Appl Microbiol Biotechnol (2008) 78:939–945
DOI 10.1007/s00253-008-1364-y

BIOTECHNOLOGICAL PRODUCTS AND PROCESS ENGINEERING

NADH- vs NADPH-coupled reduction of 5-hydroxymethyl furfural (HMF) and its implications on product distribution in *Saccharomyces cerevisiae*

João R. M. Almeida · Anja Röder · Tobias Modig · Boaz Laadan · Gunnar Lidén · Marie-F. Gorwa-Grauslund

Received: 23 November 2007 / Revised: 11 January 2008 / Accepted: 12 January 2008 / Published online: 11 March 2008

Abstract *Saccharomyces cerevisiae* alcohol dehydrogenases responsible for NADH-, and NADPH-specific reduction of the furaldehydes 5-hydroxymethyl-furfural (HMF) and furfural have previously been identified. In the present study, strains overexpressing the corresponding genes (*mut-ADH1* and *ADH6*), together with a control strain, were compared in defined medium for anaerobic fermentation of glucose in the presence and absence of HMF. All strains showed a similar fermentation pattern in the absence of HMF. In the presence of HMF, the strain overexpressing *ADH6* showed the highest HMF reduction rate and the highest specific ethanol productivity, followed by the strain overexpressing *mut-ADH1*. This correlated with in vitro HMF reduction capacity observed in the *ADH6* overexpressing strain. Acetate and glycerol yields per biomass increased considerably in the ADH6 strain. In the other two strains, only the overall acetate yield per biomass was affected. When compared in batch fermentation of spruce hydrolysate, strains overexpressing *ADH6* and mut-*ADH1* had five times higher HMF uptake rate than the control strain and improved specific ethanol productivity. Overall, our results demonstrate that (1) the cofactor usage in the HMF reduction affects the product distribution, and (2) increased HMF reduction activity results in increased specific ethanol productivity in defined mineral medium and in spruce hydrolysate.

Keywords *ADH1* · *ADH6* · Hydroxymethyl furfural · *Saccharomyces cerevisiae* · Lignocellulosic hydrolysate

J. R. M. Almeida · A. Röder · B. Laadan · M.-F. Gorwa-Grauslund (✉)
Department of Applied Microbiology, Lund University,
P.O. Box 124, 221 00 Lund, Sweden
e-mail: Marie-Francoise.Gorwa@tmb.lth.se

T. Modig · G. Lidén
Department of Chemical Engineering, Lund University,
P.O. Box 124, 221 00 Lund, Sweden

Present address:
A. Röder
Institut für Medizinische Mikrobiologie, Virologie und Hygiene,
Universitätsklinikum Hamburg-Eppendorf,
Campus Forschung, N27, 2 OG Martinistraβe 52 20246,
Hamburg, Germany

Introduction

Bioethanol production from lignocellulosic feedstock requires pretreatment and hydrolysis steps that release, in addition to sugars, a vast range of compounds known to be inhibitory to yeast metabolism (Palmqvist and Hahn-Hägerdal 2000; Almeida et al. 2007). The most investigated inhibitors include 5-hydroxymethyl-furfural (HMF) and 2-furaldehyde (furfural), which affect ethanol yield, ethanol productivity, yeast growth, and fermentation lag phase (Taherzadeh et al. 2000a, b; Liu et al. 2004) by inhibiting enzymes such as alcohol dehydrogenase, aldehyde dehydrogenase, and pyruvate dehydrogenase as well as glycolysis (Banerjee et al. 1981; Modig et al. 2002). Industrial bioethanol production would benefit from strains tolerant to HMF and furfural, as has been demonstrated for baker's yeast *Saccharomyces cerevisiae* (Nilsson et al. 2005).

S. cerevisiae is the most used microorganism in industrial bioethanol production and has the natural ability to slowly reduce HMF and furfural to less toxic compounds (Almeida et al. 2007). Under anaerobic conditions, HMF and furfural are reduced to 2,5-bis-hydroxymethylfuran [furan-2,5-dimethanol (FDM)] and 2-furanmethanol (FM), respectively (Taherzadeh et al. 1999, 2000b; Liu et al. 2004). In *S. cerevisiae*, HMF and furfural reduction are nicotinamide adenine dinucleotide phosphate (reduced form) (NADPH)- and nicotinamide adenine dinucleotide (reduced form) (NADH)-coupled, respectively (reviewed in Almeida et al. 2007). The industrial

strain TMB3000 (Linden et al. 1992) has a unique ability to catalyze NADH-dependent HMF reduction, and its in vivo and in vitro tolerance towards acid hydrolysate correlates with high HMF and furfural reducing activity (Nilsson et al. 2005; Laadan et al. 2008; Modig et al. 2008).

