ADVANCES IN APPLIED MICROBIOLOGY

VOLUME 63

Advances in
APPLIED MICROBIOLOGY

VOLUME 63

Edited by

ALLEN I. LASKIN
Somerset, New Jersey, USA

SIMA SARIASLANI
Wilmington, Delaware, USA

GEOFFREY M. GADD
Dundee, Scotland, UK

ELSEVIER

AMSTERDAM • BOSTON • HEIDELBERG • LONDON
NEW YORK • OXFORD • PARIS • SAN DIEGO
SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO
Academic Press is an imprint of Elsevier

Academic Press is an imprint of Elsevier
84 Theobald's Road, London WC1X 8RR, UK
Radarweg 29, PO Box 211, 1000 AE Amsterdam, The Netherlands
Linacre House, Jordan Hill, Oxford OX2 8DP, UK
30 Corporate Drive, Suite 400, Burlington, MA 01803, USA
525 B Street, Suite 1900, San Diego, CA 92101-4495, USA

First edition 2008

ISBN: 978-0-444-53191-9
ISSN: 0065-2164

Pinted and bound in USA
08 09 10 11 12 10 9 8 7 6 5 4 3 2 1

Advances in
APPLIED MICROBIOLOGY

VOLUME 63

CONTRIBUTORS

Joan W. Bennett
Department of Plant Biology and Pathology, Cook College, Rutgers, The State University of New Jersey, New Jersey 08901.

Ronald Bentley
Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania 15260.

Celine Cosseau
Laboratoire de microbiologie industrielle, Faculté des sciences pharmaceutiques, Université Paul Sabatier, 35 chemin des maraîchers, 31062 Toulouse cedex 09, France.

Deirdre A. Devine
Department of Oral Biology, Leeds Dental Institute, Clarendon Way, Leeds LS2 9LU, United Kingdom.

Tina K. Van Dyk
Central Research and Development Department, DuPont Company, Wilmington, Delaware 19803, USA.

Mariana L. Fazenda
Strathclyde Fermentation Centre, Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, G1 1XW, Scotland, United Kingdom.

William Gaze
Department of Biological Sciences, University of Warwick, Coventry CV47AL, United Kingdom.

Linda M. Harvey
Strathclyde Fermentation Centre, Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, G1 1XW, Scotland, United Kingdom.

Peter Hawkey
Division of Immunity and Infection, The Medical School, Edgbaston, Birmingham B152TT, United Kingdom.

Michael Horn
Phyton Biotech, Inc., East Windsor, New Jersy.

Pengju G. Luo
Department of Basic Sciences, Sherman College of Straight Chiropractic, Spartanburg, South Carolina 29304, and Microbiology and Molecular Medicine, Department of Biological Sciences, Clemson University, Clemson, South Carolina 29634.

Brian McNeil
Strathclyde Fermentation Centre, Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, G1 1XW, Scotland, United Kingdom.

John Colin Murrell
Department of Biological Sciences, The University of Warwick, Coventry CV4 7AL, United Kingdom.

Colette O'Neill
Department of Biological Sciences, University of Warwick, Coventry CV47AL, United Kingdom.

Sarad Parekh
Phyton Biotech, Inc., East Windsor, New Jersey.

Robert Seviour
Biotechnology Research Centre, LaTrobe University, Bendigo, Victoria 3552 Australia.

Venkatesh Srinivasan
Phyton Biotech, Inc., East Windsor, New Jersey.

Fred J. Stutzenberger
Microbiology and Molecular Medicine, Department of Biological Sciences, Clemson University, Clemson, South Carolina 29634.

Yuri A. Trotsenko
G.K. Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, Moscow 142290, Russia.

Elizabeth Wellington
Department of Biological Sciences, University of Warwick, Coventry CV47AL, United Kingdom.

