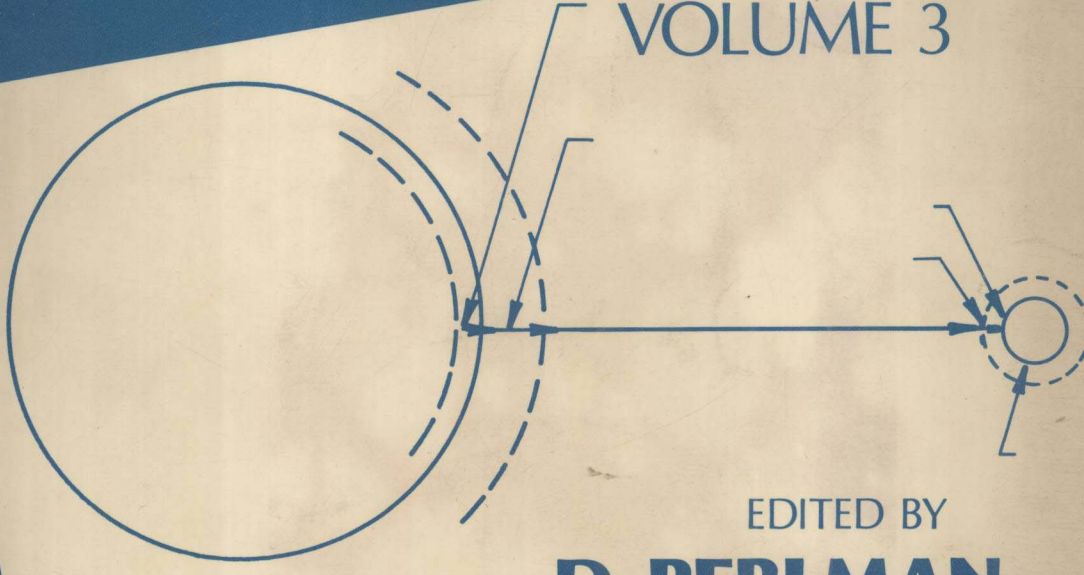
Annual Reports on Fermentation Processes

VOLUME 3



EDITED BY

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Foreword

Annual Reports on Fermentation Processes, now in its third volume, is intended to furnish readers with a timely and critical account of significant developments in fermentation processes. Only published material is included in these reviews. Each volume is designed to provide the reader with not only an account of recent developments in their own field but also, and perhaps more importantly, a means to follow developments in areas of fermentation research and development that are peripheral to their main interest. The authors of each chapter of this volume were asked to answer the question "What are the *major* developments in the field that were published this past year?" and they have provided insightful and important answers to this question.

Many persons have been involved in establishing and sustaining this series of volumes describing current developments in fermentation and we are indebted to them for their contributions. When the first volume was published in 1977, it was intended to be a three-year experiment, during which time these Annual Reports would be evaluated. The success of this experiment is overwhelming and it has been decided that this series should become a permanent chronicle for events in fermentation. The Division of Microbial and Biochemical Technology of the American Chemical Society takes great pride in having played a part in the initiation of this series and is enthusiastic about its playing a continued and active supporting role. We, as readers, are grateful to Academic Press for their help as a publisher and, most important, to Professor D. Perlman, the untiring editor of these Reports who has contributed so much to their success. As stated in past volumes, it is hoped that this volume will meet the readers' needs, and further that the editors look forward to receiving suggestions and modifications for future volumes.

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Preface

With the continued expansion in the fermentation industries and increased interest in the use of fermentation processes to produce new and useful fine chemicals, raw materials for the chemical industries, and enzymes for the food processing industry, we have expanded the scope of these volumes to include some of these topics. The wide acceptance of the earlier volumes in this series has encouraged us to consider this expansion, and we look forward to reader acceptance.

This series is sponsored by the Division of Microbial and Biochemical Technology of the American Chemical Society. In 1978 this organization undertook the sponsorship of an award to recognize contributions in fermentation microbiology and bioengineering. This award will be known as the Marvin J. Johnson Award in honor of Professor Johnson. The Upjohn Company has financially supported this program which includes a plaque and a check for $1000. The recipient is expected to present an address at the annual meeting of the Division, and we have made arrangements for an extended abstract of this address to be printed in Annual Reports on Fermentation Processes. This first awardee's lecture is included in this volume, starting on page 1. A brief biographical sketch of Professor Johnson follows. We hope that our readers will enjoy this addition to the volume.

