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Future Trends in Biotechnology

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With contributions by

M. Bardor · K. F. Chan · C. Furusawa · X. Han
R. Haryadi · T. Hirasawa · T. Horinouchi · K. Kang
Y. Lee · G. Qi · H. Shimizu · Z. Song · L. Wang · X. Xu
Y. Yong · P. Zhang · Y.-H. P. Zhang · J. J. Zhong

Springer

Editor
Jian-Jiang Zhong
State Key Laboratory of Microbial Metabolism
School of Life Sciences and Biotechnology
Shanghai Jiao Tong University
Shanghai
People's Republic of China

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Aims and Scope

This book series reviews current trends in modern biotechnology and biochemical engineering. Its aim is to cover all aspects of these interdisciplinary disciplines, where knowledge, methods and expertise are required from chemistry, biochemistry, microbiology, molecular biology, chemical engineering and computer science.

Volumes are organized topically and provide a comprehensive discussion of developments in the field over the past 3–5 years. The series also discusses new discoveries and applications. Special volumes are dedicated to selected topics which focus on new biotechnological products and new processes for their synthesis and purification.

In general, volumes are edited by well-known guest editors. The series editor and publisher will, however, always be pleased to receive suggestions and supplementary information. Manuscripts are accepted in English.

In references, Advances in Biochemical Engineering/Biotechnology is abbreviated as *Adv. Biochem. Engin./Biotechnol.* and cited as a journal.

Preface

The title of this volume may appear a bit too ambitious to some readers. Biotechnology has been developing so fast in recent decades and has had a great impact on our life and society by addressing various problems including environmental pollution, ecological protection, energy issues, and public health. It is therefore almost impossible for a single volume to provide topics covering the current development and future trends in all aspects of biotechnology. Nevertheless, for this volume we have selected a number of interesting topics from a few important areas of biotechnology. These contributions are based on talks which were held at the 1st Asian Congress on Biotechnology (ACB) in 2011 which was organized under Asian Federation of Biotechnology (AFOB) (http://www.afob.org/) [1] to acknowledge the significant advances in biotechnology innovation and applications.

In order to establish a sustainable society, not reliant on using fossil resources, bioconversion technologies which turn biomass resources into valuable materials have received great attention in recent years. To improve cellular properties for high productivity and high yield production of desired products, the metabolic engineering approach is very useful [2]. Here, optimization of metabolic pathways of cells and creation of stress tolerant cells are important [3]. Prof. Hiroshi Shimizu and his coworkers from Osaka University describe multi-omics information analyses and rational design methods for molecular breeding ("Systems Metabolic Engineering: The Creation of Microbial Cell Factories by Rational Metabolic Design and Evolution").

Recent studies indicate that bacteria usually coordinate their behaviors at population level by producing, sensing, and responding to small signal molecules. This so-called quorum sensing regulation enables bacteria to live in a 'society' with cell–cell communication, and controls many important bacterial behaviors [4]. Profs. Jian-Jiang Zhong and Yang-Chun Yong from Shanghai Jiao Tong University and Jiangsu University review quorum sensing signals and their impacts on microbial metabolism and human health ("Impacts of Quorum Sensing on Microbial Metabolism and Human Health"). Quorum sensing plays an important role both in bacteria

directly and human beings, and a better understanding of this phenomenon would lead to better control of bacteria.

The next two chapters are related to biomanufacturing, which is defined as the manufacture of desired products using living biological organisms (e.g., bacteria, yeasts, animal cells) or some components from one or several biological organisms [5]. Chinese hamster ovary (CHO) cells are the current industrial workhorse for manufacturing the majority of leading recombinant biologics. Glycosylation is an important characteristic of CHO cells, which decorate protein or lipid backbones by carbohydrate moieties, leading to a wide range of bioactive end products. Dr. Zhiwei Song and his colleagues from the Bioprocessing Technology Institute of Singapore describe CHO glycosylation mutants as potential host cells to produce therapeutic proteins with enhanced efficacy ("CHO Glycosylation Mutants as Potential Host Cells to Produce Therapeutic Proteins with Enhanced Efficacy"). In light of the critical impact of glycosylation on biopharmaceutical performances (safety and efficacy), the CHO glycosylation mutants have enormous potential in producing glycoprotein therapeutics with optimal glycosylation profiles, which result in improved safety profile and enhanced efficacy [6].

