Appl Microbiol Biotechnol (2007) 74:937–953
DOI 10.1007/s00253-006-0827-2

MINI-REVIEW

# Towards industrial pentose-fermenting yeast strains

**Bärbel Hahn-Hägerdal · Kaisa Karhumaa · César Fonseca · Isabel Spencer-Martins · Marie F. Gorwa-Grauslund**

Received: 19 October 2006 / Revised: 21 December 2006 / Accepted: 25 December 2006 / Published online: 9 February 2007


**Abstract** Production of bioethanol from forest and agricultural products requires a fermenting organism that converts all types of sugars in the raw material to ethanol in high yield and with a high rate. This review summarizes recent research aiming at developing industrial strains of *Saccharomyces cerevisiae* with the ability to ferment all lignocellulose-derived sugars. The properties required from the industrial yeast strains are discussed in relation to four benchmarks: (1) process water economy, (2) inhibitor tolerance, (3) ethanol yield, and (4) specific ethanol productivity. Of particular importance is the tolerance of the fermenting organism to fermentation inhibitors formed during fractionation/pretreatment and hydrolysis of the raw material, which necessitates the use of robust industrial strain background. While numerous metabolic engineering strategies have been developed in laboratory yeast strains, only a few approaches have been realized in industrial strains. The fermentation performance of the existing industrial pentose-fermenting *S. cerevisiae* strains in lignocellulose hydrolysate is reviewed. Ethanol yields of more than 0.4 g ethanol/g sugar have been achieved with several xylose-fermenting industrial strains such as TMB 3400, TMB 3006, and 424A(LNF-ST), carrying the heterologous xylose utilization pathway consisting of xylose reductase and xylitol dehydrogenase, which demonstrates the potential of pentose fermentation in improving lignocellulosic ethanol production.

B. Hahn-Hägerdal (✉) · K. Karhumaa · M. F. Gorwa-Grauslund
Department of Applied Microbiology, Lund University,
P.O. Box 124, Lund 22100, Sweden
e-mail: Barbel.Hahn-Hagerdal@tmb.lth.se

C. Fonseca · I. Spencer-Martins
Centro de Recursos Microbiológicos (CREM),
Biotechnology Unit, Faculty of Sciences and Technology,
New University of Lisbon,
Caparica 2829-516, Portugal

## Introduction

The energy crisis in the early 1970s initiated research and development aimed at sustainable production of fuels and chemicals from renewable lignocellulosic feedstocks from agriculture and forestry (Tong 1979; Rosenberg 1980). Such feedstocks are composed of cellulose, hemicellulose, and lignin. Cellulose is a homopolymer of glucose, while hemicellulose is composed of the hexose sugars glucose, mannose, and galactose, and the pentose sugars xylose and arabinose (Hayn et al. 1993). The relative proportion of the individual sugars depends on the raw material; the hemicellulose fraction of hardwoods and agricultural raw materials is rich in pentose sugars, while softwood hemicellulose only contains minor fractions of the pentose sugars D-xylose and L-arabinose (Hayn et al. 1993). The increased demand for crude oil, manifested in trading prices approaching $80/barrel in 2006, has renewed the interest in exploiting lignocellulosic feedstocks not only for liquid transportation fuel but also for the production of chemicals and materials, i.e., the development of carbohydrate-based biorefineries (Ohara 2003; Kamm and Kamm 2004; Farrell et al. 2006; Gray et al. 2006; Herrera 2006).

Complete substrate utilization is one of the prerequisites to render lignocellulosic ethanol processes economically competitive (Galbe and Zacchi 2002). This means that all types of sugars in cellulose and hemicellulose must be converted to ethanol, and that microorganisms must be obtained that efficiently perform this conversion under

industrial conditions. While ethanolic fermentation of glucose, mannose, and galactose using baker's yeast *Saccharomyces cerevisiae* is well established on large scale (Berg 2002), the conversion of the pentose sugars xylose and arabinose to ethanol was addressed only in the late 1970s (Chiang et al. 1981; Schneider et al. 1981). Most work focused on xylose, which is much more abundant than arabinose (McMillan and Boynton 1994). Metabolic engineering of xylose-fermenting strains has most recently been detailed by Dien et al. (2003) and by Jeffries (2006). Most engineering strategies have been only developed in laboratory strains, and less attention has been given to the development of industrially applicable solutions. The present review focuses on the development of industrial yeast strains for fermentation of hydrolyzed lignocellulose to fuel ethanol, with special emphasis on the fermentation of the pentose sugars xylose and arabinose.

## Strain benchmarking towards industrial requirements

Despite the wealth of literature in the field of pentose fermentation, relatively little attention has been given to the performance of pentose-fermenting strains in industrial substrates and environments (Hahn-Hägerdal and Pamment 2004). Here we discuss recent development in this field of research in relation to four industrial benchmarks, which were singled out to have the greatest influence on the price of lignocellulosic ethanol (Wingren et al. 2003):

- Process water economy
- Inhibitor tolerance
- Ethanol yield
- Specific ethanol productivity

Process concepts for the conversion of lignocellulosic feedstocks to ethanol generally include a pretreatment or fractionation step in which the chopped raw material is exposed to acidic or alkaline pH, at high temperature, so that the hemicellulose fraction is partially hydrolyzed to monomeric and oligomeric sugars, rendering the cellulose fraction susceptible for hydrolysis (Fig. 1). Then follows either acidic or enzymatic hydrolysis of the cellulose fraction. The hemicellulose and cellulose hydrolysates are fermented to ethanol, and the ethanol is recovered by distillation (Fig. 1). Furthermore, the lignin fraction can be used throughout the process to generate the necessary heat by burning, and the waste streams are evaporated and burned or fermented to methane to recover energy to be used in the process and commercialized as an added-value coproduct (Wingren et al. 2003).

### Process water economy

*Process water economy* means that a minimum of fresh water is permitted at any stage in the process because all water has to be removed or cleaned at a later stage and such unit operations add costs to the final product (Wingren et al. 2003). Process water economy can be improved by recirculation of process water streams (Alkasrawi et al. 2002) and by increasing the dry matter content of the substrate (Öhgren et al. 2006). Minimizing fresh water usage leads to more concentrated solutions with increased osmolality, which requires the use of osmotolerant production strains.

### Inhibitor tolerance

In addition to being tolerant to solutions with high osmolality, the fermenting organism must also *tolerate inhibitors* generated in the pretreatment and hydrolysis steps. In these steps, low molecular weight organic acids, furans, and aromatics are released, and such compounds are potent inhibitors of microbial metabolism (Larsson et al. 1999a; Palmqvist and Hahn-Hägerdal 2000b). Inhibitors can be efficiently removed by various detoxification processes (Larsson et al. 1999b; Palmqvist and Hahn-Hägerdal 2000a). However, in addition to increasing the ethanol production cost (von Sivers et al. 1995), fermentable sugars are lost (Larsson et al. 1999b) and fouling of process

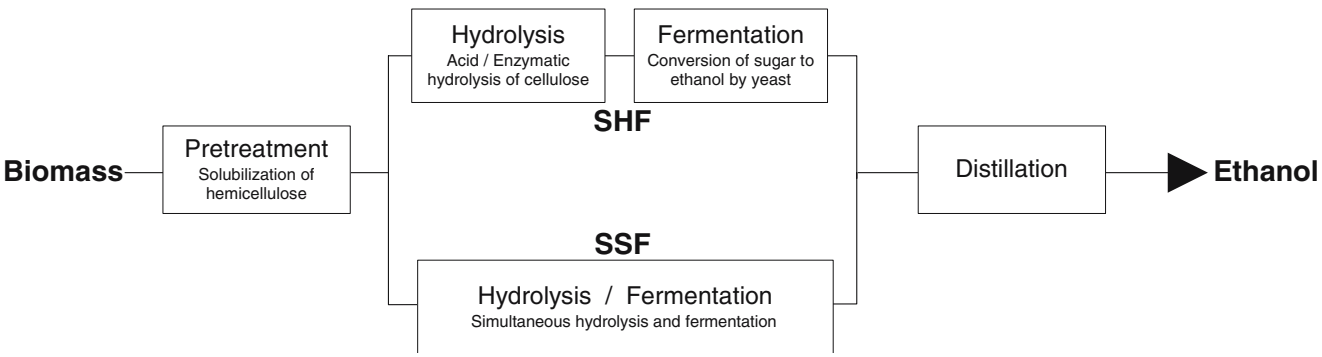


**Fig. 1** A schematic illustration of the process design for lignocellulosic bioethanol production. *SHF*: Separate hydrolysis and fermentation; *SSF*: simultaneous saccharification (hydrolysis) and fermentation

equipment may occur (Kadam et al. 2000; Schell et al. 2004). Some pretreatment and hydrolysis concepts, especially those applied to agriculture and herbaceous feedstocks, result in fermentable hydrolysates that do not require detoxification (Mosier et al. 2005). However, in view of process water economy, it is foreseeable that also hydrolysates of such raw materials must be concentrated, and then the pretreatment and hydrolysis by-products are expected to reach inhibitory levels. Therefore, in the industrial context, a robust, osmotolerant, and inhibitor-tolerant production organism remains a prerequisite.

### Ethanol yield

Ethanolic fermentation of sugar and starch generally reaches 90–95% of the theoretical yield, equivalent to 0.45–0.48 g/g sugar in the raw material. Cost analyses of the ethanolic fermentation of lignocellulose hydrolysates have estimated raw material costs to constitute as much as one third of the production cost (von Sivers and Zacchi 1996; Galbe and Zacchi 2002; Wingren et al. 2003). Conversion of all sugars to ethanol is therefore of paramount importance for the cost-effectiveness of the process. The evaluation of microbial strains for the fermentation of lignocellulose hydrolysates must be discussed in relation to the *ethanol yield* based on total amount of sugars in the hydrolysate. This means that not only must all sugars (glucose, mannose, galactose, xylose, and arabinose) be taken up and metabolized by the fermenting organism, they must also be converted to ethanol with minimal by-product formation. To achieve this goal, metabolic engineering has played an important role in the past 10–15 years of research (Dien et al. 2003; Jeffries 2006).

### Specific ethanol productivity

The productivity is the rate by which the product (ethanol) is formed. Two kinds of productivity are generally discussed: *volumetric productivity* (g ethanol $l^{-1}$ $h^{-1}$) and *specific productivity* (g ethanol g $biomass^{-1}$ $h^{-1}$). The volumetric productivity relates to the investment costs for the ethanol production plant and is a function of the amount of cells used in the fermentation step. High cell density fermentation (Thomas and Ingledew 1990) increases the volumetric productivity and reduces investment costs. However, it also increases the cost for producing the cell mass, which is a function of the price for added nutrients. The specific productivity measures the efficiency of the fermenting microorganism so that higher specific productivity translates to reduced amount of cell mass required in the fermentation step. More importantly, the specific productivity is a crucial benchmark in industrial strain development because it allows comparison of strains and experimental models.

In today's ethanol production plants the volumetric productivity is in the range of 1–3 g ethanol $l^{-1}$ $h^{-1}$ with the theoretical specific ethanol productivity, e.g., for *S. cerevisiae* being 2 g ethanol g $biomass^{-1}$ $h^{-1}$. Whereas volumetric productivities are frequently reported in the literature (Bothast et al. 1999), specific productivities mostly have to be back-traced and indirectly estimated from data on cell growth (Hahn-Hägerdal et al. 2006). However, the specific productivity is mentioned last among the four benchmarks because in bioconversion processes based on enzymatic hydrolysis of lignocellulose, the hydrolysis reaction is one to two orders of magnitude slower than the average ethanolic fermentation rate (Ljunggren 2005).

## Microorganisms considered for ethanolic pentose fermentation

Species of bacteria, yeast, and filamentous fungi naturally ferment xylose to ethanol (Jeffries 1983; Toivola et al. 1984; Skoog and Hahn-Hägerdal 1990). In the lignocellulosic context and considering modern molecular strain development strategies, each group of microorganisms has its advantages and disadvantages. In Table 1, the substrate and product ranges of microorganisms most frequently considered for ethanolic fermentation of lignocellulosic biomass are summarized. Also, parameters relating to their industrial performance are indicated.

