Microbiological Syntheses

Recent Advances

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MICROBIOLOGICAL SYNTHESES

Recent Advances

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FOREWORD

The detailed, descriptive information in this book is based on U.S. patents, issued between December 1980 and November 1982, that deal with microbiological synthesis and genetic engineering. This is the first volume in the Noyes biotechnology review series.

The book is a data-based publication, providing information retrieved and made available from the U.S. patent literature. It thus serves a double purpose in that it supplies detailed technical information and can be used as a guide to the patent literature in this field. By indicating all the information that is significant, and eliminating legal jargon and juristic phraseology, this book presents an advanced commercially oriented review of recent developments in the field of microbiological synthesis and genetic engineering.

The U.S. patent literature is the largest and most comprehensive collection of technical information in the world. There is more practical, commercial, timely process information assembled here than is available from any other source. The technical information obtained from a patent is extremely reliable and comprehensive; sufficient information must be included to avoid rejection for "insufficient disclosure." These patents include practically all of those issued on the subject in the United States during the period under review; there has been no bias in the selection of patents for inclusion.

The patent literature covers a substantial amount of information not available in the journal literature. The patent literature is a prime source of basic commercially useful information. This information is overlooked by those who rely primarily on the periodical journal literature. It is realized that there is a lag between a patent application on a new process development and the granting of a patent, but it is felt that this may roughly parallel or even anticipate the lag in putting that development into commercial practice.

Many of these patents are being utilized commercially. Whether used or not, they offer opportunities for technological transfer. Also, a major purpose of this book is to describe the number of technical possibilities available, which may open up profitable areas of research and development. The information contained in this book will allow you to establish a sound background before launching into research in this field.

Advanced composition and production methods developed by Noyes Data are employed to bring these durably bound books to you in a minimum of time. Special techniques are used to close the gap between "manuscript" and "completed book." Industrial technology is progressing so rapidly that time-honored, conventional typesetting, binding and shipping methods are no longer suitable. We have bypassed the delays in the conventional book publishing cycle and provide the user with an effective and convenient means of reviewing up-to-date information in depth.

The table of contents is organized in such a way as to serve as a subject index. Other indexes by company, inventor and patent number help in providing easy access to the information contained in this book.

16 Reasons Why the U.S. Patent Office Literature Is Important to You

1. The U.S. patent literature is the largest and most comprehensive collection of technical information in the world. There is more practical commercial process information assembled here than is available from any other source. Most important technological advances are described in the patent literature.
2. The technical information obtained from the patent literature is extremely comprehensive; sufficient information must be included to avoid rejection for "insufficient disclosure."
3. The patent literature is a prime source of basic commercially utilizable information. This information is overlooked by those who rely primarily on the periodical journal literature.
4. An important feature of the patent literature is that it can serve to avoid duplication of research and development.
5. Patents, unlike periodical literature, are bound by definition to contain new information, data and ideas.
6. It can serve as a source of new ideas in a different but related field, and may be outside the patent protection offered the original invention.
7. Since claims are narrowly defined, much valuable information is included that may be outside the legal protection afforded by the claims.
8. Patents discuss the difficulties associated with previous research, development or production techniques, and offer a specific method of overcoming problems. This gives clues to current process information that has not been published in periodicals or books.
9. Can aid in process design by providing a selection of alternate techniques. A powerful research and engineering tool.
10. Obtain licenses–many U.S. chemical patents have not been developed commercially.
11. Patents provide an excellent starting point for the next investigator.
12. Frequently, innovations derived from research are first disclosed in the patent literature, prior to coverage in the periodical literature.
13. Patents offer a most valuable method of keeping abreast of latest technologies, serving an individual's own "current awareness" program.
14. Identifying potential new competitors.
15. It is a creative source of ideas for those with imagination.
16. Scrutiny of the patent literature has important profit-making potential.