CONTENTS

CHAPTER 1

A Ferment of Fermentations: Reflections on the Production of Commodity Chemicals Using Microorganisms

Ronald Bentley,[*,1] and **Joan W. Bennett**[†]

Contents

* Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania 15260
† Department of Plant Biology and Pathology, Cook College, Rutgers, The State University of New Jersey, New Jersey 08901
[1] Corresponding author: Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260, USA

Advances in Applied Microbiology, Volume 63
ISSN 0065-2164, DOI: 10.1016/S0065-2164(07)00001-9

I. INTRODUCTION

The discovery of penicillin was a landmark event in medicine and microbiology. With its aid, many dangerous infectious diseases became controllable. The use of penicillin by the medical services of the US and UK forces facilitated the recovery of many injured combatants during World War II. For pharmaceutical companies, the success of penicillin stimulated a search for further medically useful materials derived from microorganisms. For example, the discovery of streptomycin opened a new door to the treatment of tuberculosis (Schatz *et al.*, 1944). Soon, a Golden Age of Natural Products Drug Discovery was underway as further antibiotics, antiviral and antitumor agents, immunosuppressants, and other materials were obtained in ever increasing numbers from microorganisms.

Penicillin, once a rare drug, difficult to isolate and produce, can now be considered a typical "commodity chemical"—that is, a commercially important pure chemical compound, that is bought and sold in large amounts in a competitive market. The capacity to produce large amounts of penicillin, beginning six decades ago, was a turning point in the history of the fermentation industry. The commercial production of penicillin was made possible by extensive new developments in the very large-scale growth of microorganisms. These developments have rightly formed the centerpiece of many studies in the history of what has come to be called "biochemical engineering," a term sometimes subsumed into the wider, "biotechnology." Indeed, penicillin has been termed "A Paradigm for Biotechnology" (Mateles, 1998).

The drama of the penicillin story tends to overshadow the preceding decades of development in industrial microbiology. In this essay, we look backwards at commodity chemical production by microorganisms before the 1940s. We make no attempt to be comprehensive. Essentially, this is an eclectic essay highlighting features that we feel are of special interest and significance or that have otherwise been overlooked. Our main focus is on organic acids, solvents, and penicillin. The production of amino acids, polysaccharides, vitamins, enzymes, and other commodity chemicals by fermentation is not discussed here, as many reviews on this topic are

available (Bruins *et al.*, 2001; Demain, 2000, 2006; El-Mansi *et al.*, 2006; Headon and Walsh, 1994; Lynd *et al.*, 1999; Macauley *et al.*, 2001; Magnuson and Lasure, 2004; Saha, 2003).

The progression from a concept—that penicillin might be a useful therapeutic agent—to its production as a pure, usable drug reliably manufactured on a very large scale, occurred in a period of only a few years, roughly from 1940 to 1947. It was a massive, multidisciplinary undertaking, involving biochemists, biologists, chemists, chemical engineers, clinical microbiologists, and microbiologists, with overall administration and much financial support from pharmaceutical companies and the governments of the United States and United Kingdom. In 1947, 13 USA manufacturers made 510,000 pounds (about 2.3×10^5 kg) of penicillin at a bulk price of \$3,800 per 10^9 Oxford units (\$5.67 per kg). Two decades later, the number of American manufacturers decreased to five, while the annual production increased to 1,749,000 pounds (about 7.9×10^5 kg) and the cost decreased to \$21.75 per 10^9 Oxford units (\$0.03 per kg) (Mateles, 1998). By the beginning of the 21st century, the total annual world market for β-lactam antibiotics (penicillins, cephalosporins) was about \$15 billion (Elander, 2003).

Before the discovery of penicillin, organic chemistry had dominated the pharmaceutical industry. Indeed, many distinguished scientists believed that an economically feasible chemical synthesis of penicillin would replace the use of living microbial cultures. This option was extensively pursued, in secret, both in the United Kingdom and the United States, roughly from 1943 to 1946. However, as noted by the distinguished chemist, R. B. Woodward, in his 1965 Nobel Prize lecture, "... despite the best efforts of probably the largest number of chemists ever concentrated upon a single objective the synthetic problem had not been solved when the program was brought to a close at the end of the War" (Woodward, 1972).

