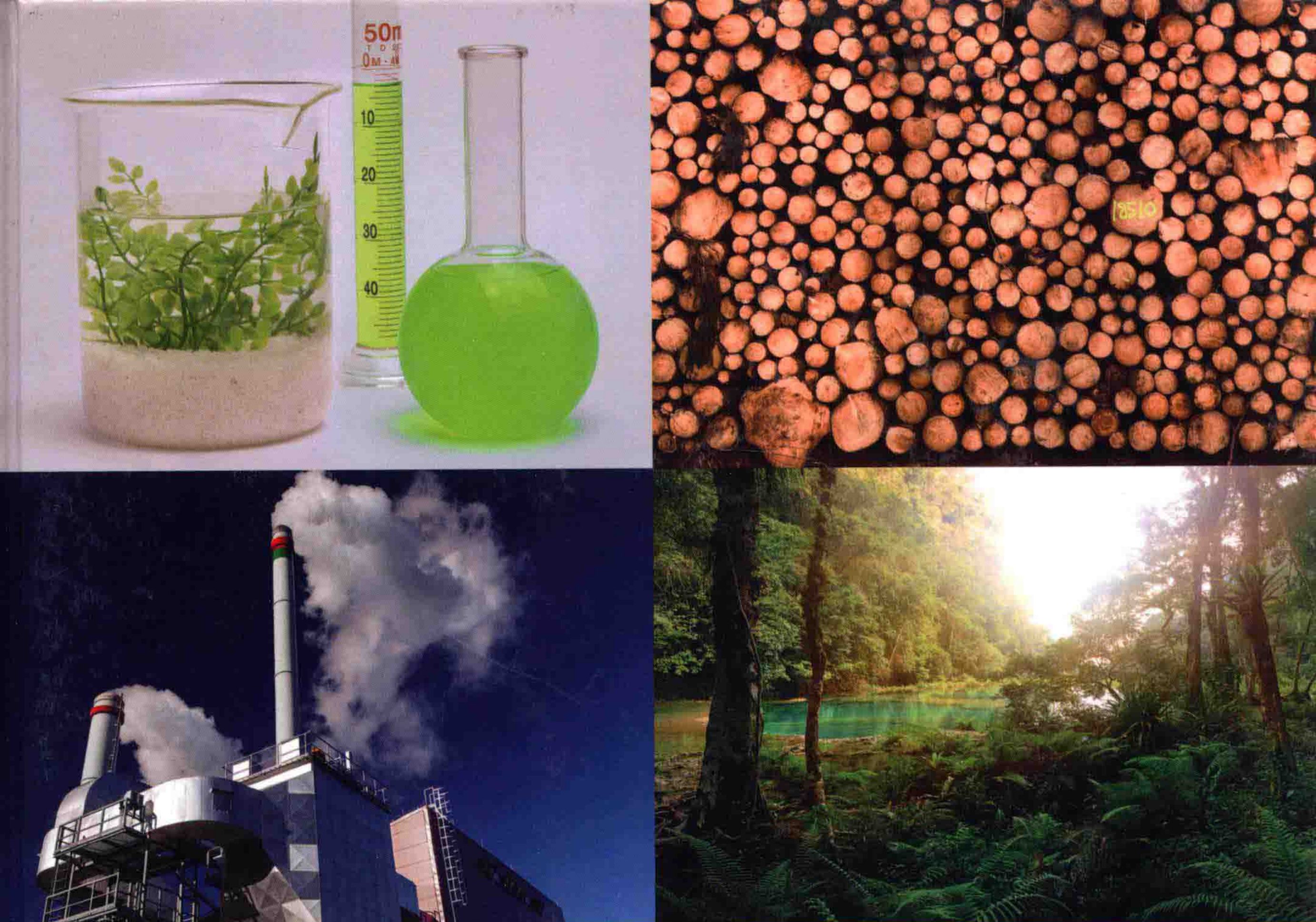

ADVANCES IN BIOENERGY

The Sustainability Challenge

Editors
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Göran Berndes • Iacovos A. Vasalos