Although HMF and furfural are chemically related compounds and qualitatively affect yeast metabolism in a similar mode, there are important differences between the inhibitory effects of these two compounds. In synthetic media, furfural inhibits the fermentation rate and yeast growth more severely than HMF. Furthermore, HMF reduction is much slower than furfural reduction and takes place only when furfural is completely reduced (Taherzadeh et al. 2000b). Consequently, the inhibitory effect of HMF lasts longer and may lead to more serious problems to the fermentation process, even if the immediate toxicity of HMF is clearly lower than that of furfural.

Genes encoding HMF-reducing enzymes in TMB3000 have been identified and overexpressed (Petersson et al. 2006; Laadan et al. 2008). Alcohol dehydrogenase 6 (ADH6p) was identified as an NADPH-dependent HMF-reducing enzyme in *S. cerevisiae* using microarray analysis and a gene overexpression collection (ExClone; Petersson et al. 2006). More recently, a mutated alcohol dehydrogenase 1 (mut-ADH1p) that catalyses NADH-dependent HMF reduction in TMB3000 was purified and characterized (Laadan et al. 2008). Both enzymes conveyed improved growth in the presence of HMF under aerobic conditions (Petersson et al. 2006; Laadan et al. 2008). In the current work, growth and product formation by *S. cerevisiae* strains overexpressing *mut-ADH1* catalyzing NADH-coupled reduction of HMF, and *ADH6* catalyzing NADPH-coupled reduction of HMF were compared under anaerobic conditions. Batch fermentation of spruce hydrolysate was also performed to assess strain robustness in an industrial medium.

Materials and methods

Yeast strains

Saccharomyces cerevisiae strains used in this work were constructed by transformation of CEN.PK 113-5D (*MAT*a ura3; van Dijken et al. 2000) with the multi-copy plasmid YEplacHXT (Karhumaa et al. 2005), carrying either the mutated alcohol dehydrogenase 1 gene (mut-ADH1 strain, TMB3206; Laadan et al. 2008) or the alcohol dehydrogenase 6 gene (ADH6 strain, TMB3286; Petersson et al. 2006). The control strain (TMB3280) carries the plasmid without a structural reductase gene (Petersson et al. 2006). In these strains, overexpression of mut-ADH1 and ADH6 is regulated by the constitutive truncated promoter HXT7 (Hauf et al. 2000). The strains were maintained on agar plates containing 6.7 g/l YNB and 20 g/l glucose.

Batch fermentation in defined medium

Inoculum cultures were grown overnight at 30°C in 1 l cotton-plugged shake-flasks with 100 ml of two times concentrated defined mineral medium (Verduyn et al. 1992) supplemented with 40 g/l glucose and 200 ml/l phthalate buffer (10.2 g/l KH phthalate, 2.2 g/l KOH). The same medium was used in batch experiments, except that 60 g/l glucose and, where indicated, 2 g/l HMF were added. The starting OD_{620} in batch fermentation was 0.5. Fermentation was performed in 1-l media at 30°C in Braun Biotech fermentors. Anaerobic conditions were maintained by continuously sparging of 0.2 l/min nitrogen gas (containing less than 5 ppm oxygen). pH was maintained at 5.5 with 3 M KOH. The stirring rate was 200 rpm.

Batch fermentation of spruce hydrolysate

Defined mineral medium was used for inoculum cultures. The hydrolysate was produced from forest residues originating mainly from spruce in a two-stage dilute-acid hydrolysis process using sulfuric acid as catalyst (Nilsson et al. 2001). The composition of the hydrolysate was 24.3 g/l glucose, 12.1 g/l mannose, 2.9 g/l galactose, 5.6 g/l xylose, 1.4 g/l arabinose, 2.0 g/l acetic acid, 1.9 g/l HMF, and 0.5 g/l furfural. The hydrolysate was adjusted to pH 5 with 6 M NaOH before use. Inoculum cultures were grown in 300 ml cotton-plugged shake-flasks containing 100 ml media supplemented with 15 g/l glucose. Precultures were incubated in a rotary shaker at 160 rpm and 30°C for 24 h. Subsequent batch fermentation experiments were inoculated with 6 ml of the preculture in Belach BR 0.5 fermentors (Belach Bioteknik AB, Solna, Sweden) at 600 rpm and 30°C. The initial working volume was 300 ml and pH was maintained at 5.0 with 0.75 M NaOH. Anaerobic conditions were achieved by sparging with 0.3 l/min nitrogen gas (containing less than 5 ppm oxygen), controlled by a mass flow meter (Bronkhurst Hi-Tec, Ruurlo, The Netherlands). Initial concentration of medium components was 2.67 times higher compared to inoculum cultures to compensate for dilution. Batch experiments were started by growing cells on 30 g glucose. When growth reached mid/late exponential phase, corresponding to cell biomass of 2.5 g/l, a single addition of 300 ml of hydrolysate was made.

