Perspective

Biochemistry–Engineering Interface in Biochemical Engineering

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Introduction

Although the roots of biochemical engineering may be traced back to brewing activities in a prehistoric era, modern biochemical engineering can probably trace its origins to the early days of the "Golden Era of Antibiotics" (Ryan, 2000). Having successfully isolated penicillin from Alexander Fleming's mold and demonstrated its chemotherapeutic effects in 1940-41, the Oxford team of Walter Florey, Boris Chain and Boris Heatley were stymied by their inability to isolate sufficient quantities of penicillin to prove its value. To solve this problem, they teamed up with scientists and engineers at the USDA's Peoria facility who successfully developed a scaleable submerged fermentation process for penicillin G production. The subsequent entry of scientists and engineers at Merck, Pfizer, and Squibb into this collaborative effort led to mass production of this miracle drug at a breathtaking pace ($>2\times10^{10}$ dosage units per month by 1943). Within the next decade, similar stories were replayed in the context of streptomycin, erythromycin, tetracycline and vitamin B_{12}. The dialog among biochemists, microbiologists, and chemical engineers has begun.

Notwithstanding this growing interdependence of industrial life scientists and process engineers, biology and chemical engineering continued to view each other from a perceived distance comparable to that between linguistics and music. Then, in the early 1980s, a series of articles were published by a chemical engineer who foresaw a new horizon between the two disciplines (Srienc et al., 1983; Dennis et al., 1983; Lee and Bailey, 1984a,b). This visionary was none other than the late Jay Bailey. What made his call for bridge-building especially unique among fellow bioengineers of his time was its emphasis on the scientific fundamentals of both disciplines. Until then, the relationship between life science and engineering operated on a need-to-know basis. Industrial biologists and chemical engineers collaborated with the barest of understanding of the underlying scientific principles of each other's fields. Bailey believed that the unique combination of molecular insight, quantitative logic and problem-solving skills, which allowed chemical engineering science to spearhead the analysis and engineering of naphtha crackers in the 1960s and 1970s, could be equally useful for the analysis and engineering of a living cell. At the same time, he also recognized that to be effective at this new scientific interface, chemical engineers would have to wholeheartedly embrace molecular biology, an emerging branch of biochemistry that sought to explain the complexities of life in molecular terms.

Two decades later, biochemical engineering science, which traces its origins to the Bailey school of thought (Bailey and Ollis, 1977), is reaching maturity. (In this article, the term "biochemical engineering science" is used to refer to the branch of chemical engineering science that is rooted in biochemistry. It can be distinguished from "biochemical engineering practice," where devices and processes involving biological components are designed and operated in analogy to their nonbiological counterparts without particular attention to the molecular uniqueness of biochemical systems. Biochemical engineers trained along both avenues play useful roles in the life science industry, and the boundaries between the two have blurred over time as new types of products and processes continue to emerge in the biotechnology marketplace.)

It is generally accepted that biology presents the chemical engineer with endless opportunities for a two-way traffic of ideas, problems and technologies. What then might the interface between biochemistry and chemical engineering look like a few decades out in the future when the field reaches its prime? I cannot claim to have a satisfactory answer to this question. I do, however, confess to asking this question periodically, both of myself and of others. Ironically, the only answer that has withstood the test of time is one that Jay Bailey gave me many years ago: biochemical engineering is, and will be, what biochemical engineers do. By this, he meant that if biochemical engineers take on challenging problems that are fundamentally rich and have practical significance, and if they develop effective solutions that gain acceptance within the broader science and engineering community, then even if these problems were not recognized as biochemical engineering at the time when they were initially undertaken, they will gradually be adopted as an integral part of the field. The challenge then lies in problem identification and in mounting a sustained attack on selected problems in the most imaginative, comprehensive and unconstrained manner possible.

Jay Bailey's own research accomplishments exemplified the above notion of biochemical engineering. Through his investigations into problem areas as diverse as metabolism, cell physiology and carbohydrate biology, he highlighted the interplay among understanding, controlling and manipulating living systems. A number of exciting areas in contemporary biochemical engineering continue to bear these fingerprints. They include the deconstruction of metabolism in a post-genomic world, the biochemistry and biotechnology of extremophilic microbes, natural product biosynthesis, and the practice of cell culture as a science rather than black art. In the last few years of Jay Bailey's life, we had several discussions on problem areas that might represent uncharted frontiers for biochemical engineering in the coming decades. A few examples are recounted in no particular order of significance or priority. By way of truth-in-disclosure, I must also emphasize that my

knowledge of these problem areas is limited. The central message is that there are two features uniting these otherwise disparate problems: (a) none of them have a satisfactory solution at present or even the beginnings of one; (b) their successful solution will depend on a combination of an intimate understanding of the underlying biochemistry and the quintessential problem-solving skills of an engineer.

