Available online at www.sciencedirect.com

SciVerse ScienceDirect

Current Opinion in Biotechnology

Metabolic engineering of yeast for production of fuels and chemicals

Jens Nielsen[1,2], Christer Larsson[1], Antonius van Maris[3] and Jack Pronk[3]

Microbial production of fuels and chemicals from renewable carbohydrate feedstocks offers sustainable and economically attractive alternatives to their petroleum-based production. The yeast *Saccharomyces cerevisiae* offers many advantages as a platform cell factory for such applications. Already applied on a huge scale for bioethanol production, this yeast is easy to genetically engineer, its physiology, metabolism and genetics have been intensively studied and its robustness enables it to handle harsh industrial conditions. Introduction of novel pathways and optimization of its native cellular processes by metabolic engineering are rapidly expanding its range of cell-factory applications. Here we review recent scientific progress in metabolic engineering of *S. cerevisiae* for the production of bioethanol, advanced biofuels, and chemicals.

Addresses
[1] Department of Chemical and Biological Engineering, Chalmers University of Technology, SE412 96 Gothenburg, Sweden
[2] Science for Life Laboratory, Tomtebodavägen 23A, SE17165 Solna, Sweden
[3] Department of Biotechnology, Delft University of Technology and Kluyver Centre for Genomics of Industrial Fermentation, Julianalaan 67, 2628 BC Delft, The Netherlands

Corresponding author: Nielsen, Jens (nielsenj@chalmers.se)

Current Opinion in Biotechnology 2013, **24**:xx–yy

This review comes from a themed issue on **Energy biotechnology**

Edited by **Eric J Toone** and **Johannes H de Winde**

0958-1669/$ – see front matter, Published by Elsevier Ltd.

http://dx.doi.org/10.1016/j.copbio.2013.03.023

Introduction

Global consumption of liquid transportation fuels amounts to about 2.9 TW and these fuels are currently mainly derived from petroleum, whereas biofuels only account for 2.7% of the total transportation energy (Key World Energy Statistics 2011; URL: http://www.iea.org). The use of petroleum for transportation results in emission of more than 5 Gt CO_2, and this represents a major contribution to the total green house gas (GHG) emissions. Currently by far the dominant biofuel is ethanol, which is being produced at 75 billion liters annually, with the majority being produced in the USA (50 billion liters) with corn as the major feedstock. The remainder of the production is concentrated in Brazil with sugar cane as feedstock. The use of biofuels is much debated due to the high costs of the corn-based process as well as the limited reduction in GHG emission by this process compared with petroleum [1]. Several predictions, however, show an increasing role of biofuels [2,3]. This is due to future production of second generation ethanol with biomass as feedstock. Another reason being the production of advanced biofuels that have improved fuel properties compared with ethanol. Such advanced biofuels will not only be cost competitive with petroleum but will also substantially reduce GHG emissions [1].

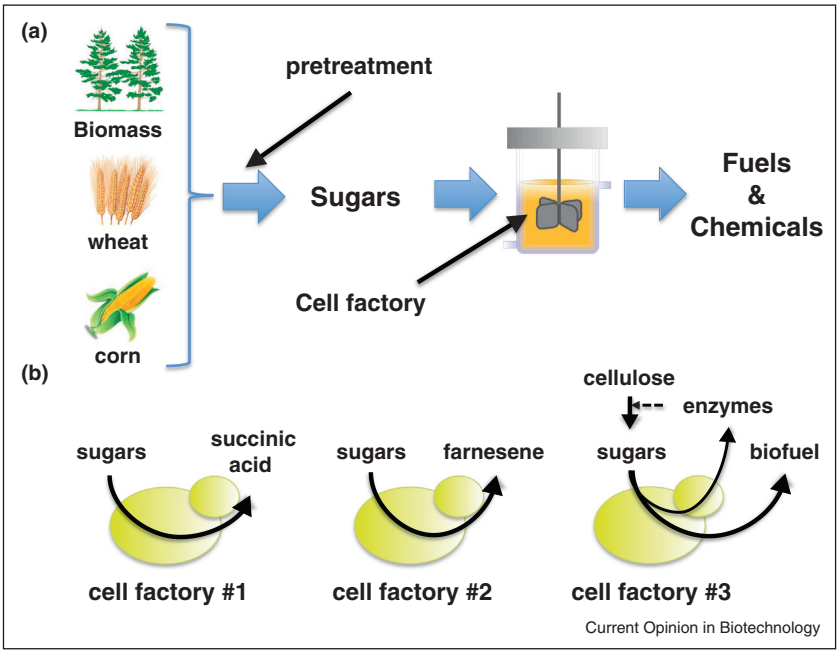
Future production of biofuels will take place in biorefineries, which may be retrofitted corn-ethanol plants, or new, dedicated plants for processing biomass. In biorefineries different types of feedstock will be processed to sugars that are subsequently converted into the desired products through microbial fermentation (Figure 1). At the heart of biorefineries is the fermentation process, in which microbial biocatalysts ensure conversion of sugars into the fuel or chemical to be produced. In order to ensure flexibility in biorefineries, industry is highly interested in so-called platform cell factories. Owing to its role in bioethanol production, the yeast *Saccharomyces cerevisiae* is already the most intensively applied microbial cell factory. In addition, robustness under process conditions, genetic accessibility and a strong fundamental knowledge base in physiology and systems biology [4,5,6•,7•] contribute to its current popularity as a 'general purpose' metabolic engineering platform [6•]. Novel synthetic biology methods, based on the unsurpassed efficiency of homologous recombination in *S. cerevisiae*, contribute to a further tremendous acceleration of genetic modification in this yeast [7•]. Here we will review the recent advances in metabolic engineering of *S. cerevisiae* for its use as a platform cell factory for the production of ethanol from conventional and lignocellulosic feedstocks and of advanced biofuels and chemicals.