Biomass and metabolite analyses

Samples for metabolites and biomass determination were regularly withdrawn from the reactor. For cell biomass, 5 ml of culture was filtered under vacuum, washed with distilled water, and dried on Gelman filters (ø 47 mm Supor-450, 0.45 μm) in the microwave oven (350 W) for

8 min. Cell weight was calculated as the difference between weight measurements of the filters with and without cells. In hydrolysate fermentation, cell biomass was determined from absorbance measurements at 610 nm and from dry-weight measurements with duplicate 10 ml samples, which were centrifuged, washed with distilled water, and dried for 24 h at 105°C. Samples for metabolite measurements were immediately centrifuged, filtered through 0.2 μm filters and stored at −20°C until analysis. Glucose, ethanol, HMF, glycerol, and acetic acid were analyzed using a high performance liquid chromatography system (Waters, Milford, MA, USA) equipped with an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) at 65°C. The mobile phase was 5 mM sulfuric acid with a flow of 0.6 ml/min. All compounds were detected with a refractive index detector, except for HMF and furfural, which were detected with a UV-detector (210 nm). The concentrations of glucose, mannose, galactose, xylose, arabinose, HMF, and furfural in hydrolysate were determined with an Aminex HPX-87P column (Bio-Rad) at 85°C, with ultra-pure water at 0.6 ml/min as mobile phase.

Carbon dioxide evolution rate

The carbon dioxide evolution rate was monitored by concentrations of carbon dioxide and oxygen in outgoing gas from the fermentor with a CP460 gas analyzer (Belach Bioteknik AB). The gas analyzer was calibrated using a mixture of 20% oxygen and 5% carbon dioxide.

Enzymatic activity measurements

Cell extracts were prepared with Y-PER reagent (Pierce, Rockford, IL, USA) following the protocol of the supplier. Cells were grown overnight at 30°C in 1 l cotton-plugged shake-flasks with 100 ml of two times concentrated defined mineral medium (Verduyn et al. 1992) supplemented with 40 g/l glucose and 200 ml/l phthalate buffer (10.2 g/l KH phthalate, 2.2 g/l KOH). Reduction of HMF and furfural reduction was measured as described previously (Petersson et al. 2006).

Results

NAD(P)H-dependent furaldehyde reductase capacity

In vitro NADH- and NADPH-dependent HMF and furfural reduction were compared in *S. cerevisiae* strains over-expressing either the mutated *ADH1* gene (TMB3206), the *ADH6* gene (TMB3286), or carrying the corresponding multi-copy plasmid without an *ADH* gene (TMB3280 control). Cells were grown aerobically overnight in defined mineral medium. The ADH6 strain displayed high NADPH-dependent HMF and furfural reduction activity, whereas considerably lower activity was detected in the two other strains (Fig. 1). In contrast, only the mut-ADH1 strain displayed high NADH-dependent activity with HMF. Furthermore, NADH-dependent furfural reduction was two times higher in the mut-ADH1 strain than in the other two strains (Fig. 1).

Batch fermentation in defined mineral medium

Fermentation performance of control, mut-ADH1, and ADH6 strains was compared under anaerobic conditions both in the absence and presence of HMF. In the absence of HMF, all strains consumed glucose in approximately 20 h (Fig. 2) and displayed a similar specific growth rate (0.29 h^{-1}). In agreement with previous results (Liu et al. 2004; Petersson et al. 2006; Laadan et al. 2008), the presence of HMF reduced the specific growth rate and decreased the glucose consumption rate in all strains, however to different extents (Table 1)(Fig. 2). The fermentation time of the control strain doubled in the presence of HMF (Fig. 2), and it displayed the lowest