WILEY

Advances in Bioenergy

The Sustainability Challenge

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Advances in Bioenergy

About the Editors

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John Byrne is Director of the Center for Energy and Environmental Policy (CEEP) and Distinguished Professor of Energy and Climate Policy at the University of Delaware. He has contributed to Working Group III of the United Nations-sponsored Intergovernmental Panel on Climate Change (IPCC) since 1992 and shares the 2007 Nobel Peace Prize with the Panel's authors and review editors. He holds several senior academic, advisory and policy advisor positions, among others in South Korea, China, India and the USA. His work in the US includes service as an advisor to the National Council for Science and the Environment (US) and the Interagency Working Group on Environmental Justice, coordinated by the US EPA. Dr Byrne created the first undergraduate and graduate degrees in the United States in the combined area of energy and environmental policy and a recent external review conducted by a committee of internationally respected scholars rated the CEEP graduate program among the three best in the field. He has published 17 books and over 150 research articles.

Göran Berndes is Associate Professor in Energy and Environment at Chalmers University of Technology in Sweden. Göran Berndes' research integrates land use and energy systems at scales ranging from local case studies to the global context. The research is in particular directed towards the production and use of biomass for food, energy and materials purposes. Important aspects include: (i) the effectiveness of different ways to produce and use biomass for energy, using long-term energy system modeling and Life Cycle/Well-to-Wheel analyses linked with land use change modeling and assessment of the associated flows of C; and (ii) the resource (e.g., land and water), environmental and socioeconomic implications of bioenergy strategies and also of the alternative strategy to use land for enhancement of biospheric carbon sequestration.

Iacovos A. Vasalos was a Professor at Aristotle University of Thessaloniki, Department of Chemical Engineering from 1979 to 2005. He is currently Emeritus Researcher at the Chemical Process and Energy Resources Institute (CPERI), Centre for Research and Technology Hellas (CERTH). He started his career in the Amoco Research Center in Naperville, Illinois, where he worked for 10 years from 1969 to 1979, as a research engineer, project manager, process specialist in catalytic cracking, research supervisor, and as a consultant until 1999. He is the author or coauthor of 132 scientific publications and 43 international patents many of which have been successfully applied in refineries. He was instrumental in founding and organizing CPERI and CERTH. He was also actively involved in a series of EU-funded projects in areas such as clean fossil fuels, syngas production, biofuels, environmental catalysis with emphasis on catalytic reaction engineering and modelling coupled with overall process simulation.

Preface

Bioenergy is one of mankind's most important natural energy sources. It dominated energy supply until the industrial revolution and still plays an important role in many countries. Today around 10 percent of the world's primary energy is derived from the use of biological materials. Its potential is far beyond the present use even when we restrict development to observe a "food first" principle and nature conservation objectives.

From a technology point of view, bioenergy is a versatile fuel as it can be converted to all final energy forms such as electricity, thermal energy or fuel. Contrary to many other renewable energy sources it can be stored for a long time. It is a local energy source which is available practically everywhere in different forms except for very harsh climatic conditions. In addition, when properly utilized, bioenergy is a highly sustainable energy source.

By nature biomass and bioenergy are strongly coupled to ecosystems and both are closely linked to biodiversity and development issues as well. Because of these linkages, bioenergy cannot be evaluated only from a technology or energy supply point-of-view, but requires a multidisciplinary approach to estimating its utilization. This book seeks to provide a multidisciplinary, "whole-picture" view while at the same time identifying advances in the different fields of bioenergy research and technology development.

Measures to reduce energy demand growth and the promotion of bioenergy and other renewable energy sources are nowadays cornerstones in climate and energy security agendas around the world. Support for bioenergy is also in many countries part of policy packages for promotion of rural development with intentions to improve energy access, increase employment, and stimulate positive development in agriculture and forestry. In this respect, bioenergy must be a part of any serious green energy economy agenda.

The use of biomass for cooking, space heating, and lighting in developing countries presently accounts for roughly 80 percent of global bioenergy use. However, recently a rapid increase in interest in other biomass uses for energy has emerged as countries contemplate steps to address concerns about energy insecurity and climate change. These so-called "modern" biomass uses for energy are so far mainly restricted to the burning of municipal organic waste, straw, wood and forest industry by-flows to provide heat and electricity, anaerobic digestion of organic waste to produce biogas, and the use of conventional agriculture crops such as cereals, oil seeds, and sugar crops to produce biofuels.

