總 說

Biochemical Engineering*-Trends and Recent Advances: Part (I) Fermentation Engineering

Doo Young Ryu**

Department of Biological Engineering,
Korea Advanced Institute of Science,
Seoul, Korea

Introduction and Scope of the Field

Biochemical Engineering may be defined as an engineering discipline that is concerned with the economic processing of materials that are of biological origin and that are processed and designed for biological application to serve useful purpose for the benefit of mankind.

Those materials of biological origin include: (1) Biological material itself (e. g. microbial cells, single cell protein, plant cells, animal tissue, viruses etc), (2) Cellular components (e. g. nucleic acids, proteins, enzymes, and extracts of other cellular components), and (3) Metabolic products (e. g. primary and secondary metabolites including antibiotics, vitamins, organic acids, alcohols, etc.) The materials that are processed and/or designed for biological application include: (1) Varieties of special pharmaceutical compounds (e. g. extracts and isolates of varieties of natural products that are of medical and clinical importance), (2) Artificial organs and devices of medical importance (e. g. artificial heart-lung machine, artificial kindney, automated diagnostic system, public health monitoring system, automated patient-aid systems and devices, assist devices for the crippled, etc.), and (3) Special type of formulation and preparation of food materials (e. g. synthetic protein and carbohydrate, etc.). The materials that are processed by such biological materials as enzymes include many compounds of biological and medical importance (e. g. steroid hormones and other compounds produced by bioconversions).

Because of the complex nature of the bioengineering field, the recent trend is to develop and specialize in one of the several subspecialty fields that could be related to and identified with industrial application or to the career-oriented specialization. These application-oriented sub-specialty fields include: fermentation engineering, enzyme engineering, health science and biomedical engineering, certain areas of pharmaceutical engineering, unconventional areas of food engineering, environmental engineering, etc.

Among these, only a few specific topics of timely interests and of special importance to future scientific and technological development are selected and their recent trends and advances will be discussed in a series. In this issue, as the first part of the series, discussion will be focussed on the Training of Bioengineers and on Fermentation Engineering. In the subsequent issues Enzyme Engineering, Single Cell Protein-microbial protein as a new food resource, and other topics of timely importance will be discussed.

Training of Bioengineers

Bioengineering field has been developed as an int-

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**柳 斗 榮 韓国科学院 生物工学科

erdisciplinary program by necessity, and the training of bioengineers require coverage of a broad range of academic disciplines including both life science and engineering science. The bioengineering program at many universities throughout the world are organized as an interdisciplinary and/or interdepartmental program and require concerted effort of several departments of academic disciplines.

The extent and range of the joint efforts of different disciplines include primarily chemical science and engineering, biological science, medical science especially human physiology, food science and engineering, mechanical engineering, and electrical engineering. A well-cordinated and well-organized bioengineering program have the primary objectives of enhancing the benefit of mankind through the contribution of trained bioengineers. This simple concept of the purpose of activities related to the bioengineering is only the culmination of long-standing man's endeavor toward the human welfare and well-being. This concept may be viewed in terms of the relationship between man and his environment and man's activities in the midst of man's biosphere. This relationship is illustrated schematically in *Fig.* 1.

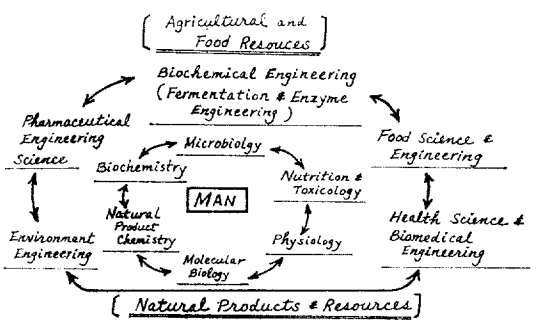

Fig. 1. Man and biosphere (bioengineer's view)

Man interact with his environment as part of the biosphere system. While maintaining nature's delicate balance of biosphere, man attempts to derive maximum benefit from the natural resources available to him from the biosphere system. In oder to achieve this, one must have a good understanding of basic sciences (i.e. nutritional chemistry, toxicology, microbiology, physiology, biochemistry, natural product chemistry, etc.). In addition, adequate training in bioengineering (biochemical engineering including fermentation and enzyme engineering, health science and biomedical engineering, food engineering, pharmaceutical engineering, environmental engineering, etc.) enables one to apply the basic knowledge to transforming or processing the natural and agricultural products to more useful and valuable products for human need and consumption. This kind of presentation of the relationship between the man and his activities as well as his natural environment in his biosphere may be considered as the bioengineer's view of biosphere.