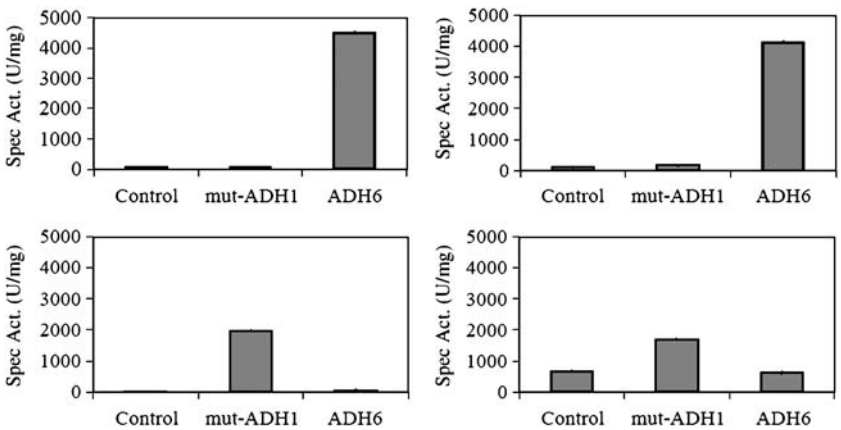

Fig. 1 HMF (*left column*) and furfural (*right column*) reduction activity measured in crude cell extracts from cells grown aerobically overnight in defined medium using NADPH (*top row*) or NADH (*bottom row*) as cofactor. The values are averages of two independent measurements. The standard deviation from the average was less than 10%

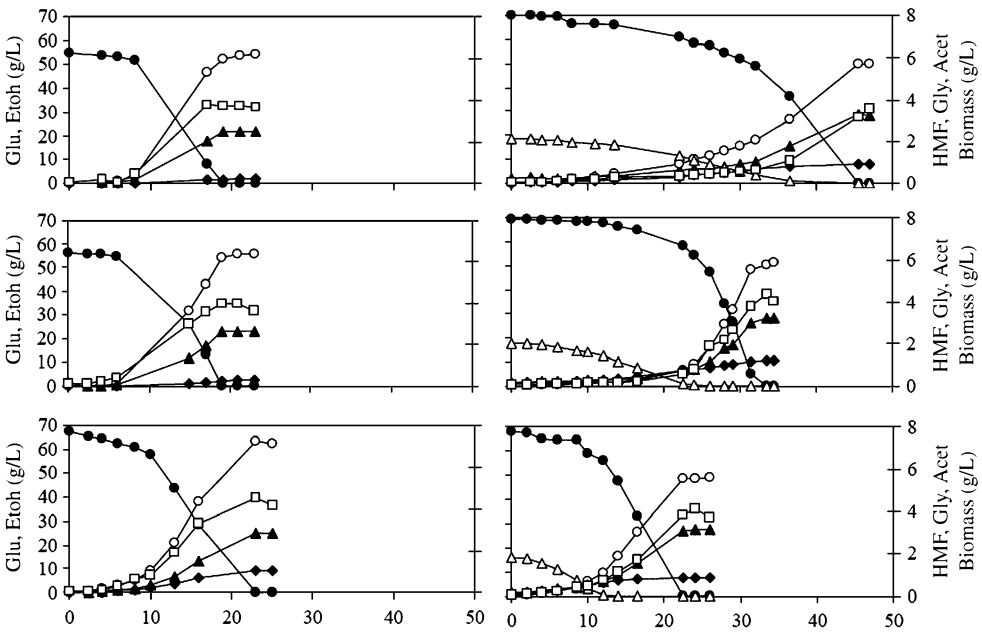

Fig. 2 Anaerobic batch glucose fermentation in defined mineral medium in absence (*left column*) or in presence of HMF (*right column*). Control strain TMB3280 (*top row*), mut-ADH1 strain TMB3206 (*middle row*), and ADH6 strain TMB3286 (*bottom row*). Glucose (*filled circle*), glycerol (*open circle*), biomass (*open square*), ethanol (*filled triangle*), acetate (*filled diamond*), HMF (*open triangle*). The experiments were done in duplicate, and the figure shows one of the profiles for each strain

specific growth rate, 0.11 h^{-1} (Table 1). The highest specific growth rate, 0.20 h^{-1}, was observed for the ADH6 strain (Table 1). The specific HMF uptake rate correlated with strain performance, i.e., higher HMF uptake rate resulted in higher specific growth rate and higher specific ethanol productivity (Table 1). The final ethanol yield 0.43 g ethanol.g $glucose^{-1}$ was not affected by HMF (Fig. 1). However, the specific ethanol productivity was 45% and 21% lower for the control and mut-ADH1 strains, respectively, in the presence of HMF, whereas the specific ethanol productivity for the ADH6 strain was unchanged (Table 1).