The search for a chemical synthesis was largely abandoned because it became apparent that not only were microorganisms capable of producing an astonishing array of useful bioactive natural products, but that the traditions of fermentation biology could be refined to meet new standards of reliability and scale. For microbiologists, whose jobs had been concentrated in hospitals and public health laboratories, the era opened lucrative new avenues of employment and required new modes of professional organization. The Society for Industrial Microbiology was founded in 1949 to provide a professional forum for the new breed of microbiologist. Like all economic and scientific revolutions, however, there were many contributing forces that led to the ascendancy of industrial fermentation; it is important to remember how much industrial scale fermentation had been conducted before penicillin was known. Coming back to the present, it is interesting to note a renaissance of natural products as drug candidates, perhaps by use of combinatorial chemistry or biochemistry

(Bentley and Bennett, 1999) and diversity-oriented chemical synthesis (Paterson and Anderson, 2005).

II. WHAT IS FERMENTATION? WHAT IS A FERMENTATION INDUSTRY?

The word, ferment, a substantive and also a verb, is derived ultimately from Latin, *fermentum*, root of *ferv-ēre*, which means "to boil," and was used to describe leaven or yeast that showed a boiling action. The noun form was used in alchemy and acquired other meanings such as agitation and excitement; the verb form was also used in metallurgy and chemistry. Samuel Johnson in his famous Dictionary quotes Boyle's work, which states that fermentation is "A slow motion of the intestine particles of a mixt body, arising usually from the operation of some active acid matter, which rarifies, exalts, and subtilizes the soft and sulphureous particles; as when leaven or yest rarifies, lightens, and ferments bread or wort." (Johnson, 1755, abridged 1843). The Oxford English Dictionary defines fermentation as a process "of the nature of that resulting from the operation of leaven on dough or on saccharine liquids."

As the biochemical processes by which yeast produced ethanol and CO_2 from carbohydrates were explored, this activity was referred to as fermentation, presumably an extension of the use of fermentation to describe the manufacture of beer and wines. Similarly, the formation of other materials, such as lactic acid, by microorganisms was also described as fermentation and qualifying adjectives were used—alcoholic fermentation, lactic fermentation, and so on.

The study of fermentation in the 19th century was long and complex, with Louis Pasteur as a major participant. In 1861, he described the transformation of sugar, mannitol, and lactic acid to butyric acid as due to a "butyric ferment" that was further described as a motile "infusorian." Remarkably, these infusoria not only lived in the absence of air, they died in its presence (Pasteur, 1861). Pasteur said this was "the first known example of animal ferments, and also of animals living without free oxygen gas." He soon named the infusoria as *Vibrion butyrique* (*sic*) (Pasteur, 1861) but in 1880 this bacterium was renamed as *Clostridium butyricum* by Adam Prazmowski. As further processes not requiring oxygen gas were recognized, Pasteur coined the words "aérobie" and "anaérobie" to designate life in the presence and absence of oxygen, respectively.

Pasteur is often credited with the aphoristic phrase, "Fermentation is life without air" (Vallery-Radot, 1960, p. 220) apparently from his famous publication, "Études sur la Bière" (Pasteur, 1876): "En résumé, la fermentation est un phénomène très-général. C'est la vie sans air, c'est la vie sans gaz oxygène libre...." In translation, "fermentation is a very general

phenomenon. It is life without air, it is life without free oxygen gas. . . ." This lengthy sentence, hardly aphoristic, continues with another 50 words of Pasteurian majesty before coming to an end.

Not everyone accepted Pasteur's view; the scientific debate was long and vituperative. Dubos, one of Pasteur's more eloquent biographers, has this commentary: "because Pasteur was convinced that fermentation could be more profitably considered as a function of life than as a chemical reaction, and because his opponents refused to meet him on this ground for reasons of scientific philosophy, there arose a battle of words in which many of the most vigorous minds of the nineteenth century took part" (Dubos, 1950).

