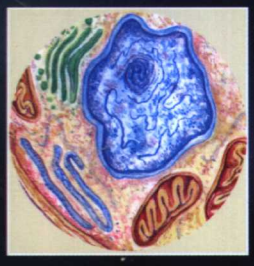
国外生命科学优秀教材

An Introduction to

Metabolic and Cellular Engineering

代谢与细胞工程导论

影印版



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内 容 简 介

本书阐述了将两大技术遗传分子生物学技术与发酵技术有机联系在一起，将各学科如微生物生理学、生物能学、热动力学和酶动力学、生物数学与生物化学、遗传学与分子生物学的方法整合协调，形成跨学科的方法，经过合理设计，以某种方式最大限度地利用微生物或细胞。内容包括：物质与能量平衡、细胞生长与代谢产物、生物过程动力学、植物细胞发育、细胞工程等等。

本书可作为生物工程、生物技术、生物医学工程等专业本科生、研究生教材，还可供生物工程技术人员以及相关科研人员参考。

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To Juan Ernesto, Nehuen Quimey, Raul and Pedro. To Gladys (in memoriam).
To Francisca, Maris and Juan Carlos. To Miguel (in memoriam).
To Silvia. To Norberto (in memoriam).

Preface

Metabolic and Cellular Engineering, although as yet only at a beginning, promises huge advances in all fields of the life sciences. The main aim of this book is to introduce students and research workers into this exciting new endeavor. To show a complete picture of the subject, we introduce the main techniques available in the field, in order to point out their power, facilitate their mastery, interpret the achievements already published, and challenge our readers with new problems.

Our own research interests have led us to the elaboration of a wider view on the emergent field of Metabolic Engineering. Thus, here we review the field in order to give a state-of-the-art account. However, in doing this we have selected examples, experiments, and puzzles that, in our opinion, accurately reflect the main advances, achievements, and unsolved problems. So a prospective for the field has also emerged. This book also pretends to be useful to those experimentalists and theoreticians who wish to project themselves into a field that offers great challenges, either experimental or theoretical, for massive integration of the available information.

Until 1960s, metabolic regulation was mainly investigated in isolated and cell-free systems. At present, biotechnology mainly deals with intact cells, and we therefore need to understand how enzymatic reactions behave and are regulated inside the cell. From this standpoint, major limitations arise from the lack of understanding of the behavior of metabolic networks. More precisely; on the one hand, geneticists and molecular biologists produce schemes to explain regulation of gene expression, e.g. by DNA-binding proteins, and on the other hand knowledge of the functioning of metabolic pathways is in some cases fairly complete. However, the link between these two aspects is poorly understood.

Metabolic and Cellular Engineering emphasizes the microorganism (e.g. enzyme function, transport, regulation) and its modification to improve cellular activities, through the use of recombinant DNA. Nevertheless, we assume that the level of performance of the recombinant cells thereby obtained must be evaluated within the context of a specific biotransformation. Thus **Metabolic and Cellular Engineering** is bred of a powerful alliance of two disciplines: Genetics-

Molecular Biology and Quantitative Biochemistry and Physiology. Both are driven by continuous refinement of basic understanding of metabolism, physiology, cellular biology (growth, division, differentiation), and the development of new mathematical modeling techniques.

We hope that, even if our aim is minimally attained, then those who have read the book will feel stimulated enough to engage in the field to themselves make new contributions.

The main material of the present book as well as its general structure originated, in part, from a series of lectures given by the authors in the framework of an international course for postgraduate students "Principles of Bioprocess and Metabolic Engineering" supported by the binational centre CABBIO (Centro Argentino-Brasileño de Biotecnología) held at the end of the year 1998 in Chascomús, Buenos Aires. We would like to gratefully acknowledge the participation in that course of Dr. Claudio Voget (Universidad Nacional de La Plata, Argentina) and Dr. Juan Carlos Aon (MIT, USA) for their contribution to the subject of mass and energy transfer and fermentation technology, respectively.

The contribution of the following people for enlightening and useful discussions is gratefully acknowledged: Sam Vaseghi (CITAG, Hamburg, Germany), late Manfred Rizzi (University of Stuttgart, Germany), Marta Cascante (Universitat de Barcelona, Spain), Francesc Mas (Universitat de Barcelona, Spain), Luis Acerenza (Facultad de Ciencias, Uruguay), Carlos E. Argaraña (Universidad Nacional de Córdoba, Argentina), Matthias Reuss (University of Stuttgart, Germany), A.H. Stouthamer (Free University Amsterdam, The Netherlands), Carlos Mignone (Universidad de La Plata, Argentina), Daniel Guebel (Universidad Nacional de Quilmes, Argentina), Nestor V. Torres Darias (Universidad de La Laguna, Spain).