However, the technologies used for converting biomass to fuels, heat and electricity continue to develop and can be expected to change the way we produce and use bioenergy products. Especially, emerging options for converting lignocellulosic biomass into refined solid, liquid and gaseous fuels gives access to new feedstock resources. New production systems for lignocellulosic biomass offer a broadened resource base. Perennial grasses and trees grown in short rotations (both coppice and single-stem plantations) represent new feedstock supply options in agriculture. Similarly, technology development in planting, silvicultural treatments and biomass extraction support an increasing biomass harvest from forests. This development will not the least be important in the case of biofuels for transport, which hitherto have been produced based on either easily degradable organic waste or food/feed crops. While different types of organic waste can be relatively cheap feedstock sources, cultivated feedstocks cost more and the ones used today make up a substantial part of the total production cost of such biofuels.

In addition to technological development and new types of feedstock for biomass, there are several other factors that contribute to the utilization of biofuels such as renewable energy related standards mandating the use of certain types of biofuels as well as tax incentives and other measures associated with low carbon fuels policies, which also limit the carbon intensity of biofuels.

The society's "footprint" on Earth will inescapably expand in order to provide food, energy and materials for an increasing human population. Yet, society expects that emerging bioenergy systems should reduce impacts caused by the existing - primarily fossil - energy systems, and that policies are developed to address risks associated with bioenergy implementation. Much attention is being directed to the possible consequences of land-use change (LUC), referring to well-documented effects of forest conversion and cropland expansion into previously uncultivated areas, possibly resulting in biodiversity loss, increased greenhouse gas emissions and degradation of soils and water bodies. There are also concerns about risks for negative social and economic impacts, including land-use conflicts, human rights violations and food-security impacts.

The management of natural resources to provide needs for human society while recognizing environmental balance is the challenge facing society. As for other human activities, governance of bioenergy development is much about balancing trade-offs between partly incompatible environmental and socioeconomic objectives. There are currently several initiatives to develop sustainability certification systems. These may hedge against some of the undesired consequences of expanding bioenergy systems and promote a positive development where implemented effectively. Complementary to sustainability certification, there is a need to develop competitive business cases that are efficient along the entire bioenergy supply chain, from feedstock production to energy markets.

The policy challenge for those wishing to utilize the planet's bioenergy potential is complex. One clear principle is balance striking the proper balance between specific energy and food needs, and more broadly between socioeconomic development and environmental sustainability goals. This challenge is fraught with uncertainties: we cannot readily know if and to what extent the delicate web of biodiverse life would be disturbed under different utilization scenarios; and we do not know if social changes in diet and family formation or international efforts to significantly alleviate poverty and hunger will succeed in enabling greater use of bioenergy of certain kinds and on certain scales. Risks embedded in these uncertainties are large, leaving many researchers unsatisfied with our statistical ability to estimate them. When policy confronts "wicked problems" like these, it is common to counsel that we observe a precautionary principle in our actions and aspirations. This second principle also has a large role in shaping policy options. For example, "food first" and other scenario evaluation approaches exemplify this thinking. Several chapters in this book contribute to this understanding, indicating that research has come a long way recently in helping us to understand the bioenergy opportunity in a precautionary manner.

It is also important that policymakers consider the bioenergy opportunity in an integrated manner as part of a multiprong strategy. There have never been "perfect" or "silver bullet" solutions in the energy field and we must be careful not to examine bioenergy in a rarefied light which would undermine its consideration altogether simply because we want an outcome free of any risk. Precaution should lead us to minimize the magnitude of adverse impacts while also balancing the need for social change sufficient to meet our pressing challenges of energy security and climate change. Precaution, balance and integrated thinking are cross themes of this book and serve as guideposts for its contribution to discussions of policy design and implementation.

This book offers an authoritative overview of opportunities and challenges associated with bioenergy utilization. In addition to up-to-date and detailed information on key issues for biomass supply and conversion to energy, the book discusses conditions for the mobilization of sustainable bioenergy supply chains and outlines governance systems to support this mobilization.