The control, mut-ADH1, and ADH6 strains produced similar yields of the by-products acetate and glycerol, in the absence of HMF (Fig. 2). The effect of HMF on by-product distribution was assessed both during the HMF conversion phase and at the end of the fermentation (Table 2). During the HMF conversion phase, the acetate yields per biomass increased significantly for all three strains, with the mut-ADH1 strain producing ten times more acetate per biomass than in the absence of HMF (Table 2). Glycerol production increased two times for the control strain, but it was not affected in the mut-ADH1 and ADH6 strains (Table 2). After HMF and glucose depletion, the glycerol yield was equivalent to the reference fermentation without HMF for the control and mut-ADH1 strains, whereas it was 24% higher for the ADH6 strain (Table 2). Similarly to glycerol, acetate production decreased after HMF and glucose depletion. However, the final acetate production was still two times higher for the three strains (Table 2).

Table 1 Specific growth, ethanol formation and HMF conversion rates in anaerobic batch fermentation in defined mineral medium supplemented with 2 g/l HMF

	μ (h^{-1})[a]	qEthanol (g.g h^{-1})[b]	qHMF (g.g h^{-1})[c]
Control	0.11±0.01	0.34±0.04	0.30±0.06
Mut-ADH1	0.17±0.00	0.53±0.09	0.83±0.04
ADH6	0.20±0.02	0.61±0.03	0.90±0.05

Glucose was used as carbon and energy source. Presented values are the mean value of two independent experiments±SD.
[a] Growth rate in absence of HMF was 0.29 h^{-1} for the three strains
[b] Overall productivity
[c] Calculated in the first 8 h of fermentation

Batch fermentation in spruce hydrolysate

The performance of the control, mut-ADH1, and ADH6 strains in spruce hydrolysate was investigated with a pulse addition of dilute-acid spruce hydrolysate to cells grown in defined medium. Glucose consumption, HMF and furfural conversion, ethanol and by-products production, and carbon dioxide evolution rate (CER) were monitored during hydrolysate fermentation. In contrast with the experiments in defined medium, the three strains did not show any significant difference in by-product formation (data not shown). Biomass was found to be more or less constant at 2.5 g/l for all strains after hydrolysate addition. In contrast, an initial approximately 50% decrease in the CER after hydrolysate addition was observed for all three strains (Fig. 3). This was followed by a gradually decreasing CER for the control strain, whereas CER remained more stable for the mut-ADH1 and ADH6 strains (Fig. 3). The initial specific ethanol productivity was also lower for the control

Table 2 Glycerol and acetate yields per biomass in anaerobic batch fermentation in defined mineral media both with and without 2 g/l HMF

	Without HMF[a]		With HMF			
	Ygly/*x*	Yacet/*x*	HMF depleted[b]		Glucose depleted[a]	
			Ygly/*x*	Yacet/*x*	Ygly/*x*	Yacet/*x*
Control	1.46±0.25	0.10±0.06	2.88±0.18	0.61±0.27	1.48±0.06	0.18±0.10
Mut-ADH1	1.51±0.13	0.11±0.07	1.31±0.21	1.19±0.09	1.53±0.09	0.21±0.10
ADH6	1.65±0.05	0.16±0.12	1.42±0.23	0.63±0.15	2.05±0.29	0.33±0.08

Glucose was used as carbon and energy source. Presented values are the mean yield value of two independent experiments±SD, expressed in g product.g biomass^{-1}.

[a] Calculated when glucose was depleted

[b] Calculated when HMF was depleted

strain than for mut-ADH1 and ADH6 strains (Table 3). The mut-ADH1 and ADH6 strains converted HMF and furfural within approximately 5 h, whereas HMF was still present in the medium after more than 20 h with the control strain (Fig. 3). The HMF conversion rate for the mut-ADH1 and ADH6 strains was four to five times higher than for the control strain (Table 3), whereas the furfural conversion rate was similar for all strains.

Discussion

In this work, the overexpression of the NADH- and NADPH-dependent furaldehyde reductases encoded by *mut-ADH1* or *ADH6* genes, respectively, was shown to improve the anaerobic performance of *S. cerevisiae* strains in the presence of the inhibitory compound HMF. This strategy also allowed faster in vivo detoxification of hydrolysate, resulting in increased initial ethanol productivity. Faster in vivo reduction of furaldehyde compounds under anaerobic conditions had previously been achieved by improving the process conditions. For example, fed-batch supply of hydrolysate kept the concentration of inhibitors at a low level, which favored yeast growth and ethanol productivity (Rudolf et al. 2005; Modig et al. 2008). Pre-cultivation of yeast in hydrolysate before simultaneous saccharification and fermentation also improved yeast fermentation (Alkasrawi et al. 2006). Constitutively high NADH- or NADPH-dependent furaldehyde reduction activities in *S. cerevisiae* by strain engineering now also enable the improvement of yeast tolerance.