Converting Carbon Dioxide and Nitrogen into Useful Synthons

Nearly a 100 years since the development of hydrocarbon cracking and the Haber-Bosch process, these processes remain the backbones of the chemical industry as the predominant sources of synthetically useful carbon and nitrogen, respectively. Their importance to modern society cannot be overestimated; yet, their inability to support sustainable growth and development is equally glaring. In contrast, most synthetically useful carbon and nitrogen atoms in the biosphere are derived through the actions of two enzymes-ribulose bisphosphate carboxylase (Rubisco) and nitrogenase. The former fixes atmospheric CO_2 into carbohydrates, whereas the latter converts atmospheric dinitrogen into ammonia. At ambient temperatures and pressures, the turnover numbers of both catalysts are ~10 s^{-1} (Lorimer et al., 1976; Rees and Howard, 2000), suggesting that at modest concentrations (~10 μM) both enzymes satisfy the Weisz rule-of-thumb for practically useful catalysts (Weisz, 1973). Despite the ecological and economic attractiveness of this proposal, the technological challenges associated with harnessing the most remarkable catalytic properties of these two unique enzymatic systems are immense, and the optimal route for developing competitive processes for the conversion of these inert atmospheric gases into useful synthons is anything but obvious. Like the proverbial onion, as individual layers of these problems are peeled back, new fundamental questions and technological challenges will become obvious. A comprehensive plan to attack such a problem might have elements of structural biology, protein engineering, redox biochemistry, microbial and/or plant biology, inorganic and organometallic chemistry, and membrane biophysics, in addition to more familiar elements of thermodynamics and reaction engineering. One thing is for certain, though. Given the scientific complexity of these systems, the ability to recapture nature's exquisite designs in a robust industrial catalytic reactor will require a strong problem-oriented focus, if we wish to replace in the next few decades century-old processes for hydrocarbon and ammonia production by environmentally benign ones.

Harnessing Stem Cells for Regenerative Medicine

After post-war chemical engineers played a vital role in the development of microbial fermentation processes for antibiotic and other fine chemical production, this cadre of biochemical engineers was presented two decades ago with a qualitatively new challenge. How can one develop and scale up the processes in which the cellular catalyst was derived from a mammalian source, rather than being a free-living microorganism? The fact that the product pipeline of the biotechnology industry today includes dozens of mammalian-cell-derived products such as growth factors, blood-clot-dissolving agents and monoclonal antibodies suggests that this problem has been solved satisfactorily, at least to a first order of approximation. Looking back though, the strategies used by biochemical engineers to solve these two problems at different points in the 20th century were remarkably similar and involved a combination of classical chemical engineering skills in reaction engineering and transport phenomena with a rudimentary understanding of cell physiology.

In contrast, recent developments in vertebrate biology have presented a fundamentally new problem/opportunity for the biochemical engineer. In particular, the isolation of human stem cells in 1998 (Thomson et al., 1998) offers unprecedented opportunities for developing new medical therapies for debilitating diseases. Stem cells are unspecialized cells that can self-renew indefinitely and also differentiate into more mature cells with specialized functions (Vogelstein et al., 2001). In spite of scientific uncertainties associated with their biological properties and ethical controversies surrounding their use, there is growing recognition that stem cells have the potential to revolutionize regenerative medicine.

Based on past experience, biochemical engineers can be expected to play an important role in the realization of this potential; however, to do so, we will have to come up with entirely new paradigms for cell culture. In contrast to traditional mammalian cell culture, which involves robust, plentiful, well-characterized and malleable cancer cells, stem cells are delicate, scarce, poorly characterized and difficult to manipulate. Biomedically useful stem cells are likely to be far more diverse than their traditional cell-culture counterparts; in the extreme case "customized" stem cells would have to be derived or engineered (and possibly even rederived or reengineered for successive use) for each patient. Notwithstanding these hurdles, to obtain regulatory approval for stem-cell-based processes, the biochemical engineer will have to assure reproducibility. To gain acceptance in the marketplace, these processes will have to be implemented on an industrial scale. Although the success of stem-cell-based therapies is far from guaranteed, it will depend heavily on the bioprocess engineer's ability to acquire a substantially deeper understanding of fields, such as developmental biology, genetics and genomics, and pharmacology than is currently the norm. Indeed, future developments in this field are likely to challenge our very notions of the term "bioprocess" in ways that were unimagined before.

Metabolic Engineering of Complex Human Diseases

A common perception in the current era of molecular medicine is that before too long complex diseases such as cancer, cardiovascular diseases, disorders of the central nervous system, and autoimmune diseases will be understood and treated at the most fundamental molecular level. While major strides have certainly been taken in our understanding of these multifactorial diseases, the outlook is more uncertain when one evaluates the cornucopia of available medicines and the pipeline of new drugs. For example, the continued widespread success of antifolates as anticancer agents and statins as cholesterol lowering drugs points to the clinical reality that, whereas genes may often cause complex diseases, the metabolism is usually the most effective point of intervention. This is because alterations in metabolic functions often lie on the causative path between defective genes and disease pathology. Whereas precision-repairing of genes from first principles is still in the realm of science fiction, pharmaceutical and clinical scientists have considerable experience in pharmacologically manipulating metabolic biochemistry to restore homeostasis.

Interestingly over the past two decades, biochemical engineers, inspired by the early work of Jay Bailey, have been honing their skills at analogous metabolic engineering of microorganisms, primarily for industrial and environmental applications. A key difference lies in the possibilities and constraints associated with the use of genetic engineering as a tool in these two forms of metabolic engineering. Whereas genetic engineering has become an essential tool in metabolic engineering of microorganisms, the challenge of metabolic engineering in the context of complex human diseases lies in the application of biochemical, genomic and physiological tools without the benefit of genetic manipulation. Thus, a biochemical engineer will have to be first and foremost an engineer and then a molecular biologist to be successful in this new endeavor. To do so, however, the biochemical engineer will first have to learn certain skills currently only taught to students of the clinical and pharmacological sciences. A combination of such training, together with knowledge of mathematics and chemistry, should open an exciting new frontier for metabolic control analysis at the interface of chemical engineering and medicine. The urgency and complexity of many contemporary problems in medicine place a hefty premium on reasonable (albeit imperfect) solutions that can be attained in a time- and resource-delineated manner. Just as the biocatalytic properties of microbes are refined via genetically driven metabolic engineering today, future generations of metabolic engineers may be able to develop effective therapeutic strategies for diseases such as multiple sclerosis, obesity, and stroke by chemically driven metabolic engineering.

Conclusions

I started this essay with the premise that Jay Bailey's notion of biochemical engineering (i.e., biochemical engineering is what biochemical engineers do) is a useful way to assess the development of this rapidly evolving discipline within the chemical engineering profession. Using selected examples from Bailey's own work, I highlighted how new frontiers in biochemical engineering science have emerged and continue to emerge when chemical engineers apply their molecular training and problem-solving skills to explore new areas of the life sciences that are rich in opportunities for practical applications. I presented three examples of challenging problem areas where chemical engineers could play a unique role in the future. While unrelated at the technical level, in each case there are certain underlying similarities. All of them lack a clear path to a satisfactory solution at present. For chemical engineers to be effective participants in the development of such solutions, they will have to leverage their molecular training to acquire a deep appreciation for new and foreign fields of science (analogous to the gradual incorporation of molecular biology into chemical engineering that has occurred over the past two decades).

Finally, since each of these problems is perhaps too complex to solve from first principles alone in the foreseeable future, a delicate balance between analytical rigor and engineering judgment will have to be struck. If biochemical engineers do succeed in these or similar endeavors, then biochemical engineering (and, by inference, chemical engineering) in 2025 will be a far broader and richer field than we know it today.

Acknowledgments

This article is dedicated to the memory of the late Jay Bailey, a visionary, mentor and friend. I am also indebted to Julio Ottino for his encouragement and valuable suggestions in the preparation of this article. The continued support of research in my laboratory by the National Institutes of Health and the National Science Foundation is gratefully acknowledged.

Literature Cited

Bailey, J. E., and D. F. Ollis, *Biochemical Engineering Fundamentals*, McGraw-Hill, New York (1977).

Dennis, K., F. Srienc, and J. E. Bailey, "Flow Cytometric Analysis of Plasmid Heterogeneity in Escherichia coli Populations," *Biotechnol. Bioeng.*, **25**, 1923 (1983).

Lee, S. B. and J. E. Bailey, "A Mathematical Model for λdv Plasmid Replication: Analysis of Wild-Type Plasmid," *Plasmid*, **11**, 151 (1984a).

Lee, S. B. and J. E. Bailey, "A Mathematical Model for λdv Plasmid Replication: Analysis of Copy Number Mutants," *Plasmid*, **11**, 166 (1984b).

Lorimer, G. H., M. R. Badger, and T. J. Andrews, "The Activation of Ribulose-1,5-Bisphosphate Carboxylase by Carbon Dioxide and Magnesium Ions. Equilibria, Kinetics, a Suggested Mechanism, and Physiological Implications," *Biochemistry*, **15**, 529 (1976).

Rees, D. C. and J. B. Howard, "Nitrogenase: Standing at the Crossroads," *Curr. Opin. Chem. Biol.*, **4**, 559 (2000).

Ryan, J. F., ed., *The Pharmaceutical Century: Ten Decades of Drug Discovery*, ACS, Washington, DC, p. 53 (2000).

Srienc, F., J. L. Campbell, and J. E. Bailey, "Detection of Bacterial β-galactosidase Activity in Individual Saccharomyces cerevisiae Cells by Flow Cytometry," *Biotechnol. Lett.*, **5**, 43 (1983).

Thomson, J. A., J. Itskovitz-Eldor, S. S. Shapiro, M. A. Waknitz, J. J. Swiergiel, V. S. Marshall, and J. M. Jones, "Embryonic Stem Cells Derived from Human Blastocysts," *Science*, **282**, 1145 (1998).

Vogelstein, B., B. R. Bloom, C. S. Goodman, P. A. King, G. M. McKhann, M. L. Weisfeldt, and K. R. Merikangas, *Stem Cells and the Future of Regenerative Medicine*, National Academy Press, Washington, (2001).

Weisz, P. B., "Zeolites. New Horizons in Catalysis," *CHEMTECH*, **3**, 498 (1973).