigenous mi-

croorganisms (biostimulation) has met with some success. This is probably due to stimulation of bacterial growth on linear alkanes and some of the smaller alicyclic and aromatic components.

The use of microorganisms to remedy other anthropogenic insults to the environment has met with more success. In each case the solution was found in the results of basic research. For example, in the 1960s, Liverpudlians in Lancashire, England, were treated to the sight of large plumes of foam drifting on the surface of the River Mersey. The culprit was found to be detergents that contained hydrophobic branched alkyl side chains. An environmental solution was found in the substitution of linear alkyl side chains for their branched-chain counterparts. Detergent activity was maintained and the visible pollution problem was eliminated. It is of interest to note that quaternary methyl-substituted alkanes are products of the petroleum industry and are not found in nature, whereas, as noted previously, linear alkanes are ubiquitous. Thus one possible explanation for the observed results is the fact that microorganisms have had millions of years to evolve enzyme systems for the degradation of linear alkanes whereas they have only been in contact with quaternary-substituted alkanes for less than a century. More recent interpretations of the evolution of biodegradation pathways are discussed in later chapters of this book.

Unfortunately, not all cases of environmental pollution are as visible as the foam from non-biodegradable detergents. DDT was used as an insecticide for many years before it was identified as the agent responsible for the almost total demise of the bald eagle and other birds of prey. In addition, analyses of environmental samples for the presence of DDT led to the identification of polychlorinated biphenyls (PCBs) as a major group of ubiquitous environmental pollutants. The identification of DDT and PCBs in soil and sediment samples was made possible by the combined techniques of gas chromatography and high-resolution mass spectrometry. Today these techniques are used routinely to monitor the concentration and fate of specific pollutants in different environments. It is worth noting, however, that a little more than 50 years ago Kern isolated chrysene from soil. This was the first polycyclic hydrocarbon to be detected in the environment. Two decades later it was possible to say that Kern's 1947 soil sample almost certainly contained individual polycyclic compounds numbering in the 10^5 range. This was due to advances in analytical resolution by several orders of magnitude. It is also an example of what the late Max Blumer called "pure ignorance," namely, "the ignorance of which we are not even aware." For instance, with each advance in analytical technique, the boundary of the unknown is not brought closer as expected, but in fact recedes rapidly, and there is no reason for us to feel complacent that the boundary will be reached in the near future. The fact that our present knowledge of the organic compounds in nature is incomplete makes it extremely difficult to predict the biological impact of chemicals in the environment. This situation is compounded by our "pure ignorance" of the composition of bacterial communities in different ecological niches. Until recently, most of our current knowledge in the fields of biodegradation and biocatalysis resulted from studies with pure cultures and single substrates. It is now widely acknowledged that more than 99% of the bacteria in a single soil or sediment sample have yet to be isolated and characterized. The presence of 4,000 different bacteria and 100,000 organic compounds of unknown structure and physiological activity in a single soil or sediment sample is a

daunting example of bacterial and chemical diversity which exposes our "pure ignorance" and challenges our imagination and perseverance.

There is no doubt that advances in analytical techniques for the isolation, separation, and identification of organic compounds have played a major role in the developing study of bacterial metabolism in general and biodegradation and biocatalysis in particular. By 1970, significant advances had been made in identifying the pathways used by bacteria to degrade compounds regarded as environmental pollutants. This was achieved by isolating and identifying intermediate compounds, followed by demonstrating the presence of enzymes responsible for their formation and further metabolism. Most of this work was done with pure cultures and single substrates. For example, aromatic hydrocarbons and related compounds were recognized as environmental pollutants emanating from the refinement and use of crude oil and the procedures used to manufacture paper products from lignocellulose. Initial studies showed that certain bacteria channel many simple aromatic compounds through arene *cis*-diols to catechol or protocatechuate. The initial reaction involves the addition of dioxygen to the aromatic nucleus by multicomponent enzyme systems belonging to a family of enzymes known as Rieske non-heme iron oxygenases. The resulting arene *cis*-diols are rearomatized by pyridine nucleotide-dependent dehydrogenases, which replace the NAD(P)H used in the initial dihydroxylation reaction. The products formed, catechols or protocatechuate, are substrates for ring-fission dioxygenases which cleave the aromatic nucleus between the hydroxyl groups (*ortho*-cleavage) or at a site adjacent to one of the hydroxyl groups (*meta*-cleavage). Ring-fission products formed by *ortho*-cleavage are channeled to a common intermediate, β -ketoadipate enol lactone, whereas in most cases *meta*-ring-fission products are converted to 2-oxo-4-hydroxyvalerate. The end products from both pathways are then metabolized to intermediates that can enter the tricarboxylic acid cycle.