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Marvin Joyce Johnson

Professor Marvin Joyce Johnson was born in McIntosh, Minnesota on November 25, 1906. Following graduation from Central High School in Superior, Wisconsin in 1924, he attended Superior Normal and then enrolled at the University of Wisconsin in Madison, where he received his bachelor's degree in 1927. He worked for a time as a chemist at the Burgess Laboratories, 1927-29. Continuing his education at Wisconsin he received his doctorate in biochemistry in 1932, his thesis being based on studies of the acetone-butanol fermentation with E. B. Fred and W. H. Peterson. This fruitful association with Professor Peterson lasted throughout Dr. Johnson's career even after the former's formal retirement in 1951 until his death in 1960. After a time at the German Technological University in Prague studying a fungal protease, Professor Johnson resumed his career at Wisconsin in the Biochemistry Department, progressing through the stages of Research Associate (1933-1940), Assistant Professor (1940-1941), Associate Professor (1941-1946). He was Professor of Biochemistry from 1946 until his retirement in 1972 culminated his academic activities after forty-eight years.

He married Gisela Hildegard ("Hilde") in 1934. They met during his stay in Prague. They raised two children, David and Edith, and continue to share a happy life together with their many mutual interests in science and wildlife. In his retirement years they divide their time between their lake cottage at Woodruff, Wisconsin and their place on the beach in Mazatlan, Mexico, residing briefly at the homestead in Madison on the way to and from.

His list of publications number 150 and range in subject matter from the anaerobic acetone-butanol, butanediol, ethanol, propionic, and lactic acid fermentations, through studies of the growth of yeast and behavior of bacterial, fungal, plant and animal enzymes, thence to the penicillin fermentation, the citric acid fermentation, and in more recent years the growth of yeast and bacteria (SCP) on hydrocarbons. During the years of World War II, he was a civilian attached to the War Production Board's Office of Production Research and Development. He was involved with a number of projects significant to the war effort. Consequently, his laboratory became the focal point for penicillin process development and the students he had in that period formed the backbone for the antibiotics industry in the United States for many years to follow. Not to be overlooked is the fact that he has been an innovator in analytical methods, notably paper chromatography, and in instrumentation. His hobby of electronics merged synergistically with his laboratory skills. His contributions to science have been many and sound.

He has been a guiding force within the ACS Division of Microbial and Biochemical Technology throughout its years, first in the Fermentation Subdivision of Agricultural and Food Chemistry, then in the Microbial Chemistry and Technology Division, having served as its chairman in 1956 and 1962. He received its Distinguished Service Award in 1968. His intolerance of sloppy science, which he never hesitated to verbalize in the society's technical sessions, succeeded immeasurably in raising the quality of the presentations.

As important as his work as a scientist has been, the value of his role as a teacher is as great or even greater. He supervised about 70 students for their master's degrees and 40 for their doctorate (often jointly with Dr. Peterson). Professor Johnson has been an inspiration to many during his career. His students look at the time spent under his tutelage as among the most significant years of their lives. They learned good science and they learned how to apply it with diligence and enthusiasm. They learned how to think incisively. And they came to know a really fine man.

William D. Maxon

Contents

ANNUAL REPORTS ON FERMENTATION PROCESSES, VOL. 3

STIMULATION OF INNOVATION IN THE FERMENTATION INDUSTRIES*

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Critical review of the Fermentation Industries evolution during the period 1850 - 1975 shows that it is almost impossible to predict the combination of circumstances that will lead to the successful introduction of new technology. There seems to be three major sources for stimulation of innovation:

1. The occurrence of crises and their resolution;
2. The availability of 'new' technology (directly or indirectly designed for the Fermentation Industries); and,
3. Interference in the 'normal pattern' by outside influences.

The occurrence of 'crises' is almost impossible to predict: These may be of the nature of 'scientific related problems' such as the accidental infection of fermentation by 'phage, the unexpected 'degeneration' of the microorganism through inadequate maintenance procedures, or the drastic change in the availability of an'essential' raw material. All of these crises can be solved by application of technology, and eventually the process will be back in 'normal operation.

The 'availability of new technology' is frequently suggested as a major stimulant to innovation. 'Spin-off technology' from the electronic industries' advances, the development of new types of analytical equipment and methodology, and better definition of engineering principles have eventually merited recognition and resulted in changes in fermentation practices. Unfortunately, the lag-time between availability of the 'new technology' and its utilization in the on-going or newly developed fermentation pro-

*Summary of remarks presented September 13,1978 by Professor D. Perlman on the occasion of receiving the first Marvin J. Johnson Award of the Division of Microbial and Biochemical Technology of the American Chemical Society.