**Table 1** Pros and cons of various natural microorganisms with regard to industrial ethanol production

| Organism | Natural sugar utilization pathways | | | | | Major products | | Tolerance | | | $O_2$ needed | pH range |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Glu | Man | Gal | Xyl | Ara | EtOH | Others | Alcohols | Acids | Hydrolysate | | |
| Anaerobic bacteria | + | + | + | + | + | + | + | − | − | − | − | Neutral |
| *E. coli* | + | + | + | + | + | − | + | − | − | − | − | Neutral |
| *Z. mobilis* | + | − | − | − | − | + | − | + | − | − | − | Neutral |
| *S. cerevisiae* | + | + | + | − | − | + | − | ++ | ++ | ++ | − | Acidic |
| *P. stipitis* | + | + | + | + | + | + | − | − | − | − | + | Acidic |
| Filamentous fungi | + | + | + | + | + | + | − | ++ | ++ | ++ | − | Acidic |

## Bacteria

Obligate anaerobic bacteria (Table 1) can ferment all lignocellulose-derived sugars, including their oligomers and polymers, to ethanol, other solvents, and acids (Wiegel and Ljungdahl 1986). Because these bacteria are more severely inhibited than other bacteria by high sugar concentrations and moderate concentrations of ethanol and acids, efforts are being made to isolate sugar- and ethanol-tolerant variants (Sommer et al. 2004; Fong et al. 2006). So far their fermentative performance has only been investigated in dilute alkali-treated hydrolysate. Nevertheless, anaerobic bacteria have an established industrial record for the production of acetone and butanol, most recently in the former Soviet Union and in South Africa. However, these processes could not compete in the market economy of the 1990s. Also, the use of obligate anaerobic bacteria is hampered by the lack of simple and efficient molecular biology tools for genetic engineering; however, protocols for thermophilic anaerobes are being developed (Tyurin et al. 2005).

Ethanol-producing bacteria (Table 1) generally display mixed acid product formation where ethanol is a minor product. Furthermore, their optimal pH around 6–7 makes bacterial fermentation susceptible to infection and their low tolerance to lignocellulose-derived inhibitors requires a detoxification step to be included in the fermentation process (Hahn-Hägerdal et al. 1994a). Nevertheless, the presently most efficient microorganisms for fermentation of detoxified lignocellulose hydrolysates are recombinant strains of *Escherichia coli* (Ingram et al. 1987; Hespell et al. 1996; Bothast et al. 1999; Dien et al. 2003).

In contrast to other bacteria, *Zymomonas mobilis* (Table 1) produces ethanol with stoichiometric yields (Swings and DeLey 1977). It also displays high specific ethanol productivity (Lee et al. 1979; Rogers et al. 1979). Despite intensive efforts over the past 20 years, the industrial exploitation of *Z. mobilis* has so far not materialized. In relation to the variety of sugars present in lignocellulosic raw materials, the substrate range of *Z. mobilis* is limited. Recombinant xylose- and arabinose-fermenting strains, capable to ferment these sugars in detoxified lignocellulose hydrolysates, have been constructed (Zhang et al. 1995; Deanda et al. 1996; Mohagheghi et al. 2002). However, *Z. mobilis* would also need pathways for the metabolism of mannose and galactose, which constitute a considerable fraction of some lignocellulosic raw materials (Hayn et al. 1993).

## Yeast

Whereas a large number of yeast species metabolize xylose and arabinose and display fermentative capacity (Barnett 2000), only approximately 1% of them are capable of fermenting xylose to ethanol (Table 1; Fig. 2a). No

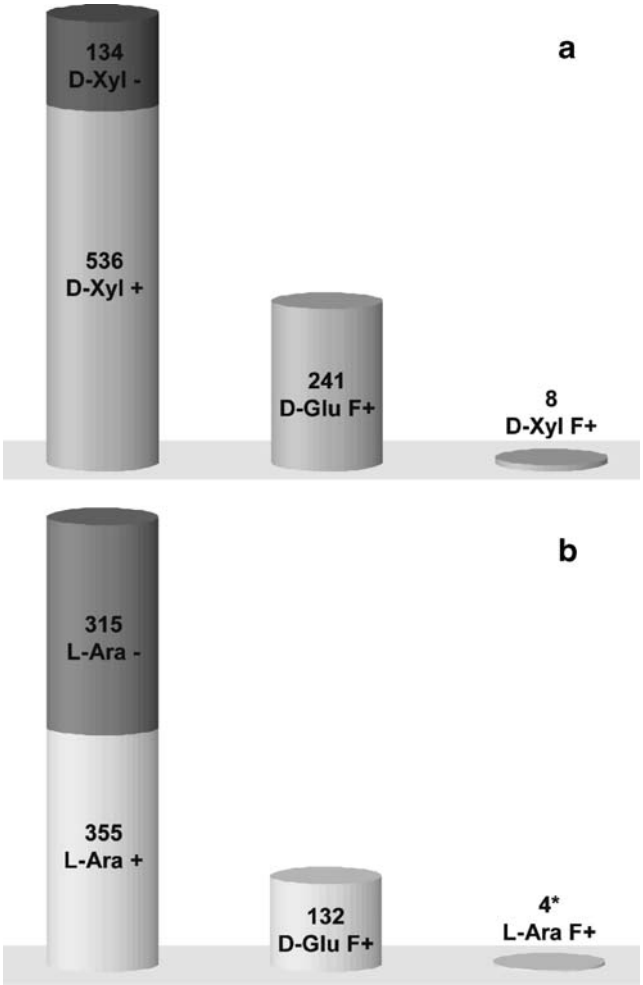


**Fig. 2** Utilization and fermentative capacity of D-xylose (**a**) and L-arabinose (**b**) among 670 yeast species (Barnett 2000). *D-Xyl* D-Xylose, *L-Ara* L-arabinose, *D-Glu* D-glucose, *F+* fermentation-positive. *Asterisk* indicates that ethanol was formed from L-arabinose (and D-xylose) under specific conditions not used in conventional taxonomic characterization (Dien et al. 1996)

arabinose-fermenting yeast was found in an early screening study (McMillan and Boynton 1994), while a subsequent study identified four yeast species able to ferment arabinose to ethanol (Fig. 2b; Dien et al. 1996). The discrepancy between these studies is most likely due to that the latter screen used a complex (YP) medium containing yeast extract and peptone, which contain compounds that may act as electron acceptors and thus aid conversion of arabinose to ethanol. The requirement for electron acceptors translates to very low, carefully controlled, levels of oxygen required for maximum ethanol production from arabinose and xylose by these yeasts (Skoog and Hahn-Hägerdal 1990; Fonseca et al. 2007). However, such precise oxygenation is technically impossible to maintain in large-scale industrial conditions, with concomitant reduced product yield. Also, the naturally pentose-fermenting yeasts are generally inhibited by industrial

substrates (Hahn-Hägerdal et al. 1991, 1994a; Olsson et al. 1992; Hahn-Hägerdal and Pamment 2004; Klinke et al. 2004) and do not grow under anaerobic conditions even on hexose sugars (Visser et al. 1990).

Filamentous fungi

Aerobic filamentous fungi tolerate industrial substrates well and ferment pentose sugars (Skoog and Hahn-Hägerdal 1988; Hahn-Hägerdal et al. 1994a), albeit with low rates of sugar consumption and product formation (Skoog and Hahn-Hägerdal 1988). Also, some species of anaerobic filamentous fungi produce ethanol, in addition to acids and hydrogen (Wu et al. 1986; Boxma et al. 2004; Panagiotou et al. 2006). Filamentous fungi also have a proven record in the industry where they are used in large scale for production of antibiotics and acids (Atkinson and Mavituna 1991). It is probably only a question of time before engineering of filamentous fungi for ethanolic fermentation will be attempted; however, the poor ethanol tolerance of these organisms is a drawback in industrial applications.

*S. cerevisiae*

*S. cerevisiae* has traditionally been used in large-scale ethanolic fermentation of sugar- and starch-based raw materials and it is therefore well adapted to the industrial context. It produces ethanol with stoichiometric yields and tolerates a wide spectrum of inhibitors and elevated osmotic pressure. Its superiority in fermenting nondetoxified lignocellulose hydrolysates has been repeatedly demonstrated (Olsson et al. 1992; Hahn-Hägerdal et al. 1994a, 2006; Hahn-Hägerdal and Pamment 2004). In favor of *S. cerevisiae* as the microorganism for fuel ethanol production speaks also the advantage of integrating large-scale lignocellulosic ethanol processes into the existing sugar cane- and starch-based ethanol plants already using this yeast. This was forecast to substantially reduce the production costs of lignocellulosic ethanol (Ångspanneföreningen 1994; Wooley 1999). Sugar- and starch-based ethanol plants today exclusively operate with *S. cerevisiae* as a production organism. The only, but major, inconvenience to use *S. cerevisiae* for lignocellulosic fermentation is its inability to metabolize and ferment the pentose sugars xylose and arabinose to ethanol.

In the following, genetic engineering of *S. cerevisiae* aiming at industrial hexose- and pentose-fermenting strains will be summarized. The engineering strategies are based on the metabolism of natural pentose-fermenting microorganisms, which will be discussed first, followed by a summary of the metabolic traits that were explored in laboratory strains of *S. cerevisiae*. Finally, the development and performance of industrial recombinant pentose-fermenting *S. cerevisiae* strains will be discussed in relation to the benchmarks discussed above.

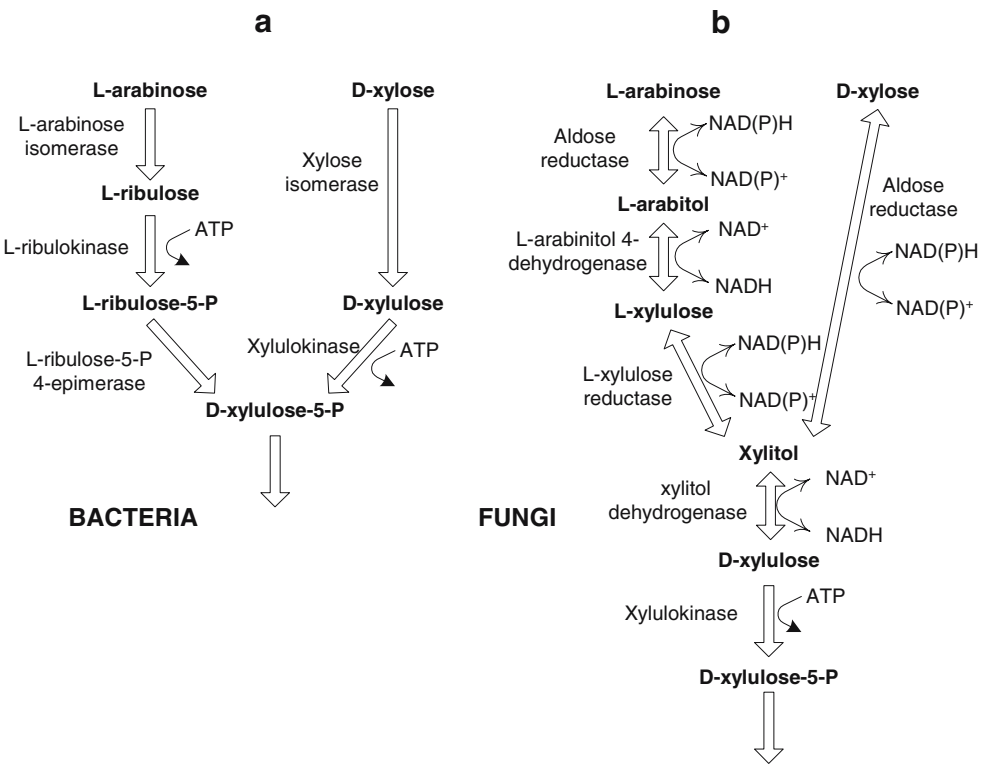


**Fig. 3** D-Xylose and L-arabinose utilization pathways in bacteria and fungi

## Metabolic engineering of *S. cerevisiae* for ethanolic pentose fermentation

### Pentose metabolism in bacteria, yeast, and fungi

Figure 3 schematically illustrates the initial metabolic pathways for D-xylose and L-arabinose utilization in bacteria, yeast, and filamentous fungi. In bacteria, D-xylose and L-arabinose are isomerized to D-xylulose and L-ribulose, respectively (Fig. 3a; Patrick and Lee 1968; Chen 1980). D-Xylulose and L-ribulose are phosphorylated to D-xylulose 5-phosphate (Anderson and Wood 1962) and to L-ribulose 5-phosphate (Lee and Bendet 1967), respectively; the latter then being epimerized to D-xylulose 5-phosphate (Lee et al. 1968), connecting the initial pentose metabolism to the central metabolism through the pentose phosphate pathway (PPP). An alternative arabinose utilization pathway in bacteria was also recently described (Watanabe et al. 2006).

