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# Some Problems on the New Horizons of Applied Microbiology*

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President Laskin, members of the Society, Ladies and Gentlemen, I thank you for your generosity in selecting me as the recipient of the Society's 1979 Charles Thom Award. I certainly appreciate the recognition and the responsibilities it implies. I am pleased that I have been judged to meet the qualifications that have been established for the Thom awardees.

When I was notified last May of my selection as the recipient of the ninth Charles Thom Award, I took the opportunity to reread the texts of the lectures presented by previous recipients of this honor. I found that several were devoted to a personalized presentation of highlights of their careers, while others focused on some aspects of their research programs which the lecturers concluded were most important to them. With these precedents before me, I decided that for my address I would include some personal history and, in my role as "unofficial chronicler" of the fermentation industries, my interpretation of the current and future "states of the art."

I had the good fortune to pursue my graduate studies at the University of Wisconsin in the Department of Biochemistry under the supervision of the team of Professors William H. Peterson and Marvin J. Johnson. Actually, my interests during my undergraduate years were focused on analytical chemistry, and even after a rather unsatisfactory year working with the Heyrovsky polarograph (an invention of the devil!), I was still planning to continue with a program in analytical chemistry. However, one day in June 1941, Professor Johnson interviewed me for a research assistantship in fermentation biochemistry, and this resulted in my switching to this field for graduate studies and a career. As I look back on this turning point in my professional life, I realize that it was a matter of chance, and I wonder if most of our major decisions in choosing careers are equally unpredictable.

My first research assignment was the study of the production of citric acid by *Aspergillus niger*. This project was supported by a grant from the Heyden Chemical Co. (now part of Newport Industries, Inc.) which had for many years conducted in-house research and development on this fermentation. Their Mr. William Eisenman made available to us information on certain techniques he had found useful and cultures for the fermentation studies, and advised periodically.

This project introduced me to some aspects of applied microbiology including: (1) The importance of culture maintenance procedures. We encountered the common problem of "strain degeneration" and were fortunate to isolate a "high producer" (*A. niger* 72/4) from the first dozen natural variants tested. (2) The importance of mineral nutrition

*Address given upon receipt of the 1979 Charles Thom Award.

in the fermentation, experience that I was able to carry over later to studies on other fermentations. (3) The potential use of ion-exchangers to remove metallic ions from nutrient media (including those based on molasses.) This was probably the first study of this application of ion-exchangers and was later exploited on an industrial scale.

The involvement of the United States in World War II brought about a shift in our research program as the laboratory group undertook a series of studies of processes for producing 2,3-butanediol. This microbial metabolite was under consideration as a starting material for chemical synthesis of butadiene for synthetic rubber, and a number of industrial laboratories, as well as several from academia, were cooperating loosely in the research and development studies on several processes. The involvement of industrial groups and visits by their staff members to Madison brought new facets of applied microbiology to my attention and helped my orientation for career preference. Our program in Madison focused on obtaining "more efficient organisms" and I eventually even considered the use of antibiotics (streptothricin from Professor S. A. Waksman) resistance as a method of selection of strains from the *Aerobacillus* culture. Other techniques that I learned during this period included: (1) Methods of selection of phage-resistant organisms; (2) the value of adapting the culture to media containing inhibitory substances (in this case wood hydrolyzates); and (3) the problems of a mixed culture fermentation (*Mucor* to hydrolyze the starch and *Aerobacter* to convert the glucose to 2,3-butanediol).

Our 2,3-butanediol program was terminated in 1943 when Professors Peterson and Johnson, at the request of the Office of Production Research and Development of the War Production Board, undertook the study of the penicillin-producing fermentations. I had previously become interested in penicillin as a result of an August 1941 visit to the Northern Regional Research Laboratory in Peoria. There I met Dr. N. G. Heatley (of the Oxford Group) and Drs. K. B. Raper, G. E. Ward, and F. H. Stodola. They were just starting the study of the submerged culture process (using roller drums), and I was fortunate to be able to see the fermentation and bioassay operations.

The penicillin research program at Madison 1943-45 focused on: (1) The nutritional requirements for antibiotic production (e.g., was there a "magic ingredient" in cornsteep liquor?); (2) the bioassay for penicillin in fermented media (eventually this led to an evaluation of paper chromatography for identification of the various penicillins in our samples); (3) the problems of production of penicillin in submerged cultures using shaken Erlenmeyer flasks and stirred jars; and (4) the evaluation of the *Penicillium chrysogenum* mutants obtained from strains 1951.B25 and X-1612 by Professors M. P. Backus and J. F. Stauffer as well as other strains sent to us from Stanford University (Professors Beadle, Tatum, and Bonner) and University of Minnesota (Dr. J. Ehrlich and Professors C. M. Christianson and E. C. Stakman). My assignments included study of the chemically defined media and an evaluation of the various cultures in stirred-jar fermentations.