These and other catabolic pathways were elucidated, as mentioned above, by studying individual reactions and their attendant enzymes. The results obtained then served as building blocks for the assembly of each pathway. Stanley Dagley was an avid proponent of this reductionist approach. This can be seen in his statement that "the most sensible questions to ask of nature are the simplest; but oversimplified answers must then be expected and these will provoke further questions." A classic example of this prediction can be seen in the convergent pathways used by bacteria to degrade aromatic compounds. These observations stimulated Gunsalus and his colleagues to explore the organization and expression of the genes encoding the enzymes responsible for aromatic metabolism, since they regarded metabolic convergence as a mechanism for reducing the total genetic load of the cell. Their studies in this area led to the discovery of the NAH, SAL, and TOL transmissible catabolic plasmids and paved the way for new molecular approaches in the fields of biodegradation and biocatalysis.

For many years pseudomonads were regarded as the most omnivorous microorganisms in the environment. Support for this conclusion can be traced back to the 1926 thesis of der Dooren de Jong in Delft, The Netherlands, which lists 80 compounds that will support the growth of *Pseudomonas putida*. Further support was provided by the detailed classification of the pseudomonads by Stanier, Palleroni, and Doudoroff at the University of California at Berkeley. Today, however, we can trace the dominance of

the pseudomonads in biodegradation studies to their preferential isolation by classical enrichment culture techniques and also to pseudomicrobiologists who identified all gram-negative motile rods as pseudomonads. Comparative sequencing of 16S ribosomal RNA has led to a recognition of the catabolic versatility exhibited by species of *Rhodococcus*, *Sphingomonas*, *Comamonas*, *Burkholderia*, *Ralstonia*, and other genera. New organisms lead to the identification of new metabolic capabilities. For example, the unwritten but general assumption that aromatic hydrocarbons cannot be degraded under anaerobic conditions has fallen under the onslaught of nitrate-, sulfate-, and iron-reducing bacteria. Another example resulted from the discovery that recalcitrant highly chlorinated biphenyls undergo reductive dehalogenation in the anaerobic sediments of the upper Hudson River and other PCB-contaminated environments. One common feature of "natural" biodegradation is the slow rate of contaminant removal. This serves to remind us that Nature marches to the beat of her own drum and is often ill prepared to withstand surges of excessive amounts of chemicals resulting from human activities.

Fortunately, the molecular techniques generated and driven by recombinant DNA technology have had a significant impact on all aspects of biodegradation and biocatalysis. Today it is possible to optimize the conditions necessary for the microbial degradation of many environmental pollutants. Examples listed by Timmis and Pieper include the isolation of new strains with desired phenotypes and the construction of novel pathways that eliminate nonproductive side reactions, reduce the formation of toxic intermediates, and, where necessary, improve enzyme performance. In addition, the advent of the World Wide Web has facilitated the development of databases that can be used to predict pathways for the biodegradation of new chemicals. Discussion of these and other aspects of biodegradation permeates the later chapters of this book and does not need to be repeated here.

Recent developments in biocatalysis stem from industry's realization of the need for environmentally benign procedures for the synthesis of commercial chemical products. The ideal synthetic system would incorporate Trost's suggestion of "atom economy," in which all atoms in the reactants are present in the product. This would favor addition reactions over elimination reactions, a situation that is not always feasible from a synthetic chemist's point of view. In contrast, there are many biocatalytic routes to commercial products that are addition reactions. These include asymmetric dihydroxylation reactions catalyzed by Rieske non-heme iron dioxygenases. It is here, and also in many chapters throughout this book, that biodegradation and biocatalysis meet. Bacteria that degrade toluene are readily detected in contaminated groundwater, and at least five distinct pathways of toluene degradation have been elucidated. The "dihydrodiol" pathway used by *Pseudomonas putida* F1 is initiated by toluene dioxygenase. This enzyme adds both atoms of dioxygen to the aromatic nucleus to form homochiral (+)-*cis*-(1*S*,2*R*)-dihydroxy-3-methylcyclohexa-3,5-diene (toluene *cis*-dihydrodiol). The dihydrodiol has found use as a chiral synthon in the synthesis of biologically active compounds such as prostaglandin E_{2α}. The most remarkable feature of toluene dioxygenase and the related enzyme naphthalene dioxygenase is their ability to oxidize more than 200 substrates to single enantiomer arene *cis*-diols. The synthetic potential of these and other chiral products from biocatalysis is virtually untapped and should serve synthetic chemists well in the future search for environmentally benign syntheses.

