
Plastics from Microbes

**Microbial Synthesis
of Polymers
and Polymer Precursors**

David P. Mobley

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Edited by David P. Mobley

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1 Introduction to Microbial Synthesis of Polymers and Polymer Precursors

David P. Mobley

In the early 1800s, the carbon-based chemicals industry offered products such as sugar, alcohol, vinegar, alizarin, indigo, and turpentine. The noteworthy feature of these chemicals is that, at that time, they were all obtained from living organisms. Carbon-based substances were thus called organic chemicals. In fact, it was widely supposed by early 19th century scientists that the formation of organic chemicals required the action of a living organism—a “vital force”—which could not be duplicated by chemical manipulations in the laboratory.

Then, in 1828, Friedrich Wöhler showed that urea, an organic chemical, could be synthesized from inorganic compounds. This discovery came to be regarded as one of the milestones in the development of organic chemistry as a scientific discipline. In the decades that followed, chemists rapidly developed elegant new methods for creating and transforming organic chemicals in the laboratory without the aid of organisms.

While Wöhler's discovery is not responsible for all the subsequent advances, it is a symbolic turning point. Since that time, the term “organic chemical” has taken on an entirely different meaning, as evidenced by the organic chemical industry. In contrast to the early 1800s, only a very small proportion of today's bulk organic chemicals are obtained from living organisms.

Since Wöhler's time, the broad direction of organic chemistry has been away from biological processes. Organic chemistry became the nonbiological manipulation and physical characterization of carbon-containing compounds. This approach has yielded many practical benefits. The composition of today's organic chemical industry is a tribute to the past successes of organic chemistry.

Over the same time, the study of the chemistry of living organisms developed into biochemistry. The discipline of biochemistry has tended to focus on the understanding of metabolic pathways, the nature and function of enzymes, and, very recently, on the chemistry of genes. Biochemistry has had a significant impact in medicine and the pharmaceuticals industry but has rarely been called upon to contribute to the commodity chemical process industry.

The plastics industry has followed the same trend as the broader organic chemical industry. Some of the earliest commercial plastics were derivatives of cellulose. Since that time, though, the development of new polymers has been overwhelmingly dominated by synthetic organic chemistry. Through most of the history of the plastics industry, there has been little attention

given to living organisms as a means of producing either polymer precursors or the polymers themselves.

The last ten to fifteen years, however, have seen some returning of organic chemistry to its biological roots. More traditionally trained organic chemists are studying and manipulating biological molecules, and, interestingly, more organic chemists are using biological transformations as a part of synthetic procedures. Concurrently, there has been renewed interest in the application of biological science and technology to the industrial production of chemicals and polymers.

A good part of the attention given to biotechnology in recent years is due to the emergence of genetic engineering in the 1970s. Genetic engineering put the tools in the hands of biologists to understand and make use of biochemical transformations as never before. Popular attention was drawn to the high-valued pharmaceuticals made by recombinant organisms, but the enthusiasm for biotechnology and the use of recombinant DNA techniques spilled over into other applications as well. A broader segment of the technical and business communities became aware of the potential of biological systems for synthesizing useful chemicals and catalyzing chemical transformations. As a result, microbial and enzymatic processes are either in use or are being studied for the production of a wider variety of materials, including agricultural chemicals, bulk chemicals, and polymers.

This book is about using microorganisms in the synthesis of polymers. It illustrates, through the use of several key examples, how microbial synthesis is being used in both the direct production of polymers and the production of polymer precursors. The examples show how microbial synthesis can yield polymers with distinct beneficial properties and describe how biotechnology makes this possible.

What are the features of microbial processes that make them attractive for the synthesis of polymers and polymer precursors? Often, the overriding benefit is that microbes carry out transformations that are impractical or impossible to accomplish with conventional chemistry. Microbial processes thus enable the production of materials that are otherwise unavailable. Added advantages are that they can make use of renewable, low-cost feedstocks, and they operate at mild process conditions with minimal environmental impact.

There are, however, formidable technical challenges to the use of microbial synthesis. The identification of a microorganism with the desired synthetic capabilities can require an extensive search. Microbes are often effective only in a narrow range of temperature, pH, and other process conditions. They are susceptible to chemical toxins, strain instability, and attack by other organisms. Compared to chemical catalysts, microbial syntheses often result in slow reaction rates and low product concentrations.

