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Fermentation performance and intracellular metabolite patterns in laboratory and industrial xylose-fermenting *Saccharomyces cerevisiae*

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Abstract Heterologous genes for xylose utilization were introduced into an industrial *Saccharomyces cerevisiae*, strain A, with the aim of producing fuel ethanol from lignocellulosic feedstocks. Two transformants, A4 and A6, were evaluated by comparing the performance in 4-l anaerobic batch cultivations to both the parent strain and a laboratory xylose-utilizing strain: *S. cerevisiae* TMB 3001. During growth in a minimal medium containing a mixture of glucose and xylose (50 g/l each), glucose was preferentially consumed. During the first growth phase on glucose, the specific growth rates were 0.26, 0.32, 0.27 and 0.30 h⁻¹ for strains TMB 3001, A (parental strain), A4, and A6, respectively. The specific ethanol productivities were 0.04, 0.13, 0.04 and 0.03 g/g-per hour, for TMB 3001, A, A4 and A6, respectively. The specific xylose consumption rates were 0.06, 0.21 and 0.14 g/g-per hour, respectively for strains TMB 3001, A4 and A6. Xylose consumption resulted mainly in the formation of xylitol, with biomass and ethanol being minor products. The metabolite profile of intermediates in the pentose phosphate pathway and key glycolytic intermediates were determined during growth on glucose and xylose, respectively. The metabolite pattern differed depending on whether glucose or xylose was utilized. The levels of intracellular metabolites were higher in the industrial strains than in the laboratory strain during growth on xylose.

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Introduction

Many countries in the world are currently committed to reduce atmospheric CO₂ levels. The view that use of bio-ethanol as an additive in fuels for transportation can help in reducing exhaust emissions is nowadays generally accepted (Wyman 1996). Cheap feedstocks such as lignocellulosic waste (sugarcane bagasse, corn stalks, wheat straw) can potentially be used for competitive ethanol production (Hahn-Hägerdal et al. 2001; Mielenz 2001). However, the utilization of sugars present in lignocellulose requires efficient hydrolytic methods and efficient fermentation microorganisms, capable of fermenting the pentoses as well as the hexoses that originate from lignocellulosic material (Ingram et al. 1999; Zaldivar et al. 2001).

Saccharomyces cerevisiae has evolved into an efficient fermentation microorganism that has acquired qualities such as high ethanol productivity, tolerance to process hardness, tolerance to fermentation by-products and is, therefore, preferred for ethanol production from crops. Moreover, the presence of extra sets of chromosomes in the industrial polyploid strain, and with that the concomitant overexpression of genes (Pretorius 2000), could be an additional advantage. A long-held belief also attributes higher genetic stability to polyploid strains, since multiple mutational events will be required in order to bring about any changes, but genetic variability in industrial strains is currently accepted to occur under strong selective pressure, although at much lower frequency than in laboratory strains (Hammond 1995; Pretorius 2000). However, applying *S. cerevisiae* for fermentation of lignocellulosic hydrolysates has the drawback that it cannot naturally utilize pentoses.

The vast accumulated knowledge regarding the physiology and genetics of *S. cerevisiae* basically originated from investigation of laboratory strains. Unfortunately, after years of handling in laboratory surroundings, laboratory strains do not display some of the traits that characterize *S. cerevisiae* strains used in industry (Wheals et al. 1999). However, when metabolic engineering goals in