Another type of biomanufacturing platform is the cell-free biosystem, which is very different from those of the above three chapters. Prof. Y.-H. Percival Zhang and Mr. Chun You from Virginia Tech summarize cell-free biosystems for biomanufacturing ("Cell-Free Biosystems for Biomanufacturing"). Cell-free biosystems are becoming an emerging biomanufacturing platform in the production of low-value biocommodities, fine chemicals, and high-value protein and carbohydrate drugs and their precursors. They believe that cell-free biosystems could become a disruptive technology to microbial fermentation, especially in the production of high-impact low-value biocommodities. This is mainly due to very high product yields and potential low-production costs [7].

"Lipid Bilayer Membrane Arrays: Fabrication and Applications" is a contribution describing the lipid bilayer of biomembranes, which is a universal component of all cell-based biological systems, forming the barrier between cytosol and the cell's exterior, as well as mediating many biological functions by providing a defined interface for cell-surface recognition, signaling, and transport. The importance of the lipid bilayer has raised much interest in fabricating artificial membrane as both free standing lipid membranes and solid supported lipid bilayer membranes. Prof. Xiaojun Han and his coworkers from the Harbin Institute of Technology describe the formation of bilayer lipid membrane arrays. The applications of lipid bilayer arrays are reviewed in the account of biosensors, protein binding studies, and lipid bilayer-based 2D electrophoresis [8].

The last chapter is about RNA aptamers, which are RNA molecules binding target molecules. Those small oligonucleotides derived from the in-vitro selection process are important candidates for therapeutics and diagnostics due to their high affinity and specificity against their target molecules. Prof. Yoon-Sik Lee and Dr. Kyung-Nam Kang from Seoul National University summarize recent trends and applications of RNA aptamers ("RNA Aptamers: A Review of Recent Trends and Applications"). As the global market for aptamers is expected to grow (about

$1.8 billion by 2014), research into the therapeutic and diagnostic applications of RNA aptamers seems to be increasing continuously [9].

Finally, I would like to thank all the authors for their excellent contributions to this book. The kind advice from Professor Thomas Scheper and great assistance from Ms. Karin Bartsch (Project Coordinator), Ms. Elizabeth Hawkins (Editor Chemistry), and other related staff at Springer are certainly appreciated. I do hope readers will enjoy this volume and provide suggestions and comments to me and the other authors.

Shanghai, 2012 Jian-Jiang Zhong

References

1. Asian Federation of Biotechnology. Asian Congress on Biotechnology, http://www.afob.org/
2. Nielsen J (ed) (2005) Biotechnology for the future. Advances in biochemical engineering/biotechnology, vol 100. Springer, Heidelberg.
3. Furusawa C, Horinouchi T, Hirasawa T and Shimizu H (2013) Systems metabolic engineering: the creation of microbial cell factories by rational metabolic design and evolution. Adv Biochem Eng Biotechnol 131:1–23
4. Yong Y-C, Zhong J-J (2013) Quorum sensing and its impact on microbial metabolism and human health. Adv Biochem Eng Biotechnol 131:25–61
5. Zhong JJ, (ed) (2004) Biomanufacturing. Advances in biochemical engineering/biotechnology, vol 87. Springer, Heidelberg.
6. Zhang P, Chan KF, Haryadi R, Bardor M and Song Z (2013) CHO glycosylation mutants as potential host cells to produce therapeutic proteins with enhanced efficacy. Adv Biochem Eng Biotechnol 131:63–87
7. You C, Zhang Y-HP (2013) Cell-free biosystems for biomanufacturing. Adv Biochem Eng Biotechnol 131:89–119
8. Han X, Qi G, Xu X, Wang L (2013) Formation of bilayer lipid membrane arrays and its application. Adv Biochem Eng Biotechnol 131:121–152
9. Kang K-N and Lee Y-S (2013) RNA aptamers: recent trends and applications. Adv Biochem Eng Biotechnol 131:153–169