The idea for this book came from the Publisher when John Wiley & Sons established a new journal, the *Wiley Interdisciplinary Reviews: Energy and Environment*, which publishes review-type articles by authoritative authors. This book is based on selected articles from this journal. The initial vision and effort of Tony Carwardine from Wiley was decisive for starting this book project. He is now retired, but he provided helpful inputs into the shaping of the book.

The Editors would also like to thank Peter Creaton, Dan Finch, Ella Mitchell, Peter Mitchell, Faith Pidduck, Kerry Powell, and Prachi Sinha Sahay from Wiley for their valuable help during the different phases of the book process.

Peter D. Lund
John Byrne
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PART I

PROMISING INNOVATION IN BIOMASS CONVERSION

1

Metabolic Engineering: Enabling Technology for Biofuels Production

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ENGINEERING THE FUTURE OF BIOFUELS

The past few years have introduced a flurry of interest over renewable energy sources. Biofuels have attracted attention as renewable alternatives to liquid transportation fuels. There are numerous potential advantages over fossil fuels: sustainable supply, diversification of energy sources, energy independence and security, rural development, and reduction in greenhouse emissions.[1] However, achieving adequate scale requires a tremendous effort in research and development beyond what has thus far been achieved. The field of metabolic engineering is well suited to develop the future technologies that will give us widespread, cost-effective, and sustainable transportation fuels.

Metabolic engineering is the improvement of cellular activities by manipulation of metabolic networks through the use of recombinant deoxyribonucleic acid technology.[2] Interdisciplinary advances in metabolic engineering have yielded powerful strategies and methods to understand and manipulate whole metabolic pathways with confidence.[3,4] To date, numerous efforts have successfully engineered and optimized metabolic networks to produce high-value targets for use in the pharmaceutical and fine chemicals industries.[5] However, attention is now being turned toward commodity-scale processes, which require both cost-efficiency and robustness.[1]

Currently, the most prevalent biofuels are ethanol produced from corn or sugarcane and biodiesel produced from vegetable oils. Under current production processes, however, neither biofuel is economically competitive or well integrable into existing petroleum-based technologies and infrastructure.[6] Two developmental challenges underpin these shortcomings: (1) the need for a better feedstock and (2) the need for a better fuel. However, these challenges also represent key opportunities to develop the next generation of biofuel technologies. A central element in these technologies will be the use of metabolic engineering to develop the biological platforms that produce these biofuels.

Engineering for Improved Feedstocks

For the past few years, production of ethanol from corn and biodiesel from vegetable oils has been increasing rapidly. Last year, the United States production capacity of corn ethanol exceeded 13 billion gallons per year (bgy), approaching 10% of the national gasoline demand.[7] Meanwhile, global biodiesel production is approaching 5.0 bgy, with a majority coming from Europe.[8]

However, production of these biofuels from plants like corn or rapeseed also competes for arable cropland needed for food. This adds undesirable price sensitivities between biofuels and food and has already shown adverse effects on food prices. Transforming forests or existing cropland can also sometimes have the effect of increasing greenhouse gas emissions, counteracting the carbon emissions benefit of biofuels.[9]

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The primary cost for producing biofuels is the cost of the feedstock: 60% in the case of corn ethanol and 80% for soybean biodiesel.[10,11] Even with gains in process yield, current crop-based feedstocks will still limit the overall profitability of biofuels. Currently, upward of half of the production cost of these biofuels needs to be supported by government subsidies.[6]

The next generation of feedstocks will need to have lower land requirements and lower production cost, yet maintain high production capacity to bring biofuels closer to economic viability. Metabolic engineering allows us to bridge the feedstock gap by enabling the utilization of cheaper and more sustainable substrates by introducing catabolic pathways and optimizing metabolic networks for the conversion of feedstock to fuel. Indeed, yield optimization has been a critical aspect of virtually all biochemical engineering processes in recent history. Metabolic engineering of organisms toward this end only serves to continue this tradition, pushing yields beyond what is naturally observed. Furthermore, microbe-based biofuel production also reduces the cropland requirements compared to crop-based methods, decreasing competition with food production.

Engineering for Improved Fuels

Although new feedstocks are explored, a simultaneous search continues for the next generation of fuel types. Current biofuels have some persistent disadvantages that limit their incorporation into existing infrastructure.