The difference in fermentation performance between the mut-ADH1 and ADH6 strains in the presence of HMF were correlated with the in vivo HMF reduction rate. Faster in vivo HMF conversion by the ADH6 strain may result from the higher in vitro HMF conversion rate by the crude cell extract of the ADH6 strain as compared with the mut-ADH1 strain at the same substrate concentration and using the preferred cofactor of the respective enzymes.

When HMF reduction activity was increased by overexpression of the NADPH-dependent enzyme Adh6p (Larroy et al. 2002), increased yields of acetate were observed, especially during the HMF conversion phase. Because acetate production is connected with redox balancing (van Dijken and Scheffers 1986), the observed increase in acetate production by the $NADP^+$-dependent

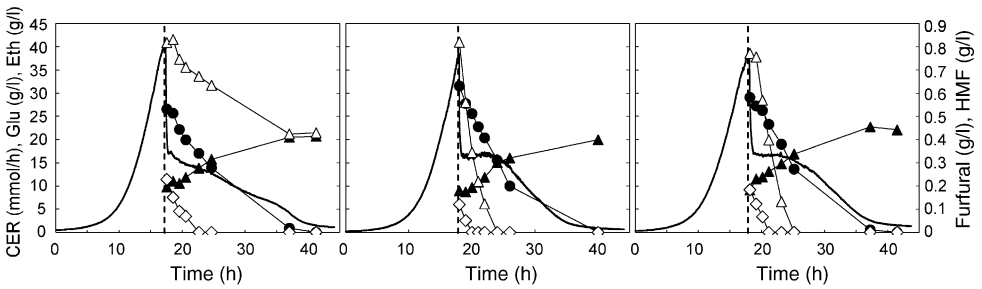

Fig. 3 Fermentation profile of control (*first column*), mut-ADH1 (*second column*), and ADH6 (*third column*) strains in batch fermentation of dilute-acid spruce hydrolysate. Strains are grown in defined medium until biomass concentration reached approximately 2.5 g/l. Then hydrolysate is added at approximately time 18 h (*dashed line*). Glucose (*filled circle*), ethanol (*filled triangle*), HMF (*open triangle*), furfural (*open diamond*), CER (*continuous line*)

Table 3 Specific rates of ethanol formation and HMF conversion in batch fermentation of dilute-acid spruce hydrolysate

Strain	qHMF ($g \cdot g \cdot h^{-1}$)[a]	qEthanol ($g \cdot g \cdot h^{-1}$)[a]
Control	0.012	0.32
Mut-ADH1	0.052	0.39
ADH6	0.049	0.40

[a] Specific rates were calculated from the first 8 h after pulse addition of hydrolysate.

acetaldehyde dehydrogenase 6 (ALD6p) could be explained by the requirement for NADPH for HMF reduction (Fig. 4).

HMF also increased acetate production in the mut-ADH1 strain where HMF reduction was NADH dependent. It corroborates previous studies in which decreased NADH pool by overexpression of H_2O-forming NADH oxidase or NADH-dependent *GPD1* resulted in higher acetate yields (Michnick et al. 1997; Remize et al. 1999; Heux et al. 2006). It has then been suggested that the increase of the NAD^+ pool resulted in acetaldehyde accumulation. Production of acetate would then favor the redox balance and avoid the toxic effects of acetaldehyde. Acetaldehyde production could not be confirmed by measurements in the current work. However, acetaldehyde is highly volatile, and the effects on the overall ethanol yields would be relatively small and difficult to assess. In contrast to acetate, the glycerol production did not vary during fermentation with the mut-ADH1 strain. Most probably, the regeneration of NAD^+ by glycerol formation was substituted to some extent by the HMF reduction by mut-Adh1p (Fig. 4).

Increased production of acetate for all strains could also induce the activity of NADPH-dependent enzymes responsible for stress response (Schneider et al. 1997; Carmel-Harel and Storz 2000). For instance, increased NADPH production by G6PDH in the PPP (Fig. 4) has been shown to increase tolerance against furfural, even if the enzyme is not able to reduce this inhibitor (Gorsich et al. 2006). Although HMF and furfural are very similar in structure and inhibition effects, further studies are necessary to validate this hypothesis.

The observed differences in acetate formation during glucose fermentation with HMF were not observed in the hydrolysate fermentation. However, only marginal changes in glycerol and acetate production could be expected during the hydrolysate fermentation due to the presence of other external electron acceptors and the absence of growth in such fermentations.