A selected list of only several academic institutions where bioengineering programs are offered is given in *Table* 1. There are many other academic and research organizations where they have modest size of bioengineering programs throughout the world. The academic training of bioengineers is very often not an easy task because of the broad nature of the

Table 1. Academic institutions with biochemical engineering program (selected list)

1. Massachusetts Institute of Technology
2. University of Pennsylvania
3. Cornell University
4. University of Wisconsin
5. University of Minnesota
6. University of California (Berkeley)
7. Columbia University
8. Rutgers University
9. Rice University
10. Texas A & M University
11. Case-Western
12. Carnegle-Mellon
13. Pennsylvania State
14. University College London (England)
15. Waterloo University (Canada)
16. Tokyo University (Japan)
17. Osaka University (Japan)
18. Eidgenossische Technische Hochschule (Switzerland)
19. University of Sao Paulo (Brazil)
20. Indian Institute of Technology (India)
21. Korea Advanced Institute of Science

scope of the field which they have to be trained in. In *Table* 2 an example of the field coverage of bioengineering programs is shown. Training one person to become a multipurpose expert is a difficult task, indeed. For this reason, the specialization in one of the application oriented bioengineering field has become a necessity as indicated earlier. Bioengineering programs in most institutions are offered only as a graduate program, but there are a few exceptions where they offer it as an undergraduate program as well.

Table 2. The field coverage for bioengineering programs

Physical Science & Engineering	Life Science
Chemical Engineering Science	Molecular Biology
Mechanical Science	Nutritional Biochemistry
Electrical Engineering	Toxicology
Instrumentation	Biophysics
Process Control (Computer)	Enzymology
Process Design	Immunology
Advanced Mathematics	Cell Biology
Physical Chemistry	Human Physiology
Physics	Biological Chemistry
Organic Chemistry	Microbial Physiology
Analytical Chemistry	Industrial Microbiology
Food Engineering	Genetics
Environmental Science	Natural Product Chemistry

Fermentation Engineering

Fermentation has been an old folk-art for many years throughout the world. To many people, fermentation processes are considered still an art rather than science because we have not yet mastered how to control the biological process or the metabolism of living organisms that are involved in the fermentation processes. Certainly, the recent advances in molecular biology have tremendously helped us broaden our understanding of life process in living organisms, but we have to go a long way still, indeed, before we will be able to control the fermentation process through the regulation of microbial metabolism.

However, some significant progress has been made recently and we are going through a transition period where fermentation art is being converted to fermentation science and engineering. We also find growing number of fermentation products that are of special economic importance. Examples of these products are antibiotics, steroids, amino acids, organic acids, nucleic acids, vitamins, enzymes, fermented beverages and foods, many fine chemicals, etc. Recent survey shows the extent of industrial involvement in fermentation business and the broad range of product lines. This survey of important fermentation product and industries are summarized in *Tables* 3, 4, 5, 6, 7 & 8.

U. S. has been very active in fermentation field and it has enjoyed a large share of world-wide market of antibiotics and her share of antibiotics market alone is estimated to be multibillion dollars annually. Japan has also been active in fermentation especially in

Table 3.

Antibiotics	(estimated sale: more than $10,000,000,000/year)
Amphotericin B	77, 78
Bacitracin	5, 6, 20, 30, 53, 56, 57, 61
Blastocidin S	38, 50
Capreomycin	22, 44
Cellocidin	38, 50
Cephalosporins	16, 27, 29, 44, 62, 80
Colistin	9, 40
Cyclohexamide	38, 81, 84
Cycloserine	20, 25
Daunomycin	25, 66
Erythromycin	1, 22, 44, 60, 68, 75
Fumagillin	1, 17
Gramicidin S	7, 46, 51
Gramicidin A	56, 85
Griseofulvin	29, 36, 52, 80
Hygromycin B	14, 44, 80
Josamycin	69, 87
Kanamycins	9, 16, 46, 51, 60, 66
Kasugamycin	9, 38, 52, 69
Leucomycin	82
Neomycin	14, 52, 56, 57, 60, 66, 68, 77, 78, 80, 84