Ethanol, although widely produced, has relatively poor fuel characteristics. Ethanol is hygroscopic, capable of absorbing water, which can lead to corrosion. The energy content is also low, containing only 70% of the energy per volume of gasoline. Also, as ethanol is produced by fermentation, the resulting beer is dilute, containing roughly 10% ethanol. Subsequent distillation to separate the ethanol is very energy intensive.[10]

Biodiesel is a better fuel, but also has some disadvantages. It is not well suited for use at low temperatures because of a high cloud point, and still often requires large quantities of petroleum-derived methanol as part of its production. It also has only 89% of the energy content of its analog, petrodiesel.[11]

Current biofuel characteristics limit their integration into existing infrastructure. Because of this, there is a high transition barrier to adoption of biofuels, and both ethanol and biodiesel are often blended only at low concentrations into conventional fuels.

Development of better fuels that have high energy density and can be integrated into existing pipelines and engines will be needed if biofuels are to be more widely adopted and have a reasonable hope to replace fossil fuels. Through metabolic engineering of production pathways, alternative products can be made that have characteristics closer to their petroleum equivalents, easing the barrier for adoption. These alternatives range from slight modifications to existing metabolites, to new pathways that create naturally unique compounds. These naturally rare products will also require extensive pathway engineering and optimization to achieve effective production capacities—one of the central strengths of metabolic engineering.

TOOLS OF METABOLIC ENGINEERING

To understand how metabolic engineering plays a role in biofuels development and how it takes an interdisciplinary approach to problem solving, it is important to first understand its main strategies and tools. The strategies of metabolic engineering can be compartmentalized into three steps: (1) understanding, (2) designing, and (3) engineering the metabolic network. Each of these steps uses tools and technologies adopted from a range of disciplines. An overview of the strategies of metabolic engineering can be found in Figure 1.1.

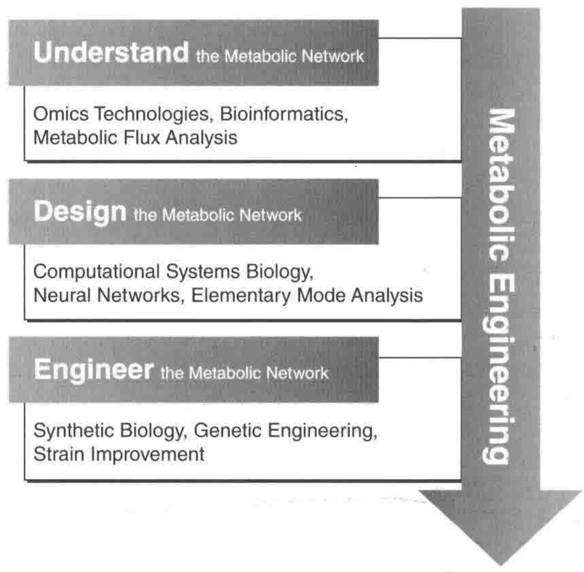

Figure 1.1 Strategies of metabolic engineering revolve around the understanding, design, and engineering of metabolic networks and pathways to produce desired molecular products from biological platforms. These strategies employ techniques and technologies from a range of disciplines, from omics technology to synthetic biology.

Understanding the Metabolic Network

The first step in metabolic engineering is to understand the complex network of enzymatic reactions that compose a cell's metabolism. In addition to the enzymology of participating enzymes, this requires information on the structure and behavior of the pathways that connect these enzymes. Knowledge of the pathway chemistry and stoichiometry allows us to calculate theoretical yields, which are often used as benchmarks for pathway engineering efficacy. Comprehensive systems-level data about these complex networks is acquired through omics technologies and bioinformatics. Omics technologies involve using genomic, transcriptomic, proteomic, or metabolomic data to quantify the system behavior of the cell along various functional axes (e.g., growth, tolerance, productivity).[12] Bioinformatics is the method of extracting biological meaning by identifying significant patterns, motifs, and connections within these large, complex data sets. These techniques enable us to develop a systems-level perspective on cellular activity and an understanding of important contributing networks.[4]

As an example, metabolic flux analysis derived from metabolomic data allows us to observe the flow of material through cellular metabolic pathways. Like a material balance, these fluxes describe the distribution of material throughout the cell's metabolic network and can help identify branch points and competing pathways relevant to our desired product. Fluxes also help to determine the degree of engagement of various enzymes in the pathway, allowing us to identify rate-limiting steps and control points.[3]

Because any biological manipulation will rarely ever produce only an isolated response, it is important to observe the system-level response of our engineering efforts. Using bioinformatics and omics technologies allows us to understand the interactions, connections, and responses between different parts of the system to predict and control the metabolic network.