In conclusion, higher specific ethanol productivity could be achieved by in vivo detoxification of lignocellulosic hydrolysate during batch fermentations with *S. cerevisiae* strains overexpressing *mut-ADH1* or *ADH6*. Usage of NADH and NADPH, respectively, by mut-Adh1p and Adh6p changed product distribution in defined mineral medium with HMF, however, without affecting ethanol yields.

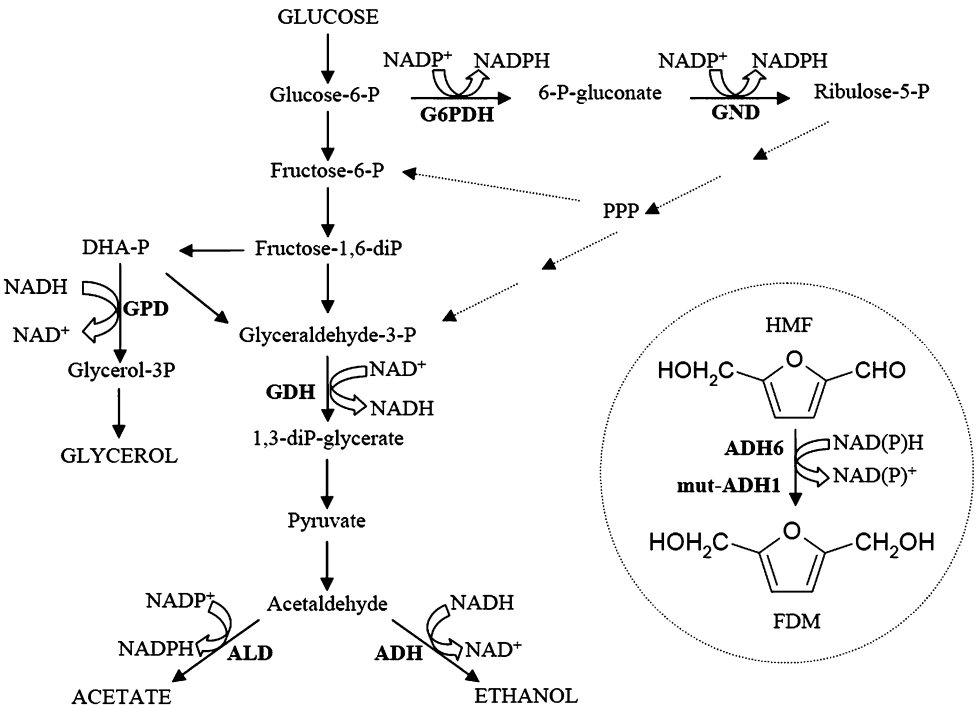

Fig. 4 Schematic illustration of the principal catabolic pathways for glucose metabolism in *S. cerevisiae* under anaerobic conditions. HMF conversion to FDM (2,5-dimethanol) by NADPH-dependent ADH6 and NADH-dependent mut-ADH1 is also shown. The enzymatic reactions and metabolites more relevant for this work appear in *bold* and *upper case*, respectively. Enzymes are: glucose-6-phosphate dehydrogenase (*G6PDH*), 6-phosphogluconate dehydrogenase (*GND*), glyceraldehyde-3-phosphate dehydrogenase (*GDH*), glycerol-3-phosphate dehydrogenase (*GPD*), aldehyde dehydrogenase (*ALD*), alcohol dehydrogenase (*ADH*)

Acknowledgments This work was sponsored by the Swedish Energy Agency and the Swedish Foundation for International Cooperation in Research and Higher Education. We would like to thank Prof. Bärbel Hahn-Hägerdal for critically reading the manuscript.

References

Alkasrawi M, Rudolf A, Lidén G (2006) Influence of strain and cultivation procedure on the performance of simultaneous saccharification and fermentation of steam pretreated spruce. Enzyme Microb Technol 38:279–287

Almeida JRM, Modig T, Petersson A, Hahn-Hägerdal B, Lidén G, Gorwa-Grauslund MF (2007) Increased tolerance and conversion of inhibitors in lignocellulosic hydrolysates by *Saccharomyces cerevisiae*. J Chem Technol Biotechnol 82:340–349

Banerjee N, Bhatnagar R, Viswanathan L (1981) Inhibition of glycolysis by furfural in *Saccharomyces cerevisiae*. Appl Microbiol Biotechnol 11:226–228

Carmel-Harel O, Storz G (2000) Roles of the glutathione- and thioredoxin-dependent reduction systems in the *Escherichia coli* and *Saccharomyces cerevisiae* responses to oxidative stress. Annu Rev Microbiol 54:439–461

