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A Prejudiced Look at Fermentation by a Microbial Biochemist

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President Litchfield, members of the Society for Industrial Microbiology, Ladies and Gentlemen. It is difficult for me to express how thrilled I was about two months ago when I received the letter from President Litchfield telling me that I was to receive the Charles Thom Award for 1971. I was particularly moved because this truly unexpected honor comes only a few weeks following my retirement as a research director for Miles Laboratories, Inc. I am especially grateful that my contributions to industrial microbiology have been judged worthy of this highest award bestowed by our Society.

When Dr. Kenneth B. Raper (1968) received the first Charles Thom Award, nothing could have been more fitting for this award address than his entertaining remarks relating to his work with Dr. Thom and his biographical reminiscences regarding Dr. Thom. Dr. Arthur M. Kaplan's (1971) Charles Thom Award address last year was an erudite discussion of the Concepts, Challenges, and Motivations of Industrial Microbiology. He called attention especially to the interdisciplinary nature of the industrial microbiological approach, and the diverse nature of industrial microbiological interests, such as fermentative production of commercially important products, enzymatic actions, protection against microbiological deterioration, waste disposal, etc.

Since my major field of interest has always been with fermentations, I have chosen to review briefly this one phase of the very broad field of industrial microbiology. My definition of fermentation is the production of useful materials by microbial action. These materials may be chemicals such as ethanol or citric acid from major metabolic conversions of substrates, biologically active materials such as antibiotics, vitamins, or enzymes, or the microbial cells themselves such as baker's yeast or "single cell protein" sources.

When I look back to my college days, I well remember how intrigued I was as a chemistry major senior at Nebraska Wesleyan University by the fascinating story of the development of the butyl-acetonic fermentation as published by Gabriel (1928) in *Industrial and Engineering Chemistry*. When I went to Iowa State College as a graduate student, I intended to become an organic chemist. But during my first graduate year I was introduced to biochemistry and decided to cast my lot in that field. While a graduate student, I also took all the courses I could in microbiology – bacteriology and mycology – as my minor subject. It was perhaps an unexpected and surprising coincidence that my major professor suggested, and I carried out as my doctorial thesis problem, an investigation of the butyl-acetonic fermentation of xylose.

As a neophyte Ph.D. and young Instructor and then Assistant Professor at Iowa State College, I turned my attention with my graduate students to practical microbial

This paper is the 1971 Charles Thom Award Address

chemistry as exemplified by fermentations. It is worthy of note that at this time in the early 1930s and until after World War II, in only three universities in the United States were there extensive fermentation research programs – those of Dr. W. H. Peterson and his group including Marvin J. Johnson at the University of Wisconsin, of Dr. S. C. Prescott including Cecil G. Dunn at Massachusetts Institute of Technology, and the group of Dr. E. I. Fulmer and myself at Iowa State College.

Fermentation and the industries based on fermentation have had an interesting history. This history constituted the "yesterday" of the intriguing discussion of "Fermentation – Yesterday and Tomorrow," published recently by Dr. Marvin J. Johnson (1971).

Looking at the past in fermentation technology, the first period may be considered to run from the beginning of human time to about 1860, that is, the pre-Pasteur period. Although the causative agents were not then known, practical fermentations had been developed and were practiced. The anaerobic fermentor was known and used for alcoholic fermentation and baker's yeast manufacture. Two types of aerobic fermentation were practiced: the vinegar generator and the semi-solid *Aspergillus oryzae* koji fermentation, mainly for Japanese sake and soy sauce production. Knowledge of techniques included pasteurization, inoculation, aeration, and cleanliness (semi-asepsis).

In the 40-year period following Pasteur, from 1860 to 1900, only one additional fermentation (lactic acid) and one additional technique (aeration in liquid fermentors) were developed. The latter was adopted for yeast production since the yeast grew better when the fermentor was aerated. The trickling filters for sewage disposal were invented, but these were only an adaptation of the known vinegar generator.