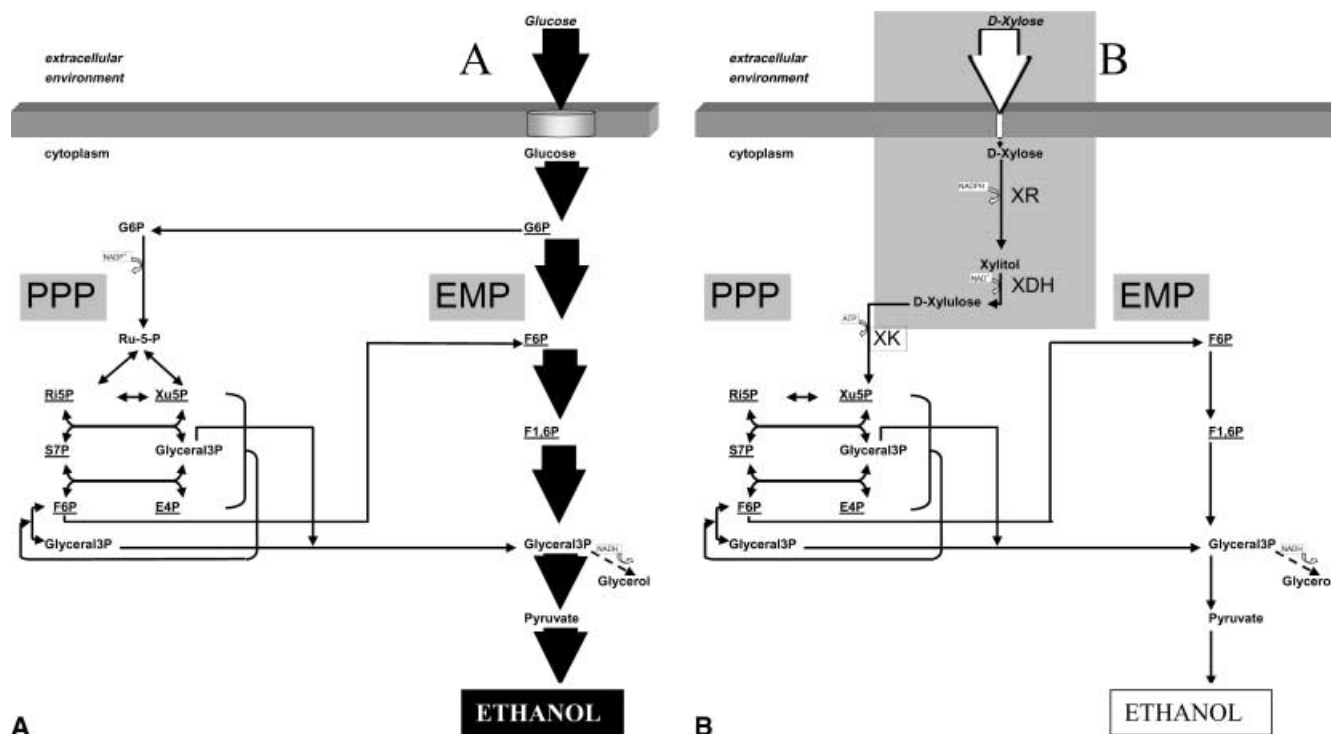


Fig. 1 Overview of metabolic pathways resulting in ethanol production from glucose (**A**) and xylose (**B**), adapted from Schaaff-Gerstenschläger and Miosga (1997); Walker (1998). PPP Pentose phosphate pathway, EMP Embden-Meyerhof-Parnas pathway (glycolysis), Ru5P ribulose-5-phosphate, Ri5P ribose-5-phosphate, Xu5P xylulose-5-phosphate, S7P sedoheptulose-7-phosphate, F6P fructose-6-phosphate, Glyceral3P glyceraldehyde-3-phosphate, Acetal acetaldehyde, G6P glucose-6-phosphate, XR xylose reductase, XDH xylitol dehydrogenase, XK xylulokinase. NADP⁺, NADPH, NAD⁺, NADH and ATP cofactors are shown. The size of the arrows has been scaled according to flux distribution based on Nissen et al. (1997)

S. cerevisiae are to be proven, laboratory strains have primarily been used, as genetic manipulations are easier. Nonetheless, to pursue an industrial application, the concept has to be proven in an industrial strain, which might have valuable additional properties. Along these lines, useful hosts for xylose-utilizing *S. cerevisiae* could be the acid-tolerant *S. cerevisiae* strains isolated from harsh environments (Lindén et al. 1992) or bred strains with industrial background, as demonstrated by Ho et al. (1998).

In metabolic engineering it is important to assess the physiological outcome of the modified metabolic pathway (Stafford and Stephanopoulos 2001). For this analysis it is highly desirable to obtain a physiological snapshot of the cell that faithfully represents its metabolic state at the very moment of harvesting. Such a snapshot can be obtained by analysis of intracellular metabolites (de Koning and van Dam 1992; Theobald et al. 1993; Gonzalez et al. 1997; Smits et al. 1998; Groussac et al. 2000).