Nystatin	17, 43, 60, 77, 78
Oleandomycin	14, 57, 58, 60
Penicillin G	7, 9, 11, 12, 13, 14, 23, 25, 27, 28, 29, 31, 32, 33, 35, 44, 46, 48, 52. 53, 57, 58, 66, 68, 77, 82, 84, 86
Penicillin V	1, 7, 9, 13, 14, 16, 22, 25, 27, 28, 31, 46, 52, 53, 57, 58, 66, 77, 78, 82, 86
Pencillin O	84
Penicillins (semisynthetic)	5, 9, 11, 12, 16, 25, 27, 28, 46, 57, 58, 62, 65, 77, 78, 80, 82, 86
Polymycins	22, 57
Rifamycin	31
Sarkomycin	9, 46
Spiramycin	42, 66
Streptomycin	23, 25, 28, 29, 33, 38, 43, 46, 48, 53, 57, 65, 66, 78, 86
Tetracylines	4, 7, 9, 16, 24, 25, 31, 33, 35, 35, 46, 53, 57, 58, 62, 64, 66, 68, 76, 78, 80
Chloro-	4, 25, 32, 35, 60, 62, 64, 65, 66, 76, 80
Dimethylchloro-	4, 25, 62, 66, 80
Oxy-	14, 28, 36, 57, 58, 60, 62. 64
Trichomycin	27, 60
Tylosin	22, 44
Tyrothricin	13. 56, 85
Viomycin	55, 57, 58
Tobramycin	
Flavomycin	
Gentamycin(derivatives)	

Table 4. Enzymes

Amylases	14, 18, 28, 30, 47, 53, 61. 63, 67, 79, 85
Amyloglucosidasee	18, 19, 29, 30, 47, 53, 85
Catalase	47, 74
Cellulase	7, 28, 47, 53, 67, 85
Glucose isomerase	18, 53
Glucose oxidase	47, 74
Hemi-cellulase	47, 53, 67
Invertase	28, 53, 74, 79, 83, 85
Lactase	21, 28, 47, 85
Lipase	47, 67, 85
Pectinase	47, 53, 67, 85
Pentosanase	67
Proteases	7, 14, 17, 28, 30, 38, 47, 53, 61, 67, 80, 85
Rennet	47, 53, 57

amino acids, and its market share is at the level of 400 million dollars annually for the amino acid catagory alone.

Table 5. Vitamins, growth factors, nucleotides, & nucleosides

Giberellic acid	1, 36, 42, 44, 48, 80
Pantoic acid	80
Riboflavin	20, 22, 30, 48, 57
5′-Ribonucleotides & nucleosides	3, 80
Vitamin B_{12}	29, 30, 48, 60, 66, 68, 87
Zearalenol	20

Table 6. Steroid hormone products (estimated sale: $1,000,000,000/year)

Fluorocinolone
Triamcinolone
Dexamethasone
Prednisolone
Hydrocortisone

Table 7. Organic acids & solvents

Amino acids	
Aspartate	3, 24, 42
Glutamate	2, 3, 6, 20, 42, 48, 54, 69, 80
Isoleucine	3, 42
Lysine	3, 42, 48, 66
Valine	3, 42
Other a. a.	3, 42
Citric acid	47, 57, 58, 66
Gluconic acid	24, 27, 30, 57, 61, 74
Itaconic acid	57, 66
2-Ketogluconic acid	27, 48, 66, 85
Kojic acid	66
Lactic acid	15, 18, 27, 66, 80
2, 3-Butanediol	28
Ethanol	28, 30, 42, 63, 80

Table 8. Other fermented products

Alcoholic beverages	(Ubiquitous)
Fermented foods	Dairy products (cheese, yogurt) Fermented grain products (miso, shoyu, natto, sake) Fermented vegetables (pickles, cabages, etc.) (Ubiquitous)

Acyloin	48
Dihydroxyacetone	85
Dextran	7, 14, 57, 80
Sorbitol oxidations	34, 48, 57, 80
Xanthan	48, 66
Bacterial insecticide	1, 37
Polysaccharides	

