A Course in

BIOCHEMICAL ENGINEERING FUNDAMENTALS (revisited)

J. E. BAILEY
California Institute of Technology
Pasadena, CA 91125

D. F. OLLIS
North Carolina State University
Raleigh, NC 27695-7905

BIOCHEMICAL ENGINEERING CONCERNS the reactors and separation systems associated with microbial cells, their enzymes and other products, and with plant and animal cells which can be propagated in an appropriate reactor outside of the whole plant or animal. In 1976, we reported on a biochemical engineering course [1] with an outline which appeared in 1977 as a textbook, *Biochemical Engineering Fundamentals*.

Spectacular changes have occured in this domain over the last ten years. Many of these changes concern methods for fusing two cells together, to produce a *hybrid* cell, and for "cutting and pasting" strands of genetic material (DNA) together to form *recombinant DNA* (rDNA) coding for a desired set of instructions. With the commercialization of processes using these two "new" biotechnology techniques, a number of new words have appeared in the biological and bioprocess literature. For the biochemical engineering teacher and practitioner, the most central vocabulary includes the following [2]

genetic engineering: technologies used at the laboratory level to alter the hereditary apparatus of the living cell so that the cell can produce more or different chemicals, or perform completely new functions.

DNA: the genetic material found in all living organisms.

clone: a group of genetically identical cells or organisms produced asexually from a common ancestor.

cloning: the amplification of segments of DNA, usually genes.

rDNA (recombinant DNA): the hybrid DNA produced by (enzymatically and extracellularly) joining together pieces of DNA from different sources.

(DNA) **vector:** a self-replicating DNA molecule (plasmid, virus) that transfers a piece of DNA from one cell host to another.

transformation: the transfer of genetic information into a cell using DNA separated from the cell as a vector.

plasmid: circular, self-replicating non-chromosomal DNA. (Because the plasmid is small [vs. the major chromosomal DNA] it is a useful "shuttle" or vector for moving rDNA into a new cell.)

virus: an infectious agent that requires a host cell in order for it to replicate. It is composed of DNA (or RNA) wrapped in a protein coat.

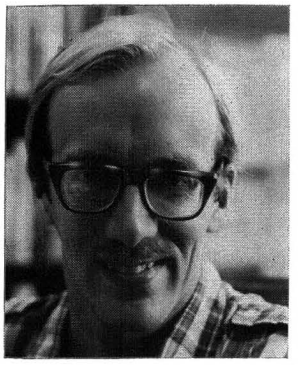
protoplast: a cell (plant, animal, microbial) without a (structural) wall (cell has "soap bubble" or plasma membrane only).

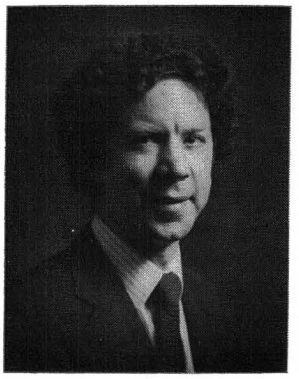
protoplast fusion: a means of achieving genetic transformation by joining two protoplasts in the laboratory to achieve a viable hybrid cell with desirable traits.

myeloma: tumor (immortal) cells of the antibody-producing system.

lymphocytes: specialized white blood cells involved in the immune response; each B lymphocyte produces a single kind of specific antibody.

hybridoma: a viable cell hybrid resulting from cell fusion of a lymphocyte (specific antibody-producing) and a myeloma ("immortal" or easily propagated cell, resulting in a cell which can be conveniently cultured (like





Jay Bailey is a professor in the chemical engineering department at Caltech. His research interests include the study of structural and functional effects of immobilization on proteins, altered metabolism and multiple product bioconversion in immobilized bacteria and yeast, production of biological polymers by microbial fermentation, and several aspects of kinetics and reactor design for recombinant microorganisms. **(L)**

David F. Ollis received his BS from Caltech in 1963, his MS from Northwestern in 1964, and his PhD from Stanford in 1969. He is currently Distinguished Professor of chemical engineering at North Carolina State University, where he is engaged in research, teaching, and biotechnology facility development. His hobbies include music and raising (mostly redheaded) boys. **(R)**

The potential impact appears to be endless: virtually any biochemical . . . or economically desirable defense mechanism can be produced or incorporated, respectively, in appropriate plant, animal, or microbial cells which can be grown in biological reactors of one sort or another.

microbes) and which produces specific antibodies.

monoclonal antibodies (MAb): Homogeneous (all alike) antibodies derived from a single clone of cells; MAb's recognize only one kind of antigen (very high specificity).