As can be seen from the foregoing discussion, I was exposed to many problems of the fermentation industries before I actually was employed in industry, and I am indebted to Professors Peterson and Johnson for this base which has served me well for the past 35 yr. It is a much broader program than we are currently able to offer our graduate students, and I feel that many are not realizing their opportunities when they confine their graduate research programs to one or two projects.

After I left Madison in 1945, I was employed as a biochemist at Hoffmann-LaRoche for some months and then as a microbiologist at Merck & Company, Inc. for 2 years.

I shifted to the Squibb Institute for Medical Research in 1947, where I spent nearly 20 years, before returning to Madison to join the Faculty of the School of Pharmacy. My assignments with these companies involved research and development programs on the production of penicillins, streptomycins, neomycins, tetracyclines, polyenes, and macrolide antibiotics; vitamins $B_{12}$, riboflavin, and ascorbic acid; microbial transformations of steroids and antibiotics; and growth of mammalian and plant cells in tissue culture.

All of these research and development projects had their challenging moments, and I would rather frequently look forward to the next day's experiments with the same anticipation we had back in the early days of penicillin research. I think it is not too difficult to find aspects of many development programs in applied microbiology which are really "fundamental research," and I have tried to promote this hypothesis in working with my associates at all levels.

My interests and activities as "chronicler of the fermentation industries" were first stimulated by Dr. A. F. Langlykke who asked me to join him in preparing a chapter on fermentations for the second edition of Sumner and Myrback's *The Enzymes* in 1949. A few years later Dr. S. B. Lee invited me to be one of the authors of the "unit process report" which he was preparing for *Industrial and Engineering Chemistry*. I participated in this activity for some years, and eventually this annual survey became the *Annual Reports on Fermentation Processes* (now in its third volume). I also took on the responsibility of editing the *Advances in Applied Microbiology* (starting with volume 10), and this opportunity, too, brought me in closer contact with the fermentation industries. Finally, Dr. H. J. Peppler and I (Peppler and Perlman 1979) have just completed the second edition of *Microbial Technology,* and participation in this effort has made me more aware of some of the problems and prospects for the fermentation industries.

I have chosen the title "Some Problems on the New Horizons of Applied Microbiology" for this 1979 Charles Thom Award lecture because I feel we are not recognizing how some of the decisions we are making today will shape the fermentation industries of the future. We are content in many instances to follow traditional pathways and other lines of least resistance rather than undertaking new approaches to solving our current and future problems and meeting the new challenges.

The fermentation industries in the United States have a record of periods of intense activity and expansion, and then periods of lesser activity. Fortunately, these latter are becoming fewer in number and the intervals between them longer.

Our industry really started in the 1850s when baker's yeast became of commercial importance. Up until that time, the bakers were satisfied to use the brewing yeast, a top yeast, for their needs. In 1848-50 there were waves of immigrants from Germany and Austria-Hungary. They brought with them their lager beer, and this was not a very suitable source for baker's yeast. Fleischmann and others started to manufacture a yeast preparation especially suited for the baking trade and this has continued until the present time. The other fermentation activities during the 1850-1910 period included manufacture of lactic acid (on a small scale) starting in 1881 in Massachusetts, and Dr. Takamine's production of enzymes for industrial and other uses. Nearly all the fermentation products (actually a very small number) were imported along with the other fine chemicals from Europe.

The need for a plentiful supply of acetone during World War I led to the eventual operation of the *Clostridium acetobutylicum* fermentation by Commercial Solvents

Corp. in Terre Haute, IN, and Peoria, IL. Although once the war was over the demand for acetone dropped, the secondary product of this fermentation, *n*-butanol, became commercially important as a solvent for lacquers used in the automobile manufacturing industry, and this fermentation product was produced in large quantities, e.g., tens of millions of pounds. Interest in production of other solvents for industrial purposes increased, and large amounts of ethanol were produced to meet these needs. Among the firms which expanded their operations to meet this market were Publicker Alcohol, Inc., U.S. Industrial Alcohol, Inc., and Commercial Solvents Corp.