In most fungi, the initial conversion of D-xylose and L-arabinose to D-xylulose 5-phosphate proceeds through a series of reduction and oxidation steps involving the cofactors $NAD(P)^+$/NAD(P)H (Fig. 3b). Whereas D-xylose is directly reduced to xylitol (Bruinenberg et al. 1984; Bolen et al. 1986; Rizzi et al. 1988), L-arabinose is reduced to L-arabitol, which undergoes additional oxidation and reduction steps to be converted to xylitol (Fig. 3b; Chiang and Knight 1960; de Vries et al. 1994; vanKuyk et al. 2001; Richard et al. 2002; Verho et al. 2004). Xylitol is oxidized to D-xylulose (Bolen et al. 1986; Rizzi et al. 1989; Wang and Jeffries 1990), which, similar to bacteria, is phosphorylated to D-xylulose 5-phosphate (vanKuyk et al. 2001). Like in bacterial metabolism, D-xylulose 5-phosphate connects the initial metabolism of arabinose and xylose to the central metabolic pathways. In addition, yeast (Vongsuvanlert and Tani 1988) and filamentous fungi (Banerjee et al. 1994; Harhangi et al. 2003) harboring xylose isomerase (XI) activity have been reported.

Based on current knowledge of pentose metabolism (Fig. 3), numerous metabolic engineering strategies have been explored in laboratory strains of *S. cerevisiae* to determine their effect on fermentation of xylose and arabinose (Table 2). In the following, metabolic engineering for pentose fermentation will first be discussed for the case of laboratory strains and then for industrial strains.

### Metabolic engineering for pentose fermentation in laboratory strains of *S. cerevisiae*

While *S. cerevisiae* naturally harbors genes for xylose utilization (Deng and Ho 1990; Kuhn et al. 1995; Richard et al. 1999; Toivari et al. 2004), these are expressed at such low levels that they do not support growth on xylose. However, *S. cerevisiae* grows on D-xylulose and ferments it to ethanol (Wang and Schneider 1980; Chiang et al. 1981) (Fig. 3), albeit with ten times lower rate than for glucose (Senac and Hahn-Hägerdal 1990). Recently, a strain of *S. cerevisiae* was described in which the endogenous xylose utilization pathway was upregulated through an adaptation protocol (Attfield and Bell 2006). However, the rate of xylose growth and fermentation of this strain has not yet reached industrially competitive levels.

The *Pichia stipitis* genes *XYL1* and *XYL2* encoding XR and xylitol dehydrogenase (XDH), respectively, have been introduced in *S. cerevisiae* (Kötter and Ciriacy 1993; Tantirungkij et al. 1993; Fig. 3), which resulted in growth on xylose. It was recognized that also the endogenous *XKS1* gene encoding xylulokinase (XK) had to be overexpressed for xylose fermentation to occur (Ho et al. 1998; Eliasson et al. 2000; Toivari et al. 2001). Bacterial and fungal XI pathways have been also established in *S. cerevisiae* (Walfridsson et al. 1996; Kuyper et al. 2003).

Arabinose-fermenting *S. cerevisiae* strains have been generated only recently. A functional bacterial pathway could only be established when the *E. coli* L-arabinose isomerase (Sedlak and Ho 2001) was replaced by the corresponding *Bacillus subtilis* enzyme (Becker and Boles 2003), resulting in arabinose fermentation. Also, a fungal arabinose pathway was expressed in *S. cerevisiae* but this did not result in ethanolic fermentation (Richard et al. 2003), in agreement with the inability of natural arabinose-utilizing yeast to produce ethanol in the absence of external electron acceptors (McMillan and Boynton 1994; Dien et al. 1996).

The rate of pentose utilization by recombinant *S. cerevisiae* is generally one to two orders of magnitude lower than the utilization of hexose sugars (Hahn-Hägerdal et al. 2005). Therefore, additional metabolic engineering strategies were elaborated in *S. cerevisiae* strains harboring the initial pentose-utilization pathways. Most engineering strategies have aimed at increasing the metabolic flux of xylose and increasing the ethanol yield. While tested in xylose-utilizing strains, many of the strategies developed should also apply to the more recently constructed arabinose-utilizing strains (Becker and Boles 2003; Richard et al. 2003; Karhumaa et al. 2006). The engineering approaches are summarized in Table 2, including engineering of sugar transport, initial conversion of the pentose sugars, utilization of redox cofactors, enhancing the PPP, and random improvement by evolutionary methods. Generally, the physiological results of these endeavors are in accordance with metabolic control analysis (MCA) (Cornish-Bowden et al. 1995), which predicts that the flux increases by "pulling" rather than "pushing" the metabolic flow and that the demand for the end-product controls the flux (Hofmeyr and Cornish-Bowden 2000).

As for other sugars, pentose uptake by yeasts requires transport proteins. Xylose is transported by the high- and

**Table 2** Metabolic engineering strategies applied in laboratory strains of *S. cerevisiae* to improve xylose fermentation under laboratory (academic) conditions

| Category | Modification | Reference |
|---|---|---|
| Transport | Xylose transport | (Hamacher et al. 2002)<br>(Leandro et al. 2006) |
| Xylose utilization pathways | XR/XDH | (Kötter and Ciriacy 1993)<br>(Tantirungkij et al. 1993)<br>(Walfridsson et al. 1997)<br>(Ho et al. 1998)<br>(Toivari et al. 2001) |
| | Mutated XR | (Jeppsson et al. 2006) |
| | XI | (Walfridsson et al. 1996)<br>(Kuyper et al. 2003) |
| | Mutated XI | (Lönn et al. 2002) |
| | XK | (Deng and Ho 1990)<br>(Toivari et al. 2001)<br>(Johansson et al. 2001)<br>(Jin et al. 2003) |
| Arabinose utilization pathways | Fungal arabinose pathway | (Richard et al. 2003) |
| | *E. coli* arabinose pathway | (Sedlak and Ho 2001) |
| | *B. subtilis/E. coli* arabinose pathway | (Becker and Boles 2003) |
| Xylose and arabinose pathways combined | XR/XDH/XK and *B. subtilis/E. coli* arabinose pathways | (Karhumaa et al. 2006) |
| Reducing xylitol formation | Aldose reductase *Gre3* deletion | (Träff et al. 2001)<br>(Lönn et al. 2003)<br>(Träff-Bjerre et al. 2004)<br>(Kuyper et al. 2005a) |
| | Oxidative PPP deletion | (Jeppsson et al. 2002)<br>(Verho et al. 2003) |
| | GDH1-GDH2, GsGoGAT | (Roca et al. 2003) |
| | NADP(H)-dependent G3PDH | (Verho et al. 2003)<br>(Bro et al. 2006) |
| | Transhydrogenase | (Jeppsson et al. 2003a) |
| | Optimization of XR/XDH levels | (Eliasson et al. 2001)<br>(Jin and Jeffries 2003)<br>(Jeppsson et al. 2003b)<br>(Karhumaa et al. 2007) |
| Improving the efficiency of metabolism | Overexpression of TAL | (Walfridsson et al. 1995)<br>(Jin et al. 2005) |
| | Overexpression of the non-oxidative PPP | (Johansson and Hahn-Hägerdal 2002b)<br>(Johansson and Hahn-Hägerdal 2002a)<br>(Karhumaa et al. 2005)<br>(Kuyper et al. 2005a) |
| | Phosphoketolase pathway | (Wahlbom 2002)<br>(Sonderegger et al. 2004c) |
| | *Mig1*/*Mig2* deletion | (Roca et al. 2004)<br>(Thanvantri Gururajan et al., submitted for publication) |
| | Evolutionary engineering/ adaptation/mutagenesis | (Wahlbom et al. 2003)<br>(Kuyper et al. 2004)<br>(Kuyper et al. 2005b)<br>(Pitkänen et al. 2005)<br>(Attfield and Bell 2006) |
| Anaerobic growth on xylose | Evolutionary engineering | (Sonderegger and Sauer 2003)<br>(Kuyper et al. 2004) |

intermediate-affinity hexose transporters in *S. cerevisiae* (Hamacher et al. 2002; Lee et al. 2002). A MCA study indicated that xylose transport controls the xylose flux at low sugar concentration in strains with high activity of the subsequent xylose-utilization pathway (Gárdonyi et al. 2003a). The affinity of the hexose transporters for xylose is one to two orders of magnitude lower than the affinity for glucose (Kötter and Ciriacy 1993). Therefore, in sugar mixtures, xylose is usually consumed only after depletion of hexose sugars.

Naturally xylose-utilizing yeasts transport xylose by a facilitated diffusion mechanism, for which the sugar gradient is the only driving force, or through an active transport system, usually sugar/proton symports, driven by the proton motive force that allows the intracellular accumulation of substrate (Spencer-Martins 1994). They are strongly regulated by the extracellular substrate concentration (Gárdonyi et al. 2003a; Rodrigues de Sousa et al. 2004). At first glance a heterologous active transport protein would be the prime choice for metabolic engineering of xylose-fermenting *S. cerevisiae*. However, this transport mechanism concomitantly consumes 1 mol of ATP for each proton cotransported with the sugar to pump out the proton and prevent intracellular acidification (Fig. 4). This functions under aerobic conditions without creating major energetic constraints; however, the depletion of a significant fraction of the conserved ATP considerably reduces anaerobic growth (Weusthuis et al. 1993). Therefore, better prospects for the development of pentose-fermenting *S. cerevisiae* are to consider heterologous expression of specific pentose-transporting facilitated diffusion proteins. The recently reported heterologous expression in *S. cerevisiae* (Leandro et al. 2006) of both the facilitated diffusion and the proton symporter proteins from the xylose-utilizing yeast *Candida intermedia* PYCC 4715 (Gárdonyi et al. 2003b) offers great promise for future development of pentose fermenting strains of *S. cerevisiae*.

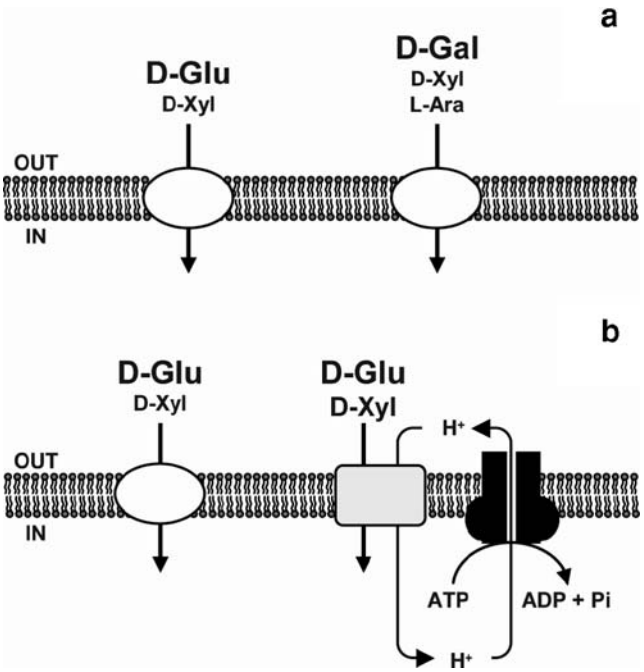


**Fig. 4** Illustration of transport mechanisms for sugars present in hemicellulosic hydrolysates and found in **a** *S. cerevisiae* and in **b** other yeasts and filamentous fungi. *D-Glu* D-glucose, *D-Xyl* D-xylose, *D-Gal* D-galactose, *L-Ara* L-arabinose

In defined mineral medium (Hahn-Hägerdal et al. 2005), almost all recombinant D-xylose- and L-arabinose-utilizing strains of *S. cerevisiae* produce the corresponding sugar alcohols xylitol and L-arabitol, which translates into reduced ethanol yield—one of the four benchmarks. In the absence of oxygen and other electron acceptors, xylitol and arabitol formation is caused by the inability of the cell to provide $NAD^+$ for the XDH reaction and the additional $NAD^+$-consuming reactions in the arabinose pathway (Fig. 3). Xylitol formation has been reduced by introducing the XI pathway (Walfridsson et al. 1996; Kuyper et al. 2003; Lönn et al. 2003; Fig. 3) instead of the XR/XDH pathway. So far, XI enzymes have been only expressed under strong promoters in multicopy plasmids, suggesting that the specific activity of the present XIs is insufficient to generate stable chromosomally integrated recombinant strains.