CONTENTS AND SUBJECT INDEX

INTRODUCTION

This book is a survey of over three hundred patents granted since January 1, 1981 and having to do with synthesis of various substances by microorganisms or by the enzymes formed by microorganisms. Included also is the subject of genetic engineering, which must certainly be considered today's most exciting area of scientific research.

Most syntheses by living organisms may be looked upon as fermentation–chemical changes induced in various substrates by microorganisms such as bacteria, yeasts, molds or fungi, or the enzymes which such microorganisms produce. Some fermentation processes have been known and used since the beginning of man's history. Fermentation is essential in the manufacture of alcoholic beverages and the preparation of most breads. It has been used for the recovery of copper from the drainage water of mines since 1000 B.C.

The bulk of the processes reviewed in this book deal with the formation of new antibiotics. Since of all the antibiotics synthesized since the discovery of penicillin very few are now in use, it may seem surprising that in the past two years so many patents have been granted for totally new or chemically modified antibiotics, but two things must be recognized in this field that make it necessary for the search for new antibiotics to continue. First of all, no antibiotic known has antibacterial activity for all clinically important pathogenic bacteria, particularly those of Gram-negative type. Secondly, the problem of acquired resistance of bacteria to existing antibiotics has become very serious. It may also be mentioned that certain antibiotics have been shown in in-vivo animal studies to exhibit potent antitumor and antileukemic characteristics. Other antibiotics have antifungal, anthelmintic and/or antimildew characteristics.

This book has been divided into ten chapters. The first four concern products made primarily by the fermentation of various strains of microorganisms. The products include antibiotics, other pharmaceuticals and certain organic compounds–primarily acids. A few products, such as interferon in the third chapter, are produced by cell culture rather than fermentation.

The fifth, sixth and seventh chapters deal primarily with enzymatically catalyzed reactions–for the preparation of drugs and organic chemicals (fifth chapter), carbohydrates and artificial sweeteners (sixth chapter) and ethanol and other fuels (seventh chapter).

It will be obvious that the patents could have been arranged other ways and that many of the explained processes fall into more than one of the listed categories. For example, most of the processes for fermentation of starches could be carried to completion for the production of ethyl alcohol.

The polysaccharides described in the eighth chapter are produced either by microorganisms or by enzyme fermentations. They are of a particular type, being gums or thickeners used to control the flow properties of aqueous systems. They are of particular interest at this time because of their use in oil well drilling and fracturing, although they have utility also as food and paint additives and in textile printing.

The ninth chapter contains descriptions of the treatment of waste materials by various microorganisms and a collection of misceilaneous processes which do not fit comfortably in any other chapter.

The last chapter is the longest and most complicated. It deals with the most discussed and least understood field of research since the splitting of the atom and is considered by some people to have almost as much potential for the benefit and the ruin of the human race. It was felt that this chapter must contain the words "genetic engineering," despite the fact that to some persons the words conjure up visions of the creation of Frankensteins or Supermen, since those who are involved in this work use the words as the overall term to describe their research.

Twenty-two of the thirty patents deal with specific procedures devised for use in recombinant DNA technology. The others concern specific products which can be made using such techniques. The first patent in the chapter is the only one in the book which was granted before January 1, 1981 and has been included because of its importance as an historical first.

The study of the molecular structure of genes and the knowledge which has been gained by these studies have already produced new tools for the diagnosis and treatment of the diseases of plants and animals, as will be shown by some of the patented processes herein.

Another of the patents in this chapter involves production of genetically engineered microorganisms which demonstrate remarkable improvement in their degradation of complex hydrocarbons. It is hoped that this type of microorganism may provide a solution to the terrible ecological problem of oil spills.

It may well be that, when the next edition of this volume is published, the major thrust of the research in microbial biosynthesis and the larger number of patents granted will be in this field now covered by only one chapter.

ANTIBIOTICS PRODUCED BY MICROORGANISMS

In most of the patents in this chapter, the morphological characteristics and physiological properties of the microorganism strain used for the fermentation are described in detail. The description may include their appearance, particularly their color characteristics; their growth characteristics on various media; their utilization of various carbon sources, etc. In many cases, especially when a new strain of microorganism is described, the source of the new strain is given and the steps taken to classify the genus and species of the strain are thoroughly delineated.