In this work, an *S. cerevisiae* strain used in the industrial production of ethanol was transformed with an inte-

grating plasmid harboring the endogenous gene encoding xylulokinase (*XKS1*) and genes for xylose reductase (*XYL1*) and xylitol dehydrogenase (*XYL2*) from *Pichia stipitis*, which enabled the utilization of xylose (Eliasson et al. 2000; Fig 1B). The fermentation capabilities of the strains constructed were evaluated in minimal media under anaerobic conditions in terms of growth, substrate consumption, product and by-product formation during batch growth. The metabolite levels for the pentose phosphate pathway (PPP) were measured since this is the primary metabolic pathway for xylose (Lighthouse 1988). Furthermore, the connection between the metabolic profile and factors such as genotype, background (laboratory or industrial) of the strains, and sugars consumed (glucose or xylose) was investigated.

Materials and methods

Media for batch cultivations

The strains of *S. cerevisiae* were cultivated in minimal media according to Verduyn et al. (1992). The composition of the medium (in g/l) was: (NH₄)₂SO₄ 5.0, KH₂PO₄ 3.0, MgSO₄·7H₂O 0.5. The medium was supplemented with 1 ml of trace metal solution, 1 ml of vitamins solution and 1 ml ergosterol/Tween 80. The trace metal solution had the following composition (in g/l): EDTA 15, ZnSO₄·7H₂O 4.5, MnCl₂·2H₂O 0.84, CoCl₂·6H₂O 0.30, CuSO₄·5H₂O 0.30, Na₂MoO₄·2H₂O 0.40, CaCl₂·2H₂O 4.5, FeSO₄·7H₂O 3.0, H₃BO₃ 1.0, KI 0.10. The composition of the vitamin solution (in g/l) was: D-biotin 0.05, calcium pantothenate 1.0, nicotinic acid 1.0, myoinositol 25.0, thiamine hydrochloride 1.0, pyridoxal hydrochloride 1.0, *p*-aminobenzoic acid 0.20. A mixture of 50 g/l glucose and 50 g/l xylose was used as carbon source. Ergosterol, a precursor of cellular membrane not synthesized by *S. cerevisiae* in anaerobic cultures (Andreasen and Stier 1953, 1954) was added as a solution containing ergosterol (15 mg/l) and Tween 80 (660 mg/l) (Verduyn et al. 1992).

S. cerevisiae strains

CEN.PK113-7A MATa *his3-Δ1 MAL2-8^c SUC2* was used as the recipient for the integrating plasmid YIpXR/XDH/XK (Entian and Kötter 1998). This plasmid harbors the *XYL1* and *XYL2* genes from *P. stipitis* and an endogenous *XKS1* gene under the control of the *PGK1* promoter. The constructed strain, TMB 3001 (Eliasson et al. 2000), was used as a xylose-utilizing reference strain in this study. Industrial strain A was transformed with the plasmid YIpLoxZEO (Jeppsson et al. 2002). This plasmid contains a homologous xylulokinase *XKS1*, and heterologous *XYL1* and *XYL2*. In addition it contains two selection markers: zeocin and ampicillin (for selection in *Escherichia coli*), and a fragment of an *S. cerevisiae* gene to promote recombination (*HIS3*). Plasmid DNA (10 µg) was linearized with *NdeI*. From this preparation, 5 µg was used to transform *S. cerevisiae* A appropriately treated with lithium acetate (Schiestl and Gietz 1989). Screening was made on YPD plates containing 50, 150 or 300 µg/ml zeocin, respectively. Plates were incubated at 30°C and after 24–48 h colonies were transferred to fresh plates having a similar formulation. Colonies that grew in this second selection were evaluated in 50 ml YPX (xylose 20 g/l) in baffled 300-ml Erlenmeyer flasks and incubated in a shaker at 150 rpm, at 30°C. Potentially good candidates were grown in YPD (20 g/l glucose), then washed twice in distilled water and finally transferred to YPX (20 g/l xylose). To enable a fair comparison, inoculum was normalized, i.e., initial biomass was similar in all cases, equivalent to 20 mg DW/l. Growth was evaluated at 600 nm in a spectrophotometer (Hitachi U-1100, Tokyo, Japan).