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Chascomús, Buenos Aires
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List of Abbreviations

AcCoA	acetyl Coenzyme A
ADPGlc	ADP glucose
ADPGlcPPase	ADP glucose pyrophosphorylase
αKG	α ketoglutarate
ADH	alcohol dehydrogenase
ATPase	ATP hydrolase
BST	Biochemical system theory
CDC	cell division cycle
CER	CO_2 evolution rate
CSTR	continuously stirred tank reactor
CAM	crassulacean acid metabolism
2DG	2-deoxyglucose 2DG
DAHP	3-deoxy-D-arabino-heptulosonate-7-phosphate
DHAP	dihydroxyacetone-P
D	dilution rate
DBA	Dynamic bifurcation analysis
E4P	erythrose 4 phosphate
EPSP	5-enolpyruvylshikimate-3-phosphate
C_{Ek}^{Ji}	Flux control coefficient
FCH	Flux coordination hypothesis
FBPase	fructose-1,6-bisP phosphatase
G6P	glucose 6 phosphate
GAP	glyceraldehyde-3P
GC/MS	gas chromatography/mass spectrometry
GAPDH	glyceraldehyde 3 phosphate dehydrogenase
HK	hexokinase
K_m	Michaelis-Menten constant in enzyme kinetics
MCE	Metabolic and cellular engineering
MCA	Metabolic control analysis
MFA	Metabolic flux analysis

C_{Ek}^{Mi}	Metabolite concentration control coefficient
μ	growth rate
MTP	microtubular protein
m_S	maintenance coefficient
NMR	nuclear magnetic resonance
NADP GDH	NADP dependent glutamate dehydrogenase
OAA	oxalacetate
ODE	ordinary differential equations
OUR	oxygen uptake rate
PP pathway	pentose phosphate pathway
PEP	phosphoenolpyruvate
PEPCK	phosphoenolpyruvate carboxy kinase
PEPCase	phosphoenolpyruvate carboxylase
PFK	phosphofructokinase
PGI	phosphoglucoisomerase
3PG or 3PGA	3 phosphoglycerate
PGK	phosphoglycerokinase
PGM	phosphoglyceromutase
PTS	phosphotransferase system
PEG	polyethylene glycol
PY	pyruvate
qCO_2	specific rate of carbon dioxide production
qEtOH	specific rate of ethanol production
qGlc	specific rate of glucose consumption
qO_2	specific rate of oxygen consumption
R5P	ribose 5 phosphate
RPPP	reductive pentose phosphate pathway
RQ	respiratory quotient
Cp	specific heat
TP	triose phosphate
TDA	Transdisciplinary approach
TCA cycle	tricarboxylic acid cycle
V_{max}	maximal rate in enzyme kinetics
Y_{ATP}	yield of biomass on ATP
Y_{O2}	yield of biomass on oxygen
Y_{PC}	yield of product on carbon substrate
Y_{XC} or Y_{XS}	yield of biomass on carbon substrate
γ_B	degree of reduction of biomass
γ_S	degree of reduction of substrate

Contents

Chapter 1

Introduction

Introductory Outlines

In the present book we aim to develop ideas that allow us to confront and solve many problems in Metabolic and Cellular Engineering (MCE). This is not yet a mature speciality, as it is a new area at a meeting point of several disciplines. Evidence for this is provided by the fact that many of the qualitative and quantitative methodologies used are still under development, although in the past ten years striking progress has been achieved (Stephanopoulos *et al.*, 1998; Lee and Papoutsakis, 1999). Nevertheless, there is not yet a well established link between the different disciplines and techniques that are employed in these problems; major inputs come from Molecular Biology, Fermentation Technology and Mathematical Modeling.

We intend to go beyond the assertion that "At present, metabolic engineering is more a collection of examples than a codified science" (Bailey, 1991). However, a valuable and rich experience has accumulated following the explosive development of MCE in the last years. This experience not only reflects the specific achievements (e.g. production of metabolites and heterologous proteins, introduction of heterologous metabolic pathways into microorganisms to give them the ability to degrade xenobiotics, modification of enzymatic activities for metabolite production) but has also allowed us to understand previously unknown aspects of cell function and the regulation of networks of chemical reactions inside cells. The latter places MCE at the interphase between basic and technological research through an iterative, self-correcting and self-fed process for solving the multiple challenges posed by the present developments in biotechnology.

Metabolic and Cellular Engineering in the Context of Bioprocess Engineering

Traditionally, bioprocesses are the bases of the food and pharmaceutical industries. The operation of bioprocesses deals with microbial, plant or mammalian cells, or their components such as enzymes, which are used in the manufacturing of new products, or for the degradation of toxic wastes. The use of microorganisms for the production of fermented foods has a very long history. Since early times, many different bioprocesses have been developed to give an enormous variety of commercial products from cheap ones (e.g. ethanol or organic solvents) to expensive ones (e.g. antibiotics, therapeutic proteins or vaccines). Enzymes and microorganisms such as bakers' yeast are also commercial products which are obtained through different bioprocesses.

Recently, the bases of the broad and highly multidisciplinary field of Metabolic Engineering (ME) have been established (see Bailey, 1991; Cameron and Tong, 1993; Farmer and Liao, 1996; Cameron and Chaplen, 1997; for reviews). ME within the context of Bioprocess Engineering constitutes a thorough transdisciplinary effort toward the development of rationally-designed cells with specific biotransformation capabilities. This transdisciplinary effort requires the participation of scientists with different strengths or abilities in their scientific backgrounds.