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Systems Metabolic Engineering: The Creation of Microbial Cell Factories by Rational Metabolic Design and Evolution

Chikara Furusawa, Takaaki Horinouchi, Takashi Hirasawa and Hiroshi Shimizu

Abstract It is widely acknowledged that in order to establish sustainable societies, production processes should shift from petrochemical-based processes to bioprocesses. Because bioconversion technologies, in which biomass resources are converted to valuable materials, are preferable to processes dependent on fossil resources, the former should be further developed. The following two approaches can be adopted to improve cellular properties and obtain high productivity and production yield of target products: (1) optimization of cellular metabolic pathways involved in various bioprocesses and (2) creation of stress-tolerant cells that can be active even under severe stress conditions in the bioprocesses. Recent progress in omics analyses has facilitated the analysis of microorganisms based on bioinformatics data for molecular breeding and bioprocess development. Systems metabolic engineering is a new area of study, and it has been defined as a methodology in which metabolic engineering and systems biology are integrated to upgrade the designability of industrially useful microorganisms. This chapter discusses multi-omics analyses and rational design methods for molecular breeding. The first is an example of the rational design of metabolic networks for target production by flux balance analysis using genome-scale metabolic models. Recent progress in the development of genome-scale metabolic models and the application of these models to the design of desirable metabolic networks is also described in this example. The second is an example of evolution engineering with omics analyses for the creation of stress-tolerant microorganisms. Long-term culture experiments to obtain the desired phenotypes and omics analyses to identify the phenotypic changes are described here.

Keywords Systems Biotechnology · Strain improvement · Constraint-based flux balance analysis · Experimental evolution

C. Furusawa · T. Horinouchi · T. Hirasawa · H. Shimizu (✉)
Department of Bioinformatic Engineering, Osaka University, 1-5 Yamadaoka, Suita Osaka, 565-0871, Japan
e-mail: shimizu@ist.osaka-u.ac.jp

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1 General Introduction

Microorganisms are used as cell factories for producing valuable chemical compounds such as various primary and secondary metabolites [5, 43, 53, 55, 65] and recombinant proteins [62, 67], all of which play important roles in the pharmaceutical, chemical, and agricultural industries. One of the goals of microbial cell factory development is the establishment of cheap and high-yield bioprocesses to meet commercial requirements. To achieve this, modifications of the metabolic system of the host microorganism are typically required to improve the productivity of target products. This is because the metabolic systems of natural microorganisms are already tuned to optimize their growth in their natural habitat [83]. To acquire the cellular properties necessary for achieving high productivity and high production yield of the target product, the microorganism can be tailored so that it has the following two characteristics [81]. First, the metabolic system of the microorganism should be optimized to maximize production yield and eliminate byproduct formation. Moreover, the balance of coenzyme production and consumption—for example, the recycling of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (NAD)—should also be designed so that optimal target production yield is achieved [16, 60, 101]. Second, the cells should be able to tolerate the environmental stresses encountered during the production process. Such environmental stresses include exposure to acids, high/low temperature, high osmotic pressure, and so forth—all of which can reduce cellular growth and target productivity. Therefore, tolerance to environmental stresses is essential for improving bioprocesses [3, 12, 44, 114]. Classical strain improvement techniques have traditionally been used to modify metabolic systems and achieve the desired properties. For example, microorganisms with superior production capabilities have been developed through random mutagenesis following appropriate screening procedures [80]. The properties of metabolic systems have also been improved by molecular biology techniques, resulting in increased target productivity. However,

conventional approaches have not always been successful due to unexpected changes in the metabolic systems caused by various perturbations. Such unexpected responses could arise from the complexity of intracellular systems in which networks of metabolites, genes, and proteins are interconnected to form multi-hierarchical complex networks. Thus, for the rational design of microbial metabolic systems suitable for bioproduction, proper understanding of the systems is necessary.