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cesses has often been delayed and this may be an indication of the Fermentation Industries' conservative planning. Ofcourse, economics always influences introduction of new technology,though at times it may be a secondary influence.

A number of innovations introduced in recent years have been stimulated by politically related factors. These include:

1. Major confrontations involving government regulations on pollution of the environment both within the manufacturing area and the surrounding property; and,
2. Changes in the standards of acceptable quality of the product for distribution through normal commercial channels.

Another politically-related factor has been the rather unpredictable and apparently manipulated price of components frequently used in fermentation media, e.g. sucrose (or molasses), soybean meal, vegetable oils, etc. Other changes in practices have been related to changes in patent laws, availability of consulting services, and emergence of conglomerate corporation structures. All of these have been considered as 'outside the usual pattern' for the Fermentation Industries.

Most Research and Development managements have become accustomed to handling the 'crisis' situations with some degree of confidence. The disastrous effects of 'phage infection in the fermentations production n-butanol and acetone taught us how to cope with 'phage problems in this and other fermentations. (We have now found 'phages for most of the bacterial fermentations including the streptomycete-produced antibiotics, the amino acid - producing bacteria, and the bacteria used for steroid conversions).

Other crises attributed to the microorganism have involved lack of proper precautions in maintenance of the special strains. This results in 'degeneration' and in practical terms results in a low-yielding fermentation. Since it has been observed in antibiotic - producing fermentations as well as those producing organic acids or enzymes, we now expect such problems on almost a routine basis.

Microbial contamination as a hazard in most industrial fermentations has been a major concern. Over the years we have perfected a number of engineering-based techniques for sterilization of media and equipment to reduce the chances of major contamination to less than 2% of inoculated fermentations. This has often been a long and costly development program, and perhaps not entirely necessary as in a number of fermentations there is a 'self protection factor' and complete sterility may not be needed.

Crises that are of economic origin often evolve so slowly that preventive measures can be developed to avoid them. Some

years ago when the price of sucrose (and molasses) increased about 8-fold due to manipulation of the market, most fermentation companies were able to substitute cheaper grades of carbohydrate without marked effect on production rates. In a few instances a shift was made to an alternative substrate such as acetic acid and microbial strains were found which were effective producers of the desired product when grown in these media where the carbon source came primarily from this acid instead of carbohydrate.

If the crisis results from major increases in labor costs, coping is sometimes an impossibility. Substitution of data-logging systems for plant operational personnel may be feasible but only if adequate sensors are available. Alternative energy use patterns can also be developed, especially in the aerated fermentations where the use of oxygen-enriched air for short periods may be both practical and economic and reduce the total amount of air required for maximum productivity of the process.

Product surveys of the Industry show that a number of companies have found it more profitable to concentrate on production of a few products that yield them a 'comfortable profit margin' than to to develop to manufacturing stage processes where the margin is quite questionable. This pattern is now being challenged in the USA as mergers of fermentation companies into conglomerates results in the blurring of the identification of the fermentation operation with only a few products. These mergers frequently bring new technology into operation and modification of the traditional practice results.

The 'availability of new technology' argument to justify innovation in the Fermentation Industries is not easily substantiated by historical review. In many instances, the technology was at hand and was not use until an emergency or need arose in a specific fermentation process. Some of this delay might be avoided by having multi-disciplinary teams working the fermentation development rather than assigning process development to the microbiologists.

The politically-related factors sometimes stimulate innovations and often have the opposite effect. The considerable concern in recent years with toxic or obnoxious substances occurring in the fermentations or in the residues has forced several companies to revise their processes. These revisions have included use of 'special strains' which do not produce the toxins or odors, changes in the composition of the fermentation media, and in some instances moving the manufacturing facility to new geographic locations.

The rather involved problems of SCP from petrochemicals in Italy and other countries increases our concerns about artificial

standards for some fermentation products. The cost of the effort in Italy to construct the plant and have the large scale testing of the product was far more than originally budgeted, and now the prospect is to terminate the project and abandon the proposal,all due to some political entanglements.

In many instances innovation in the Fermentation Industries has been markedly stimulated by competition from or within the Chemical Industries: This was the case with our original interest in finding microorganisms capable of specific transformation of steroids and thus eliminating some of the costly operations in the chemical synthesis of cortisone and hydrocortisone. A number of the innovations which have this origin have been successful and others have not been economically attractive. In nearly every instance it was necessary for the Fermentation Industries to revise previously held concepts about microbial operations, and to accept some new hypotheses on what might be feasible with selected microorganisms. Although considerable interest has been generated by reports on the biosynthesis of somatostatin, insulin (chains A and B), and ovalbumin by'genetically engineered' special strains of Escherichia coli, it still remains to be determined how soon these will be commercialized as fermentation products.