The examples in this book feature a range of useful polymer products made available by the use of microbes and describe the other benefits that result from microbial synthesis. They also demonstrate how the challenges inherent in microbial processes are being overcome.

Chapters 2 and 3 present examples of the direct microbial synthesis of polymers. Chapter 2 reviews polyhydroxyalkanoates, microbial polymers that combine the desirable physical properties of widely used thermoplastics with the uncommon characteristics of biodegradability and biocompatibility. Chapter 3 describes protein polymers, synthetic proteins composed of specific

repeating units, and shows how the tools of genetic engineering can be used to specify the detailed structure of a polymer at the molecular level, thereby determining mechanical properties as well as biological activity.

The next four chapters deal with the use of microbes to make polymer precursors. This can be accomplished through biosynthesis or biocatalysis. In biosynthesis, the organism generates the product from the nutrients on which it grows. This method is illustrated in Chapter 4 by the biosynthesis of lactic acid, which is chemically converted to polylactide, a biocompatible and biodegradable polymer with current biomedical uses and broad potential applications in biodegradable packaging. Using the microbial synthesis route, polylactide can be made from cheap agricultural byproducts.

In biocatalysis, the microbe is used to catalyze the conversion of a chemical feedstock to the desired product. Examples of biocatalysis are presented in Chapters 5 through 7.

Each of these chapters deals with the oxidative transformation of aromatic compounds to produce precursors for high performance polymers. In each instance, the key benefit of the microbial catalyst is that it can perform a transformation that is not readily carried out with nonbiological chemistry. The result of the microbial processes is a set of precursors to polymers with special performance properties. Chapter 5 describes the biocatalytic production of *cis*-dihydroxycyclohexadiene and the chemical conversion of this intermediate into polyphenylene, a material with extremely high thermal stability that is well-suited for electrically insulating coatings. Chapter 6 details the combined biological and chemical synthesis of *m*-hydroxyphenylacetylene and 4-hydroxybenzocyclobutene and explains how these intermediates are used to produce tough, thermooxidatively stable polymers for aerospace applications. Chapter 7 tells how a microbial *para*-hydroxylation activity can be used to produce a variety of hydroxylated aromatic monomers that are difficult to produce chemically and demonstrates how the use of these monomers leads to improvements in thermoplastic properties such as solvent resistance and toughness.

This book presents an overview of the state of technology in applying microbial processes to polymer synthesis. It does not attempt, however, to cover all of the applications of biological sciences in polymer technology. In particular, this book does not deal with polysaccharides, a number of which are produced microbially at a commercial scale. These materials have been omitted because they are suited for a different range of uses than the plastics applications that are emphasized here and because they have recently been reviewed elsewhere. A related topic is the application of isolated enzymes to the synthesis of polymers and polymer precursors. While interesting advances are being made in this technical area, they are beyond the scope of this book.

Historically, there has been limited contact between biological scientists and technologists and polymer scientists and technologists. Individuals in these broad disciplines are usually trained in separate academic departments. They use different techniques, work on different sorts of applications, use different terminology, and report their work in distinct sets of the technical literature.

A primary aim of this book is to foster greater communication between biological scientists and technologists and polymer scientists and technologists. It is hoped that individuals in the biological disciplines will gain a better appreciation of the kinds of polymers and polymer properties that are important to the polymer technologist. Similarly, it is hoped that polymer

scientists and technologists will be made aware of the range of polymer building blocks that are made available through biotechnology.

The contributors to this book come from a variety of backgrounds, including microbiology, biochemistry, molecular biology, organic chemistry, polymer sciences, and chemical engineering. They have brought together information that is usually dispersed through the technical literature of their specific disciplines. The attempt has been made to produce a book that is accessible to readers with a general technical background. The primary literature has been extensively referenced for the reader who seeks more specialized or detailed information. It is recognized, however, that the reader may occasionally feel a need for further background in technical areas outside of his or her own discipline. The following section is a short list of texts and references recommended for this purpose.