In 1949 Marjory Stephenson predicted that in 25 to 50 years a biochemical description of cell growth would be almost complete, leading her to suggest that "biochemistry and microbiology as we know them will become cold stars (black holes)," to be replaced with "fresh fields whose character we can only dimly guess at." One can only wonder at the words of this prescient scientist in light of the fact that June 26, 2000, saw the announcement of the DNA sequence of the human genome. This came just 5 years after the publication of the first complete genome, that of *Haemophilus influenzae*. The entire genomes of more than 30 organisms have been determined to date. These include the genome of *Enterococcus faecium* (2.98 million base pairs), which was sequenced in a single day. Robotic nucleotide sequencing has clearly revolutionized biology and given birth to genomics, structural genomics, proteomics, toxicogenomics, and no doubt many more "-omics" still to come. These qualify as the fresh fields Stephenson envisaged. They are also powerful tools that will enable scientists to determine a clear picture of bacterial growth in terms of the activity of regulatory proteins that control the timing and levels of gene expression throughout the cell growth cycle. The ability to conduct these and related experiments is extolled weekly in the scientific press—accompanied by articles warning of the difficulties to be encountered in the emerging fields of postgenomic biology.

One of the many attractive features of this book is the integration of the birth and growth of biodegradation and biocatalysis into the pre- and potential postgenomic eras. It thus seems appropriate to conclude this introduction with a few words on Arthur Kornberg's "Ten Commandments: Lessons from the Enzymology of DNA Replication," a Guest Commentary published in the July 2000 issue of the *Journal of Bacteriology*. Kornberg presents a powerful statement for the value of studies on pure enzymes in biology and cites Frederick Gowland Hopkins (1931) as an early spokesman for this approach (see above). I have always been hesitant to invoke the thoughts and commandments of a higher being in my experiments. Nevertheless, Kornberg's statement that poly P is "an inorganic polymer of hundreds of phosphate residues linked by 'high-energy' anhydride bonds" suggests an 11th commandment: *Thou shalt not ignore thermodynamics when considering the birth and future development of any field of biology.*

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Preface

This book takes the view that it is more important to define the emerging questions in biodegradation and biocatalysis than to contribute to the pretense that there are answers to many of the most important questions in the field. James Thurber expressed it more simply, "It is better to know some of the questions than all of the answers." This book frames questions such as, "What is the extent of microbial metabolism on Earth?"; "What microorganisms participate in certain types of metabolic transformations?"; "How does one define microbial metabolism in light of widespread genome sequencing?"; "How will microbial biocatalysis and biodegradation be applied commercially in the future?" Some of the answers will be found in the short term; some may remain elusive for a long time. In fact, it is important to question whether microbial metabolism can ever be fully appreciated; might not microbes evolve new catabolic activities faster than they can be studied by scientists?

To begin to answer the global questions posed in this book, a reductionist approach is taken. This goes against the perception of some people who feel that global questions require non-reductionist, or global, methods of experimentation. Throughout this book, the underlying molecular basis of microbial biocatalysis is detailed. One must reduce the complexity of a system, understand its parts, and then see how the parts act together to make a functional whole. To understand microbial biocatalysis globally, it is important both to delve deeply *and* to step back periodically for the broad view.

A reductionist approach also flows naturally from the duality of biocatalysis and biodegradation: the chemistry of the substances being transformed and the microbiology of how those transformations occur in the world. This necessitates going back and forth between microbiology and chemistry. The first part of the book, chapters 2 through 4, focuses on microorganisms and laboratory methods for their isolation and study. Then, chapter 5 focuses on chemical compounds, their origins, and their

distribution on Earth. In chapters 6 through 8, microbiology and chemistry blend with coverage of enzymes, evolution, and metabolic logic. Last, in chapters 9 to 13, issues currently attracting strong interest are covered: predicting biodegradative metabolism, genomics, and industrial applications of biocatalysis and biodegradation.

The book also seeks to deal with the complexity of biocatalysis and biodegradation, viewing the global microbial ecosystem and its collective metabolic activities as part of the beautiful tapestry of nature. Edward O. Wilson described complexity in human terms as follows, "The love of complexity without reductionism makes art; the love of complexity with reductionism makes science." This book stresses the complexity of organic molecules, over ten million of which are currently known, and the complementary complexity of microbial metabolism that has evolved to transform those organic compounds.

The book's focus on events occurring at the molecular level differentiates it from a number of other recent books on biodegradation which stress bioremediation engineering or other aggregate processes such as microbiological aspects of soils and waters that influence biodegradation. Other treatments of biodegradation stress different chemical compounds and how each is metabolized by microorganisms. The present book differs in that it seeks to describe the *logic* of microbial biocatalysis. By logic, we mean the conceptual framework at the molecular level: how enzymes work, how pathways interact, and how physiological systems support biodegrading organisms. Overall, we envision this book to be useful for graduate students, for whom it might be suitable as a textbook, and for specialists in academia and industry interested in microbial biocatalysis and biodegradation. With microbial genome sequencing projects providing a new approach for studying individual prokaryotes, we anticipate that microbial catalysis will enter a new golden age. It is more important than ever, for better annotation of gene sequences and for applying biocatalysis to practical problems, that the underlying logic of microbial metabolism be better appreciated.

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