Table 9. Index to fermentation industries

1. Abbott Laboratories, North Chicago, Illinois
2. Ac'cent International, San Jose, California
3. Ajinomoto Company, Tokyo, Japan
4. American Cyanamid, Wayne, New Jersey
5. Apothekernes Lab fur Specialpraeparater A/S, Oslo, Norway
6. Asahi Chemical Industry, Osaka, Japan
7. Aktiebolaget Astra, Sodertalje, Sweden
8. Ayerst Laboratories, New York, New York
9. Banyu Pharmaceutical Company, Tokyo
10. Bayer AG, Leverkusen, West Germany
11. Beecham Research Laboratories, Brentford, England
12. Beecham Inc., Clifton, New Jersey
13. Biochemie Kundl GmbH, Kundl, Austria
14. Biogal, Debrecen, Hungary
15. Bowmans Chemicals, London, England
16. Bristol Laboratories, Syracuse, New York
17. Chinoin, Budapest, Hungary
18. Clinton Corn Processing Company, Clinton, Iowa
19. CPC International Inc., Argo. Illinois
20. Commercial Solvents Corporation, Terre Haute, Indiana
21. Dairyland Food Industries, Inc., Waukesha, Wisconsin
22. Dista Products, Ltd., Liverpool, England
23. Dumex A/S, Kobenhavn, Denmark
24. Carlos Erba, Milano, Italy
25. Farmitalia S. p. A., Milano, Italy
26. Fermentfarma S. p. A., Milano, Italy
27. Fugisawa Pharmaceutical Company, Osaka, Japan
28. Gist-Brocades n. v., Delft, Netherlands
29. Glaxo Laboratories Ltd., Greenford, England
30. Grain Processing Corporation, Muscatine, Iowa
31. Gruppo Lepetit, Milano, Italy
32. Hindustan Antibiotics Ltd., Pimpri, India
33. Hoechst A. G., Frankfurt(Main), West Germany
34. Hoffmann-LaRoche Inc., Nutley, New Jersey
35. Icar S. p. A., Roma, Italy
36. Imperial Chemical Industries Ltd., Manchester, England
37. International Minerals and Chemicals Corporation, Libertyville, Illinois
38. Kaken Chemical Company, Tokyo, Japan
39. Kanegafuchi Chemical Company, Osaka, Japan
40. Kayaku Antibiotics Research Company, Tokyo, Japan
41. Kowa Company, Nagoya, Japan
42. Kyowa Hakko Kogyo Company, Tokyo, Japan
43. Leo Pharmaceutical Products, Ballerup, Denmark
44. Eli Lilly and Company, Indianapolis, Indiana
45. H. Lundbeck and Company, Valby, Denmark
46. Meiji Seika Kaisha Ltd., Tokyo, Japan
47. Miles Laboratories Inc., Elkhart, Indiana
48. Merck and Co. Inc., Rahway, New Jersey
49. Musashino Chemical Laboratories, Tokyo, Japan
50. Nihon Nohyaku Company, Tokyo, Japan
51. Nikken Chemicals Compay, Ltd., Tokyo, Japan
52. Nippon Kayaku Company, Tokyo, Japan
53. Novo Industri A/S, Kobenhavn, Denmark
54. Orsan S. A., Paris, France
55. Parke Davis and Company, Detroit, Michigan
56. S. B. Penick and Company, Orange, New Jersey
57. Chas. Pfizer and Company, New York, New York
58. Pfizer International, New York, New York
59. Pharmacosmos, Viby, Denmark
60. Pierrel S. p. A., Milano, Italy
61. Premier Malt Products, Milwaukee, Wisconsin
62. Proter S. p. A., Opera, Italy
63. Publicker Industries, Inc. Philadelphia, Pennsylvania
64. Rachelle Laboratories, Inc., Long Beach, California
65. Recherche Industrie Therapeutique, Genval, Belgium
66. Rhone-Poulenc, Paris, France
67. Rehm and Haas, Philadelphia, Pennsylvania
68. Roussel UCLAF, Romainville, France
69. Sanraku Ocean, Tokyo, Japan
70. Sankuo Company, Ltd., Tokyo, Japan
71. Sarbhai Chemicals, Madrad, India
72. Schering AG., Berlin, West Germany
73. Schering Corporation, Bloomfield, New Jersey
74. Searle Biochemics, Arlington Heights, Illinois
75. Shionogi and Company, Ltd., Osaka, Japan
76. Societa Prodotti Antibiotici, Milano, Italy
77. E. R. Sqibb and Sons, Princeton, New Jersey
78. Squibb International, New York, New York
79. Standard Brands, Inc., Stamford, Connecticut

80. Takeda Chemical Industries, Ltd., Osaka, Japan
81. Tanabe Seiyaku Company, Ltd., Osaka, Japan
82. Toyo Jyozo, Ohito, Tagata-gun, Shizuoka-ken, Japan
83. Universal Foods Corporation, Milwaukee, Wisconsin
84. Upjohn Company, Kalamazoo, Michigan
85. Wallerstein Laboratories, Deerfield, Illinois
86. Wyeth Laboratories, Philadelphia, Pennsylvania
87. Yamanouchi Pharmaceutical Company, Tokyo, Japan

Fermentation engineering is an engineering discipline that is based primarily on molecular biology, microbiology, and chemical engineering. Large scale industrial application of microorganisms require good knowledge of not only the microbiological aspect of the process but also the chemical engineering principles of the fermentation operation.