Very little xylitol is formed by XR/XDH-carrying strains in lignocellulose hydrolysate (Moniruzzaman et al. 1998; Martin et al. 2002; Sedlak and Ho 2004; Katahira et al. 2006; Öhgren et al. 2006), in contrast to defined mineral medium. Similarly, xylitol formation has been reduced by adding electron acceptors such as ketones and aldehydes in the medium (Bruinenberg et al. 1983; Alexander 1986; Wahlbom and Hahn-Hägerdal 2002). Such compounds are present also in lignocellulose hydrolysates (Larsson et al. 1999a; Palmqvist and Hahn-Hägerdal 2000b) and most likely also in YP medium (Dien et al. 1996; Ho et al. 1998). Xylitol formation is also significantly lower when xylose is cofermented with glucose (Meinander et al. 1999; Karhumaa et al. 2007; Öhgren et al. 2006). Under these conditions, the rate of xylose utilization is increased suggesting that xylitol formation is not only a consequence of an insufficient pool of oxidized cofactors but also a function of an insufficient xylose flux (Hofmeyr and Cornish-Bowden 2000).

Other strategies to reduce xylitol formation and to increase the ethanol yield are (1) to alter the pool of oxidized cofactors by engineering the levels of XR and XDH (Walfridsson et al. 1997; Eliasson et al. 2001; Jeppsson et al. 2003b; Karhumaa et al. 2007), (2) to express a mutant XR with reduced affinity for NADPH (Jeppsson et al. 2006), and (3) to downregulate the *ZWF1* gene encoding the NADPH producing glucose 6-phosphate dehydrogenase enzyme (Jeppsson et al. 2002) (Table 2). In addition,

*S. cerevisiae* harbors a number of endogenous reductases, such as the aldose reductase GRE3 (Kuhn et al. 1995; Träff et al. 2002), which naturally convert xylose to xylitol (Kuhn et al. 1995). Strains in which the *GRE3* gene was deleted produce less xylitol (Träff et al. 2001; Träff-Bjerre et al. 2004), which is of particular importance for XI-carrying strains because xylitol is a potent inhibitor of this enzyme (Yamanaka 1969; Lönn et al. 2003; Kuyper et al. 2005a).

The activity of PPP in *S. cerevisiae* is lower than in other yeast species (Gancedo and Lagunas 1973; Fiaux et al. 2003), most likely due to the domestication of *S. cerevisiae* for ethanolic fermentation of hexose sugars. Overexpression of the gene encoding transaldolase (Walfridsson et al. 1995) and overexpression of the entire non-oxidative PPP (Johansson and Hahn-Hägerdal 2002a,b; Karhumaa et al. 2005, 2007; Kuyper et al. 2005a) concomitantly increased the rate of xylose consumption. Overexpression of PPP in *S. cerevisiae* is probably one of the most illustrative examples of the advantage of a pulling strategy over a pushing strategy for redirecting metabolism (Cornish-Bowden et al. 1995; Eliasson et al. 2001). The importance of high PPP activity was recently verified in a proteome analysis of recombinant xylose-fermenting *S. cerevisiae* (Karhumaa et al., submitted for publication).

Anaerobic growth reflects cell viability and is thus a desirable quality in industrial fermentation. Neither natural nor recombinant xylose-metabolizing yeast grow anaerobically on xylose, the molecular reasons for which are not entirely known. Therefore, evolutionary engineering strategies (Sauer 2001) have been applied to achieve growth on xylose in the absence of oxygen (Sonderegger and Sauer 2003; Kuyper et al. 2004) (Table 2). Transcription analysis performed on a mutant strain indicated that a concerted upregulation of central metabolism was necessary to permit anaerobic growth on xylose (Sonderegger et al. 2004a).

The phosphoketolase pathway is generally considered to be present only in bacteria. However, phosphoketolase activity was detected in a *Rhodotorula* sp. strain (Evans and Ratledge 1984). This pathway can theoretically increase the ethanol yield from xylose from 0.51 g ethanol/g xylose to 0.61 g ethanol/g xylose. However, when the bacterial genes encoding phosphoketolase and phosphotransacetylase were expressed in *S. cerevisiae* and the metabolism was further manipulated with a deletion of a acetaldehyde dehydrogenase, the ethanol yield increased only slightly (Sonderegger et al. 2004c).

In addition to xylitol, glycerol is another by-product that reduces the ethanol yield. Glycerol is formed by *S. cerevisiae* during anaerobic growth to oxidize surplus NADH formed in biosynthesis of amino acids (Oura 1977; Albers et al. 1996). NADH accumulates because *S. cerevisiae* does not harbor a transhydrogenase to convert NADH into NADPH in response to cellular demand (Bruinenberg et al. 1983). Artificial transhydrogenase activities have been introduced in *S. cerevisiae* by engineering endogenous reactions of ammonia and amino acid metabolism (Moreira dos Santos et al. 2003; Roca et al. 2003), which reduced xylitol formation in recombinant xylose-utilizing strains of *S. cerevisiae* (Roca et al. 2003; Verho et al. 2003). Also, the choice of nitrogen source can affect xylitol formation (Panagiotou et al. 2006; Slininger et al. 2006). However, industrial media contain various nitrogen sources, which may considerably limit the industrial exploitation of such metabolic engineering strategies (Bro et al. 2006).

*S. cerevisiae* consumes glucose preferentially to any other sugar, which results from carbon catabolite repression (Gancedo 1998) and allosteric competition of the sugars for transport. However, simultaneous consumption of all sugars in the lignocellulose hydrolysates would enhance the specific ethanol productivity. Several recombinant strains were reported to consume xylose in the presence of glucose. For example, the recombinant *S. cerevisiae* strains TMB 3057 (Karhumaa et al. 2007) and 1400(pLNH32) (Ho et al. 1998) coconsume glucose and xylose at glucose concentrations below 10 g/l. Furthermore, signaling elements of the carbon catabolite repression cascade have been engineered to overcome glucose repression, resulting in increased coconsumption of glucose and xylose with a concomitantly increased ethanol production (Roca et al. 2004; Thanvantri Gururajan et al., submitted for publication).

## Industrial pentose-fermenting strains of *S. cerevisiae*

Fermentation of lignocellulosic hydrolysates requires production strains that combine osmotolerance with tolerance towards lignocellulose degradation products as recently reviewed by Liu (2006) and by Almeida et al. (2007). These traits are generally summarized in the term *inhibitor tolerance*, which is defined in relation to a particular set of hydrolysates from a particular raw material prepared with a particular fractionation and hydrolysis procedure. Inhibitor tolerance has not been rationalized in molecular terms (Liu 2006), which has called for empiric selection of fermentation microorganisms with improved tolerance in relation to particular hydrolysates (Nigam 2001; Hahn-Hägerdal et al. 2005). Rational engineering approaches to improve inhibitor tolerance have so far been limited to overexpression of specific enzymes including laccase (Larsson et al. 2001a), phenylacrylic acid decarboxylase (Larsson et al. 2001b), glucose 6-phosphate dehydrogenase (Gorsich et al. 2006), and alcohol dehydrogenase 6 (Petersson et al. 2006). The influence of lignocellulose hydrolysate on growth of a number of xylose-fermenting strains has been described (Sonderegger et al. 2004b), confirming the general observation that lignocellulose hydrolysate inhibits growth before it

**Table 3** Characteristics and fermentation performance of industrial xylose-fermenting *S. cerevisiae* strains in laboratory conditions in defined mineral medium

| Strain | Genotype | Strain background | Anaerobic specific xylose consumption rate | Ethanol yield/ xylose | Xylitol yield | Setup | Aerobic growth rate on xylose | Reference |
|---|---|---|---|---|---|---|---|---|
| TMB 3400 | XR/XDH/XK integrated and random mutagenesis | USM21 (van der Westhuizen and Pretorius 1992) | 0.18[a] (Sonderegger et al. 2004b) | 0.18 | 0.25 | Batch 8 g/l xylose | 0.14 | (Wahlbom et al. 2003) |
| TMB 3006 | XR/XDH/XK integrated in | TMB 3000 (Linden et al. 1992) | 0.04[a] | – | 0.30[a] | Batch 20 g/l glucose + 50 g/l xylose | – | (Johansson 2001) |
| Adapted TMB 3006 | improved hydrolysate tolerance by evolutionary engineering | TMB 3000 (Linden et al. 1992) | – | – | – | – | – | (Hahn-Hägerdal et al. 2005) |
| F 12 | XR/XDH/XK integrated | *S. cerevisiae* F | 0.18 | 0.26 | 0.40 | Batch 50 g/l glucose + 50 g/l xylose | – | (Sonderegger et al. 2004b) |
| BH42 | bred strain | – | 0.17 | 0.28 | 0.36 | Batch 50 g/l glucose + 50 g/l xylose | – | Isabel Spencer-Martins/ (Sonderegger et al. 2004b) |
| A4 | XR/XDH/XK integrated in *S. cerevisiae* A+ | *S. cerevisiae* A | 0.21 | 0.19[b] | 0.26[a] | Batch 50 g/l glucose + 50 g/l xylose | – | (Zaldivar et al. 2002) |
| 424A LNH-ST | XR/XDH/XK multiply integrated | *S. cerevisiae* ATCC 4124 | – | – | 0.11[a] | Batch | – | (Sedlak and Ho 2004) |
| 1400 (pLNH32) | XR/XDH/XK on plasmid | *Saccharomyces diastaticus*/ *Saccharomyces uvarum* hybrid | – | 0.3[a] | 0.1[a] | Batch 50 g/l xylose in YPD | – | (Ho et al. 1998) |

*YPD* Yeast, peptone, and dextrose
[a] Approximated from figure
[b] Recalculated from reference

inhibits ethanolic fermentation (Hahn-Hägerdal et al. 1994b). Industrial yeast strains tolerate many hydrolysates better than laboratory strains (Sonderegger et al. 2004b), which is why generating pentose-fermenting *S. cerevisiae* strains with an industrial background is necessary.

Whereas laboratory strains of *S. cerevisiae* are useful for evaluating metabolic engineering strategies, they do not possess the robustness required in the industrial context (Garay-Arroyo et al. 2004; Sonderegger et al. 2004b; Nilsson et al. 2005). Laboratory strains are usually haploid and have a defined genetic composition, and their genetic modification has been eased by the introduction of auxotrophic selection markers, which facilitates metabolic engineering strategies involving plasmids (Entian and Kötter 1998; Walker 1998). However, multicopy plasmids are incompatible with the industrial context primarily because of their inherent instability (Meinander and Hahn-Hägerdal 1997). Furthermore, legal regulations and public perception will create obstacles to the acceptance of antibiotic selection markers on the industrial scale, even though these can be removed (Guldener et al. 1996; Johansson and Hahn-Hägerdal 2002b). Instead of plasmid usage, conserved genomic regions, such as ribosomal DNA (rDNA) sequences, can be useful targets for chromosomal gene integration in industrial yeast strains. In the pentose fermentation context, where the necessary heterologous enzymes have low specific activities, an advantage of the rDNA locus is the possibility to chromosomally integrate multiple gene copies (Szostak and Wu 1979; Lopes et al. 1996; Wery et al. 1997; Ho et al. 1999; Klabunde et al. 2003; Karhumaa et al. 2006).

In contrast to the well-defined genetic make-up of laboratory strains, industrial strains are diploid, polyploid, or aneuploid, and often contain genetic segments from several yeast species (Groth et al. 1999; Marinoni et al. 1999; Gonzalez et al. 2006; Karhumaa et al., submitted for publication). The presence of multiple chromosome sets makes gene deletions in particular challenging because the target gene must be deleted from all chromosomes containing it. Only recently have auxotrophic mutants in a diploid strain of *S. cerevisiae* been generated (Hashimoto et al. 2005), but such strains will most likely only be used for exploratory metabolic engineering approaches rather than for the design of industrial production strains for reasons given above. However, genomic integration of genes encoding the pentose utilization pathway enzymes into industrial strains is relatively straightforward because positive selection for growth on xylose or arabinose can be used (Ho et al. 1998; Johansson 2001; Wahlbom et al. 2003; Karhumaa et al. 2006).