First generation bioethanol production: status and perspectives

Considerable efforts have been made to minimize or completely abolish formation of glycerol, the major by-product during current bioethanol production. During anaerobic growth of *S. cerevisiae*, glycerol serves as an essential electron sink for reoxidizing reduced redox cofactors (NADH) generated in biosynthesis. Glycerol formation can be prevented or reduced by deleting one or both genes encoding cytosolic NADH-dependent glycerol-3-phosphate dehydrogenases, *GPD1* and *GPD2* [8]. However, a double deletion renders cells unable to grow anaerobically. Deletion of, for example, *GPD2*,

Please cite this article in press as: Nielsen J, et al.: Metabolic engineering of yeast for production of fuels and chemicals, Curr Opin Biotechnol (2013), http://dx.doi.org/10.1016/j.copbio.2013.03.023

Figure 1



Overview of a biorefinery. **(a)** Different types of feedstock, that is, corn, biomass and agricultural waste, are processed to generate sugars. These sugars are subsequently converted into fuels and chemicals by a biocatalyst, for example, the yeast *S. cerevisiae*. **(b)** By metabolic engineering different yeast strains can be generated and this allows for production of a range of different products using the same infrastructure, that is, a plug-and-play solution. Yeast can also be engineered to express enzymes that can degrade the polymers present in the feedstock, and hereby it is possible to reduce the overall processing costs, as enzymes used for degradation of starch, cellulose or lignocellulose are quite expensive. A process where yeast is secreting enzymes for polymer degradation is referred to as a consolidated bioprocess.

results in an increased ethanol yield and decreased glycerol formation, but severely hampers growth and ethanol productivity [9]. Alternative approaches aim at engineering cellular redox metabolism to reduce formation of cytosolic NADH. Nissen *et al.* [10] deleted the NADPH-dependent glutamate dehydrogenase, *GDH1*, while overexpressing *GLN1* and *GLT1* (encoding glutamine synthetase and glutamate synthase), respectively. The resulting ammonium assimilation pathway consumed NADH as well as ATP and led to a reduction in glycerol yield by 38% while the yield of ethanol was increased by 10%. Reducing energy conservation in alcoholic fermentation of sugars can by itself increase the ethanol yield in *S. cerevisiae*, as illustrated by Basso *et al.* [11]. These authors substituted the dominant extracellular invertase with a cytoplasmic version relying on proton-symport for transport of sucrose into the cell. The increased energy expenditure was shown to be compensated by an increased flux toward ethanol. An alternative approach to reduce both the surplus of cytosolic NADH and produce less ATP has been done by replacing the natural glyceraldehyde-3-phosphate dehydrogenase with a non-phosphorylating, $NADP^+$-dependent counterpart (GAPN) from *Bacillus cereus* or *Streptococcus mutans* [12–15]. All these attempts were successful in reducing the glycerol yield and increasing the ethanol yield. Zhang *et al.* [15] took this one step further and combined expression of $NADP^+$ dependent GAPN with introduction of novel NADH-reoxidizing pathways. They used either a NAD^+ dependent fumarate reductase or an acetaldehyde dehydrogenase and in both cases impressive ethanol yields above 95% of the theoretical maximum were reported [15]. An earlier redox engineering study by Guadalupe Medina *et al.* [16•] demonstrated that expression of an acetylating NAD^+-dependent acetaldehyde dehydrogenase from *Escherichia coli* enabled acetate-dependent, glycerol-negative anaerobic growth of a *gpd1Δgpd2Δ* mutant. Since the resulting strain converted acetate into ethanol, this concept may be valuable for conversion of acetate-containing biomass-based feedstocks. Jain *et al.* [17] expressed alternative oxidoreductase genes for consumption of excess NADH in a *gpd1Δgpd2Δ* background which partly restored the ability to grow under anaerobic conditions.

A problem associated with reduced ability to produce glycerol in *S. cerevisiae* is that the osmosensitivity as well as the general robustness is reduced [18]. Maintenance of an osmotolerant phenotype is crucial when moving toward using high or very high gravity fermentations, which are attractive in terms of productivity and titer, and hence lower capital and energy requirements. Efforts

Please cite this article in press as: Nielsen J, et al.: Metabolic engineering of yeast for production of fuels and chemicals, Curr Opin Biotechnol (2013), http://dx.doi.org/10.1016/j.copbio.2013.03.023