Growth over the last ten years of both new and established commercial efforts in biotechnology has been astonishing. This rate of change is nicely chronicled by the following sequence [2]

1973 First gene cloned.

1974 First expression (production of genetically coded product) of a gene cloned from a different species in bacteria.
rDNA experiments first discussed in a public forum.

1975 Science conference at Asilomar, Calif. held; support for guidelines for recombinant DNA research voted.
First hybrid cell created by the fusion of two animal cells.

1976 First recombinant DNA-based company founded: Genentech.

1980 New life forms are patentable: Diamond vs. Chakrabarty.
Wall Street selling record set: Genenetch goes public, stock price moves from $20 to $89/share in 20 minutes.
United Kingdom, West Germany target biotechnology for development
Patent (Cohen-Boyer) issued for rDNA methodology

1981 First monoclonal antibody test kit marketed
First automated gene synthesizer marketed
Japan, France target biotechnology R and D
Cetus Corp. raises record $115 million on first public offering
DuPont dedicates $120 million to life sciences R & D
80 new biotechnology companies founded

1982 First rDNA animal vaccine (colibacillosis) marketed (Europe)
First rDNA pharmaceutical (human insulin/Lilly) marketed (England)

1983 First plant gene transferred to different species of plant
$500 million raised (total) by new U.S. biotechnology companies

1984 First plant cells genetically modified to be resistant to a broad spectrum herbicide (glyphosate).

Additional developments include both the "new" and the 'old' biotechnologies:

- Renewable resources engineering has demonstrated a number of biological (enzymatic, microbial) and non-enzymatic means of converting cellulosic materials into fermentable feedstocks.
- Ethanol production, largely through strain and process fermentation/separation improvements, has become commercial for gasohol (<10% ethanol in gasoline) production and is now competitive with ethanol from ethylene on a new plant basis.
- Single cell (microbial) protein has been produced on a mammoth scale (Imperial Chemical Industries) using a novel (airlift) bioreactor configuration and a major petrochemical (rather than agricultural) feedstock: methanol.
- Bovine growth hormone has been produced by cultured rDNA bacteria (E. coli): this product could enhance milk production in dairy cows and growth rates in beef cattle.
- A soil microbe (Pseudomonas) has been genetically engineered to contain bacterial genes coding for a toxin against a nematode root predator, indicating potential for a perpetual biocide-generating soil inoculant.
- Wholly synthetic genes have been constructed by wet biochemical techniques, inserted into a bacterium and the newly coded product obtained, thus verifying the ability to "type" any biochemical production instruction, by wet chemistry, into the genes of bacteria.
- Plant cells (tobacco) have been manipulated to contain microbial (non-plant) genes for a toxin against a specific pest (not commercially useful example, but importantly suggestive for engineering plant pest resistance).

The potential impact appears to be endless: virtually any biochemical (enzyme, antibody, hormone, vitamin, growth factor) or economically desirable defense mechanism can be produced or incorporated, respectively, in appropriate plant, animal, or microbial cells which can be grown in biological reactors of one sort or another. Different cell types will be preferred for different products; each will impose specific requirements on bioprocess reaction and separation systems. As new products and new organisms for current products multiply, the biochemical engineer must provide more systematic, predictive guidance to select among available options and bring optimized organism-process systems into timely and efficient production.

In our view, these new opportunities and challenges in biotechnology will require biochemical engineering to move beyond the empirical, macroscopic approaches which were adequate in the past. We believe that biochemical engineers must understand, model, and productively exploit the fundamental biological, chemical, and physical mechanisms which interact to determine process perform-

ance. Previously, we have often viewed cells only from a "black-box", "input-output" perspective, and seen aeration primarily in terms of an overall volumetric mass transfer coefficient. For the future, we need to apply known features of cellular pathways and their regulation to improve reactor design, and to include detailed studies of hydrodynamics and interfacial phenomena to enhance mass transfer rates with minimal damage to cells and products. Armed with quantitative methods for reaction engineering and description of multicomponent partitioning, the biochemical engineer should seek an increasing role earlier in the process research and development sequence, aiding in definition of organisms and product forms which have the characteristics needed to enable or to facilitate large-scale manufacture.