Cultivation conditions

Batch fermentations were performed in in-house manufactured fermenters with a total volume of 5 l, equipped with two Rushton turbines, containing 4 l minimal medium. Technical quality nitrogen (AGA, Copenhagen, Denmark) containing less than 5 ppm O₂ was flushed through the vessels (0.4 l/min) to obtain an anaerobic environment, and the exhaust gas passed through a reflux cooler maintained at 2°C to minimize ethanol evaporation. The pH was maintained at 5.0 with 2 M NaOH. The fermentations were run at 30°C at a stirring speed of 600 rpm. Carbon dioxide was monitored during fermentation with an acoustic gas-analyzer (Brüel & Kjaer type 1308, Nærum, Denmark). Reactors were inoculated to an initial biomass concentration of 2 mg DW/l with precultures grown in unbaffled flasks at 30°C and 150 rpm for 15 h.

Analytical methods

Cell growth was monitored by absorbance measurements at 600 nm in a spectrophotometer (Hitachi U-1100, Tokyo, Japan) and gravimetric determination by dry weight as described by Olsson and Nielsen (1997). Samples for determination of sugars and extracellular metabolites were filtered through 0.45-µm-pore-size acetate filters (Osmonics, Minnetonka, Minn.) and the filtrates were frozen at –20°C and later used for analyses. Glucose, arabinose, galactose, mannose and xylose were determined by high-performance anion exchange chromatography (Dionex, Sunnyvale, Calif.), using pulsed amperometric detection. Sugars were separated in a CarboPac PA1 column at 30°C. Two eluents, A and B, 200 mM NaOH and 1 mM NaOH/0.03 mM NaAc, respectively, were used as the mobile phases, at a flow of 1 ml/min. The gradient was established as follows: from 0–40 min eluent A was used at 100% of the flow rate; from 40–45 min, the flow of A was decreased to zero, whereas the flow of B was increased to 100%; from 45–50 min the flow of B was kept constant, and from 50–55 min the flow of B was decreased to 0%, whereas the flow of A was increased to 100% to finish the cycle.

Sugar alcohols, xylitol and arabitol, were separated in a CarboPac MA1 column at 30°C, utilizing 612 mM NaOH as the mobile phase, at a flow rate of 1 ml/min.

Extracellular metabolites, ethanol, succinate, pyruvate, acetate, and glycerol were measured by high-performance liquid chromatography (Waters, Milford, Mass.), using an Aminex ion-exclusion HPLC-87H cation exchange column (Bio-Rad, Calif.) at 65°C and 5 mM H₂SO₄ at a flow rate of 0.6 ml/min as the mobile phase. Succinate, glycerol and ethanol were determined by RI-detection and pyruvate and acetate by UV-detection.

For the analysis of intracellular metabolites, 5 ml broth was harvested in duplicate from the reactors, before glucose exhaustion (at 22 and 26 h of cultivation) and after glucose exhaustion (42, 79 and 131 h of cultivation). Procedures for metabolic arrest, solid-phase extraction of metabolites and analysis have been described in detail by Smits et al. (1998). However, the analysis by high-pressure ion exchange chromatography coupled to pulsed amperometric detection used to analyze cell extracts, was slightly modified. Solutions used were eluent A, 75 mM NaOH, and eluent B, 500 mM NaAc. To prevent contamination of carbonate in the eluent solutions, a 50% NaOH solution with low carbonate concentration (Baker Analysed, Deventer, The Netherlands) was used instead of NaOH pellets. The eluents were degassed with He for 30 min and then kept under a He atmosphere. The gradient pump was programmed to generate the following gradients: 100% A and 0% B (0 min), a linear decrease of A to 70% and a linear increase of B to 30% (0–30 min), a linear decrease of A to 30% and a linear increase of B to 70% (30–70 min), a linear decrease of A to 0% and a linear increase of B to 100% (70–75 min), 0% A and 100% B (75–85 min), a linear increase of A to 100% and a linear decrease of B to 0% (85–95 min). The mobile phase was run at a flow rate of 1 ml/min. Other conditions were according to Smits et al. (1998).