The laboratory studies of Dr. Thom and his associate Dr. J. N. Currie on the conversion of carbohydrate to citric acid by *Aspergillus niger* cultures encouraged the Chas. Pfizer Co. to undertake large-scale production of this acid in 1923. This company has maintained a leading position in the manufacture of this product for more than 50 years. They and others found that some strains of *A. niger* would convert the sugar glucose to gluconic acid, and this, too, became a major fermentation product.

Toward the end of the 1930s it was realized that fermentation processes for riboflavin and thiamin might be commercially feasible, and a number of pharmaceutical companies became interested in these fermentations. Other developments attracting the interest of the pharmaceutical industry included the microbial conversion of D-sorbitol to L-sorbose (useful in the Reichstein synthesis of ascorbic acid), the stereospecific acetylation of benzaldehyde to form L-phenylacetyl carbinol (an intermediate in the synthesis of L-ephedrine), and the use of microbial acylases to prepare L-amino acids from D,L mixtures.

Although the involvement of the pharmaceutical industry with fermentation processes was rather minimal, many had sufficient contact to accept the challenges encountered in the production of penicillin and other antibiotics. In a practical sense, we note that the number of fermentation products increased from 1940 to 1960 by a factor of nearly 3, with about nine products produced on a manufacturing scale in 1940 and 30 in the early 1950s. By 1960 the total approached 60, and we presently have over 190 fermentation processes in operation (this is considering the semi-synthetic antibiotics operations as single fermentation processes). Many of these processes are related to either antibiotic production (96 products) or amino acids (21 products), and the number of new fermentations expanded to commercial scale is approx. 15 every 10 yr. Of course, this rate of increase will change with economic and political circumstances.

There are at least three important changes from a management viewpoint that have evolved in the fermentation industries in the past 10 to 20 yr.

First, we have come to realize the importance of the pilot plant as a research and development tool. We no longer see many companies carrying out their process development entirely in the manufacturing plant. It is both too expensive and not easily accomplished in these days when the FDA and other regulatory agencies want full explanations of why manufacturing processing was changed from one batch to the next.

Second, most managements have adopted the operational plan in which representatives from microbiology, bioengineering, chemistry, and perhaps pharmacology (if pharmaceuticals are being manufactured) are part of the development and research teams. This has proved efficient for processes where many different technologies are involved. I doubt that there will be a return to compartmentalization.

Third, many managements have concluded that it is more economical to purchase technology than to develop expertise within their own organization. This is especially

true with the antibiotic processes where Panlabs, Inc., and Cetus Corp. have served as sources of "new" microorganisms and related technology. It also is interesting to note that with the increase in government regulation in countries outside the United States, several companies have found it economic and practical to share manufacturing facilities and to license processes.

This emphasis on "group research," which was so successful in developing processes for manufacture of antibiotics, was carried over to the development of processes for microbial transformation of steroids and now sterols. We find greater acceptance of the hypothesis that microbial reactions can be substituted for chemical steps in many situations, that they can serve as supplements to the chemical reaction sequence, and/or they can serve as model systems for both chemical and physiological (animal) systems. This has led to the involvement of many persons trained formally as organic and analytical chemists in devising processes where microbial steps are part of the strategy. Unfortunately, since only a few of these chemists have had formal training in microbiology, they sometimes expect too little or too much of our microbial slaves. It is the opportunity of the microbiologist to educate these chemists to respect the advantages of the microbial reactions and also their limitations.

There have been many major accomplishments in the past decade on which we can build for the next 20 yr. I think the most important are: (1) The use of immobilized cells for transformation of organic substrates. Mosbach and his associates and Chibata et al. (1975) showed the principles involved, and the latter group have commercialized several of the processes. Of course, the commercial processes for production of high-fructose syrups have been the most important application of this technology. One of the consequences of this development has been quite a revolution in the sucrose/molasses market. (2) The new methods of strain and culture selection described in recent conferences on genetics of microorganisms. These have replaced "blind, brute force" techniques for culture improvement programs although the latter approach is still successfully operated. (3) The demonstration of recombinant DNA strategy for the production of protein hormones by *Escherichia coli* and potential use of this technique with other organisms. (4) The potential application of computer control for fermentation processes. At present, we are still in the "data logging and analysis phase." I expect we may soon hear of (experimental) programs for certain fermentation process controls.

We have been encouraged, impressed, and jealous of the success our biochemist colleagues have realized in introducing the capability for "unusual protein" biosynthesis in microorganisms. Cape (1980) has discussed some of these in a symposium at this meeting. I gather that many more "special strains" are being developed, and soon there will be a real problem in finding new goals for this technology. We are uncertain of the economic implications of some of these new developments. It is likely only a few fermentation companies will be involved in these new strategies for manufacturing protein hormones and other pharmaceuticals.