The research field of ME was highlighted as an exciting new endeavor in biotechnology in the 1998 International Conference. As a new approach for rationally designing biological systems it is becoming ever more important for biotechnological production processes and medicine. ME can be defined as the introduction of specific modifications to metabolic networks for the purpose of improving cellular properties. Because the challenge of this interdisciplinary effort is to redesign complex biosystems, a rigorous understanding of the interactions between metabolic and regulatory networks is critical. At this point we will adopt the notation Metabolic and Cellular Engineering (MCE) all throughout the book, since it describes more accurately the panoply of activities being undertaken in the field (see below). As such, an important component of MCE is the emphasis of the regulation of metabolic reactions in their cellular entirety (i.e. in the whole organism). This goal differentiates the field from those related areas of life science that adopt the reductionistic approach. Concepts and methodologies of MCE have potential value in the direct application of metabolic design of cellular systems for biotechnology production processes. These include cell-based processes as well as gene therapies, and degradation of recalcitrant

pollutants. They promise a great impact on many areas of medicine (Yarmush and Berthiaume, 1997).

At present the following subjects are actively researched in MCE (Cameron and Tong, 1993; Lee and Papoutsakis, 1999) (see below):

1. Experimental and Computational Tools.
2. Applications to the Production of Pharmaceuticals.
3. Applications to Fuels and Chemicals.
4. Biomaterials.
5. Applications to Plants.
6. Applications to Production of Proteins.
7. Evolutionary Strategies for Strain Improvement via ME
8. Medical Applications and Gene Therapy.
9. Higher Level Metabolic Engineering through Regulatory Genes.
10. Environmental Applications.

Tools for Metabolic and Cellular Engineering

MCE requires the development of several tools in the various disciplines contributing to the field. The area of molecular biology needs:

1. Transformation systems for microorganisms used in industrial production, or in bioprocesses (e.g. for *Corynebacterium*, commonly used for the production of aminoacids, or for Pseudomonads, currently used in the degradation of xenobiotics) (see Keasling, 1999, for a review of gene expression tools in bacteria).

2. Promoters and special vectors used in such transformations: e.g. the yeast retrotransposon Ty3 employed for site-specific integration of heterologous genes with the advantages of stability and high copy number (Wang and Da Silva, 1996), or the filamentous fungus vector *Agrobacterium* able to transform the genera *Neurospora*, *Trichoderma*, *Aspergillus* and *Agaricus* (de Groot *et al.*, 1998).

3. Multicistronic expression vectors to allow one-step multigene metabolic engineering in mammalian cells (Fussenegger *et al.*, 1999).

4. Methods for stabilizing cloned genes, e.g. by integration into the chromosomes of host organisms.

5. Markers to search for and analyze metabolic pathways

The microbiological and analytical tools allow the evaluation of the effectiveness of the modified metabolic pathway:

1. Culture of the modified microorganism. In this respect, the ideal culture system enabling the application of mathematical and computational tools (see below) is continuous culture. However, it may happen that the stability of the genetically modified microorganism precludes the possibility of continuous maintenance of the culture in the long-term. In this case either fed-batch or batch systems have to be used.

2. Optimization of growth medium suitable for the operation of the desired metabolic pathway. For instance, an organism (*Serratia* spp.) modified with the bacterial hemoglobin gene (vgb) that is supposed to improve growth and to avoid by-product formation, has been reported to display various fermentation patterns according to the medium composition (Wei *et al.*, 1998).

3. Mass balance. This allows calculation of the yield of the desired product with respect to various substrates, and by comparison with maximal theoretical yields, evaluation of how far from the thermodynamic limit the metabolic pathway operates.

4. Isotopic labeling and analysis of blocked mutants as well as the determination of enzyme activities and metabolites, allow the determination of the effectiveness of operation of a given metabolic pathway contributing to the consumption of a certain substrate, or the formation of a required product.

5. The employment of non-invasive methods such as nuclear magnetic resonance and flow cytometry are preferred since they allow a direct evaluation of the performance of the microorganism under conditions similar to or identical with those in the industrial bioprocess.

With respect to the mathematical and computational tools the following considerations are important:

1. DNA data bases and software (Overbeek *et al.*, 2000; Covert *et al.*, 2001; see also below).

2. Metabolic pathways data bases, including kinetic and thermodynamic enzyme data. In this respect, several Internet sites are now available (Karp, 1998; Overbeek *et al.*, 2000; Covert *et al.*, 2001).

3. Tools designed for estimation of theoretical yields (e.g. from the metabolic pathway stoichiometry). This point is developed in Chapters 2 and 4.

4. Tools for the design of metabolic pathways. Several algorithms have been proposed for this purpose (Hatzimanikatis *et al*, 1996).

5. Quantitative tools for the simulation and prediction as well as analysis of the performance of the modified microorganism (e.g. Metabolic Control Analysis (MCA), Biochemical System Theory (BST) and Metabolic Flux Analysis (MFA)). These methods encompas a series of stoichiometric and linear