Calculations

Ethanol evaporation

Due to its low boiling point, ethanol evaporates when the reactor is sparged with nitrogen. To compensate for ethanol loss in the calculations, evaporation rate was determined experimentally. The reactor was set up as used in the batch cultivations and ethanol was added. The ethanol evaporation was estimated by measuring the ethanol concentration in the liquid phase over time. The evaporation at ethanol concentrations obtained in this work followed the equation $dC_{\text{ethanol}}/dt = -k C_{\text{ethanol}}$, where the rate constant was $k = 0.1134 \text{ h}^{-1}$.

Yields of biomass and ethanol

Yields of biomass and ethanol on sugars were calculated based on the time of glucose exhaustion from the reactor (36 h) for the reference strain A, since it is unable to consume xylose. For the xylose-utilizing strains, the end of the fermentation (191 h) was used for the calculations.

Specific ethanol productivity

Specific ethanol productivity was based on the volumetric productivity (g/l-per hour) divided by the biomass at the time of glucose exhaustion (for the reference strain) and at the end of the cultivation for the recombinant strains. Specific ethanol productivity was expressed in g/g DW per hour.

Sugar uptake rate

Sugar (glucose or xylose) uptake rate was calculated based on the equation: uptake rate = $dC_{\text{sugar}}/dt \times 1/DW$, where DW is the biomass concentration. Sugar uptake rate was expressed in g/g DW per hour.

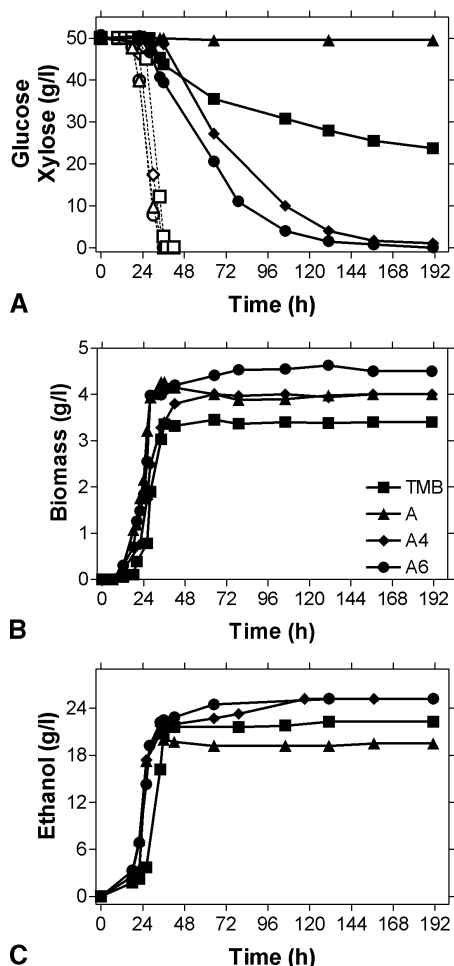


Fig. 2A–C Fermentation performance of *Saccharomyces cerevisiae* strains TMB 3001 (□,■), A (△,▲), A4 (◇,◆) and A6 (○,●). TMB 3001 has a laboratory background, whereas A, A4 and A6 have industrial backgrounds; TMB 3001, A4 and A6 are xylose-utilizing strains. Time course of glucose (hollow symbols) and xylose (filled symbols) consumption (A), biomass concentration (B), and ethanol production (C) in anaerobic batch cultures employing minimal medium

Results

Sugar consumption, growth, and formation of extracellular metabolites

The recombinant strains were able to utilize xylose and only those reactors containing strains with an industrial background exhausted xylose during the 191 h of cultivation (Fig. 2A). Sugar consumption occurred sequentially: first glucose and then xylose. Glucose exhaustion occurred at 36 h and the specific glucose consumption rates were 2.7, 2.8, 2.9 and 2.9 g/g per hour, for strains TMB 3001, A, A4 and A6, respectively. The specific xylose consumption rates were 0.06, 0.21 and 0.14 g/g per hour, respectively, for strains TMB 3001, A4 and A6 (Table 1), i.e., 3.5 and 2.4-fold higher in the recombinant strains with industrial background compared to TMB 3001 with a CEN.PK laboratory strain background.