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2 Polyhydroxyalkanoates

David Byrom

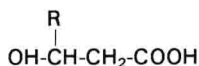
2.1 Introduction

Polyhydroxyalkanoates (PHA) are naturally occurring biodegradable polymers that can be thermoformed to fabricate articles in a similar manner to conventional nonbiodegradable petrochemical-based plastics. Polyhydroxybutyrate (PHB) was the first member of the PHA family to be discovered. It was first detected in 1926 and was the subject of academic interest for some years. Industrial interest, until relatively recently, has been sporadic. For example, it was not until 1962 that a patent mentioning PHB was filed [1]. After a period during which little academic or industrial work was reported in the field, there has been a resurgence of activity stimulated by environmental pressures. Objects such as bottles, film, fibers, nonwoven fabric, biomedical devices such as bone plates and screws, and many other products can be formed from the material. Several articles, including biodegradable bottles, razors, and food trays already on the market are made from a copolymer of PHB and polyhydroxyvalerate (PHV), and others are in development. The current production of PHB/HV is about 300 tonnes per annum. This figure will about double in 1993 and is scheduled to be several thousand tonnes by the mid to late 1990s. The level of commercial interest in biodegradable polymers can be judged by the fact that there are at least four other materials (non-PHA) announced or available at development scale. Some are based on natural polymers, such as starch, and others are chemically produced.

Many aspects of the microbiology, biochemistry, genetics, and industrial production of PHA have been reviewed recently by several authors [2–4]. In this chapter, it is intended that developments since these publications appeared will be discussed, and earlier work is only included for the sake of clarity and context. The term “polyhydroxyalkanoate” is used generically for all polyester reserve materials produced by bacteria. Individual polymers such as PHB will be referred to by name where appropriate.

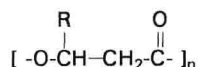
PHA are intracellular carbon and energy storage materials that are accumulated by a very wide range of bacteria. The compounds are polyesters of 3-hydroxyalkanoic acids and have the general formula given in Figure 2.1. The monomers in the polymer are all in the D(–) configuration, implying specificity in the biosynthetic route. The polymers have molecular weights in the range 2×10^5 to 3×10^6 , depending on the source of the material. Until recently, the most well-known members

3-hydroxyalkanoic acids



where R = 1 to 11

Polymer structure



where n = 4000 to 20,000

Figure 2.1 General structure of monomers and PHA polymers.

of the PHA family were PHB and PHV, which are composed of the monomers 3-hydroxybutyrate (3HB) and 3-hydroxyvalerate (3HV), respectively. However, polymers containing monomers of 3-hydroxy acids of carbon chain length up to C₁₄ have now been reported. All naturally occurring PHA are composed of straight chain monomers, but since the polyester chain is formed through the 1- and 3-positions of the hydroxy acid monomers, a methylene side chain projects from the polymer backbone (see Figure 2.1). Unsaturation can be present in the methylene side chain in naturally occurring PHA. A large number of nonnatural PHA have been made by supplying a variety of compounds to microorganisms in feeding experiments. This work will be described in more detail below.

The range of types of bacteria that are known to synthesize PHA is very large. Although PHA is not generally considered a ubiquitous storage compound, representatives of autotrophs, heterotrophs, phototrophs, aerobes, anaerobes, eubacteria, and archaeobacteria have been reported to accumulate granules of PHA-like material. See [4] for a comprehensive reference list. PHA storage, therefore, appears to be a very successful strategy for survival, since the ability to accumulate the polymer has been conserved in so many diverse organisms.

PHA appears in the bacterial cell as discrete granules, the number of which varies from species to species. In the high molecular weight form, PHA are exclusively prokaryotic materials. A fascinating development in recent years has been the discovery of the very wide distribution of PHB as a low molecular weight oligomer 120–200 monomers long in microorganisms, plants, and animals, including humans [5–7]. Calcium polyphosphate is associated with the PHB in membranes in many of the cases studied by Reusch and colleagues. The PHB/calcium polyphosphate complex was found in the plasma membrane of both Gram-positive and Gram-negative microorganisms. In *Escherichia coli*, the complex appeared in the membrane of cells made competent for genetic transformation [8]. A structure was proposed whereby the PHB molecule formed a helical membrane-spanning pore lined with another helix of polyphosphate. Calcium ions link the two helices. Reusch and Sadoff (1988) discussed the role of this proposed structure in calcium, phosphate, and DNA transport.