When one considers the fermentation engineering as an academic discipline and/or industrial operation he has to take into consideration of several important aspects involved. Partial list of these may include:

(1) Cellular growth, structure, and function, (2) Cell's interaction with its environment, (3) Cellular physiology, (4) Cell's metabolic regulation and methods of oversynthesis and overproduction (5) Kinetics of growth and product formation, (6) Theory and methodology of continuous culture technique, (7) Sterilization of air and media, (8) Oxygen mass transfer operation, (9) Process control and intrumentation, (10) Biological separation technique and product recovery, (11) Scale-up techniques, (12) Fermentation plant design problems, and (13) Special topics (Including problems related to specific products and processes, primary metabolites, secondary metabolises, enzymes, organic acids, nucleic acids, amino acids, viral vaccines, bioconversion products, etc.).

Among these varieties of the topics our attention will be focussed on only a few specific areas. The most important recent advances in fermentation are in the following areas:

1) Better understanding of microbial regulatory mechanisms: By exploiting the metabolic regulatory mechanisms the productivity may be increased by a large factor.

2) Better understanding of oxygen mass transfer phenomena: Based on the better understanding of quantitative physiology and oxygen mass transfer phenomena, an optimal process control may be designed and implemented for an improved fermentation productivity.

1) Exploitation of Metabolic Regulation

We can achieve very high productivity of fermentation processes by eliminating or circumventing normal metabolic regulation in the microorganisms. Manipulation and exploitation of regulatory mechanisms may be accomplished through genetic mutation and control of cellular environment.

Regulatory mechnisms in living cells have evolved over the course of many years in order that cells may be able to regulate their metabolic rate, to maintain cellular economy, and to adapt themselves to the changes in their environmental conditions. These control mechanisms include induction, inhibition, repression, feedback inhibition and repression, catabolite repression, etc. These regulatory mechanisms are essential to the living cells if the living system containing hundreds of enzymes is to function effectively in a well-coordinated manner. Relatively short doubling time of microorganisms, order of one hour, is manifestation of the effectiveness and very good coordination of such regulatory mechanisms in living microorganisms.

Yet, we endeavor to eliminate or bypass such metabolic regulation of microorganisms to increase primary and secondary metabolites of medical, nutritional and industrial importance. Thus, bioengineers continue to battle against the control mechanisms of the cells and try to outsmart microorganisms.

Biosyntheses of primary metabolites such as amino acids, nucleotides, and vitamins are controlled by feedback inhibition or feedback repression. Overproduction of these metabolites can be accomplished by two methods: (1) By decreasing the concentration of inhibitory or repressive end-product and (2) By mutation of the enzyme system that will be less sensitive to the feedback regulation effect.

The first methods is based on the isolation of an auxotrophic mutant in the desired pathway and feeding low concentration of the required nutrient. Arginine auxotrophs produce as much as 16 gram/liter of citrulline or 26 gram/liter of ornithine when the feeding of arginine is restrictrd. Adenine auxotrophs can produce 13 gram/liter of inosinic acid, and guanine auxotrophs can accumulate as much as 6 gram/liter of xanthylic acid. Inosinic acid and xanthylic acid are flavor enhancers.

The organisms used for production of glutamic acid is deficient in α-ketoglutarate dehydrogenase and blocking of this enzyme in the tricarboxylic acid cycle ensures the shunting of carbon source to glutamate. The broth concentration of glutamate produced by certain mutant strains reach as high as 60 gram/liter. End-product of branched pathways are produced in a similar manner. When a mutants requiring threonine and methionine are fed at low levels of these amino acid, the feedback inhibition of third amino acid, namely, lysine is bypassed and this mutant produces as much as 42 grams/liter of lysine. The aspartokinase which is the first enzyme in this pathway and it can be inhibited by the concerted feedback mechanism by a combined effect of both threonine and lysine.

The second method is to isolate a mutant that is resistant to toxic analogues of the desired endproduct. We can find resistant mutants that is inhibition resistnt due to an alteration in the structure of a feedback inhibited enzyme. We can also find resistant mutants that is repression resistant due to an alteration in the enzyme forminig system. Some mutants resistant to an analogue of leucine, trifluoroleucine, can overproduce leucine. Similarly, the productivity of riboflavin (Vitamin B_2) and cyanocobalamin (Vitamin B_{12} derivative) can be increased to 5 gram/liter and 30 mg/liter level respectively.