Table 1 Summary of the growth characteristics of strains of *Saccharomyces cerevisiae* during anaerobic batch fermentation of a mixture of 50 g/l glucose and 50 g/l xylose

	TMB 3001 ^a	A	A4	A6
μ_{Max}^b	0.26	0.32	0.27	0.30
Biomass _{max} ^c	3.5	4.4	4.0	4.6
Ethanol _{max} ^d	23.3	20.8	25.2	25.2
Glycerol _{max} ^e	8.7	6.5	8.3	8.3
Xylitol _{max} ^f	4.1	0.4	13.6	15.9
Y_{sx}^g	0.034	0.090	0.040	0.046
Y_{se}^h	0.23	0.42	0.27	0.27
r_{Ethanol}^i	0.04	0.13	0.04	0.03
r_{Xylose}^j	0.06	0	0.21	0.14

^a TMB 3001, A4 and A6 are xylose-metabolizing strains

^b Maximum specific growth rate (h⁻¹)

^{c,d,e,f} Maximal concentrations of biomass, ethanol, glycerol and xylitol (g/l), respectively

^g Biomass yield (g biomass/g sugar consumed). For the calculation of yields of biomass and ethanol, coefficients were calculated on glucose (50 g/l) only for strain A, whereas for the recombinant strains calculations were based on glucose + xylose (100 g/l)

^h Ethanol yield (g ethanol/g sugar consumed)

ⁱ Specific ethanol productivity (g ethanol/g biomass per hour). The volumetric productivity, i.e., ethanol produced per unit time (g/l per hour), divided by the biomass concentration at the time of glucose exhaustion for strain A, and the end-point for the recombinant strains

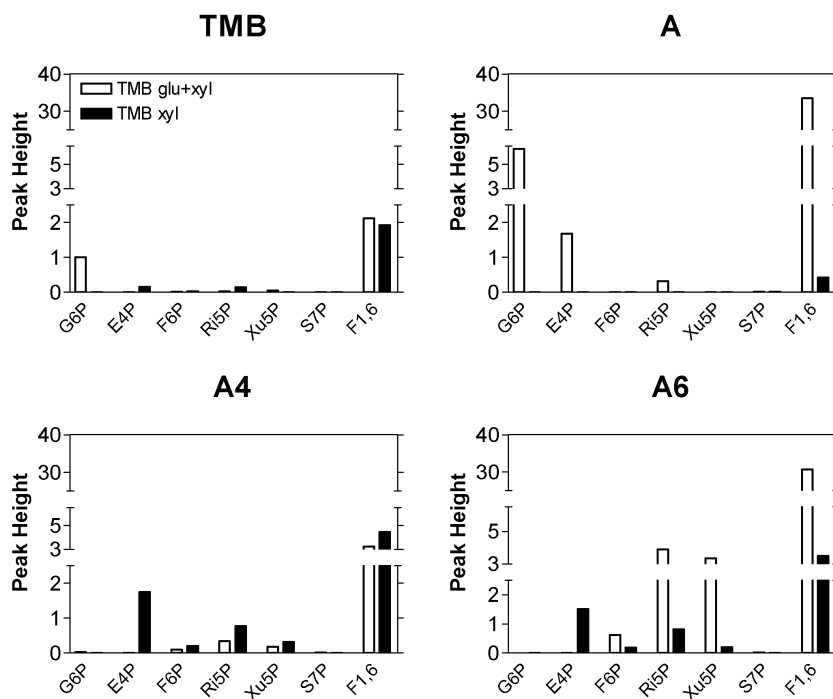
^j Specific xylose consumption rate (g xylose/g biomass per hour), calculated based on the equation: uptake rate = $dC_{\text{xylose}}/dt \times 1/DW$, where DW is the biomass concentration

Growth (and ethanol production) occurred predominantly during the initial 36 h, in which interval glucose was predominantly consumed. A minimal growth of the recombinant strains on xylose occurred afterwards (Fig. 2B). Thus, the values presented in Table 1 for strains TMB 3001, A4 and A6 represent increases of 3%, 15% and 13%, respectively, over growth on glucose. No growth on xylose occurred in strain A. The yield of biomass on sugars was 0.09 g/g on the parental strain A, which is close to the typically found value of 0.1 g/g for anaerobic *S. cerevisiae* cultures. The highest Y_{sx} among recombinant strains was 0.046 g/g (Table 1), i.e., 50% lower than in the parental strain, caused by the minimal growth during the xylose consumption phase. As the reference strain was unable to utilize xylose, yield coefficients were based on glucose (50 g/l), whereas for the recombinant strains glucose and xylose (100 g/l) were considered for the calculations (Table 1).