Only a limited number of industrial pentose-fermenting strains have been described in literature, and all genetic engineering has been limited to the introduction of the initial xylose and arabinose utilization pathways. Further improvement of the recombinant strains has been achieved by adaptation strategies, including random mutagenesis (Wahlbom et al. 2003), evolutionary engineering (Sonderegger et al. 2004b), and breeding (Sonderegger et al. 2004b). Table 3 summarizes the fermentative performance of publicly known xylose-fermenting industrial strains in laboratory conditions, and Table 4 in lignocellulose hydrolysates. The parental strains of the strains summarized in Tables 3 and 4 originated

**Table 4** Fermentation performance of industrial xylose-fermenting *S. cerevisiae* strains in lignocellulose hydrolysates

| Strain | Hydrolysate | Detoxification | Setup | Ethanol productivity | Ethanol yield on total sugar | Reference |
|---|---|---|---|---|---|---|
| TMB 3400 | Spruce | Nondetoxified | Fed-batch | 0.25 | 0.43 | (Hahn-Hägerdal and Pamment 2004) |
| TMB 3006 | Spruce | Nondetoxified | Fed-batch | 0.66 | 0.37 | (Hahn-Hägerdal and Pamment 2004) |
| 424A LNH-ST | Corn stover | Overliming | Batch | – | 0.41 | (Sedlak and Ho 2004) |
| 424A LNH-ST | Corn stover | Not known | Batch | – | 0.44 | (Sedlak and Ho 2004) |
| Adapted TMB 3006 | Northern spruce 70% | Nondetoxified | continuous, D 0.1 | – | 0.41 (on glucose) | (Hahn-Hägerdal et al. 2005) |
| MT8-1/Xyl/BGL | wood chip hydrolysate | Overliming | Batch | 0.42 | 0.41 | (Katahira et al. 2006) |
| F 12 | Still bottoms fermentation residue (vinasse) | Not known | Batch | 0.005–0.24 | 0.27[a] | (Olsson et al. 2006) |
| TMB 3400 | Corn stover, steam pretreatment | Nondetoxified | Batch SSF | – | 0.32 | (Öhgren et al. 2006) |
| TMB 3400 | Corn stover, steam pretreatment | Nondetoxified | Fed-batch SSF | – | 0.30 | (Öhgren et al. 2006) |

[a] Recalculated from reference

either from the ethanol industry and were obtained by hybridization (D'Amore et al. 1989; van der Westhuizen and Pretorius 1992) or by natural selection in industrial environments (Linden et al. 1992). All strains are genetically modified to carry the *P. stipitis* XR and XDH enzymes and to overexpress the endogenous XK (Ho et al. 1998; Johansson 2001; Wahlbom et al. 2003). XI-carrying industrial strains or the performance of such strains in lignocellulose hydrolysate have not yet been reported. More recently, the first xylose and arabinose coutilizing industrial *S. cerevisiae* strain was described (Karhumaa et al. 2006). The performance of this strain in lignocellulose hydrolysate has not yet been reported.

The existing data enables us to assess the strains summarized in Tables 3 and 4 with regard to the four criteria discussed in the beginning of this review. Only strain TMB 3400 was exposed to a protocol of improved process water economy, when it was used in a simultaneous saccharification and fermentation (SSF) setup with 12% water insoluble solids (Öhgren et al. 2006). With respect to inhibitor tolerance, strains TMB3006 and TMB3400 ferment both spruce and corn stover hydrolysates without prior detoxification (Hahn-Hägerdal and Pamment 2004), while strain 424A(LNF-ST) requires detoxification of the hydrolysate (Sedlak and Ho 2004). Only when a mildly treated and dilute hydrolysate was investigated could the detoxification step be omitted for this strain (Mosier et al. 2005). Strains TMB 3006 and 3400 were used for fermentation of nondetoxified lignocellulose hydrolysate both in batch and fed-batch mode (Hahn-Hägerdal and Pamment 2004; Öhgren et al. 2006). The fed-batch mode of fermentation has proven to be a very valuable technology making use of the intrinsic capacity of yeast to convert the inhibitory compounds present in hydrolysates (Taherzadeh et al. 2000) because the slow addition of hydrolysate minimizes the concentrations of the inhibitory compounds at a given time. Furthermore, the concentration of glucose is kept at a low enough level to allow simultaneous xylose utilization.

Ethanol yields above 0.4 g ethanol/g consumed sugar have been obtained both with strain 424A(LNF-ST), strain TMB3006, and strain TMB 3400 in lignocellulose hydrolysates (Table 4; Hahn-Hägerdal and Pamment 2004; Öhgren et al. 2006). For strain 424A(LNF-ST) this can partially be ascribed to high levels of yeast extract and peptone in the medium (Sedlak and Ho 2004; Hahn-Hägerdal et al. 2005), whereas relatively high hexose content of the spruce hydrolysate most likely contributed to the ethanol yield obtained with TMB3006. The ethanol yields observed in lignocellulose hydrolysates (Table 4) are generally higher than those observed in defined mineral medium due to the presence of electron acceptors and the absence of xylitol production in most lignocellulose hydrolysates (Moniruzzaman et al. 1998; Martin et al. 2002; Sedlak and Ho 2004; Katahira et al. 2006; Öhgren et al. 2006). However, the specific ethanol productivity for the industrial xylose-fermenting strains is an order of magnitude lower than what is achieved in hexose fermentation. Still, this may not pose a problem in the bioconversion of lignocellulosic raw material to ethanol, because the rate of the enzymatic hydrolysis of the raw material is considerably lower than the rate of hexose consumption by the yeast (Ljunggren 2005).

In conclusion, inhibitor-tolerant strains capable of fermenting nondetoxified hydrolysates are an absolute requirement for the development of lignocellulosic fermentation processes. In addition, such strains must ferment all hemicellulose-derived monosaccharides, including the pentose sugars xylose and arabinose, to ethanol. Industrial xylose-fermenting strains of *S. cerevisiae* are now reaching levels of fermentation performance that approach economically feasible ethanol production from lignocellulose, as demonstrated for strain TMB 3400 in a fed-batch SSF fermentation setup of nondetoxified corn stover hydrolysate (Öhgren et al. 2006). Only recently has the development of industrial arabinose-fermenting *S. cerevisiae* strains been initiated (Karhumaa et al. 2006). Moreover, the simultaneous cofermentation of hexose and pentose sugars constitutes a major strain engineering challenge.

**Acknowledgement** The authors acknowledge the financial support from the Swedish Energy Agency (STEM), Sweden, and the Fundação para a Ciência e a Technologia (FCT), Portugal.

## References

Albers E, Larsson C, Liden G, Niklasson C, Gustafsson L (1996) Influence of the nitrogen source on *Saccharomyces cerevisiae* anaerobic growth and product formation. Appl Environ Microbiol 62(9):3187–3195

Alexander NJ (1986) Acetone stimulation of ethanol production from D-xylose by *Pachysolen tannophilus*. Appl Microbiol Biotechnol 25:203–207

Alkasrawi M, Galbe M, Zacchi G (2002) Recirculation of process streams in fuel ethanol production from softwood based on simultaneous saccharification and fermentation. Appl Biochem Biotechnol 98–100:849–861

Almeida JR, Modig T, Petersson A, Hahn-Hägerdal B, Liden G, Gorwa-Grauslund M-F (2007) Increased tolerance and conversion of inhibitors in lignocellulosic hydrolysates by *Saccharomyces cerevisiae*. J Chem Technol Biotechnol (in press)

Anderson RL, Wood WA (1962) Purification and properties of L-xylulokinase. J Biol Chem 237:1029

Ångspanneföreningen (1994) IPK system study—techno/economic reviews of process combinations of ethanol processes and other relevant industrial processes. Report: P23332-1. NUTEK, Stockholm, Sweden

Atkinson B, Mavituna F (1991) Biochemical engineering and biotechnology handbook. Stockton, New York, NY, USA

Attfield PV, Bell PJ (2006) Use of population genetics to derive nonrecombinant *Saccharomyces cerevisiae* strains that grow using xylose as a sole carbon source. FEMS Yeast Res 6 (6):862–868

Banerjee S, Archana A, Satyanarayana T (1994) Xylose metabolism in a thermophilic mould *Malbranchea pulchella* var. *sulfurea* TMD-8. Curr Microbiol 29:349–352

Barnett JA (2000) Yeasts, characteristics and identification. Cambridge Univ Press, Cambridge

Becker J, Boles E (2003) A modified *Saccharomyces cerevisiae* strain that consumes L-arabinose and produces ethanol. Appl Environ Microbiol 69(7):4144–4150

Berg C (2002) World ethanol production 2001. Available at http://www.distill.com/world_ethanol_production.htm

Bolen PL, Roth KA, Freer SN (1986) Affinity purifications of aldose reductase and xylitol dehydrogenase from the xylose-fermenting yeast *Pachysolen tannophilus*. Appl Environ Microbiol 52(4):660–664

Bothast RJ, Nichols NN, Dien BS (1999) Fermentations with new recombinant organisms. Biotechnol Prog 15(5):867–875

Boxma B, Voncken F, Jannink S, van Alen T, Akhmanova A, van Weelden SW, van Hellemond JJ, Ricard G, Huynen M, Tielens AG, Hackstein JH (2004) The anaerobic chytridiomycete fungus *Piromyces* sp. E2 produces ethanol via pyruvate:formate lyase and an alcohol dehydrogenase E. Mol Microbiol 51(5):1389–1399

Bro C, Regenberg B, Forster J, Nielsen J (2006) *In silico* aided metabolic engineering of *Saccharomyces cerevisiae* for improved bioethanol production. Metab Eng 8(2):102–111

Bruinenberg PM, Peter HM, van Dijken JP, Scheffers WA (1983) The role of redox balances in the anaerobic fermentation of xylose by yeasts. Eur J Appl Microb Biotech 18:287–292

Bruinenberg PM, de Bot PHM, van Dijken JP, Scheffers WA (1984) NADH-linked aldose reductase: the key to anaerobic fermentation of xylose by yeasts. Appl Microbiol Biotechnol 19:256–260

Chen WP (1980) Glucose isomerase (a review). Process Biochem 15:30–41

Chiang C, Knight SG (1960) A new pathway of pentose metabolism. Biochem Biophys Res Commun 3:554–559

Chiang LC, Gong CS, Chen LF, Tsao GT (1981) d-Xylulose fermentation to ethanol by *Saccharomyces cerevisiae*. Appl Environ Microbiol 42(2):284–289

Cornish-Bowden A, Hofmeyr J-HS, Cardenas ML (1995) Strategies for manipulating metabolic fluxes in biotechnology. Bioorg Chem 23:439–449

D'Amore T, Celotta G, Russell I, Stewart GG (1989) Selection and optimization of yeast suitable for ethanol production at 40°C. Enzyme Microb Technol 11:263–274

de Vries RP, Flipphi MJ, Witteveen CF, Visser J (1994) Characterization of an *Aspergillus nidulans* L-arabitol dehydrogenase mutant. FEMS Microbiol Lett 123(1–2):83–90

Deanda K, Zhang M, Eddy C, Picataggio S (1996) Development of an arabinose-fermenting *Zymomonas mobilis* strain by metabolic pathway engineering. Appl Environ Microbiol 62(12):4465–4470

Deng XX, Ho NW (1990) Xylulokinase activity in various yeasts including *Saccharomyces cerevisiae* containing the cloned xylulokinase gene. Appl Biochem Biotechnol 24–25:193–199

Dien BS, Kurtzman CP, Saha BC, Bothast RJ (1996) Screening for L-arabinose fermenting yeasts. Appl Biochem Biotechnol 57–58:233–242

Dien BS, Cotta MA, Jeffries TW (2003) Bacteria engineered for fuel ethanol production: current status. Appl Microbiol Biotechnol 63 (3):258–266

Eliasson A, Christensson C, Wahlbom CF, Hahn-Hägerdal B (2000) Anaerobic xylose fermentation by recombinant *Saccharomyces cerevisiae* carrying *XYL1*, *XYL2*, and *XKS1* in mineral medium chemostat cultures. Appl Environ Microbiol 66(8):3381–3386

Eliasson A, Hofmeyr J-HS, Pedler S, Hahn-Hägerdal B (2001) The xylose reductase/xylitol dehydrogenase/xylulokinase ratio affects product formation in recombinant xylose-utilising *Saccharomyces cerevisiae*. Enzyme Microb Technol 29:288–297