Production of secondary metabolites such as antibiotics are also controlled by the regulatory mechanisms. Certain enzymes involved in biosynthesis of secondary metabolites are controlled by catabolite regulation as demonstrated by the inhibition of the synthesis of many antibiotics by glucose. Phenoxazinone synthetase, an obligatory enzyme of actinomycin formation, is subject to catabolite repression.

Feedback regulation also affect secondary metabolism as illustrated for penicillin by lysine and for cephalosporin by methionine. Derepression of enzymes involved in the biosynthesis of antibiotics has also been demonstrated with certain key enzymes. These are amidinotransferase for streptomycin biosynthesis, acyltransferase for penicillin biosynthesis, and phenoxazinone synthetase for actinomycin biosynthesis.

Production of microbial enzymes is also controlled by the regulatory mechanisms. The enzyme contents of cells may vary by a large factor as a result of genetic and environmental changes. The activity of biosynthetic enzymes in a given living organism can be varied over a range of hundred-fold while catabolic enzymic activity can be varied several thousand-fold.

The methods of increasing enzymatic activity include: (a) addition of inducers, (b) decreasing the concentration of feedback repressors and catabolite repressors, (c) mutation to constitutivity by eliminating the requirement for inducer, (d) increasing gene copies by means of episome transfer or transduction.

These approaches to over production of enzymes should be of great importance to industrial microorgauisms tdat are used for fermentations, since the yield of desired product that can be synthesized bʎ the enzyme would be proportional to the concentration of the same enzyme in the biosynthetic system.

2) Oxygen mass Transfer and Process Control Based on Quantitative Physiology

As the demand for fermentation products increases, those products, namely, (antibiotics, steroid hormones, enzymes, biologicals, etc.) considered heretofore as the "specialty fine chemicals" are gradually becoming the bulk chemicals. This trend combined with the increasing demand for the development of fermentation processes for production of more complex and valuable metabolic products is an important reason for the development of more sophisticated

fermentation process technology. In fact, the computer-aided optimal process control of fermentation processes is rapidly becoming an economic necessity. So far, however, the fermentation industry has not been able to take full advantage of the technology of process control and automation, in large part, due to the lack of good understanding of the complex and intricate nature of biological system. At best, the heuristic method of process control has been applied with a modest degree of success.

There is an urgent need for a development of sound mathematical models and the process control logic that are solidly based on a good understanding of biological and physiological properties of microbial cells used in the fermentation system. Once such models for simulation are developed, the productivity of fermentation processes can be optimized and eventually the automatic process control based on the feedback control can be implemented.

We have recently begun a study of penicillin fermentation system as a model system as part of our effort to further our understanding of microbial physiology with an ultimate objective of optimizing the penicillin process. As an example, the results and findings of our preliminary study will be discussed. We will also suggest further work to be pursued and a practical method by which the fermentation process can be optimally controlled with the aid of a computer system.

The approach of quantitative physiology may be briefly described as follows. The most important state variables (variables that describe the state and condition of microbial cells in the fermentation system) and the control variables (operational process variables that would have to be controlled) must be identified and selected through experimental work or based on theoretical evaluation. The objective function that is either the productivity function or the cost function must be well defined.

The optimization becomes a mini-max problem, that is, minimization of the production cost and/or maximization of productivity. The objective function is related to the state function. The state function includes both the state and/or control variables. These state and control variables belong to one or more of the following three distinct groups, namely, (1) concentrations or potentials, (2) rates, and (3) the specific rates. The variables that belong to any one of these three groups could be biological, chemical, or physical parameters. The special importance of the parameter, specific rate, should be noted. The specific rates reflect the biological and physiological condition including the productivity of the cells, since it is defined as the rate of production or the metabolic rate per unit amount of cells.

For example, a possible simulation model may take a functional form of

$$P_{ijk}=f(C_i,\ R_j,\ S_k,\ t) \tag{1}$$

where P represents the productivitiy function, C_i the concentration function, R_j the rate function, S_k the specific rate function, t the time, and i, j, k, the subscripts of those variables. Each subscripted variable represents a set of variables in each group. We may have n_1, n_2, and n_3 number of variables for the subscripts i, j, and k respectively.

The concentration, C_i, is basically a function of time, since it changes with time. In most cases the concentration is also function of the rate function, R_j, and the specific rate function, S_k, due to their interactions.

$$C_i=f(t, R_j, S_k) \tag{2}$$

The rate equation includes the parameters for material flow in and out of the system, M_j, and the metabolic rates of microorganisms. By taking into consideration of the material balance, the metabolic rates, and the specific rates, the functionality of the rate equation may be written as

$$R_j=f(M_j, R_j, S_k) \tag{3}$$

Because of the complexity involved in the fermentation process, this kind of work will have to be carried out in part with the aid of a computer. The functional relationship between the productivity function and the state or control variables must be deter-

mined quantitatively from experimental work.