The final ethanol concentration was 23.3, 20.8, 25.2 and 25.2 g/l, in strains TMB 3001, A, A4 and A6, respectively (Fig. 2C, Table 1). For the recombinant strains, these values represent increases of 7%, 15% and 12%, respectively, compared with production on glucose.

Peak concentrations of glycerol were 8.7, 6.5, 8.3 and 8.3 g/l in strains TMB 3001, A, A4 and A6, respectively, whereas the production of xylitol reached 4.1, 0.4, 13.6 and 15.9 g/l, respectively (Table 1).

Fig. 3 Intracellular metabolite profiles determined during growth on glucose + xylose (open bars) and xylose (filled bars) in *S. cerevisiae* strains TMB 3001, A, A4 and A6. Bars represent peak heights and not absolute concentrations. Samples were harvested at 26 and 79 h, respectively. G6P Glucose-6-phosphate, E4P erythrose-4-phosphate, F6P fructose-6-phosphate, Ri5P ribose-5-phosphate, Xu5P xylulose-5-phosphate, S7P sedoheptulose-7-phosphate, F1,6 fructose-1,6-diphosphate



Intracellular metabolites formation

To evaluate the phenotype of the recombinant strain further, intracellular metabolites were analyzed. Samples were collected from the reactor during the glucose consumption phase (at 22 and 26 h) and during the xylose consumption phase (samples 42, 79 and 131 h). After quenching and adequate sample preparation, they were analyzed for metabolites from the PPP and the Embden-Meyerhof-Parnas (EMP) pathway. Erythrose-4-P, ribose-5-P, xylulose-5-P, and sedoheptulose-7-P, are exclusive to the PPP, fructose-6-P and glucose-6-P can be present in both pathways, and fructose-1,6-diP is exclusive to the EMP pathway (Fig. 1A, B). It should be noted that the metabolite profiles are not based on absolute concentrations, but only peak heights are represented (Fig. 3). However, the same sample volume was applied during analysis, making it possible to compare the height as a representation of the amount of the component in the samples. To facilitate the discussion, samples corresponding to 26 h and 79 h were chosen as representatives of the effect of glucose and xylose, respectively, on the metabolism of the strains.

During the glucose consumption phase, the laboratory strain (TMB 3001) had low levels of PPP metabolites, and the levels of glucose-6-P and fructose-1,6-diP were also low compared to the levels in strains A and A6. In the industrial strain A, unable to metabolize xylose, intermediates such as glucose-6-P, erythrose-4-P and ribose-5-P appeared as expected only when glucose was available in the broth (PPP intermediates erythrose-4-P and ribose-5-P are used in biosynthetic reactions). The level of fructose-1,6-diP was 20-fold higher than that of erythrose-4-P. In strain A6, the level of fructose-6-P was

as high as in strain A, but PPP intermediates ribose-5-P and xylulose-5-P were one order of magnitude lower than fructose-1,6-diP. The level of fructose-6-P, the precursor of fructose-1,6-diP in the EMP pathway, was 40-fold lower than the latter. In strain A4, metabolite levels (including fructose-1,6-diP) were 10-fold lower than in strains A and A6.

During the xylose consumption phase (after glucose exhaustion) the levels of PPP intermediates in strain TMB 3001 did not increase and levels of the glycolytic intermediate fructose-1,6-diP did not change dramatically. In strain A, there was a minimal level of fructose-1,6-diP, 65-fold lower than during growth on glucose, possibly due to gluconeogenesis (Gancedo and Gancedo 1997). In strain A6 the level of fructose-1,6-diP was reduced by one order of magnitude compared to the glucose phase. Furthermore, in the recombinant strains with industrial background, erythrose-4-P levels in each strain were in the same range. Similarly, the levels of ribose-5-P in each strain, or xylulose-5-P in each strain, were in the same range. No accumulation of septulose-7-phosphate could be shown, challenging earlier studies (Kötter and Ciriacy 1993).