The current biochemical engineering lecture course additions and alterations, summarized below, are driven by our firm conviction that a major refinement and refocusing of biochemical engineering education (and research, but that is another story) is needed to meet the technical challenges ahead and to achieve the most productive role for the biochemical engineer in future commercial biotechnology enterprises.

We have, consequently, restructured our course and our text (second edition in press) to include the following emphases in the topic sequence indicated

1. **Microbiology** becomes Microbiology and Cell (plant, animal) Biology.
2. **Biochemistry** includes additional sections on DNA structure, plasmids, and enzymes need for DNA manipulation via rDNA techniques.
3. **Enzyme kinetics** includes reversible reactions (e.g., for high fructose syrup via glucose isomerase) and more detailed treatment of enzyme deactivation mechanisms and kinetics.
4. **Enzyme applications** include new immobilized enzyme processes, and preparation methods, materials, catalyst characteristics including deactivation and mass transport-reaction interactions.
5. **Bioenergetics and metabolism** is restructured to show clearly how energy balances work to give biosynthetic engine efficiencies and microbial heat release, and how stoichiometries of microbial conversions are deducible directly from understanding the major metabolic flow routes.
6. **Genetics and (microbial) control** systems altered enormously to indicate techniques of the "new" biotechnology: recombinant DNA manipulations and cell fusion approaches for hybridomas. This section also emphasizes the need to understand lower (procaryotic) and higher (eucaryotic) life form biochemical control systems and cell cycles to allow proper rDNA catalyst preparation and to provide a basis for kinetic models.
7. **Cell kinetics** is enlarged to include not only unstructured models (constant biomass composition) but structured (multicomponent) models which allow inclusion of known internal metabolic details which vary with cell environment or culture growth stage. Segregated models for kinetics, incorporating cell-to-cell heterogeneity (population balances) are also included. Similarly, product formation kinetics can be empirically or metabolically modeled.
8. **Transport phenomena** (aeration, agitation, power input, mixing) is modified to include transport correlations for recent configurations (air-lift reactor), and materials (viscous fluids) as well as data for CO_2 gas-liquid transfer (coupled to fermentation broth pH). Aeration bubble coalescence is discussed, and both Weber number and Kolmogoroff theories presented as determinants of bubble size and thus interfacial area/vol, **a**, of the mass transfer conductance, $k_l a$.
9. **Reactor design** for pure cultures is revised to include a number of non-ideal reactors (including circulation in imperfectly mixed batch reactors). Formulation, characterization, and application of immobilized cell catalysts are examined. Multiphase reactors, including packed beds, fluidized beds, and trickle reactors are treated, and an entire section is devoted to reactors for plant and animal cell propagation.
10. **Instrumentation and Control** is an entirely new topic, encompassing process sensors for dissolved oxygen, substrates, temperature, pH, etc., and biological parameters needed for on-line data acquisition and calculation of instantaneous mass and energy balances. This discussion leads naturally to application of state estimation and process control.
11. **Separation and purification processes** are also isolated in a new, unified treatment, beginning with the fermentation broth or biological product source, and surveying techniques for cell and particle removal (filtration, sedimentation, coagulation, centrifugation), product concentration (solvent extraction, aqueous 2-phase extraction, precipitation, adsorption, ultrafiltration, etc.) and product purification (large scale chromatographies, fractional thermal and salt-driven precipitations, electrophoresis, affinity chromatography and immunosorbent columns, reverse osmosis). Examples of bulk chemical (ethanol), enzyme, antibiotic, polysaccharide and organic acid recovery are discussed.
12. **Bioprocess economics** is introduced as a new subject. A complete case study due to Bartholomew and Reisman is first covered, including process inception, flow sheets, equipment sizing, capital equipment, operating, utility, labor, and raw materials costs, and return-on-investment and sensitivity determinations. Subsequent examples include a number of major bioproducts (antibiotics, ethanol, enzymes, proteins from recombinant cells, organic acids, polysaccharides, single-cell protein, monoclonal antibodies, vaccines).
13. **Mixed populations** discussion has been enlarged to include mixed cultures arising naturally from propagation of unstable recombinant microbial strains.
14. **Biological waste treatment** continues to be taught be-