The distinguished physiologist, Claude Bernard, became interested in fermentation late in life. Bernard stated that fermentation "se fait sans fixation d'oxygène" (proceeds without fixation of oxygen) (d'Arsonval, 1937), and came to believe that alcoholic fermentation did not require a living cell. His evidence for this was not straightforward nor did he publish it. However, after Bernard's death in 1878, various notes, thoughts, and unpublished data were found by d'Arsonval. To Pasteur's distress they were published by M. Berthelot (a rival who doubted Pasteur's conclusions) under the title, "La Fermentation Alcoolique. Dernières Expériences de Claude Bernard" (Berthelot, 1878). The final section of this paper referred directly to Pasteur's theory and was titled "Théorie de la Fermentation Alcoolique." He listed five objections and stated that the theory was destroyed. The first objection was as follows: "Ce n'est pas la vie sans air; car à l'air comme à l'abri de son contact, l'alcool se forme sans levure." (It is not life without air, as, in contact with air or not, alcohol is formed without yeast). Needless to say, the evercombative Pasteur vigorously attacked both Berthelot and Bernard in the Académie des Sciences. Given that Bernard was dead, it was a weird polemic, "in which one of the main protagonists was in the grave and appeared only in the form of a few posthumous notes" (Dubos, 1950).

Pasteur quickly demonstrated that Bernard's experimental techniques were deficient. Bernard had claimed, for example, that although fermentation occurred in the juice of crushed grapes, he could not find evidence for the presence of yeast. Bernard concluded that yeast was a consequence of, and not the originator, of fermentation. Looking back with hindsight and generosity one can conclude that Bernard had, perhaps, to some extent foreseen that the conversion of sugar to alcohol could, in fact, be accomplished by a collection of ferments (enzymes) even in the absence of living yeast. This concept was finally verified by Buchner's famous discovery of "zymase" in 1897 (Cornish-Bowden, 1997).

Pasteur had investigated the manufacture of vinegar; in the process, he identified a microorganism, "*Mycoderma aceti*," as the causative agent and thereby showed that the process was aerobic. In a lecture before the Mayor

and President of the Chamber of Commerce at Orleans, November 11, 1864, he was recorded as follows: "The Mayor and the President of the Chamber of Commerce having heard that I had studied the fermentation which produces vinegar have asked me to lay before the vinegar makers of this town the results of my work" (Vallery-Radot, 1960, p. 148). It appears, therefore, that Pasteur's view of fermentation encompassed an aerobic transformation carried out by a microorganism. This wider meaning of fermentation as almost any microbial transformation under either aerobic or anaerobic conditions persisted in Pasteur's lifetime and to the current era, existing side by side with the more narrow, "microbial transformation of substrates under anaerobic conditions."

Nowadays in industrial microbiology, the term fermentation is used in a broad way to describe all processes that are carried out in large tanks similar to those used in ethanol fermentations. Writing in his classic text, "Chemical Activities of Fungi" in 1949, the distinguished mycologist, Jackson Foster, noted that he used "fermentation," in the colloquial sense, "meaning the formation of some product by a microbiological process." He added, however, that the formation of citric acid by fungi was "an oxidation, not a fermentation, in the Pasteurian or scientific sense" (Foster, 1949).

In contemporary technical dictionaries, both meanings are attached to fermentation. One meaning emphasizes the anaerobic breakdown of glucose to lactate or ethanol while the second more broadly encompasses "the use of microorganisms or cultured cells to produce useful materials, such as antibiotics, beverages, enzymes, and some commodity chemicals" (Smith *et al.*, 2000). In describing the early application of microbes to the production of commodity chemicals, we will use the big-tent definition.

III. WHEN DID THE PRODUCTION OF COMMODITY CHEMICALS BY MICROORGANISMS BEGIN ?

The rapid development of penicillin as a commodity chemical produced by microbial fermentation owed its success to two great traditions in applied microbiology. The older tradition is the application of microorganisms since antiquity for the production and preservation of food and fluids (e.g., bread, cheese, sauerkraut, vinegar, yogurt, beer, cider, kumiss, saké, wine). The pleasures afforded by the various fermented foods and beverages are due not only to the inebrietory potential of the alcoholic beverages but also to the fact that they are complex and savory mixtures of many components. Clearly, a 50% solution of absolute ethanol in distilled water would never substitute for a single malt scotch whisky! Food and beverage fermentations are usually produced by empirical operations. However, two of the ancient technologies forming complex