Discussion

Sugar consumption, growth, and formation of extracellular metabolites

This work verified that the recombinant strains consumed sugars sequentially, xylose being utilized after glucose exhaustion. The recombinant strains A4 and A6 with industrial background were superior to TMB 3001

(with laboratory background), as indicated by the enhanced xylose consumption, biomass and ethanol production (Fig. 2A–C). It has been shown previously that overexpression of XR led to an increased xylose consumption rate and, concomitantly, a higher xylitol yield (Johansson 2001). The yields of xylitol on consumed xylose were 0.27 and 0.31 g/g for strains A4 and A6, respectively, which were 1.5 to 2-fold higher than the yield of 0.16 g/g for TMB 3001. The higher xylitol yields in the industrial recombinant strains might be a consequence of the increased xylose consumption rates. This might be caused by increased XR activity in the recombinant industrial strains since, due to their polyploid nature, several integrations of the plasmid might have occurred.

As a result of the increased xylose consumption, biomass and ethanol concentration in strains A4 and A6 increased. In spite of the better performance of strains A4 and A6 compared to TMB 3001, the slow consumption of xylose in comparison with glucose consumption is a problem in direct implementation. A specific xylose transporter is absent in *S. cerevisiae*; uptake is carried out by the hexose transporter (Walsh et al. 1994; Özcan and Johnston 1999), which has an affinity for xylose in the range 98–137 mM (Singh and Mishra 1995) or even lower (170 mM is indicated by Kotyk 1967). The slow metabolism of xylose results in long fermentation times, lengthening the exposure of the cells to stressing conditions in the reactor such as increased levels of ethanol and by-products, affecting cell viability. Viability would be further reduced by the limited biosynthetic capability after glucose exhaustion, as discussed below. Furthermore, a significant percentage of consumed xylose is directed towards xylitol formation, due to cofactor imbalance in xylose metabolism (Bruinenberg et al. 1983) as well as to unfavorable thermodynamics (Rizzi et al. 1988, 1989). In this regard, approximately 30% of the xylose consumed was directed towards xylitol formation.

Intracellular metabolite levels

To gain further insight into the metabolism of laboratory and industrial *S. cerevisiae* strains, key intermediates in the PPP and EMP pathway were analyzed in this work. The results verified that the profiles of metabolites are related to: (1) the genetic background of the strains; the laboratory strains had levels of metabolites lower than the strains with industrial background, (2) strain genotype; only strains transformed with the genes for xylose utilization possess, to a lower (TMB 3001) or higher (A4 and A6) extent, a profile of PPP metabolites after glucose exhaustion, (3) the type of carbon source present in the reactor; the presence (and utilization) of xylose resulted in differences in metabolite profiles between recombinant and non-transformed strains.

As verified in this work, it is a critical issue that in batch cultivations employing minimal media, xylose is predominantly consumed after glucose exhaustion and

that the non-oxidative PPP gains a catabolic role (Fig. 1B). Since reducing power results from the oxidative PPP (Nogae and Johnston 1990) and ATP in anaerobic cultures results from glycolytic activity, cellular growth is minimized when these pathways are bypassed. Because ATP (and ethanol) formation relies on a high glycolytic flux, a low efflux of the intermediates glyceraldehyde-3-P and fructose-6-P from PPP to EMP pathway results in both minimal growth and minimal ethanol formation. Thus, with the aim being a high production of ethanol, metabolic engineering demands an accelerated flux through the sequence of “pipelines” in the PPP to generate high levels of the EMP pathway intermediates glyceraldehyde-3-P and fructose-6-P. It is expected that such influx will fuel a high flux through the EMP pathway, yielding ethanol levels comparable to those achieved with glucose. In this study, we showed that by introduction of xylose metabolizing genes into an industrial strain, increased xylose consumption rate and elevated levels of PPP metabolites were achieved in comparison with those determined for xylose utilization in a laboratory strain. The increased levels of PPP metabolites could be an indication of higher flux through this pathway or to limitations in, or downstream of, the PPP. However, further studies would be required for clarification.

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