Recent advances in experimental techniques have facilitated the comprehensive analysis of different cellular states. Vast amounts of omics data are now available, which have improved our understanding of cellular processes [6, 8, 106, 115]. For example, rapid developments in sequencer and DNA handling technologies have allowed routine sequencing of the whole genome of microorganisms [15, 52, 54]. The availability of such rapid and inexpensive genome sequencing technology has had a great impact on the analysis of genotype-phenotype relationships; for example, it has enabled the identification of mutations responsible for specific phenotypes such as stress tolerance [11]. Similarly, comprehensive gene expression analysis by microarrays and protein expression analysis by 2D electrophoresis or mass spectroscopy have provided detailed information on the cellular state and its dynamics [2, 40–42, 99]. Therefore, it is reasonable to expect that appropriate integration of these high-throughput omics data would yield a system-level understanding of complex cellular processes and that this would lead to a new era of biotechnologically useful microorganisms capable of high productivity of target products [22, 33, 63, 69, 81, 84].

In this review, we present two recent approaches in systems biotechnology. Both studies are based on omics data analysis and are aimed at strain improvement for the purpose of bioproduction. The first approach is constraint-based flux balance analysis using genome-scale metabolic models [77]. This allows the prediction of metabolic profiles of microorganisms by in silico simulations and permits the screening of candidate genes that may be manipulated to improve target productivity [30]. The second approach is strain improvement through experimental evolution [87]. After obtaining the evolved microbial strains, omics analysis can be used to analyze genetic/phenotypic changes that have occurred in the evolutionary process. This analysis provides an in-depth understanding of cellular processes, such as the mechanism of stress tolerance. Such systems analyses provide new information on metabolic systems and in turn facilitate the engineering of strains suitable for industrial applications.

2 Genome-Scale Metabolic Models for Strain Improvement

2.1 Introduction

Each microorganism differs in terms of its metabolic capabilities for producing target metabolites because each has evolved for survival in its natural habitat. Therefore, when using microorganisms to produce valuable metabolites, it is often necessary to

retrofit the metabolic system of the host to obtain the desired phenotype. Genetic engineering techniques permit the modification of metabolic systems through the addition or deletion of genes responsible for metabolic reactions. Engineered microorganisms can be obtained through such gene modifications, and these can be used to improve the productivity of target products. This approach is referred to as the metabolic engineering approach [59, 100]. However, due to the complexity of the metabolic system, it is still difficult to "design" an appropriate metabolic network by gene modifications. For example, several key metabolites such as ATP, NADH, and glutamate act as "hubs" of complex metabolic networks, and the effects of local perturbations by gene modifications on the metabolic network can be propagated throughout the network by these key metabolites [14]. Therefore, to design a metabolic network with high productivity, metabolic systems must be analyzed as a whole, which would require huge inputs in terms of experiments, knowledge, and intuition. Against this background, it is evident that to accelerate strain improvement by metabolic engineering, it would be highly desirable to use genome-wide in silico simulations to predict the metabolic state after genetic modification and under varying environmental conditions.

Recently, on the basis of whole-genome information, genome-scale metabolic networks of cells have been reconstructed for many organisms, including representatives of each of the three major domains of life, namely, archaea [35], bacteria [36, 73, 75, 95], and eukarya [28, 29, 94]. At present, more than 50 genome-scale metabolic reconstructions have been published [72]. Using genome-scale metabolic models, it is possible to reliably estimate metabolic fluxes by the flux balance analysis (FBA) method [34, 76, 77]. FBA is an analysis of metabolic flux profiles in which a steady state of metabolic flux is assumed, and the profile of metabolic fluxes is calculated by optimizing an objective function using linear programming. Although genome-scale metabolic models cannot compute the detailed kinetic dynamics of metabolic reactions in a cell, they permit the description of a range of possible metabolic states on the basis of constraints defined by the stoichiometry of metabolic reactions and transport steps at the steady state. It is also possible to obtain a solution (i.e., a set of all metabolic fluxes) that maximizes or minimizes an objective function using linear programming. The biomass production rate is generally adopted as the objective function. The metabolic profiles calculated by the maximization of biomass production can describe those obtained experimentally in many organisms and under various environmental conditions, suggesting that organisms can maximize their growth rate by adaptation and evolution [38, 46]. By using the appropriate genome-scale metabolic network and objective function to be maximized, FBA can be used to predict the relationship between the genotype, environmental conditions, and product yields at the steady state; this data can then be used to improve microbial production [3, 61].