Designing the Metabolic Network

Once we have sufficient understanding of the organism and its cellular activities, we are then able to develop and design specific strategies to obtain our desired product. Although we can introduce, remove, or otherwise modify pathways, identifying the most effective actions *a priori* can help save much time and effort. Modern methods to do so are found in the field of computational systems biology.

A main goal of computational systems biology is to reconstruct cellular networks *in silico*, which can model the behavior of the cell. Starting with a cellular model, one is able to simulate and characterize how possible pathway manipulations will affect the system overall. Evaluation of these changes can help identify the ideal genetic targets that will maximize our objectives.

One such method of evaluation is called elementary mode analysis, which uses a systems engineering approach to decompose metabolic networks into uniquely organized pathways that can be used to evaluate cellular phenotypes, metabolic network regulation, network robustness, and network fragility.[13] As an extension, neural networks can also be used to make sense of exceptionally difficult systems and to subsequently predict future behavior.[14]

Engineering the Metabolic Network

Once targets and pathways are identified, the next task is to implement these changes *in vivo*. This involves genetic manipulation of the host organism using molecular biology. The term synthetic biology describes the systematic approach to pathway manipulation through standardized biological components for the purpose of increasing their programmability and robustness.[15] Under this framework, genetic elements are modularized to simplify the process of genetic engineering. These elements can then be used to introduce new genes, knockout existing genes, or modify existing deoxyribonucleic acid sequences. Modules can be built up to produce whole pathways and can also be rearranged to optimize expression.

Numerous nonrational techniques are also available that extend the reach of traditional strain improvement: high throughput screening, directed evolution, gene shuffling, and combinatorial engineering. These techniques increase the efficiency of strain improvement by sampling a much larger phenotypic search space, opening up the possibility to select for changes in less-intuitive or distal targets. An interesting example is the use of transcriptional engineering to achieve phenotypic diversity.[16] This technique involves mutagenesis of transcription factors to produce global changes in the transcription and expression of genes in the cell, which would not be feasible through isolated point mutations.[17]

Finally, optimizing to maximize flux through the production pathway is often the most difficult engineering task. Tuning the expression of genes many times is necessary as intermediates and cofactors need to be balanced within the pathway. Furthermore, troubleshooting the pathway often involves alleviating any number of potential bottlenecks that may impede flux, such as competing pathways, lack of enzymatic driving force, cofactor imbalances, insufficient enzyme activity, unbalanced enzyme expression, transport issues, enzyme regulation, and toxicity.

Because of its interdisciplinary nature, metabolic engineering will be limited to the available tools and technologies of the fields from which it draws. However, as the

