BIOMOLECULAR ENGINEERING SOLUTIONS FOR RENEWABLE SPECIALTY CHEMICALS

MICROORGANISMS, PRODUCTS, AND PROCESSES

EDITED BY
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WILEY

Biomolecular Engineering Solutions for Renewable Specialty Chemicals

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Preface

Biocommodity Engineering

Microorganisms have been realized as promising sources for production of biocommodities such as biofuels, pharmaceuticals, organic acids, amino acids, vitamins, biopolymers, surfactants, detergents, and enzymes. They offer several advantages over the conventional chemical processes including mild operating conditions, stereospecificity of the products, environmentally benign nature, and ecofriendly. Translating the bioproducts from laboratory to the industry remains a bottleneck. Biocommodity and biomolecular engineering approaches help in overcoming these limitations, developing new products, and improving the processes.

Considering the importance of the field, this book is specifically focused on potential technologies that can help in commercializing the processes. The objective of the book is to provide advanced technologies in producing different products using improved microorganisms/enzymes. This book will also discuss on improving the microbes or enzymes using protein engineering, metabolic engineering, and systems biology approaches for converting the wastes to value-added products.

Overall, this would be an ideal textbook for bioprocess, biorefinery, biomolecular, and biocommodity engineering courses for chemical, biochemical, and environmental engineering students. We have also included glossary and reasoning type questions at the end of each chapter. This book will also help the scientists to understand the advanced concepts in biomanufacturing. This book discusses the promising strategies that will help overcome the current limitations in the biochemical synthesis processes. The book will help the readers working in industry or research to know about the new ways for improving the efficiency of the biochemical synthesis processes.

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Engineered Microorganisms for Production of Biocommodities

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1.1 Introduction

As we are going toward becoming more developed, we tend to see our transition toward more sustainable resources and knowing and understanding the life form more. This leads to the use of the living system and engineer them to produce biocommodities such as fuels, polymers, hormones, therapeutic proteins and peptides, and neurotransmitters, which is termed as biocommodity engineering. It basically deals with the need of society. Biotechnology, genetic engineering, and biocommodity engineering can be combined to meet these needs. The foundation of biocommodity engineering lies in molecular biology, which is also the foundation of genetic engineering or recombinant DNA technology (rDT). Therefore, it can be said that these terms are interrelated to each other. The majority of the biocommodities consumed by humans were earlier isolated from plants and animals, posing the threat of activation of immune reactions in humans. So, the machinery of the synthesis of these biocommodities can be engineered in microorganisms.

Its main aim is to engineer microorganisms to get a high yield of the product, use cheap raw material as a substrate so that cost of the product can be minimized, easy downstream processing, increasing robustness of the microorganism, etc. All this can be achieved by genetically modifying the organisms using genetic toolkits. This chapter deals with the basics of genetic engineering, giving details about the enzymes used, transformation techniques, and how to select a transformant from non-transformants. Further sections compile the comprehensive data of the problems in the production of biopolymers, organic acids, and therapeutic proteins from conventional methods and development of mutant strains for the synthesis of these biocommodities. The last section of the chapter gives an insight about the biofuel production

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from photoautotrophic organisms such as cyanobacteria and microalgae, which utilizes sunlight and carbon dioxide as energy and carbon source, respectively.

1.2 Fundamentals of Genetic Engineering

The advent of genetic engineering, also called rDT, started in 1952 with the discovery of Hershey and Chase, stating DNA as the genetic material (Hershey and Chase, 1952). Cohen and Boyer in the early 1970s were the first to show that the genetic material of one organism can be easily expressed in the other. Genetic engineering (Figure 1.1), in general, is the process in which the DNA is extracted, modified, transformed into a host cell, and a new organism is formed. The DNA from the desired organism is extracted and purified. It is then cleaved using restriction enzymes to get the gene of interest from it. The DNA fragment is then ligated into a vector, which acts as a driving vehicle for the DNA molecule to the host cells. This chimeric DNA molecule is then transformed into the host cells, and selection procedure under suitable stress conditions takes place. Finally, after numerous generations, the organism growing in the stress conditions is said to be recombinant or genetically modified. Genetic engineering has emerged as a crucial step in the development of industrial bioprocesses.

Each and every organism has a different genetic (DNA) makeup, which in turn makes the whole organism different with respect to their carbohydrates, lipids, and proteins. This is due to the fact that DNA transcribes and translates to mRNA and proteins, respectively (central dogma). This makes DNA the choice for manipulation in genetic engineering as manipulating it leads to the generation of a whole new organism. This postulation gives rise to many other disciplines of genetic engineering like recombinant protein production, protein engineering, metabolic engineering, etc.

Every organism being different makes it difficult to use proteins and other biomolecules of one organism to the other. This was the main reason why proteins/enzymes from animals cannot be used by humans. Earlier, insulin was extracted from the pancreas of slaughtered pigs, posing a threat to human health. This leads to the discovery of the first recombinant product, Insulin, approved by the US Food and Drug Administration (FDA) in 1982 (Goeddel et al., 1979). Now synthetic insulin is easily being produced by yeast worldwide as *Escherichia coli* does not perform post-translational modifications required to form functional insulin.

Similarly, genetic engineering is now used to produce several other biocommodities. Modifying DNA and getting it expressed inside the host organism requires several steps, as shown in Figure 1.1 and the number of enzymes. These enzymes are explained in further sections with other requirements for genetic engineering.

1.2.1 DNA-altering Enzymes

The basis of rDT is the manipulation of DNA molecules with the help of molecular biology tools and biocatalysts. The available purified enzymes that can

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