One of the most important parameters that we have studied recently, for instance, was the specific rate of penicillin production, Q_p. Q_p is then one of the variables that belong to the group of variables, S_k.

$$S_k=f(Q_p, \ldots\ldots) \quad (4)$$
or $S_i=f(Q_p,\ t)$

The fermentation process being the biological process, the functionality among these variables becomes very complex when the interactions among these variables are taken into consideration. In large part, the interactions are the results of biological response of microorganisms as an effect of cellular environment of physical and/or chemical nature. Thus, as an example, the functionality of Q_p may be expressed as,

$$Q_p=f(\mu,\ x,\ Q_o,\ Q_c, \ldots) \quad (5)$$

where μ represents the specific growth rate of microorganism, x the cell concentration, Q_o the specific uptake rate of oxygen, and Q_c the specific rate of carbohydrate assimilation.

The equation (5) indicates that the specific rate of antibiotics production, Q_p, is related to at least four parameters, namely, μ, x, Q_o, and Q_c. The effects of these parameters on the specific rate of production of antibiotics, Q_p, have been experimentally studied and

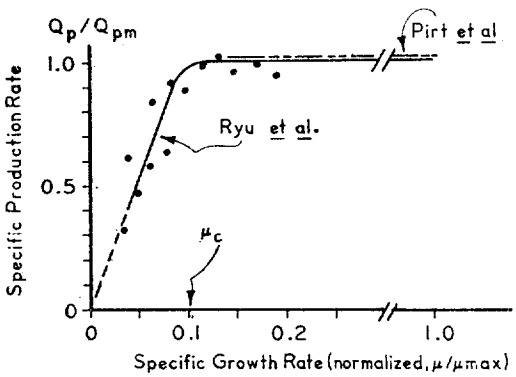

Fig. 2. Specific production rate (normalized, Q_p/Q_{pm}) and specific growth rate

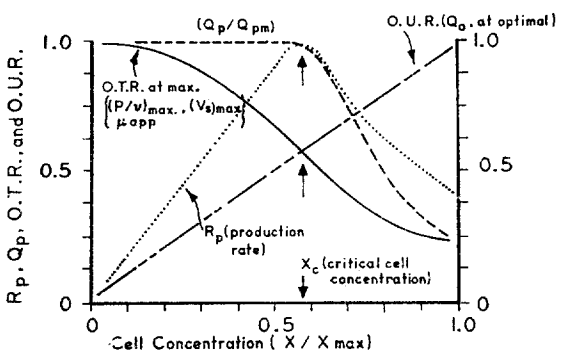

Fig. 3. Relationship between antibiotic production rate (R_p) and Q_p, O.T.R, O.U.R., and X(All values are normalized with respect to their maximum values)

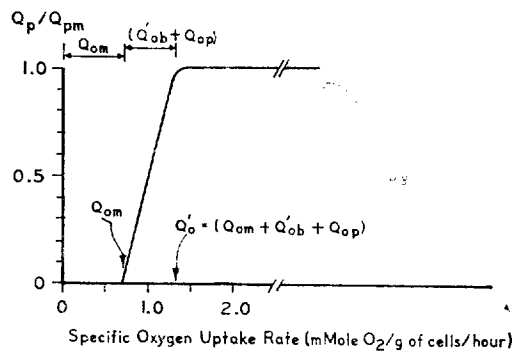

Fig. 4. Specific production rate (Q_p/Q_{pm}, as normalized value) and specific oxygen-uptake rate

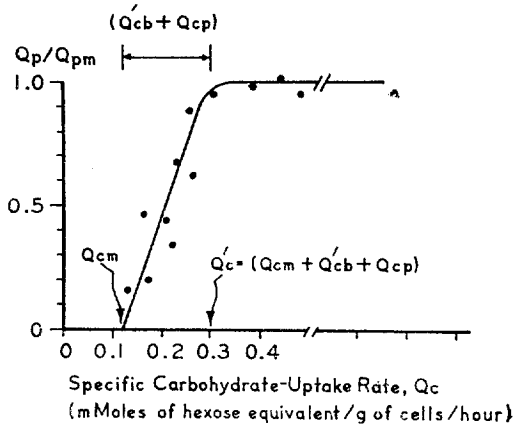

Fig. 5. Specific production rate (normalized, Q_p/Q_{pm}) and specific carbohydrate-uptake rate, Q_c

the results are illustrated in *Figures* 2, 3, 4 and 5.