In the next section, we focus on in silico metabolic simulations using genome-scale metabolic models and experimental evaluations. We also address the possible applications of model prediction to metabolic engineering.

2.2 Reconstruction of Genome-Scale Metabolic Models

In general, genome-scale metabolic models of target organisms have been manually built by the following two steps. First, a draft metabolic model is constructed based on public databases such as KEGG [51] and the BioCyc database collection [21], which collects metabolic reactions occurring in the target organism based on gene annotation data. Second, the draft model is further tuned based on available literature because a draft model constructed using only public databases generally contains incorrect and insufficient metabolic pathways due to the incompleteness of database information. The most frequently observed problem is that of missing enzymes in metabolic pathways producing essential components. Therefore, the resulting network is next subjected to the gap-filling process to adequately represent cellular growth [103]. To fill the gaps, data from literature and homology search results of genomes that are closely related to the target organism are generally used. In some cases, metabolic reactions are added to the metabolic model without any background information to realize cellular growth. It should be noted that the benefit of the gap-analysis process is that it can provide indicators of missing enzymes, i.e., enzymes that probably exist in the target organism but have not been identified. After the construction of the metabolic network, to calculate metabolic fluxes as presented below, it is converted into a stoichiometric matrix SS containing the stoichiometry of all reactions in the network. If a given network is composed of m molecular species involved in n reactions, then the stoichiometry matrix is an $m \times n$ matrix. The element S_{ij} of the stoichiometric matrix represents the contribution of the jth reaction to the ith metabolite.

In addition to reconstructing the metabolic network of a target organism, several parameters representing cellular processes are also necessary for in silico simulations using the genome-scale metabolic model [103]. One such essential parameter is the coefficient of the biomass synthesis reaction. This reaction is hypothetical and represents the requirements of precursors and coenzymes for biomass formation. Biomass synthesis involves the linear combination of dozens of components, including amino acids, DNA, RNA, lipids, and cell envelope components. In general, the biomass composition is determined from reported cellular data such as the compositions of amino acids and other macromolecules (DNA, RNA, protein, lipids, and so forth). Other important parameters that are representative of cellular processes, such as the ATP maintenance costs and P/O ratio, are also determined from the reported data. In the FBA scheme, the reconstructed genome-scale metabolic model and parameters for cellular processes are used to estimate the metabolic flux profiles by the linear algebra-based method presented below.

2.3 Constraint-Based Flux Analysis

Detailed information on the kinetic parameters of enzymatic reactions is unavailable at the genome-scale level; therefore, in silico simulations of the kinetic behavior of metabolic reactions have been difficult so far. Instead, in the FBA

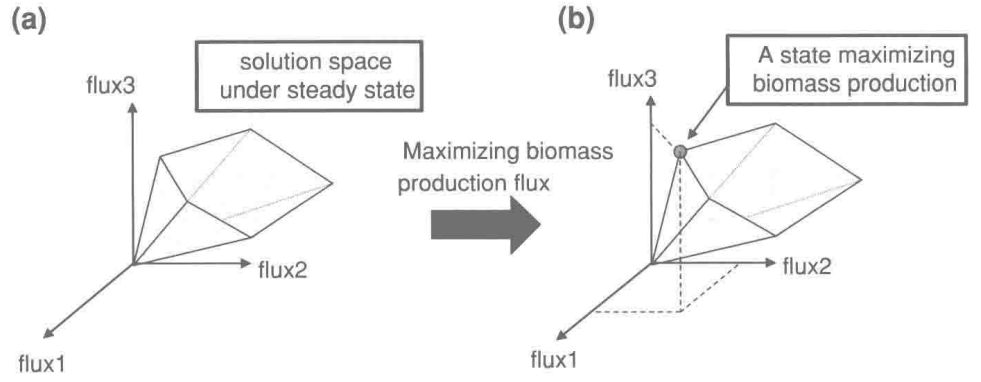

Fig. 1 Schematic representation of the constraint-based flux balance analysis. The axes represent metabolic fluxes. **a** By applying the steady state assumption, we can obtain the feasible solution space. **b** If the biomass production flux is used for an objective function, optimal solutions that maximize the objective function can be calculated by linear programming

scheme, the metabolic flux profile is determined by employing the following assumptions [30].