During the first 20 years of the present century, partially due to the needs of World War I, there was considerable fermentation activity. Efficient fermentative production of industrial ethyl alcohol (from grains and from molasses) was developed from the alcoholic beverage fermentations early in this period. Fermentative production of glycerol, acetone, and butanol, and of microbial enzymes (fungal and bacterial) was industrialized. The aerobic activated sludge process and the anaerobic Imhoff tank for sewage disposal were introduced. The aerobic production of yeast with continuous sugar addition was also made commercial during this period.

The next 20 years, from 1920 to 1940, were particularly a period of improvement of the existing important fermentation processes. The citric acid fermentation became an industrial reality, and fermentation gluconic acid and sorbose production were introduced. Two important techniques were invented: the shaken flask laboratory aeration procedure coupled with the agitated-aerated fermentors, and the sterilization of air by use of fibrous filters.

It was about the middle of this period, in the early 1930s that my research career began, resulting in 15 publications by the end of 1940. Our work related to certain aspects of yeast ethanol and bacterial butanol-acetone fermentations, and to the oxidation of sugar alcohols by *Acetobacter suboxydans*. With the latter we did pioneering laboratory work on fermentation of sorbitol to sorbose, which was subsequently scaled up by United States Department of Agriculture workers and adopted by the industry to make sorbose as the starting material for vitamin C synthesis. We also developed dihydroxyacetone production from glycerol and I eked out my meager college salary by producing this material for several fine chemical marketing companies. In the course of this work I frequently had very brown fingers and hands, but it did not occur to me then that this could have any interest as a chemical to produce a nice brown skin pigment

without the sun. Cosmetic products containing dihydroxyacetone for this purpose were quite a lucrative fad for certain fermentation companies a few years ago.

A tremendous metamorphosis in the fermentation industry occurred during the decade 1940 to 1950. The early part of this period, due to the exigencies of World War II, saw tremendous expansions and significant yield improvements in the conventional anaerobic fermentations, particularly to produce ethanol for synthetic rubber and other war uses. Also grain fermentations for butanol and acetone returned to prominence. However, after the war, the petrochemical industries completely captured many of the former fermentation markets, except for beverage alcohol products, with synthetic ethanol, butanol, acetone, and other easily synthesized chemicals. But the submerged aerobic fermentation processes for penicillin, other antibiotics, vitamins (riboflavin and vitamin B_{12}), and microbial enzymes exploded in importance. This was possible through adopting and perfecting techniques developed toward the end of the previous decade. These were: (1) use of the shaken flask as an aerobic laboratory tool; (2) use of aerated-agitated vessels as aerobic fermentors; and (3) efficient sterilization of air by passage through fibrous filters.

During this decade my students and I had 31 fermentation publications, and I became Associate Professor and then full Professor. During the early part of the period we worked particularly on perfecting methods for producing efficient fungal amylase saccharifying agents for use in ethanol fermentation of grain. This was necessary since war demands increased ethanol production from just under 300 million proof gallons in 1941 to almost a billion proof gallons in 1944, and 1.1 billion proof gallons in 1945, with use of about 10 million bushels of grain in 1945. Malt for the saccharification was in very short supply. Our work led to establishing a manufacturing plant for mold bran production which supplied part of the saccharifying needs at the government's alcohol plant at Omaha, Nebraska, during 1945. I also served, on leave from Iowa State College, as Chief Chemist at this alcohol plant 1944-45. The original design capacity for the plant was 50,000 gallons of 190-proof alcohol per day. We routinely turned out 75,000 gallons per day, and the record single day production was a little over 95,000 gallons. Most of our production went for making synthetic rubber.

I might also mention a three-month interlude during the winter of 1942-43 when I went to Central America as an emissary of our Coordinator of Inter-American Affairs to investigate the potential fermentation of bananas. The U.S. government had taken the banana boats for other essential war uses, and billions of bananas were rotting to waste. After my laboratory work was well along, I was joined at the United Fruit Company research laboratories at La Lima, Honduras, by Dr. Donald Othmer, Chemical Engineer from Brooklyn Polytechnic Institute. We were able to develop successful alcoholic fermentation of bananas and recovery of the product in the laboratory. We could ferment and recover excellent alcohol yields from either ripe bananas (carbohydrate mainly sucrose) or green bananas with amylase saccharification (carbohydrate mainly starch). However, requirements for critical metals and equipment made it impossible to justify building any processing plant which could have only temporary use. So there was no practical outcome from our work, except that Don and I escaped in a tropical climate the rigors of one winter in the United States.

