PRINCIPLES OF METABOLIC ENGINEERING

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Contents

Summary

During the last two decades the design of a number of industrial bio-processes has been greatly advanced by a systematic application of modeling of the cell metabolic network. The results of the modeling are tested in laboratory experiments, and point to genetic modifications in the pathway reaction that will lead to higher yield and productivity of the desired product. The concept is named *Metabolic Engineering*.

The combination of mathematical and experimental tools that will lead to a shorter path from conceiving a process to large scale production is viewed as a key methodology in modern biotechnology. The main mathematical tools of Metabolic Engineering (MFA, MCA for steady state and dynamic cultivation) are reviewed using a few toy-examples to illustrate how the mathematical tools are applied.

Several important classes of application of Metabolic Engineering are shown as examples to illustrate the versatility of the concept: Synthesis of Bio-Fuels and the systematic approach to improve product qualities that are only indirectly determined by the metabolic network reactions.

1. Introduction

The concept of Metabolic Engineering was formulated in just two papers, published

back to back in *Science* in 1991, one by Bailey and the other by Stephanopolous. Since then the meaning of the term Metabolic Engineering has been reiterated time and again, in review articles by Stephanopolous in 1998 and in 1999, and in 1999 by Lee and Papoutsakis. Also there are countless original papers published in the major journals, of which one, *Metabolic Engineering*, founded in 1999 is entirely devoted to the application of the concept, in Industrial Biotechnology, in the Health Sciences and in Bio-Remediation.

The ideas, succinctly formulated by Bailey and Stephanopolous in 1991, started to penetrate the biotechnology community already during the two decades leading up to 1991. Terms like Quantitative Physiology and Physiological Engineering were coined by an enthusiastic group of (especially) Chemical Engineers who had discovered the rich opportunities of using the tools of Mathematical Modeling, Chemical Reaction Engineering,

Transport Phenomena and Chemical Thermodynamics to solve challenging quantitative problems in the Biosciences. Metabolic Engineering is concerned with the flow, or *flux* of mass through the metabolic network of living organisms with the ultimate purpose of *redirecting* the (carbon) flux towards desired metabolic products.

The desired products can be metabolites of pharmaceutical importance derived from the primary metabolism, pharmaceutical proteins, bulk chemicals for use in the solvents industry and in the polymer industry, and they can be any of a selection of compounds to substitute oil based raw materials for the transportation sector.

Redirection of the carbon flux is obtained by application of a combination of a deep understanding of fundamental Biology (characterized by the use of a number of new words such as genomics, proteomics, metabolomics and fluxomics), and an equally solid appreciation of the power of efficient treatment of large sets of data by a range of mathematical tools (calculus, linear algebra, numerical and statistical methods, and with increasing urgency also some of the "advanced" topics of mathematics such as group theory, topology and graph theory).

The objective of Metabolic Engineering was always to supplement, and perhaps even supplant the huge strain development programs with *modeling*, guided by a few key experiments. This concept should eventually lead from random mutagenesis to *in silico* studies of the metabolism on the whole genome scale of the organism. Thereby, the direct path to the desired improvement of the organism would be guaranteed.

There would, however, never be any chance of achieving this goal without the availability of to-days vast inventory of analytical tools that were not even envisioned in 1990: Machines in which rates of metabolic reactions can be studied in the millisecond time frame, or give a total, genome-scale overview of which genes are silenced at given operation conditions.

Micro-reactors are used to study steady state metabolism at a pace which was unheard of, even with the best "ideal laboratory reactor" practice of 1990 Thereby the validation of the biological results becomes so fast, that the progress from an idea, conceived in

the laboratory to a patented industrial process is now counted in terms of a few months.

In the perspective of the rapid development of new machinery for laboratory investigations, and the equally rapid development of new modeling tools since 1990, one can indeed claim that the first generation of engineers-*cum*-biologists who initiated the new level of quantitative studies of microbial physiology in the 1980s were somewhat naïve.

Twenty years ago it was exciting to publish papers where *Metabolite Balancing* was used to quantify, at a certain steady state operating condition (*e.g.* at a given dilution rate *D*), the flux of carbon through different branches of the primary pathways of organisms such as *E. coli, Saccharomyces cerevisiae* and *Lactococcus lactis.* The results immediately showed where improvements in the metabolic network could be made, and industry could modify their strain selection work based on the calculations.

Next came *Metabolic Control* which emphasized the importance of whole pathways rather than individual pathway reactions, and the *sensitivity* of the flux through a pathway to changes in the enzyme levels of all the reactions in the pathway were calculated.

This led to an increased emphasis on the *regulatory structure* of the network, a topic much more difficult to address than the mass-flow network structure, and slowly an experimental foundation is being built up to cope with the effect of rapid as well as more slow dynamics in the network.

As part of the quest for an ever deeper insight in the functioning of living organisms, even the name Metabolic Engineering appears to merge into Systems Biology or Systems Biotechnology.

Apart from realizing that the limits of a quantitative understanding of the system are continuously expanding to encompass ever more complicated modeling studies, supported by deep level experimental techniques, the new names given to what in this paper is discussed as Metabolic Engineering hardly signal a basically new approach. In a monograph from 2006 Palsson describes how metabolic networks can be "reconstructed" based on genome-wide data sets and "wiring diagrams" of genetic circuits.

These data comprise many layers of cell behavior, from DNA replication and translation to carbon flux analysis in the metabolic network. The concepts are used for microorganisms as well as for multicellular organisms, cell tissue, and even special phenomena such as cell motility are incorporated in the model.

The complexity that characterizes the leading-edge research in quantitative Bio-Science can never hope to be captured in a Topic-Level contribution on the subject of Metabolic Engineering. What can, hopefully, be done is to review some basic *tools*, both theoretical and experimental.

The importance of these tools must necessarily be appreciated in order to understand

what can, at least in principle, be achieved by Metabolic Engineering, also in its more recent guise as Systems Biology.

The review of these basic tools will be supplemented with brief discussions of some examples where Metabolic Engineering has been used to generate a systematic, quantitative understanding of cell physiology that has led to improved technical processes.

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Biographical Sketch

John Villadsen (born June 12, 1936) received his MSc (Chem Eng) in 1959 from the Technical University of Denmark (DTU) and a PhD from the same university in 1963.

He worked in Brazil as a plant engineer for the Danish NIRO company, and after a post doc at U. Wisconsin (Madison) returned in 1966 to academia as Associate Professor in Chemical Engineering. In 1970 dr.techn on a dissertation on Orthogonal Collocation applied in Chemical Engineering. Full Professor (Chem Eng) 1976-1983 at U. Houston, Texas. Returned to Denmark to become director of the newly established Center for Process Biotechnology (1985-2001) at DTU. In 2004 he became the founding professor for the Novo Nordisk chair in Bioengineering at DTU. Since 2006 he is Senior Professor at DTU.

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