Entian KD, Kötter P (1998) Yeast mutant and plasmid collections. In: Brown JPA, Tuite MF (eds) Yeast gene analysis, vol. 26. Academic, San Diego, California, pp 431–449

Evans CT, Ratledge C (1984) Induction of xylulose-5-phosphate phosphoketolase in a variety of yeasts grown on D-xylose-the key to efficient xylose metabolism. Arch Microbiol 139:48–52

Farrell AE, Plevin RJ, Turner BT, Jones AD, O'Hare M, Kammen DM (2006) Ethanol can contribute to energy and environmental goals. Science 311(5760):506–508

Fiaux J, Cakar ZP, Sonderegger M, Wuthrich K, Szyperski T, Sauer U (2003) Metabolic-flux profiling of the yeasts *Saccharomyces cerevisiae* and *Pichia stipitis*. Eukaryot Cell 2(1):170–180

Fong JC, Svenson CJ, Nakasugi K, Leong CT, Bowman JP, Chen B, Glenn DR, Neilan BA, Rogers PL (2006) Isolation and characterization of two novel ethanol-tolerant facultative–anaerobic thermophilic bacteria strains from waste compost. Extremophiles 10(5):363–372

Fonseca C, Spencer-Martins I, Hahn-Hägerdal B (2007) L-arabinose metabolism in *Candida arabinofermentans* PYCC $5603^T$ and *Pichia guilliermondii* PYCC 3012: influence of sugar and oxygen on product formation. Appl Microbiol Biotechnol DOI 10.1007/s00253-066-0830-7

Galbe M, Zacchi G (2002) A review of the production of ethanol from softwood. Appl Microbiol Biotechnol 59(6):618–628

Gancedo JM (1998) Yeast carbon catabolite repression. Microbiol Mol Biol Rev 62(2):334–361

Gancedo JM, Lagunas R (1973) Contribution of the pentose phosphate pathway to glucose metabolism in *Saccharomyces cerevisiae*: a critical analysis on the use of labelled glucose. Plant Sci Lett 1:193–200

Garay-Arroyo A, Covarrubias AA, Clark I, Nino I, Gosset G, Martinez A (2004) Response to different environmental stress conditions of industrial and laboratory *Saccharomyces cerevisiae* strains. Appl Microbiol Biotechnol 63(6):734–741

Gárdonyi M, Jeppsson M, Liden G, Gorwa-Grauslund MF, Hahn-Hägerdal B (2003a) Control of xylose consumption by xylose transport in recombinant *Saccharomyces cerevisiae*. Biotechnol Bioeng 82(7):818–824

Gárdonyi M, Osterberg M, Rodrigues C, Spencer-Martins I, Hahn-Hägerdal B (2003b) High capacity xylose transport in *Candida intermedia* PYCC 4715. FEMS Yeast Res 3(1):45–52

Gonzalez SS, Barrio E, Gafner J, Querol A (2006) Natural hybrids from *Saccharomyces cerevisiae*, *Saccharomyces bayanus* and *Saccharomyces kudriavzevii* in wine fermentations. FEMS Yeast Res 6(8):1221–1234

Gorsich SW, Dien BS, Nichols NN, Slininger PJ, Liu ZL, Skory CD (2006) Tolerance to furfural-induced stress is associated with pentose phosphate pathway genes ZWF1, GND1, RPE1, and TKL1 in *Saccharomyces cerevisiae*. Appl Microbiol Biotechnol 71(3):339–349

Gray KA, Zhao L, Emptage M (2006) Bioethanol. Curr Opin Chem Biol 10(2):141–146

Groth C, Hansen J, Piskur J (1999) A natural chimeric yeast containing genetic material from three species. Int J Syst Bacteriol 49(Pt 4):1933–1938

Guldener U, Heck S, Fielder T, Beinhauer J, Hegemann JH (1996) A new efficient gene disruption cassette for repeated use in budding yeast. Nucleic Acids Res 24(13):2519–2524

Hahn-Hägerdal B, Pamment N (2004) Microbial pentose metabolism. Appl Biochem Biotechnol 113–116:1207–1209

Hahn-Hägerdal B, Linden T, Senac T, Skoog K (1991) Ethanolic fermentation of pentoses in lignocellulose hydrolysates. Appl Biochem Biotechnol 28–29:131–144

Hahn-Hägerdal B, Jeppsson H, Olsson L, Mohagheghi A (1994a) An interlaboratory comparison of the performance of ethanol-producing micro-organisms in a xylose-rich acid hydrolysate. Appl Microbiol Biotechnol 41:62–72

Hahn-Hägerdal B, Jeppsson H, Skoog K, Prior BA (1994b) Biochemistry and physiology of xylose fermentation by yeasts. Enzyme Microb Technol 16:933–943

Hahn-Hägerdal B, Karhumaa K, Larsson CU, Gorwa-Grauslund M, Görgens J, van Zyl WH (2005) Role of cultivation media in the development of yeast strains for large scale industrial use. Microb Cell Fact 4:31

Hahn-Hägerdal B, Galbe M, Gorwa-Grauslund M-F, Liden G, Zacchi G (2006) Bio-ethanol—the fuel of tomorrow from the residues of today. Trends Biotechnol 24(12):549–556

Hamacher T, Becker J, Gárdonyi M, Hahn-Hägerdal B, Boles E (2002) Characterization of the xylose-transporting properties of yeast hexose transporters and their influence on xylose utilization. Microbiology 148(Pt 9):2783–2788

Harhangi HR, Akhmanova AS, Emmens R, van der Drift C, de Laat WT, van Dijken JP, Jetten MS, Pronk JT, Op den Camp HJ (2003) Xylose metabolism in the anaerobic fungus *Piromyces* sp. strain E2 follows the bacterial pathway. Arch Microbiol 180(2):134–141

Hashimoto S, Ogura M, Aritomi K, Hoshida H, Nishizawa Y, Akada R (2005) Isolation of auxotrophic mutants of diploid industrial yeast strains after UV mutagenesis. Appl Environ Microbiol 71 (1):312–319

Hayn M, Steiner W, Klinger R, Steinmüller H, Sinner M, Esterbauer H (1993) Basic research and pilot studies on the enzymatic conversion of lignocellulosics. In: Saddler JN (ed) Bioconversion of forest and agricultural plant residues. CAB International, Wallingford, UK, pp 33–72

Herrera S (2006) Bonkers about biofuels. Nat Biotechnol 24(7):755–760

Hespell RB, Wyckoff H, Dien BS, Bothast RJ (1996) Stabilization of pet operon plasmids and ethanol production in *Escherichia coli* strains lacking lactate dehydrogenase and pyruvate formate-lyase activities. Appl Environ Microbiol 62(12):4594–4597

Ho NW, Chen Z, Brainard AP (1998) Genetically engineered *Saccharomyces* yeast capable of effective cofermentation of glucose and xylose. Appl Environ Microbiol 64(5):1852–1859

Ho NW, Chen Z, Brainard AP, Sedlak M (1999) Successful design and development of genetically engineered *Saccharomyces* yeasts for effective cofermentation of glucose and xylose from cellulosic biomass to fuel ethanol. Adv Biochem Eng Biotechnol 65:163–192

Hofmeyr JS, Cornish-Bowden A (2000) Regulating the cellular economy of supply and demand. FEBS Lett 476(1–2):47–51

Ingram LO, Conway T, Clark DP, Sewell GW, Preston JF (1987) Genetic engineering of ethanol production in *Escherichia coli*. Appl Environ Microbiol 53(10):2420–2425

Jeffries TW (1983) Utilization of xylose by bacteria, yeasts, and fungi. Adv Biochem Eng Biotechnol 27:1–32

Jeffries TW (2006) Engineering yeasts for xylose metabolism. Curr Opin Biotechnol 17(3):320–326

Jeppsson M, Johansson B, Hahn-Hägerdal B, Gorwa-Grauslund MF (2002) Reduced oxidative pentose phosphate pathway flux in recombinant xylose-utilizing *Saccharomyces cerevisiae* strains improves the ethanol yield from xylose. Appl Environ Microbiol 68(4):1604–1609

Jeppsson M, Johansson B, Jensen PR, Hahn-Hägerdal B, Gorwa-Grauslund MF (2003a) The level of glucose-6-phosphate dehydrogenase activity strongly influences xylose fermentation and inhibitor sensitivity in recombinant *Saccharomyces cerevisiae* strains. Yeast 20(15):1263–1272

Jeppsson M, Träff K, Johansson B, Hahn-Hägerdal B, Gorwa-Grauslund MF (2003b) Effect of enhanced xylose reductase activity on xylose consumption and product distribution in xylose-fermenting recombinant *Saccharomyces cerevisiae*. FEMS Yeast Res 3(2):167–175

Jeppsson M, Bengtsson O, Franke K, Lee H, Hahn-Hägerdal B, Gorwa-Grauslund MF (2006) The expression of a *Pichia stipitis* xylose reductase mutant with higher K(M) for NADPH increases ethanol production from xylose in recombinant *Saccharomyces cerevisiae*. Biotechnol Bioeng 93(4):665–673

Jin YS, Jeffries TW (2003) Changing flux of xylose metabolites by altering expression of xylose reductase and xylitol dehydrogenase in recombinant *Saccharomyces cerevisiae*. Appl Biochem Biotechnol 105–108:277–286

Jin YS, Ni H, Laplaza JM, Jeffries TW (2003) Optimal growth and ethanol production from xylose by recombinant *Saccharomyces cerevisiae* require moderate D-xylulokinase activity. Appl Environ Microbiol 69(1):495–503

Jin YS, Alper H, Yang YT, Stephanopoulos G (2005) Improvement of xylose uptake and ethanol production in recombinant *Saccharomyces cerevisiae* through an inverse metabolic engineering approach. Appl Environ Microbiol 71(12):8249–8256

Johansson B (2001) Metabolic engineering of the pentose phosphate pathway of xylose fermenting *Saccharomyces cerevisiae*. Ph.D. thesis, Department of Applied Microbiology, Lund University

Johansson B, Hahn-Hägerdal B (2002a) The non-oxidative pentose phosphate pathway controls the fermentation rate of xylulose but not of xylose in *Saccharomyces cerevisiae* TMB3001. FEMS Yeast Res 2:277–282

Johansson B, Hahn-Hägerdal B (2002b) Overproduction of pentose phosphate pathway enzymes using a new CRE-loxP expression vector for repeated genomic integration in *Saccharomyces cerevisiae*. Yeast 19(3):225–231

Johansson B, Christensson C, Hobley T, Hahn-Hägerdal B (2001) Xylulokinase overexpression in two strains of *Saccharomyces cerevisiae* also expressing xylose reductase and xylitol dehydrogenase and its effect on fermentation of xylose and lignocellulosic hydrolysate. Appl Environ Microbiol 67(9):4249–4255

Kadam KL, Wooley RJ, Aden A, Nguyen QA, Yancey MA, Ferraro FM (2000) Softwood forest thinnings as a biomass source for ethanol production: a feasibility study for California. Biotechnol Prog 16(6):947–957

Kamm B, Kamm M (2004) Principles of biorefineries. Appl Microbiol Biotechnol 64(2):137–145

Karhumaa K, Hahn-Hägerdal B, Gorwa-Grauslund MF (2005) Investigation of limiting metabolic steps in the utilization of xylose by recombinant *Saccharomyces cerevisiae* using metabolic engineering. Yeast 22(5):359–368

Karhumaa K, Wiedemann B, Boles E, Hahn-Hägerdal B, Gorwa-Grauslund MF (2006) Co-utilization of L-arabinose and D-xylose by laboratory and industrial *Saccharomyces cerevisiae* strains. Microb Cell Fact 5(1):18

Karhumaa K, Fromanger R, Hahn-Hägerdal B, Gorwa-Grauslund MF (2007) High activity of xylose reductase and xylitol dehydrogenase improves xylose fermentation by recombinant *Saccharomyces cerevisiae*. Appl Microbiol Biotechnol 73(5):1039–1046

Katahira S, Mizuike A, Fukuda H, Kondo A (2006) Ethanol fermentation from lignocellulosic hydrolysate by a recombinant xylose- and cellooligosaccharide-assimilating yeast strain. Appl Microbiol Biotechnol 72:1136–1143

Klabunde J, Kunze G, Gellissen G, Hollenberg CP (2003) Integration of heterologous genes in several yeast species using vectors containing a *Hansenula polymorpha*-derived rDNA-targeting element. FEMS Yeast Res 4(2):185–193