We also developed, at Iowa State College, a greatly improved and efficient glycerol fermentation process, employing insoluble sulfites to fix acetaldehyde and drive the yeast fermentation reaction to glycerol. However, synthetic glycerol from propylene became

available at lower cost before our fermentation process could be industrialized by the company which licensed our patients.

Subsequent to 1950, we may say that the fermentation industries peaked in importance around 1955. It was at the end of 1954 that the two volume authoratative work *Industrial Fermentations,* with chapters by specialists devoted to each of the then important fermentations, was edited by me and my former graduate student, Richard J. Hickey (1954). During the early 1950s, my research program at Iowa State College gradually shifted from other fermentations to enzymology. It was a very natural transition, therefore, when I joined Takamine Laboratory in 1955 as Director of Research. Takamine Laboratory was acquired by Miles Laboratories in 1956, and I served as Director of Enzymology Research for Miles until 1968. After that, until my retirement last May, I was Director of Molecular Biology Research at Miles.

Since 1950, the new fermentations developed and industrialized have included production of amino acids, steroid conversions, several new antibiotics, some new enzymes, gibberellins, and production of nucleotides. As far as new techniques are concerned, the advances have been mostly in measurement and control: pH measurement and control during fermentation; foam control; automated chemical analyses; and oxygen and carbon dioxide analyses.

Also during the period since 1950, genetic manipulation of fermentation cultures has been fruitful. Selection and use of high yielding mutants have increased greatly the production of citric acid, antibiotics, and enzymes, for example, Also the efforts to produce superior cultures by genetic recombination have been successful in some cases, and the work in this area is surely increasing.

While the techniques are not new, major improvements, at least in fermentations I am familiar with for producing enzymes, have come through recognition and application of techniques of continuous environmental control. Less dependence is being placed on the components of the initial medium to control the conditions during the fermentation. Instead, the environmental conditions are continuously regulated, including continuous pH control and possible variation of pH during the fermentation cycle, variation and control of temperature, variation and control of agitation and aeration, and feeding of nutrient and precursor materials during the fermentation cycle.

Progress during the very recent past includes the development of processes for new, important commercial enzymes. These include glucoamylase now universally used industrially to produce dextrose from starch, for microbial rennet used in cheese making, for alkaline protease used in detergent products, and glucose isomerase for syrup modification. Also much progress has been made using hydrocarbons or other substrates, with processes for producing microbial cells of high protein quantity and quality having potential for animal and human nutrition.

The increasing problem of environmental pollution by chemical insecticides has called for the attention of fermentation microbiologists. Fermentative production of *Bacillus thuringiensis* to control insect pests in agricultural crops, or of *Bacillus popilliae,* causing milky disease for control of Japanese beetles, has resulted. On the horizon appears the possibility of producing specific viruses for the control of undesirable insects or other pests without affecting man or desirable animals. While viruses, as well as plant and animal cell cultures, have not been the purview of industrial microbiologists in the past, all of these are surely coming to the forefront. Plant and animal tissue cultures promise to become important sources of valuable products – enzymes, hormones, vaccines, viruses, antigens, and antibodies.

This brings me to the end of my story of the history of fermentation and some of the things that I and my co-workers attempted in this field. I do not forsee any immediate new boom in fermentation. Present-day fermentation industries can, of course, be expected to continue and to expand. Only time can tell what new fermentations may be developed for producing natural products of such complexity that chemical synthesis is impractical, or what new useful techniques may be developed. Industrial microbiologists can be depended upon to develop such new techniques and to further exploit old ones. Now that I am retired and "out to pasture," I shall look on from the sidelines with much interest to the future progress in fermentation as well as in other important areas of industrial microbiology engaging the attention of members of our Society.

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