The rate of development of innovations in the Fermentation Industries is practically impossible to predict. The major 'stumbling block' to a continuously supported program appears to be related to difficulty in definition in technical terms of the parameters of the problem. Once the problem is well defined, the possibility for review by non-fermentation trained technologists, e.g. electronic engineers, systems analysts, bioengineers, etc., is increased and solutions are usually proposed.

It seems that the Fermentation Industries have a'conservative image' as far as innovation is concerned. Most of the manufacturing facilities are constructed with multi-purpose use in prospect and many are expected to have a 20-year replacement schedule. Under these circumstances it is not surprising the innovations are often introduced by companies new to the field or consulting and contracting groups. Unfortunately, both the laboratories of the U.S. government, e.g. U.S.D.A., D.O.E., and D.O.I., and those of the universities are not encouraged to try innovations and study the parameters involved which will lead to progress in our understanding of the control of microorganisms for production of useful metabolites. Perhaps this will change in the USA(as it has already in Japan). We await with impatience these changes.

GENETICS OF INDUSTRIAL MICROORGANISMS

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I. INTRODUCTION

In the first volume of this series, Elander, Chang and Vaughan (1) reported on the genetics of industrial microorganisms through early 1977. In the intervening two years, the development of new, very broadly applicable methods of gene transfer has proceeded so rapidly that some may now question the traditional classification of organisms as "academic" or "industrial." Indeed, the classic academic microorganism, *Escherichia coli*, may soon become an important industrial microorganism for producing human insulin. With the new methodologies of protoplast fusion, protoplast transformation, gene cloning or "recombinant DNA," transposon manipulations, gene synthesis and gene sequencing, virtually all of the tools for genetic manipulation are now potentially available for any microorganism.

It is in this broad context that we attempt to assess the current state of the art on the genetics of industrial microorganisms. In doing so we realize that we cannot be comprehensive; therefore, we have chosen in general to emphasize studies which illustrate the techniques having broad applications to many microorganisms. This emphasis has meant that many excellent examples of genetic manipulations of microorganisms currently producing industrial products have been omitted. In addition, because of our particular interests in antibiotics produced by streptomycetes and fungi, we have

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emphasized studies with these microorganisms at the expense of others. Topics recently reviewed elsewhere are dealt with when possible by referring readers to those articles.

II. MUTATION

A. *General*

Mutation induction and mutant isolation continues to be an extremely valuable genetic tool in the fermentation industry both from the standpoints of new product discovery and yield improvement in ongoing fermentation processes. Many specific applications, selection techniques and methodologies have been discussed in detail in the first volume of this series (1) and elsewhere (2).

Recent reviews dealing, in part, with mutation in industrial organisms have emphasized the increased use of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) in strain programs (3,4), have described the effectiveness of induced mutation to develop industrial yeasts (5) and corynecin-producing strains (6), and have summarized the selection and utility of conditional mutants of industrial microorganisms (7).

Recent articles have described the use of MNNG to induce mutants of *Aspergillus niger* with enhanced organic acid yield (8), mutagenesis with other N-nitroso compounds to induce mutations in *Aspergillus oryzae* which improve α-amylase production (9), induction by UV or gamma irradiation of mutants of *Streptomyces atroolivaceus* blocked in the synthesis of mithramycin (10), application of an improved method for nitrous acid treatment of *Claviceps purpurea* to induce mutations which enhance ergot yield (11), and mutagenesis with nitrous acid, UV light, and gamma irradiation to produce mutants of *Streptomyces galilaeus* with improved production of anthracycline antibiotics (12).

Although much is known about the genetics and biochemistry of the various mutational processes (13-19) from numerous studies in a variety of "academic" microorganisms, little can be said about the specifics of these processes in, for example, *Streptomyces*. Two general observations, however, are pertinent. The first is that *Streptomyces* are susceptible to high frequency mutation induction by the potent chemical mutagen MNNG (20-22), which appears in *E. coli* to induce mutation by a complex mechanism which includes direct interaction with DNA and indirect interaction(s) involving a cytoplasmic (protein?) component (23,24). The second observation is that UV light induces mutations in *Streptomyces coelicolor* by an apparent