Klinke HB, Thomsen AB, Ahring BK (2004) Inhibition of ethanol-producing yeast and bacteria by degradation products produced during pre-treatment of biomass. Appl Microbiol Biotechnol 66(1):10–26

Kötter P, Ciriacy M (1993) Xylose fermentation by *Saccharomyces cerevisiae*. Appl Microbiol Biotechnol 38:776–783

Kuhn A, van Zyl C, van Tonder A, Prior BA (1995) Purification and partial characterization of an aldo-keto reductase from *Saccharomyces cerevisiae*. Appl Environ Microbiol 61(4):1580–1585

Kuyper M, Harhangi HR, Stave AK, Winkler AA, Jetten MS, de Laat WT, den Ridder JJ, Op den Camp HJ, van Dijken JP, Pronk JT (2003) High-level functional expression of a fungal xylose isomerase: the key to efficient ethanolic fermentation of xylose by *Saccharomyces cerevisiae*? FEMS Yeast Res 4(1):69–78

Kuyper M, Winkler AA, Van Dijken JP, Pronk JT (2004) Minimal metabolic engineering of *Saccharomyces cerevisiae* for efficient anaerobic xylose fermentation: a proof of principle. FEMS Yeast Res 4(6):655–664

Kuyper M, Hartog MM, Toirkens MJ, Almering MJ, Winkler AA, van Dijken JP, Pronk JT (2005a) Metabolic engineering of a xylose-isomerase-expressing *Saccharomyces cerevisiae* strain for rapid anaerobic xylose fermentation. FEMS Yeast Res 5(4–5): 399–409

Kuyper M, Toirkens MJ, Diderich JA, Winkler AA, van Dijken JP, Pronk JT (2005b) Evolutionary engineering of mixed-sugar utilization by a xylose-fermenting *Saccharomyces cerevisiae* strain. FEMS Yeast Res 5(10):925–934

Larsson S, Palmqvist E, Hahn-Hägerdal B, Tengborg C, Stenberg K, Zacchi G, Nilvebrant N-O (1999a) The generation of fermentation inhibitors during dilute acid hydrolysis of softwood. Enzyme Microb Technol 24:151–159

Larsson S, Reimann A, Nilvebrant N-O, Jönsson LJ (1999b) Comparison of different methods for the detoxification of lignocellulosic hydrolysates of spruce. Appl Biochem Biotechnol 77–79:91–103

Larsson S, Cassland P, Jönsson LJ (2001a) Development of a *Saccharomyces cerevisiae* strain with enhanced resistance to phenolic fermentation inhibitors in lignocellulose hydrolysates by heterologous expression of laccase. Appl Environ Microbiol 67 (3):1163–1170

Larsson S, Nilvebrant NO, Jönsson LJ (2001b) Effect of overexpression of *Saccharomyces cerevisiae* Pad1p on the resistance to phenylacrylic acids and lignocellulose hydrolysates under aerobic and oxygen-limited conditions. Appl Microbiol Biotechnol 57(1–2):167–174

Leandro MJ, Gonçalves P, Spencer-Martins I (2006) Two glucose/xylose transporter genes from the yeast *Candida intermedia*: first molecular characterization of a yeast xylose-H+ symporter. Biochem J 395(3):543–549

Lee N, Bendet I (1967) Crystalline L-ribulokinase from *Escherichia coli*. J Biol Chem 242(9):2043–2050

Lee N, Patrick JW, Masson M (1968) Crystalline L-ribulose 5-phosphate 4-epimerase from *Escherichia coli*. J Biol Chem 243(18):4700–4705

Lee KJ, Tribe DE, Rogers PL (1979) Ethanol production by *Zymomonas mobilis* in continuous culture at high glucose concentration. Biotechnol Lett 1:421–426

Lee WJ, Kim MD, Ryu YW, Bisson LF, Seo JH (2002) Kinetic studies on glucose and xylose transport in *Saccharomyces cerevisiae*. Appl Microbiol Biotechnol 60(1–2):186–191

Linden T, Peetre J, Hahn-Hägerdal B (1992) Isolation and characterization of acetic acid-tolerant galactose-fermenting strains of *Saccharomyces cerevisiae* from a spent sulfite liquor fermentation plant. Appl Environ Microbiol 58:1661–1669

Liu ZL (2006) Genomic adaptation of ethanologenic yeast to biomass conversion inhibitors. Appl Microbiol Biotechnol 73(1):27–36

Ljunggren M (2005) Kinetic analysis and modeling of enzymatic hydrolysis and SSF. MSc thesis, Department of Chemical Engineering, Lund University

Lopes TS, de Wijs IJ, Steenhauer SI, Verbakel J, Planta RJ (1996) Factors affecting the mitotic stability of high-copy-number integration into the ribosomal DNA of *Saccharomyces cerevisiae*. Yeast 12(5):467–477

Lönn A, Gardonyi M, van Zyl W, Hahn-Hägerdal B, Otero RC (2002) Cold adaptation of xylose isomerase from *Thermus thermophilus* through random PCR mutagenesis. Gene cloning and protein characterization. Eur J Biochem 269(1):157–163

Lönn A, Träff-Bjerre KL, Cordero Otero RR, van Zyl WH, Hahn-Hägerdal B (2003) Xylose isomerase activity influences xylose fermentation with recombinant *Saccharomyces cerevisiae* strains expressing mutated *xylA* from *Thermus thermophilus*. Enzyme Microb Technol 32:567–573

Marinoni G, Manuel M, Petersen RF, Hvidtfeldt J, Sulo P, Piskur J (1999) Horizontal transfer of genetic material among *Saccharomyces* yeasts. J Bacteriol 181(20):6488–6496

Martin C, Galbe M, Wahlbom CF, Hahn-Hägerdal B, Jönsson LJ (2002) Ethanol production from enzymatic hydrolysates of sugarcane bagasse using recombinant xylose-utilising *Saccharomyces cerevisiae*. Enzyme Microb Technol 31:274–282

McMillan JD, Boynton BL (1994) Arabinose utilization by xylose-fermenting yeasts and fungi. Appl Biochem Biotechnol 45–46:569–584

Meinander N, Hahn-Hägerdal B (1997) Fed-batch xylitol production with two recombinant *Saccharomyces cerevisiae* strain expressing XYL1 at different levels, using glucose as a cosubstrate: a comparison of production parameters and strain stability. Biotechnol Bioeng 54(4):391–399

Meinander N, Boels I, Hahn-Hägerdal B (1999) Fermentation of xylose/glucose mixtures by metabolically engineerred *Saccharomyces cerevisiae* strains expressing XYL1 and XYL2 from *Pichia stipitis* with and without overexpression of TAL1. Bioresour Technol 68:79–87

Mohagheghi A, Evans K, Chou YC, Zhang M (2002) Cofermentation of glucose, xylose, and arabinose by genomic DNA-integrated xylose/arabinose fermenting strain of *Zymomonas mobilis* AX101. Appl Biochem Biotechnol 98–100:885–898

Moniruzzaman M, Dien BS, Skory CD, Chen ZD, Hespell RB, Ho NW, Dale BE, Bothast RJ (1998) Fermentation of corn fibre sugars by an engineered xylose utilizing *Saccharomyces* yeast strain. World J Microbiol Biotechnol 13:341–346

Moreira dos Santos M, Thygesen G, Kotter P, Olsson L, Nielsen J (2003) Aerobic physiology of redox-engineered *Saccharomyces cerevisiae* strains modified in the ammonium assimilation for increased NADPH availability. FEMS Yeast Res 4(1):59–68

Mosier N, Hendrickson R, Ho N, Sedlak M, Ladisch MR (2005) Optimization of pH controlled liquid hot water pretreatment of corn stover. Bioresour Technol 96(18):1986–1993

Nigam JN (2001) Ethanol production from wheat straw hemicellulose hydrolysate by *Pichia stipitis*. J Biotechnol 87(1):17–27

Nilsson A, Gorwa-Grauslund MF, Hahn-Hägerdal B, Liden G (2005) Cofactor dependence in furan reduction by *Saccharomyces cerevisiae* in fermentation of acid-hydrolyzed lignocellulose. Appl Environ Microbiol 71(12):7866–7871

Ohara H (2003) Biorefinery. Appl Microbiol Biotechnol 62(5–6):474–477

Öhgren K, Bengtsson O, Gorwa-Grauslund MF, Galbe M, Hahn-Hägerdal B, Zacchi G (2006) Simultaneous saccharification and co-fermentation of glucose and xylose in steam-pretreated corn stover at high fiber content with *Saccharomyces cerevisiae* TMB3400. J Biotechnol 126(4):488–498

Olsson L, Linden T, Hahn-Hägerdal B (1992) Performance of micro-organisms in spent sulphite liquor and enzymatic hydrolysate of steam-pretreated *Salix*. Appl Biochem Biotechnol 34–35:359–368

Olsson L, Soerensen HR, Dam BP, Christensen H, Krogh KM, Meyer AS (2006) Separate and simultaneous enzymatic hydrolysis and fermentation of wheat hemicellulose with recombinant xylose utilizing *Saccharomyces cerevisiae*. Appl Biochem Biotechnol 129–132:117–129

Oura E (1977) Reaction products of yeast fermentations. Process Biochem 12:19–21, 35

Palmqvist E, Hahn-Hägerdal B (2000a) Fermentation of lignocellulosic hydrolysates I: Inhibition and detoxification. Bioresour Technol 74:17–24

Palmqvist E, Hahn-Hägerdal B (2000b) Fermentation of lignocellulosic hydrolysates II: Inhibitors and mechanisms of inhibition. Bioresour Technol 74:25–33

Panagiotou G, Christakopoulos P, Grotkjaer T, Olsson L (2006) Engineering of the redox imbalance of *Fusarium oxysporum* enables anaerobic growth on xylose. Metab Eng 8(5):474–482

Patrick JW, Lee N (1968) Purification and properties of an L-arabinose isomerase from *Escherichia coli*. J Biol Chem 243(16):4312–4318

Petersson A, Almeida JR, Modig T, Karhumaa K, Hahn-Hägerdal B, Gorwa-Grauslund MF, Liden G (2006) A 5-hydroxymethyl furfural reducing enzyme encoded by the *Saccharomyces cerevisiae* ADH6 gene conveys HMF tolerance. Yeast 23(6): 455–464

Pitkänen JP, Rintala E, Aristidou A, Ruohonen L, Penttilä M (2005) Xylose chemostat isolates of *Saccharomyces cerevisiae* show altered metabolite and enzyme levels compared with xylose, glucose, and ethanol metabolism of the original strain. Appl Microbiol Biotechnol 67(6):827–837

Richard P, Toivari MH, Penttilä M (1999) Evidence that the gene YLR070c of *Saccharomyces cerevisiae* encodes a xylitol dehydrogenase. FEBS Lett 457(1):135–138

Richard P, Putkonen M, Väänänen R, Londesborough J, Penttilä M (2002) The missing link in the fungal L-arabinose catabolic pathway, identification of the L-xylulose reductase gene. Biochemistry 41(20):6432–6437

Richard P, Verho R, Putkonen M, Londesborough J, Penttilä M (2003) Production of ethanol from L-arabinose by *Saccharomyces cerevisiae* containing a fungal L-arabinose pathway. FEMS Yeast Res 3(2):185–189

Rizzi M, Elrlemann P, Bui-Thahn N-A, Dellweg H (1988) Xylose fermentation by yeasts. 4. Purification and kinetic studies of xylose reductase from *Pichia stipitis*. Appl Microbiol Biotechnol 29:148–154

Rizzi M, Harwart K, Erlemann P, Bui-Thahn N-A, Dellweg H (1989) Purification and properties of the NAD+ xylitol-dehydrogenase from the yeast *Pichia stipitis*. J Ferment Bioeng 67:20–24

Roca C, Nielsen J, Olsson L (2003) Metabolic engineering of ammonium assimilation in xylose-fermenting *Saccharomyces cerevisiae* improves ethanol production. Appl Environ Microbiol 69(8):4732–4736

Roca C, Haack MB, Olsson L (2004) Engineering of carbon catabolite repression in recombinant xylose fermenting *Saccharomyces cerevisiae*. Appl Microbiol Biotechnol 63(5):578–583

Rodrigues de Sousa H, Spencer-Martins I, Gonçalves P (2004) Differential regulation by glucose and fructose of a gene encoding a specific fructose/H+ symporter in *Saccharomyces* sensu stricto yeasts. Yeast 21(6):519–530