The first is the steady state assumption in which the concentrations of all metabolites are assumed to remain unchanged over time. In other words, the net sum of all production and consumption fluxes for each internal metabolite is set to zero. This assumption results in a feasible space that is a convex set in the N-dimensional space of metabolic fluxes (where N represents the total number of fluxes), as schematically shown in Fig. 1a. This steady state assumption can be mathematically represented as $S \cdot v = 0$, where S is the stoichiometric matrix and v is a vector of fluxes through the metabolic network. Furthermore, upper and lower bounds are generally employed and are mathematically represented as $v_{\min} \leq v \leq v_{\max}$, where $v_{\min}$ and $v_{\max}$ indicate the minimum and maximum constraints on the fluxes. These bounds of fluxes are used to define the constraints for the maximal enzymatic rate, irreversibility of the reaction, or constant uptake from the environment.

Based on the steady state assumption, the possible flux profile is bound within a closed finite space. However, it is not possible to determine a unique solution for the flux profile by employing only the steady state assumption. Therefore, to determine the flux profile, the second assumption (i.e., maximization or minimization of an objective function) is generally used to obtain a unique solution. The most popular objective function in FBA for obtaining the metabolic flux profile is the biomass synthesis flux, which is represented as a linear combination of metabolic fluxes of building blocks and coenzymes required for biomass synthesis, as mentioned above. Mathematically, maximization of the biomass synthesis flux is solved by linear programming, which corresponds to obtaining the optimal solution at one corner in the feasible flux space (Fig. 1b). This assumption for maximizing biomass synthesis flux is based on the understanding that organisms have evolved toward growth maximization under a given environment. Other objective functions are also used to estimate the metabolic flux profile. For example, to

calculate the metabolic shift after knocking out a metabolic reaction, the Euclidian distance to the wild-type flux profile is often used as an objective function that has to be minimized [93]. Minimization of regulatory on–off switching is also used to predict metabolic changes after gene knock-out [97].

2.4 Example: Flux Balance Analysis of Corynebacterium Glutamicum

For more detailed examination of the processes of reconstruction and experimental verification of genome-scale metabolic models, we study the flux balance analysis of *Corynebacterium glutamicum* as an example [95].

C. glutamicum is a facultatively aerobic, gram-positive bacterium that can grow on various sugars or organic acids [53, 105]. This organism shows high-efficiency production of various amino acids such as glutamate and lysine and is thus widely used for the large-scale production of amino acids [57]. The production of ethanol and organic acids such as lactate and succinate by *C. glutamicum* under oxygen deprivation conditions has been recently proposed [74]. Because of its importance in bioproduction, *C. glutamicum* is an interesting microbial host for metabolic engineering purposes. Therefore, it is highly desirable to construct and explore appropriate in silico metabolic models that can help to predict both cellular behavior and target compound production by this organism.

A genome-scale metabolic model of *C. glutamicum* was developed earlier [95], which can be described as follows. First, a draft model of this microorganism was constructed by collecting metabolic reactions from public databases such as BioCyc and KEGG as well as scientific publications. After performing the gap-filling procedure based on published data and a homology search with genomes of closely related species, a genome-scale metabolic model consisting of 277 genes, 502 metabolic reactions, and 423 metabolites was constructed. Of the total reactions, 428 from the BioCyc and KEGG database collections were included in the model, while the remaining 74 were added based on previously published studies. From the entire set of metabolic reactions, 470 corresponded to intracellular reactions, while 32 were fluxes for transport through the membrane. The biomass synthesis reactions consisted of linear combinations of 43 components, including amino acids, DNA, RNA, lipids, and cell envelope components. The biomass composition was determined from reported data; for example, the requirements of precursors such as pyruvate, acetyl-CoA, and oxaloacetate for biomass production were based on previously reported data [31]. The macromolecular composition was also determined from earlier studies [20]; the total biomass was 52 % protein, 5 % RNA, 1 % DNA, 13 % lipid, 19 % cell wall components, and 10 % other components. *C. glutamicum* cells have a characteristic cell membrane called MAPc that consists of mycolic acids and the polysaccharides peptidoglycan and arabinogalactan. The pathway for the synthesis of this membrane structure was