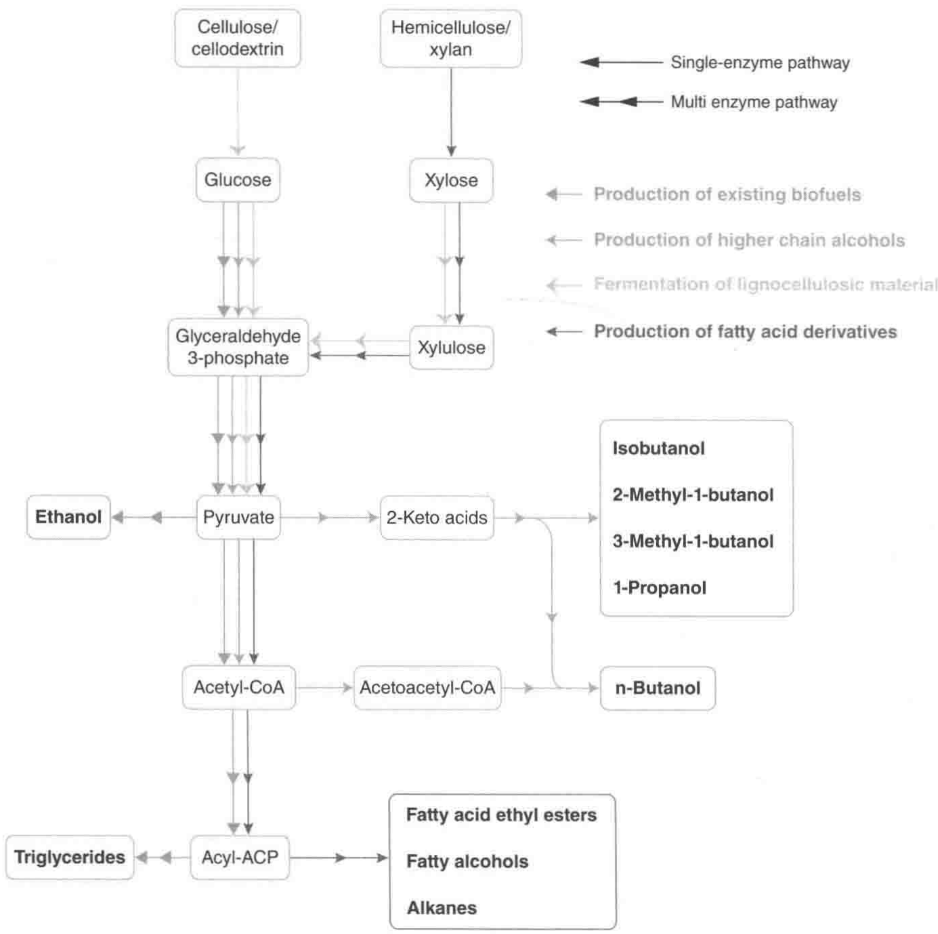

Figure 1.2 Metabolic network of biofuel production pathways and intermediates for the conversion of feedstocks to fuels (bold text): current biofuels (←), higher chain alcohols (←), lignocellulosic fermentation (←), and fatty acid derivatives (←). Engineering the desired biofuel pathway requires maximizing flux through the relevant nodes while minimizing metabolite flux to competing branches. This can involve tuning expression of intermediate reaction steps, deletion of competing pathways, or manipulation of distal enzymatic or regulatory targets.

state of the art progresses and development improves at the interface of these related disciplines, metabolic engineers will have increased power to evaluate, design, and manipulate cells to produce the desired products that will be in demand in the future. These abilities will most certainly be necessary for the development of the next generation of biofuels.

METABOLIC ENGINEERING ENABLES BIOFUELS DEVELOPMENT

In the following section, we will review various researches that attempt to address these biofuel challenges and how metabolic engineering is central to enabling these technologies. A schematic of relevant metabolic pathways for biofuels production can be found in Figure 1.2.

Production of Higher Chain Alcohols

Because ethanol has several undesirable fuel properties, higher chain alcohols have received attention as possible fuels. For example, *n*-butanol has 20% higher energy content than ethanol. In addition, it is more hydrophobic and thus less susceptible to inducing corrosion.[18] Traditionally, butanol is produced through refining petroleum or from acetone-butanol-ethanol fermentation. The ability to robustly produce higher chain alcohols represents a step toward biofuels with characteristics approaching that of gasoline.

Utilizing metabolic engineering, pathways for the production of higher chain alcohols have been introduced into organisms such as *Escherichia coli*.[18,19] The use of the model bacteria allows for the study and efficient development of novel pathways. Two pathways have recently emerged to successfully produce C4 or C5 alcohols: coenzyme A (CoA)-mediated and nonfermentative pathways.

