From: Metabolic Engineering in the Post Genomic Era
Edited by: *Boris N. Kholodenko and Hans V. Westerhoff*

Chapter 1

Introduction

Hans V. Westerhoff and
Boris N. Kholodenko

Abstract

After a brief discussion of the history of metabolic engineering, the existing definition of the field is brought to the fore and some of its pre-genomics methodologies are described. Then the paradigm shift in molecular cell biology towards biocomplexity is noted. This paradigm shift is taken to imply that metabolic engineering should consider the cell as a production *organism* rather than as a production *line*. Control of a metabolic pathway may well reside outside the pathway, such as in its supply lines, in the demand for its products, in its management or in its regulation. In addition, the organism is highly plastic and this can show in a rich response to engineering attempts. The organism may largely do away with much of the engineering by invoking its homeostatic control mechanisms. Metabolic engineering is therefore redefined as the approach that alters not only metabolism itself but also the regulatory and gene-expression networks of the target organism. It should be directed at optimising both the production flux and the functioning of the organism itself, thus preventing much of the opposing homeostatic response and the reduced metabolic performance. Subsequently, developments in metabolic engineering that are strongly related to genomics and will be highly useful for post genomic

metabolic engineering, are reviewed. This will lead us to introducing the subsequent chapters of this book in the context of the new definition of metabolic engineering.

Introduction

The Origin

Biotechnology may well be as old as civilization. Very early on, yeast helped bake bread and brew beverages of improved taste or quality. *Lactococcus lactis* did so for milk products. The origin of this biotechnology resided in the apparently spontaneous fermentation of food. Grape juice appeared to become wine without external intervention. These were not just chemical processes, but they required living organisms that could not generate spontaneously. Leading to the end of vitalism, Pasteur's discoveries also led to the emergence of biotechnology as a discipline distinct from chemical technology. Biotechnology would focus on the biological aspects of the technologies used to make living organisms produce chemical substances of interest.

Important aspects of early biotechnology included: (i) the conditioning (*e.g.* sterilization) of food in order to reduce the probability or rate of microbiological spoilage, (ii) the analysis of fermentation substrates, products and conditions in terms of the presence of microorganisms and their products, and (iii) the selection and identification of microbial strains with improved industrial properties. Modern genomics offers great potential in improving the latter two aspects of biotechnology, but this is not what this book will be about. This book focuses on metabolic engineering.

The aim of metabolic engineering is to optimize a biotechnologically important process carried out by organisms. The advent of Metabolic Engineering as a scientific discipline became possible with the onset of biochemistry and, subsequently, molecular biology. Biochemistry showed that: (i) chemical work by microorganisms involves a series of processes, (ii) the rate of each of these processes is catalyzed by a protein sometimes with the aid of prosthetic groups derived from vitamins synthesized by other organisms, and (iii) coenzymes such as ADP and NAD communicate important commodities such as free energy and redox equivalents between processes. Together, these features are referred to as metabolism. Biochemistry also developed the techniques for isolating the enzymes and for characterizing them kinetically.

This led metabolic engineering to physiology (*i.e.* to influencing biochemistry by altering growth conditions) and classical genetics (identifying mutant enzymes with enhanced catalytic properties, or identifying strains or

organisms with more useful pathways). Since the eighties of the last century, the classical genetic methods have been amplified by molecular genetics. After genetics had shown that functional properties are determined by genes, molecular biology showed that these genes were in the DNA and made the DNA accessible for manipulation. Accordingly and in spite of what its name may suggest, metabolic engineering has *not* been limited to the study of metabolism. The DNA, considered being the virtually autonomous determinant of living cells, was just used as an engineering tool. Metabolic engineering was the engineering of metabolism, using both physiology and the DNA as its tools.

The Prevalent Definition of Metabolic Engineering: The Cell as a Production Line

Already before the genomics revolution, metabolic engineering had become a field of its own, with a journal called 'Metabolic Engineering', with its own regular conferences and with regular penetration into other major journals and conferences. Together, the developments in biochemistry and molecular biology had suggested that the living organisms involved in industrial processes were much like a production line consisting of many machines, each of which could be optimized for the overall function. The optimization should take place by modifying the DNA of each of the machines, or perhaps by replacing a machine by a more efficient one. This then defined the field of metabolic engineering as the field using molecular genetics to change the machines of living organisms in such a way that they produce (more of) the desired material. In the first issue of the journal Metabolic Engineering, Stephanopoulos wrote: "Metabolic engineering is the directed improvement of cellular properties through the modification of specific biochemical reactions or the introduction of new ones, with the use of recombinant DNA technology." [1].

These definitions of metabolic engineering focused on altering the biochemical reactions in the metabolic pathway in order to improve the cell as a factory. They started from the concept of the cell as a *production line* only. In this concept, each of the processes in the production line was completely specified by a gene. Engineering of the metabolic process of interest should be done by engineering the sequence or expression level of these genes.

Having reviewed the ambient definition of metabolic engineering, it may now be useful to assess critically what the limits of that definition are and whether the definition of the activities of metabolic engineering optimally befits its purpose, *i.e.* the optimization of a metabolic pathway in a production organism. We shall do this in three steps. First, we shall review a number of useful aspects of existing metabolic engineering. Then, we shall note a number of issues where metabolic engineering if practiced strictly according to the

above definition would fail to provide useful answers. And as the third step we shall note the paradigm shift in the cell biology sciences that occurred in the genomics era.

This will lead us to the formulation of a new definition of metabolic engineering. Subsequently we shall refer the reader to the chapters in this book that are all leading into the new 'post-genomic' metabolic engineering.

Metabolic Engineering in the Pregenomic Era

Tools and Concepts

Yield

Much of metabolic engineering *avant la lettre* was concerned with the growth of microorganisms. This was in part because of the interest in biomass itself as the product. It was thought that microorganisms grown in a bioreactor could subsequently serve as fodder in animal production facilities. In the context of the oil/energy crisis, photosynthetic organisms were seen as potential harvesters of light energy in the form of biomass. In addition, many of the so-called primary metabolites of microorganisms, *i.e.* substances the production of which is coupled to growth, are important industrial products. These include ethanol, acetic acid and carbon dioxide. And third, although the production of so-called secondary metabolites such as penicillin and amino acids begins when growth stops or slows down, the rate of this production depends on the amount of active biomass accumulated in the preceding growth phase of the culture.

For all these processes then, the growth yield, defined as the amount of biomass in grams produced per amount of substrate consumed (in grams of substrate), appeared to be the important property to maximize. In terms of its composition, biomass is similar to carbohydrates. A chemical engineer might therefore write a chemical reaction equation for growth as follows:

$$C_6H_{12}O_6 \rightarrow 6/m(CH_2O)_m$$

In this equation, the yield in terms of moles of carbon in the biomass and moles of carbon in the substrate equals 1. This should make it possible to calculate the expected growth yield on the basis of the molecular formulas of the substrate and the biomass (which implies a correction away from the otherwise expected yield of 1, for the nitrogen, phosphorous, sulfur, etc. present in the biomass but not in the substrate). In Chapter 9 of this book we shall encounter a modern variant of this procedure.

Experimental growth yields were much lower than this. This lack of yield was thought to be due to three reasons. First, growth does not only require