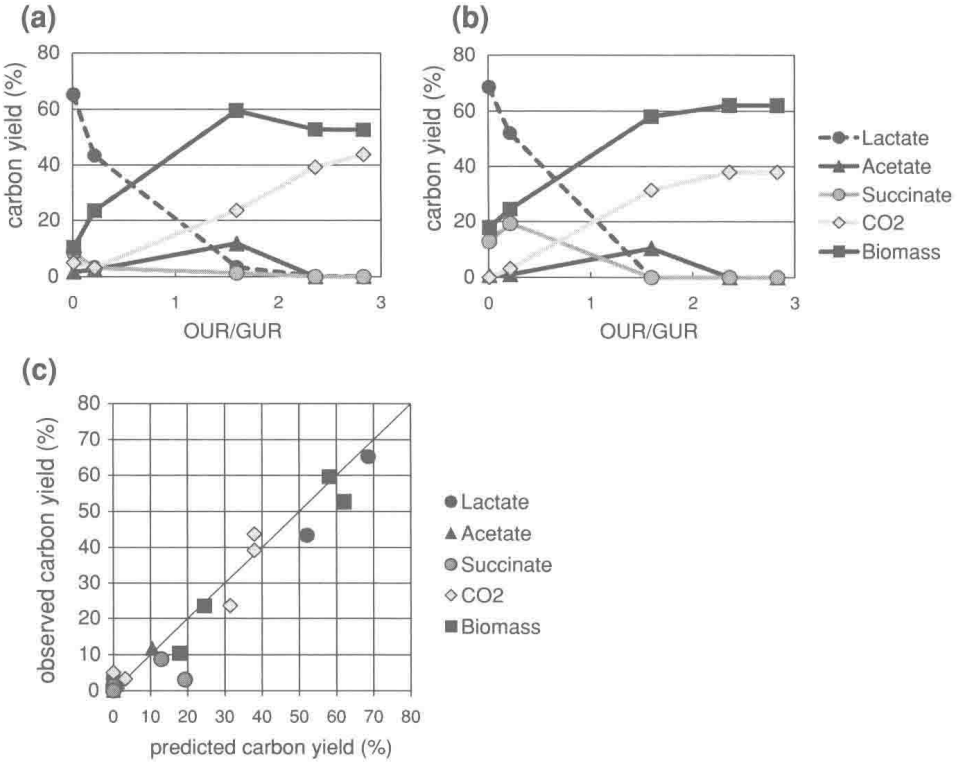

Fig. 2 Changes in the yields of organic acids, biomass, and carbon dioxide when the OUR/GUR ratio is altered. **a** Experimental results with different OUR/GUR ratios. GUR, OUR, and the production rates of CO_2, lactate, acetate, succinate, and biomass are represented in mmol/gDW/h. The values in parentheses represent the carbon yields. **b** Predictions by FBA simulations. The simulation results were obtained using the GUR and OUR values from the experimental data. **c** A scatter plot of carbon yield. The x-axis corresponds to the result of FBA simulation, while the y-axis shows the experimentally observed carbon yield. The carbon yields in the five sets of experimental and simulation results are presented. The line corresponding to $y = x$ is also shown

included in the model, while the MAPc demand in biomass production was regarded to be consistent with the cell wall composition of *C. glutamicum*.

In general, the reconstructed metabolic models and in silico simulations by these models should be verified experimentally. To construct a genome-scale metabolic model of *C. glutamicum*, a series of culture experiments were performed under different environmental conditions. In the study, the oxygen uptake rate (OUR) was used as the parameter for changing the metabolic profiles of *C. glutamicum*. This is because OUR is known to drastically alter the metabolic profile and is a key factor controlling the productivity of various compounds, such as organic acids, by the microorganism. Culture experiments with five different OURs were performed, and the glucose uptake rate (GUR), OUR, and production rates of CO_2 and organic acids were quantified, as summarized in Fig. 2a. The experimental results showed that under anaerobic and microaerobic conditions (i.e., under conditions of low OUR/GUR ratios), the cells converted most of the