Rogers PL, Lee KJ, Tribe DE (1979) Kinetics of ethanol production by *Zymomonas mobilis* at high sugar concentration. Biotechnol Lett 1:165–170

Rosenberg SL (1980) Fermentation of pentose sugars to ethanol and other neutral products by microorganisms. Enzyme Microb Technol 2:185–193

Sauer U (2001) Evolutionary engineering of industrially important microbial phenotypes. Adv Biochem Eng Biotechnol 73:129–169

Schell DJ, Riley CJ, Dowe N, Farmer J, Ibsen KN, Ruth MF, Toon ST, Lumpkin RE (2004) A bioethanol process development unit: initial operating experiences and results with a corn fiber feedstock. Bioresour Technol 91(2):179–188

Schneider H, Wang PY, Chan YK, Maleszka R (1981) Conversion of D-xylose into ethanol by the yeast *Pachysolen tannophilus*. Biotechnol Lett 3:89–92

Sedlak M, Ho NW (2001) Expression of *E. coli* araBAD operon encoding enzymes for metabolizing L-arabinose in *Saccharomyces cerevisiae*. Enzyme Microb Technol 28(1):16–24

Sedlak M, Ho NW (2004) Production of ethanol from cellulosic biomass hydrolysates using genetically engineered *Saccharomyces* yeast capable of cofermenting glucose and xylose. Appl Biochem Biotechnol 113–116:403–416

Senac T, Hahn-Hägerdal B (1990) Intermediary metabolite concentrations in xylulose- and glucose-fermenting *Saccharomyces cerevisiae* cells. Appl Environ Microbiol 56(1):120–126

Skoog K, Hahn-Hägerdal B (1988) Xylose fermentation. Enzyme Microb Technol 10:66–80

Skoog K, Hahn-Hägerdal B (1990) Effect of oxygenation on xylose fermentation by *Pichia stipitis*. Appl Environ Microbiol 56(11): 3389–3394

Slininger PJ, Dien BS, Gorsich SW, Liu ZL (2006) Nitrogen source and mineral optimization enhance D-xylose conversion to ethanol by the yeast *Pichia stipitis* NRRL Y-7124. Appl Microbiol Biotechnol

Sommer P, Georgieva T, Ahring BK (2004) Potential for using thermophilic anaerobic bacteria for bioethanol production from hemicellulose. Biochem Soc Trans 32(Pt 2):283–289

Sonderegger M, Sauer U (2003) Evolutionary engineering of *Saccharomyces cerevisiae* for anaerobic growth on xylose. Appl Environ Microbiol 69(4):1990–1998

Sonderegger M, Jeppsson M, Hahn-Hägerdal B, Sauer U (2004a) Molecular basis for anaerobic growth of *Saccharomyces cerevisiae* on xylose, investigated by global gene expression and metabolic flux analysis. Appl Environ Microbiol 70(4):2307–2317

Sonderegger M, Jeppsson M, Larsson C, Gorwa-Grauslund MF, Boles E, Olsson L, Spencer-Martins I, Hahn-Hägerdal B, Sauer U (2004b) Fermentation performance of engineered and evolved xylose-fermenting *Saccharomyces cerevisiae* strains. Biotechnol Bioeng 87(1):90–98

Sonderegger M, Schumperli M, Sauer U (2004c) Metabolic engineering of a phosphoketolase pathway for pentose catabolism in *Saccharomyces cerevisiae*. Appl Environ Microbiol 70(5):2892–2897

Spencer-Martins I (1994) Transport of sugars in yeasts: implications in the fermentation of lignocellulosic materials. Bioresour Technol 50:51–57

Swings J, DeLey J (1977) The biology of *Zymomonas*. Bacteriol Rev 41:1–46

Szostak JW, Wu R (1979) Insertion of a genetic marker into the ribosomal DNA of yeast. Plasmid 2(4):536–554

Taherzadeh MJ, Gustafsson L, Niklasson C, Liden G (2000) Inhibition effects of furfural on aerobic batch cultivation of *Saccharomyces cerevisiae* growing on ethanol and/or acetic acid. J Biosci Bioeng 90(4):374–380

Tantirungkij M, Nakashima N, Seki T, Yoshida T (1993) Construction of xylose-assimilating *Saccharomyces cerevisiae*. J Ferment Bioeng 75:83–88

Thomas KC, Ingledew WM (1990) Fuel alcohol production: effects of free amino nitrogen on fermentation of very-high-gravity wheat mashes. Appl Environ Microbiol 56(7):2046–2050

Toivari MH, Aristidou A, Ruohonen L, Penttilä M (2001) Conversion of xylose to ethanol by recombinant *Saccharomyces cerevisiae*: importance of xylulokinase (XKS1) and oxygen availability. Metab Eng 3(3):236–249

Toivari MH, Salusjärvi L, Ruohonen L, Penttilä M (2004) Endogenous xylose pathway in *Saccharomyces cerevisiae*. Appl Environ Microbiol 70(6):3681–3686

Toivola A, Yarrow D, van den Bosch E, van Dijken JP, Scheffers WA (1984) Alcoholic fermentation of d-xylose by yeasts. Appl Environ Microbiol 47(6):1221–1223

Tong GE (1979) Industrial chemicals from fermentation. Enzyme Microb Technol 1:173–179

Träff KL, Otero Cordero RR, van Zyl WH, Hahn-Hägerdal B (2001) Deletion of the *GRE3* aldose reductase gene and its influence on xylose metabolism in recombinant strains of *Saccharomyces cerevisiae* expressing the xylA and XKS1 genes. Appl Environ Microbiol 67(12):5668–5674

Träff KL, Jönsson LJ, Hahn-Hägerdal B (2002) Putative xylose and arabinose reductases in *Saccharomyces cerevisiae*. Yeast 19(14): 1233–1241

Träff-Bjerre KL, Jeppsson M, Hahn-Hägerdal B, Gorwa-Grauslund MF (2004) Endogenous NADPH-dependent aldose reductase activity influences product formation during xylose consumption in recombinant *Saccharomyces cerevisiae*. Yeast 21(2):141–150

Tyurin MV, Sullivan CR, Lynd LR (2005) Role of spontaneous current oscillations during high-efficiency electrotransformation of thermophilic anaerobes. Appl Environ Microbiol 71(12): 8069–8076

van der Westhuizen TJ, Pretorius IS (1992) The value of electrophoretic fingerprinting and karyotyping in wine yeast breeding programmes. Antonie Van Leeuwenhoek 61(4):249–257

vanKuyk PA, de Groot MJ, Ruijter GJ, de Vries RP, Visser J (2001) The *Aspergillus niger* D-xylulose kinase gene is co-expressed with genes encoding arabinan degrading enzymes, and is essential for growth on D-xylose and L-arabinose. Eur J Biochem 268(20):5414–5423

Verho R, Londesborough J, Penttilä M, Richard P (2003) Engineering redox cofactor regeneration for improved pentose fermentation in *Saccharomyces cerevisiae*. Appl Environ Microbiol 69(10):5892–5897

Verho R, Putkonen M, Londesborough J, Penttilä M, Richard P (2004) A novel NADH-linked l-xylulose reductase in the l-arabinose catabolic pathway of yeast. J Biol Chem 279(15): 14746–14751

Visser W, Scheffers WA, Batenburg-van der Vegte WH, van Dijken JP (1990) Oxygen requirements of yeasts. Appl Environ Microbiol 56(12):3785–3792

von Sivers M, Zacchi G (1996) Ethanol from lignocellulosics: a review of the economy. Bioresour Technol 56:131–140

von Sivers M, Zacchi G, Olsson L, Hahn-Hägerdal B (1995) Cost analysis of ethanol production from willow using recombinant *Escherichia coli*. Biotechnol Prog 10:556–560

Vongsuvanlert V, Tani Y (1988) Purification and characterisation of xylose isomerase of a methanol yeast, *C. boidinii*, which is involved in sorbitol production from glucose. Agric Biol Chem 52:1817–1824

Wahlbom CF (2002) Metabolic engineering and random mutagenesis for improved xylose utilisation of *Saccharomyces cerevisiae*. Ph. D. thesis, Department of Applied Microbiology, Lund University

Wahlbom CF, Hahn-Hägerdal B (2002) Furfural, 5-hydroxymethyl furfural, and acetoin act as external electron acceptors during anaerobic fermentation of xylose in recombinant *Saccharomyces cerevisiae*. Biotechnol Bioeng 78(2):172–178

Wahlbom CF, van Zyl WH, Jönsson LJ, Hahn-Hägerdal B, Otero RR (2003) Generation of the improved recombinant xylose-utilizing *Saccharomyces cerevisiae* TMB 3400 by random mutagenesis and physiological comparison with *Pichia stipitis* CBS 6054. FEMS Yeast Res 3(3):319–326

Walfridsson M, Hallborn J, Penttilä M, Keränen S, Hahn-Hägerdal B (1995) Xylose-metabolizing *Saccharomyces cerevisiae* strains overexpressing the TKL1 and TAL1 genes encoding the pentose phosphate pathway enzymes transketolase and transaldolase. Appl Environ Microbiol 61(12):4184–4190

Walfridsson M, Bao X, Anderlund M, Lilius G, Bulow L, Hahn-Hägerdal B (1996) Ethanolic fermentation of xylose with *Saccharomyces cerevisiae* harboring the *Thermus thermophilus xylA* gene, which expresses an active xylose (glucose) isomerase. Appl Environ Microbiol 62(12):4648–4651

Walfridsson M, Anderlund M, Bao X, Hahn-Hägerdal B (1997) Expression of different levels of enzymes from the *Pichia stipitis* XYL1 and XYL2 genes in *Saccharomyces cerevisiae* and its effects on product formation during xylose utilisation. Appl Microbiol Biotechnol 48(2):218–224

Walker GM (1998) Yeast physiology and biotechnology. Wiley, New York

Wang VW, Jeffries T (1990) Purification and properties of xylitol dehydrogenase from the xylose-fermenting *Candida shehatae*. Appl Biochem Biotechnol 26:197–206

Wang PY, Schneider H (1980) Growth of yeasts on D-xylulose 1. Can J Microbiol 26(9):1165–1168

Watanabe S, Shimada N, Tajima K, Kodaki T, Makino K (2006) Identification and characterization of L-arabonate dehydratase, L-2-keto-3-deoxyarabonate dehydratase and L-arabinolactonase involved in an alternative pathway of L-arabinose metabolism: novel evolutionary insight into sugar metabolism. J Biol Chem 281:33521–33536

Wery J, Gutker D, Renniers AC, Verdoes JC, van Ooyen AJ (1997) High copy number integration into the ribosomal DNA of the yeast *Phaffia rhodozyma*. Gene 184(1):89–97

Weusthuis RA, Adams H, Scheffers WA, van Dijken JP (1993) Energetics and kinetics of maltose transport in *Saccharomyces cerevisiae*: a continuous culture study. Appl Environ Microbiol 59(9):3102–3109

Wiegel J, Ljungdahl LG (1986) The importance of thermophilic bacteria in biotechnology. Crit Rev Biotechnol 3:39–108

Wingren A, Galbe M, Zacchi G (2003) Techno-economic evaluation of producing ethanol from softwood: comparison of SSF and SHF and identification of bottlenecks. Biotechnol Prog 19 (4):1109–1117

Wooley RJ (1999) Lignocellulosic biomass to ethanol processing design and economics utilizing co-current dilute acid prehydrolysis and enzymatic hydrolysis current and futuristic scenarios. US Department of Energy, USA, NREL/TP-580-26157

Wu JF, Lastick SM, Updegraff DM (1986) Ethanol production from sugars derived from plant biomass by a novel fungus. Nature 321:887–888

Yamanaka K (1969) Inhibition of D-xylose isomerase by pentitols and D-lyxose. Arch Biochem Biophys 131(2):502–506

Zaldivar J, Borges A, Johansson B, Smits HP, Villas-Boas SG, Nielsen J, Olsson L (2002) Fermentation performance and intracellular metabolite patterns in laboratory and industrial xylose-fermenting *Saccharomyces cerevisiae*. Appl Microbiol Biotechnol 59(4–5):436–442

Zhang M, Eddy C, Deanda K, Finkelstein M, Picataggio S (1995) Metabolic engineering of a pentose metabolism pathway in ethanologenic *Zymomonas mobilis*. Science 267:240–243