cause: (i) it represents (still) the largest, successfully operating microbial reactor systems in existence, (ii) it provides, in the aerated (activated) sludge process, a clearly studied, mixed population system (iii) it illustrates beautifully, with an anaerobic reactor simulation, the enormous sensitivity of actual mixed culture systems to process upsets of flow rate and feed composition.

15. **Homework problems** for every topic have been extensively revised, especially to add a number of brief calculational essays and to edit or remove some unduly long problems.

In spite of the apparent burden of covering an increasing number of topics in increasing depth, the biochemical engineering course has actually been strengthened and streamlined by two pedagogical approaches: (a) the topics build on the preceding topics, thus the early material contains primarily those key items needed in latter chapters, and (b) the nomenclature has been shifted and recodified into a vocabulary most familiar to chemical engineers. For example, metabolism leads to stoichiometry and energy balances, biological products are recovered in separation unit operations, and bioprocess economics is based on standard chemical plant cost estimating terminology.

Throughout, original presentations of biological background have been pruned and revised to present major concepts as clearly and concisely as possible. Introductory summaries are provided for all of the less familiar topics (*e.g.*, microbiology and cell biology, biochemistry, enzyme kinetics and structure, metabolism, genetics and DNA, biochemical control systems). As before, no chemical engineering material not already available to the chemical engineer by the end of the junior year (stoichiometry, energy balances, transport phenomena, thermodynamics, chemical kinetics) is assumed. Our experience indicates that eager students, willing to undertake new vocabularies in short order and to absorb the considerable amount of qualitative material prior to "comfortable quantitation" in equation form, will find the subject and its organization fascinating and exciting.

REFERENCES

1. J. E. Bailey and D. F. Ollis, "Biochemical Engineering Fundamentals," *Chem. Eng. Education*, Fall, 1976.
2. "Commercial Biotechnology; An International Analysis" (OTA-BA-214) Office of Technology Assessment, Washington, D.C. 20510 (January, 1984). A larger glossary appears on pages 586-597. (For sale by Superintendent of Documents, U.S. Gov't. Printing Office, Washington, D.C. 20402.)

ChE letters

"NONADIABATIC" A MISNOMER?

Dear Editor:

I noted in a recent issue of the AIChE Journal (April '85) an article with the word "Nonadiabatic" in the title.

I have always (for the past 25 years) called such a term simply "diabatic." The term nonadiabatic is redundant since the *a* prefix in adiabatic means nonadiabatic, i.e. without heat transfer.

The prefixes *a, ab,* or *an* in English all infer a negative connotation as in:

aneroid	=	**without fluid (in a barometer)**
ascorbic acid	=	**anti-scorbutic (Vitamin C)**
anhydrous	=	**without water**
anesthesia	=	**without feeling**
asymmetrical	=	**not symmetrical**
anisotropic	=	**not isotropic**
agnostic	=	**without knowledge (of God)**
atheist	=	**does not believe in God**
abnormal	=	**not normal**
etc.		

Thus to use the term non-adiabatic, literally means non-non-diabatic and the two negatives cancel each other to yield the simpler term diabatic. No one would think of using non-abnormal to replace the word normal, or non-anisotropic for isotropic, so why not diabatic for non-adiabatic?

I would appreciate your publishing this letter and maybe someone will know the answer to these questions:

1. Have you ever heard of a reactor or a process with heat transferred to or from it being called "diabatic"?
2. Any references in books or journals to a diabatic reaction or other process?

In any case, I propose that Chemical Engineers use the simple term "diabatic" to replace this awkward, redundant and more complex expression non-adiabatic, at least for chemical reactors.

I have not coined a new word since Merriam-Webster's Unabridged Dictionary lists *diabatic* and defines it as involving the transfer of heat (opposed to adiabatic).

Allen J. Barduhn
Syracuse University