In most cases, the processes and procedures for isolation and purification of the compound made by the fermentation are also carefully described. The chemical and physical characteristics of the product antibiotic are also given in detail. Such information may include the empirical formula, elemental analysis, optical rotation measurement, solubilities, antibacterial activity both in vitro and in vivo, etc. In many cases the patent contains tables or graphs giving the infrared and ultraviolet absorption spectra of the product.

When the antibiotics are capable of forming acid addition salts, the caveat that such salts must be nontoxic and pharmaceutically acceptable is understood.

Because of the space limitations of this book, the above data are not usually included in the abstract given for each patent. If any process is of particular interest to the reader the patent is, of course, obtainable to provide the details.

Reference is also made in these abstracts to "conventional" or "normal" fermentation procedures. Such procedures consist of the cultivation of the microorganism under aerobic conditions, for example in a stationary surface culture or, preferably, in a submerged culture that is supplied with oxygen, normally in the form of atmospheric oxygen, by shaking and/or stirring in shaking flasks or fermenters of known construction. Suitable temperatures are between about 20° and 35°C, preferably 28°C. The cultures are usually carried out at a pH of from 6.0 to 8.0 which normally needs no adjustment during fermentation. The time for completion of the fermentation varies, but seems usually to be between two and seven days.

The nutrient medium used for the cultivation of the microorganism must contain assimilable carbon and nitrogen sources and essential mineral salts. As sources of carbon starch, disaccharides such as lactose and saccharose, monosaccharides like glucose, and suitable carbohydrate-containing industrial raw materials, such as molasses may be used. Examples of nitrogen sources are amino acids, peptides and proteins and their decomposition products, ammonium salts and nitrates, and suitable industrial nitrogen-containing raw materials, such as meat extracts, yeast autolysate and extract, casein hydrolysate, soy protein, cereal seed fractions, corn steep liquor, etc. Apart from ammonium salts and nitrates, the nutrient medium may contain as inorganic salts chlorides, carbonates, sulfates, and especially phosphates of alkali metals and alkaline earth metals, and also trace elements, such as magnesium, iron, zinc and manganese.

To avoid repetition, the abstracts do not usually specify the carbon and nitrogen sources used in the culture, unless a preferred source is used for the microorganism in question. Quite detailed and typical examples of the procedure used to cultivate and ferment the microorganisms to produce antibiotics are given in the first section of this chapter under the two headings, "Production of Rachelmycin" and "Antibiotic SM-173B."

In a further means of saving space and avoiding repetition, names and locations of the most commonly used repositories for microorganisms and enzymes, together with the abbreviations used for their accession numbers, are listed below rather than each time they are used in the book. Repositories which are used only once or twice are fully identified in the patent.

Accession Number Prefix	Repository
ATCC	American Type Culture Collection, Rockville, Maryland
NRRL	Northern Regional Research Laboratory, U.S. Department of Agriculture, Peoria, Illinois
FERM	Fermentation Research Institute, Agency of Industrial Science and Technology, Chiba City, Japan
DSM	Deutsche Sammlung von Mikroorganismen, Goettingen, West Germany
NCIB	National Collection of Industrial Bacteria, Torry Research Station, Aberdeen, Scotland
IFO	Institute for Fermentation, Osaka, Japan
CBS	Centraalbureau voor Schimmel-cultures, Baarn, The Netherlands
ICPB	International Collection of Phytopathogenic Bacteria, University of Caliornia at Davis, California

BY STREPTOMYCES SPECIES

Northienamycin

A.J. Kempf and K.E. Wilson; U.S. Patent 4,247,640; January 27, 1981; assigned to Merck & Co., Inc. describe a fermentation process for preparing 6-hydroxymethyl-2-(2-aminoethylthio)-1-carbadethiapen-2-em-3-carboxylic acid which is useful as an antibiotic, and which has the formula: