Perspective

Metabolic Engineering: Developing New Products and Processes by Constructing Functioning Biosynthetic Pathways *in vivo*

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In a recent *AIChE Journal* perspective on Metabolic Engineering[1] we asked rhetorically whether the microbial world is so diverse as to allow one to isolate some microbe capable of producing *any* desired molecule. It turns out that this is very likely the case, a manifestation of the enormous diversity of molecules and reaction processes resident in a microbe. These actually constitute the mechanisms by which cellular functions are being carried out. The problem with most organisms is that they may make only traces of any single desired molecule and under conditions that may be difficult to implement on an industrial scale. These microbes must be improved before their potential is realized. Furthermore, after demonstrating that it is possible to cross the species barrier, pathways that are incomplete for the production of a molecule in one organism can now be completed by transferring the missing pieces from another microbe. Thus, the enormous diversity present in an array of species can now be commandeered for accomplishing a specific purpose, such as the production of entirely new products or the construction of new synthesis routes for existing products. This is the goal and essence of metabolic engineering.

Metabolic engineering was developed in the previous decade to improve industrial strains using modern genetic tools. While microorganisms were modified before by random mutation and selection methods, the development of recombinant technologies in early 80s allowed *directed* strain modification by introducing specific genes conferring desirable properties to cells for industrial, medical and environmental applications. Metabolic engineering, thus, emerged as the scientific discipline occupied with the improvement of cellular properties through introduction to cells of specific transport, enzymatic or regulatory reactions using, primarily, recombinant technologies.[2,3] In less than a decade an impressive number of metabolic engineering applications have appeared in diverse areas including aminoacid fermentations,[4] polyketide and novel antibiotic synthesis,[5] indigo and aromatic aminoacid synthesis in *Escherichia coli*,[6] golden rice,[7] ethanologenic *E. coli,*[8] isoprenoid (lycopene) overproduction,[9] indene biocatalysis for the synthesis of chiral pharmaceuticals,[10] tricistronic gene expression in Chinese Hamster Ovary cells for foreign protein overproduction under no growth and high viability conditions,[11] manipulation of the glycosylation pathway in mammalian cells,[12] 1,3-propanediol and succinate production in *E. coli* as monomers for polymer production from renewable resources, and many, many others along with numerous applications in the medical and environmental areas.[13,14]

Metabolic engineering makes extensive use of applied molecular biological methods in order to introduce pathway modifications and controls at the genetic level. As such, its experimental implementation has a strong molecular orientation. However, metabolic engineering is much more than just an industrial variant of genetic engineering. Since the goal is the overproduction of a product, we must be concerned with the function of the entire pathway, as well as its optimal configuration in terms of adequate precursor supply and kinetic controls. This means that one needs to examine a broader bioreaction network that extends beyond the strict collection of those reactions just necessary for product synthesis. For example, many biosynthetic pathways are net producers or consumers of energy (ATP) and reducing equivalents (NADH, NADPH). These resources are produced and consumed by many other reactions and for many functions in a cell. The introduction of a new pathway or the amplification of its rate should be done in a way that does not disturb the cellular molecules too far away from their normal (physiological) steady state. This means that proper consideration must be given to the entire bioreaction network and this is the main differentiating characteristic of metabolic engineering: It concerns itself with a biosynthetic route *in its entirety* instead of isolated cellular reactions.[15] As mentioned, it is often necessary to transfer some reactions from a different organism in order to complete a pathway. This opens many possibilities as to the possible biosynthetic routes that may be used for product

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synthesis.[16] Some may be thermodynamically infeasible and some others may incur particularly high costs in terms of precursor use or energetics. Finally, the ultimate carbon and energy source(s) (glucose, oxygen, minerals, vitamins) must be transferred into the cell from the medium. The rate of reactant import and, similarly product export, are important steps in the overall process.

It is clear from the above simple outline of a cell factory that its design, optimization and control make heavy use of the principles and methods of chemical engineering developed for the design and operation of chemical plants. This is the basis for the critical role of the discipline in the genesis and continued growth of metabolic engineering.

While attention is often focused on pathway optimization, the importance of using the diversity of microorganisms for *new product synthesis* should not be underestimated. This can be done either by introducing specific new enzymes (through the expression of their genes) in a cell, or by the introduction of *enzyme libraries* creating large diversities of entirely new molecules from which those with desired properties must be selected. Efficient methods for creating large libraries along with creative ideas for the selection of desired products are critical elements in this approach.

Rational, or model-based, and combinatorial methods can be used in the design of optimal pathways.[17] Rational approaches make use primarily of stoichiometric approaches as stoichiometric models are the only type of models that can be used with reasonable reliability on a cell-wide basis. Kinetic models are not as useful because models of enzymatic kinetics that are valid under intracellular conditions are rare. In addition, the regulation of these reactions at the transcriptional and enzymatic levels is largely unknown. Combinatorial approaches, whereby a cell is transformed with random genomic libraries and well-defined mutants are selected, compliment the rational approaches. A key concept here is that of *inverse metabolic engineering,*[18] whereby a desired mutant is selected from a library and then its specific genetic modification, typically gene deletion or overexpression, are well characterized. This is a powerful approach to identifying specific genes that materially impact the product phenotype either by their stoichiometric, kinetic or (primarily) regulatory effects.

Biotechnological routes are presently preferable to chemical ones in the production of chiral pharmaceuticals and complex fine chemicals, in precision chemistry and addition of new functionalities to existing molecules, and in the utilization of renewable resources, all areas with high expected growth rate. Furthermore, various problems that impaired the development of biological processes in the past (such as low titer, product inhibition, and slow rates) are rapidly being solved by a variety of newly developed methods. For example, product inhibition is minimized or altogether avoided by engineering enzyme mutants resistant to high product levels, while osmo-tolerant strains allow accumulation of continuously higher product concentrations. Continued levels of strong R&D support by the U.S. Federal Government and industry will be creating an increasing number of opportunities for biotechnological applications. As a result of these developments, life sciences will impact the chemical industry in a very profound way. Evidence of this assertion is widespread: an approximate market of $60B for chiral pharmaceuticals; a robust and growing biotechnological industry (with more than $40B in sales and hundreds of new products in the regulatory approval pipeline); and an increasing number of biotechnologically produced products such as 1,3-propanediol, polylactic acid and an array of new biopolymers. This trend is very likely to continue in the future and metabolic engineering will be providing the enabling technologies for harnessing the potential of microbes for an expanding portfolio of new applications.

So, why might chemical engineers be interested in metabolic engineering? First, metabolic engineering combines the intellectual framework and implementation tools required to capture the enormous potential of biology for industrial and medical applications. Its concepts and tools should be familiar to chemical engineers as metabolic engineering borrows heavily from chemical reaction engineering. Second, the importance of metabolic engineering in materials, fuels, and specialty chemicals (pharmaceuticals and chiral compounds) is undeniable as evidenced by a growing number of applications in these areas.[19] In the medical field, the greatest impact of metabolic engineering will be in the development of methods for the rigorous assessment of the physiological state and determination of reasonable enzymatic targets for the treatment of disease. This will be implemented either by direct therapeutic intervention or screening programs for the discovery of new drugs. Finally, this is an excellent entry for chemical engineers into a very rich field of scientific inquiry.[20] Biological systems owe their exceptional properties to specific chemical reactions catalyzed by enzymes that, in a growing number of cases, can be uniquely prescribed from genomic information. In other words, genomics provides the means to define the specific steps of the chemical reaction system that can be subsequently analyzed using the tools of metabolic engineering. This is a profound difference from typical chemical reacting systems where defining the actual reaction steps is a major challenge.

Chemical reactions are, for the most part, responsible for the wonders of biology. Metabolic engineering combines the tools and concepts of reaction engineering and molecular biology for the analysis and purposeful modification of bioreaction networks.[21] It also provides a framework for integrating and quantifying genomic information and cell-wide data generated from modern technologies. As such, it is the natural vehicle for capturing the enormous potential of biology and transforming it into the enabling science of many new industrial and medical applications. Chemical engineers are in a unique position to extend their educational and research paradigm into the most exciting field of scientific inquiry. This will require that they embrace biology as a foundational science equal to chemistry and modify the curriculum to reflect this fundamental change into chemical and biological engineering.

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