The CoA-mediated pathway involves utilizing the native pathway of the butanol-producing organism, *Clostridium acetobutylicum*. Introduction of five *Clostridium* genes into *E. coli* is sufficient for production of *n*-butanol from acetyl-CoA. However, initial titers were at best about 1 g/L, which is much lower than the 10 g/L typically produced by native *n*-butanol producers.[19]

More recently, extensive engineering of the pathway has led to insights into maximizing the production through this pathway. Three major bottlenecks were discovered that could be alleviated by (1) balancing the expression of upstream and downstream enzymes, (2) balancing cofactor utilization and generation, and (3) engineering driving force to increase flux toward the product.[20,21] Indeed, these bottlenecks seem to be recurring obstacles in many efforts to engineer high production pathways. After addressing these bottlenecks, titers of 30 g/L could be achieved at about 70% theoretical maximum yield.[20]

The second pathway utilizes a creative nonfermentative approach, producing C4 and C5 branched chain alcohols from intermediates in the amino acid metabolic network.[22] The introduction of a 2-keto-acid decarboxylase and an alcohol dehydrogenase allows the conversion of a variety of 2-keto acid metabolites found in amino acid synthesis pathways into their analogous branched chain alcohols: 1-propanol, isobutanol, *n*-butanol, 2-methyl-1-butanol, 3-methyl-1-butanol, and 2-phenylethanol. Branched chain alcohols have higher octane numbers than their straight-chained counterparts, making them better fuels. This process has the advantage of avoiding CoA-mediated chemistry, while also leveraging the wealth of understanding from decades of research on metabolic engineering for amino acid production. As such, they were able to obtain isobutanol production of over 20 g/L at 86% of the theoretical maximum yield.[22] Optimization of cofactor imbalances produced strains that achieved 100% theoretical maximum yield, suggesting some of the same bottlenecks may exist in this alternative pathway.[23]

Fermentation of Lignocellulosic Material

In the search for improved feedstocks, the push toward cellulosic biofuels is a clear choice. Cellulosic biomass eliminates the need to compete with food crop production as an estimated 1.3+ billion dry tons per year of biomass is potentially available in the United States.[24] Two issues highlight how metabolic engineering can enable industrial utilization of this feedstock: xylose utilization and cellulose utilization.

Because hydrolysis of lignocellulosic biomass results in 20–30% carbohydrates in the form of xylose, utilization of pentose sugars is one of the first steps toward efficiently using cellulosic materials. *Saccharomyces cerevisiae*, the most productive of ethanologenic organisms, cannot ferment xylose; it lacks the ability to convert xylose into xylulose, although xylulose is metabolized within the pentose phosphate pathway (PPP). Transferring the xylose reductase (XR) and xylitol dehydrogenase (XDH) enzymes from *Scheffersomyces stipitis* (formerly *Pichia stipitis*) enables the growth of yeast on xylose and production of ethanol.[25]

However, growth and production are considerably slower than on glucose, and significant amounts of xylitol are often produced. Xylitol is the intermediate of the XR/XDH pathway, and most understand this to result from differences in cofactor specificity between reduced nicotinamide adenine dinucleotide phosphate (NADPH)-dependent XR and nicotinamide adenine dinucleotide (NAD)-dependent XDH.[26] The cofactor imbalance has been addressed in two different ways: use of (1) xylose isomerase pathway or (2) protein mutagenesis to switch cofactor specificity.

Additional factors to the limited productivity are the lack of dedicated pentose transporters, low PPP flux, and inability for the cell to identify xylose as a fermentable sugar.[25–27] However, more recently, progress has been made in these areas through additional metabolic engineering strategies: introducing heterologous xylose transporters, overexpressing PPP enzymes, engineering cofactor specificity, and evolutionary engineering (for a comprehensive review see Matsushika et al.).[26]

Cellulose on the contrary is a polysaccharide composed of $\beta(1\rightarrow4)$ linked glucose molecules. Enzymatic digestion is most commonly used to break these chains down into free glucose molecules. However, this process is somewhat inefficient, requiring a separate enzymatic unit operation. To improve the efficiency of this process, recently, cellodextrin transporters from *Neurospora crassa* were introduced into *S. cerevisiae* allowing for utilization of cellobiose, cellotriose, and cellotetraose.[28] Upon cellodextrin uptake, β-glucosidase breaks down cellodextrin into monomeric glucose, allowing for immediate catabolism. When coupled with enzymatic digestion, this process improves utilization efficiency and allows for the simultaneous saccharification and fermentation of cellulose.

Because of its preference for glucose, *S. cerevisiae* will natively repress the utilization of alternative substrates as