One of the more encouraging indicators of the continued economic success for the fermentation industries is the continued investment in new equipment and facilities. I have summarized in Table 1 a number of reports on expansion of manufacturing facilities as well as construction of new manufacturing plants. Some of the expansion is also replacement, but much of it is real expansion. This suggests continued confidence of the management in the fermentation operations and also satisfaction with previous performance.

TABLE 1. *Recent manufacturing facility construction (USA)*

| Company | Location and Kind of Construction* | |
|---|---|---|
| G-B Fermentation Industries | South Carolina | (P) |
| Hoffmann-LaRoche, Inc. | New Jersey | (X) |
| Eli Lilly and Company | Indiana | (X) |
| Miles Laboratories, Inc. | Ohio | (P) |
| Novo Industri A/S | South Carolina | (P) |
| Rachelle Laboratories | California | (X) |
| Schering Corporation | Puerto Rico | (X) |
| Upjohn Company | Michigan | (X) |
| Wyeth Laboratories | Pennsylvania | (X&P) |

*(P) = new plant construction; (X) = plant expansion.

The fermentation industries are generally considered to be "conservative" in their approach to market planning strategy. Thus, there are a number of limitations to evolution. I have listed a few in Table 2, and of these I think the more important are slow replacement of facilities, lack of innovation incentives, and limited number of training programs for personnel.

The slow replacement of facilities really means that new processes have to fit into equipment that was perhaps optimal for some other process. And when expansion is approved, the new equipment frequently is very nearly identical with that used in the older unit.

The lack of stimulants to innovation can be reversed by several forces including: (1) A new discovery; basic science (such as microbial genetics); (2) competition from a chemical process (as seen in lactic acid); (3) new government regulations (EPA and related problems); (4) some political problem (change in tariffs on manufactured products); and (5) economic parameters (shift in cost sucrose/molasses). However, we cannot always look to these outside forces to sustain and stimulate the research and development, and there has to be some conviction that a continuous innovation program is both desirable and essential to the success of the company. Of course, some companies meet these needs by licensing and other means of acquisition; these approaches may solve an immediate problem but do not result in the long-range commitment that is frequently needed.

There have been some "partial successes" in the past 10 to 20 yr to which we should take note. (1) *Continuous fermentations*. We find that after much study by our bioen-

TABLE 2. *Limitations to evolution in fermentation industries*

| |
|---|
| Tradition |
| Slow replacement of facilities |
| Low profit items |
| Competition from chemical industry |
| Lack of innovation incentives |
| Limited amount of research and development |
| Government regulation |
| Limited number of training programs |

gineering colleagues, it is probably feasible to carry out any fermentation on a continuous basis. However, in practically all instances this capability is not realized due to problems in operating a continuous recovery process in seriatum, increased labor costs, etc. In fact, the only fermentation I know that is currently operated on a continuous basis is the conversion of D-sorbitol to L-sorbose. (2) *Gasahol*. I am not convinced that it is likely to be economic, though it will maintain the high price of grain. We shall have to expand the alcohol production facilities about a thousand-fold to supply enough alcohol for supplemenation of only part of the gasoline used for motor fuel. Who is going to finance this, and what will the fermentation substrate be, etc., etc.? (3) *Hydrocarbons as substrates*. We found that most fermentation processes could be adapted so that hydrocarbons can replace carbohydrates and lipids as energy sources. Now, with the higher costs for hydrocarbons, we cannot make use of this technology. (4) *Single cell protein*. The fermentation operation is technically feasible, but political complications frequently interfere with marketing the product. At the moment, I gather AMOCO is still manufacturing yeast (Tortuin) from ethanol and several companies are using other substrates with similar strains of yeast. The acceptance as a feed and food ingredient is growing, but very slowly. (5) *Washing powder enzymes*. Their use may be revived, but it will take a long time to overcome the negative image in the United States. (6) *Computer-controlled fermentations*. Still to be exploited. (7) *Mixed culture fermentations*. Here is a real challenge not very successfully met on either laboratory or pilot plant scale to date. These are only a few of the fermentation innovations that have had some evaluation. I think most will merit further study.

I regret to conclude that in nearly all of our currently operated fermentations, we are partially or totally ignorant of many parameters which are controlling the fermentation. These are listed in Table 3. I think fermentation microbiologists have had experience with one or more of these, and realize that their effects often interact to such an extent that it is not easy to isolate one at a time. This is no reason to neglect them completely! On the other hand, is it possible to study these parameters in groups? This may show their relative importance.