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 71:339–349

Hauf J, Zimmermann FK, Muller S (2000) Simultaneous genomic overexpression of seven glycolytic enzymes in the yeast *Saccharomyces cerevisiae*. Enzyme Microb Technol 26:688–698

Heux S, Cachon R, Dequin S (2006) Cofactor engineering in *Saccharomyces cerevisiae*: Expression of a H_2O-forming NADH oxidase and impact on redox metabolism. Metab Eng 8:303–314

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 22:359–368

Laadan B, Almeida JR, Rådström P, Hahn-Hägerdal B, Gorwa-Grauslund MF (2008) Identification of an NADH-dependent 5-hydroxymethylfurfural-reducing alcohol dehydrogenase in *Saccharomyces cerevisiae*. Yeast 25:191–198

Larroy C, Fernandez MR, Gonzalez E, Pares X, Biosca JA (2002) Characterization of the *Saccharomyces cerevisiae* YMR318C (ADH6) gene product as a broad specificity NADPH-dependent alcohol dehydrogenase: relevance in aldehyde reduction. Biochem J 361:163–172

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, Slininger PJ, Dien BS, Berhow MA, Kurtzman CP, Gorsich SW (2004) Adaptive response of yeasts to furfural and 5-hydroxymethylfurfural and new chemical evidence for HMF conversion to 2,5-bis-hydroxymethylfuran. J Ind Microbiol Biotechnol 31:345–352

Michnick S, Roustan J-L, Remize F, Barre P, Dequin S (1997) Modulation of glycerol and ethanol yields during alcoholic fermentation in *Saccharomyces cerevisiae* strains overexpressed or disrupted for *GPD1* encoding glycerol 3-phosphate dehydrogenase. Yeast 13:783–793

Modig T, Liden G, Taherzadeh MJ (2002) Inhibition effects of furfural on alcohol dehydrogenase, aldehyde dehydrogenase and pyruvate dehydrogenase. Biochem J 363:769–776

Modig T, Almeida JRM, Gorwa-Grauslund MF, Lidén G (2008). Variability of the response of *Saccharomyces cerevisiae* strains to lignocellulose hydrolysate. Biotech Bioeng (in press) DOI 10.1002/blt.21789

Nilsson A, Taherzadeh MJ, Liden G (2001) Use of dynamic step response for control of fed-batch conversion of lignocellulosic hydrolyzates to ethanol. J Biotechnol 89:41–53

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 71:7866–7871

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

Petersson A, Almeida JRM, 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 23:455–464

Remize F, Roustan JL, Sablayrolles JM, Barre P, Dequin S (1999) Glycerol overproduction by engineered *Saccharomyces cerevisiae* wine yeast strains leads to substantial changes in by-product formation and to a stimulation of fermentation rate in stationary phase. Appl Environ Microbiol 65:143–149

Rudolf A, Alkasrawi M, Zacchi G (2005) A comparison between batch and fed-batch simultaneous saccharification and fermentation of steam pretreated spruce. Enzyme Microb Technol 37:195–205

Schneider R, Brors B, Burger F, Camrath S, Weiss H (1997) Two genes of the putative mitochondrial fatty acid synthase in the genome of *Saccharomyces cerevisiae*. Curr Genet 32:384–388

Taherzadeh MJ, Gustafsson L, Niklasson C, Liden G (1999) Conversion of furfural in aerobic and anaerobic batch fermentation of glucose by *Saccharomyces cerevisiae*. J Biosci Bioeng 87:169–174

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

Taherzadeh MJ, Gustafsson L, Niklasson C, Liden G (2000b) Physiological effects of 5-hydroxymethylfurfural on *Saccharomyces cerevisiae*. Appl Microbiol Biotechnol 53:701–708

van Dijken JP, Scheffers WA (1986) Redox balances in the metabolism of sugars by yeasts. FEMS Microbiol Lett 32:199–224

van Dijken JP, Bauer J, Brambilla L, Duboc P, Francois JM, Gancedo C, Giuseppin ML, Heijnen JJ, Hoare M, Lange HC, Madden EA, Niederberger P, Nielsen J, Parrou JL, Petit T, Porro D, Reuss M, van Riel N, Rizzi M, Steensma HY, Verrips CT, Vindelov J, Pronk JT (2000) An interlaboratory comparison of physiological and genetic properties of four *Saccharomyces cerevisiae* strains. Enzyme Microb Technol 26:706–714

Verduyn C, Postma E, Scheffers WA, Van Dijken JP (1992) Effect of benzoic acid on metabolic fluxes in yeasts: a continuous-culture study on the regulation of respiration and alcoholic fermentation. Yeast 8:501–517