The effect of μ, Q_o, and Q_c, on the specific rate of production may be represented by a functional form of the type,

$$Q_p = \frac{Q_{pm}\,\alpha_i}{K_i + \alpha_i} \tag{6}$$

where α_1 represents the specific growth rate, μ, α_2 the specific oxygen uptake rate or $(Q_o - Q_{om})$, α_3 the specific carbohydrate uptake rate of $(Q_c - Q_{cm})$, and functions also require the conditions;

$$\begin{aligned} &Q_o \geq Q_{om} \quad \text{and} \\ &Q_c \geq Q_{cm} \end{aligned} \tag{7}$$

otherwise,

$$Q_p = 0 \text{ if } Q_o < Q_{om} \text{ and } Q_c \leq Q_{cm} \tag{8}$$

These functional relationship can be incorporated into the simulation models of the process. These empirical correlations and the simulation models are useful in estimating the effects of changes in the μ, Q_o, and Q_c on the productivity either as control variables or estimates of deviation from the optimal productivity. In practice, however, the set-point control might be most useful, namely, the optimal values of μ_s, Q'_o and Q'_c can be used as set-points and these variables are controlled in such a way that the following conditions are satisfied. These optimal values correspond to the maximum specific productivity, Q_{pm}.

$$\begin{aligned} &\mu \geq \mu_s \\ &Q_o \geq Q'_o \\ &Q_c \geq Q'_c \end{aligned} \tag{9}$$

In considering cell concentration x, a set-point control may also be used in such a way that the condition in Equation (10) is satisfied,

$$(X_c - \Delta X) \leq X \leq (X_c + \Delta X) \tag{10}$$

since the maximum production rate can be achieved in the range of x as specified by the Equation (10).

The complexity of fermentation system is multiplied not only by the interactions among the state and control variables but also by the dynamic nature of biological systems. The fermentation system is highly non-linear and it is a dynamic system. Certain linearization technique will have to be selected and used.

Table 10. Process Variables in fermentation processes

Temperature	pH	Respiratory quotient
Pressure	Oxidation reduction	Cell concentration
Agitation speed	Dissolved oxygen	Cellular components;
Power input	Dissolved CO_2	Protein (Enzymes)
Air flow rate	Effluent gas oxygen	DNA
Feed Rate of;	Effluent CO_2	RNA
Nutrients	Concentrations of;	Specific Activity of enzyme
Precursors	Carbohydrate	Specific rates of;
Inducers	Nitrogen	Product Formation
Weight	Mineral ions	
Volume of liquid	Precursor	Growth
Viscosity (Apparent)	Inducer	Oxygen uptake
Cumulative amount of;	Product	Nutrient uptake
Acid		Oxygen transfer rate
Base	Metabolites	ATP
Antifoam	Flow characteristics*	NADH
	Power characteristics*	
	Energy Balance*	

Because the complexity of the fermentation system is enormous and it will be an insurmountable task to study the biological system completely, we have to select several key parameters that are considered most important to the productivity and optimize the process with respect to those key parameters. The parameter optimization may then be extended to a larger number of state and/or control variables in order to optimize the process. One and each parameter optimization may be considered as a unit of a building block for a large control system of the fermentation process. We will have to examine and optimize other parameters in the order of importance and priorities. A typical list of the parameters that are considered very important in fermentation is shown in *Table* 10.

Once the process control logic for the entire process is developed, the computer-aided process control can be implemented with an adequate interfacing of the fermentation facilities. A lay-out of such an interfacing of fermentor-computer system is also shown in *Figure* 6. The optimal process control logic so developed will have to be tested experimentally using a specific fermentation process as a model system, and the applicability of the optimal process control will have to be demonstrated.

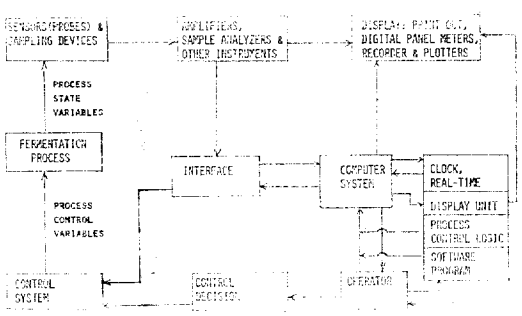

Fig. 6. Process lay-out for fermentor-computer system

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