If the fermentation industries are to continue to expand (we now have some dozen processes where more than 50 million pounds of product are produced each year), new manufacturing sites have to be located. I listed in Table 1 a few companies where plant expansion commitments have been announced. Among the current problems to be considered in siting new facilities are those listed in Table 4. It is a formidable list, and making the decisions may be a very difficult assignment. Perhaps this is why these decisions are so often postponed. Even the decision on fermentor fabrication can be

TABLE 3. *Insufficiently explored parameters*

| |
|---|
| Absolute nutritional requirements |
| Use of selective inhibitors |
| Optimal dissolved oxygen levels |
| Optimal dissolved carbon dioxide levels |
| Viscosity |
| Optimal temperature profile |
| Optimal pH profile |
| Optimal sterilization procedure |

TABLE 4. *Current/future problems in siting fermentation manufacturing facilities*

| |
|---|
| Transportation: raw materials; finished products |
| Water: quality; quantity |
| Power: continuous/intermittent |
| Disposal: waste fermentation solids; solvents |
| Labor: pool of skilled and semi-skilled workers |
| Environmental impact: water table; odor control; waste product disposal (marketable) |
| Tax privileges |
| Potential nationalization |

debated at length. The industries started with wooden fermentors (cypress was preferred) and then shifted to iron. Aluminum was used for constructing some tanks, and some were made of iron with plastic or glass linings. We seem to have "standardized" on stainless steel, although I doubt that such expense is needed for all fermentations. If we build a facility in the next decade, should we consider concrete vessels, especially if the fermentation does not have to be operated under 100% aseptic conditions?

One problem that results from a decision concerning fabrication materials is that the more expensive the initial equipment, the more likely management will be reluctant to have a single purpose facility, and consider replacement before 15 to 20 yr use. This "fixes" many of the fermentation parameters and I suspect limits fermentation performance.

Nevertheless, I foresee a few major changes in the next 20 yr. I think we can expect some new fermentor designs, partly because we are more knowledgeable now concerning the microbial requirements, and partly due to the economic pressure (to reduce power costs, etc.). I think we can also expect some new uses for fermentation products, especially in the agricultural and food industries, and this should lead in time to some new fermentation processes being operated on a large scale. Exploitation of microbial genetics is just beginning, and new fermentation substrates are being sought.

Unfortunately, a major question for fermentation expansion is "Where are we going to find the trained personnel?" Our schools are graduating fewer and fewer qualified persons to undertake many of the research and development assignments mentioned earlier. We have considered having nondegree programs (set up especially for those now in industry) to supply the academic and practical background. These are not easily arranged, and if the academic institution cannot obtain some sort of guaranteed enrollment, there is no incentive to have such programs arranged.

The alternative of having special short courses has not been altogether satisfactory. The program at Massachusetts Institute of Technology, now in its 20th yr, has been the leader in the field. We have had 4 yr experience at the University of Wisconsin, and the Center for Professional Advancement in New Jersey has had about the same experience as we have. Short courses are of limited value to the person who has very minimal understanding of the field, and often are of limited value to those who have too much knowledge. The net result is a major reliance on publications.

We find that in recent years, especially the past 2 yr, there has been a marked increase in the publication of texts, proceedings of symposia, and journals dealing with fermentation topics. The Wang et al. (1978) volume is a partial distillation of the lectures given in the M.I.T. course. The second edition of *Microbial Technology* (Peppler and

Perlman 1979) covers the major fermentations, some economic parameters, and some possibilities for the future. The same ground is being covered more extensively by Rose (1977) where, in six volumes of *Economic Microbiology* he is evaluating the past, present, and future of industrial fermentations.

I also must mention the increase in journals dealing with fermentation topics, and the many symposia whose published proceedings have been helpful to those unable to attend the sessions. If the professional has some of these resources available and consults them, then I think attendance at scientific meetings and short courses will be immeasurably more valuable.

In this lecture I have tried to assess the "current state of the art," and project some ideas about the future. I am confident we will be able to meet some of the challenges easily and can handle the more difficult ones in due time. Of course, we must have confidence in our profession and our partners. The "laws of applied microbiology" (Table 5) must be observed for success.

TABLE 5. *The laws of applied microbiology for 1979*

1. The microorganism is { always right / your friend / a sensitive partner }
2. There are *no* stupid microorganisms
3. Microorganisms { can / will } do anything
4. Microorganisms are { smarter / wiser / more energetic } than { chemists, / engineers, etc. }
5. If you take care of your microbial friends, they will take care of your future (and you will live happily ever after)

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