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RESEARCH ARTICLE

Enhancement of Simultaneous Xylose and Glucose Utilization by Regulating *ZWF1* and *PGI1* in *Saccharomyces Cerevisiae*

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Abstract Xylose utilization is one of the key issues in lignocellulose bioconversion. Because of glucose repression, in most engineered yeast with heterogeneous xylose metabolic pathway, xylose is not consumed until glucose is completely utilized. Although simultaneous glucose and xylose utilization have been achieved in yeast by *RPE1* deletion, we regulated *ZWF1* and *PGI1* transcription to improve simultaneous xylose and glucose utilization by controlling the metabolic flux from glucose into the PP pathway. Xylose and glucose consumption increased by approximately 80 and 72%, respectively, whereas *ZWF1* was overexpressed by multi-copy plasmids with a strong transcriptional promoter. *PGI1* expression was knocked down by promoter replacement; the glucose and xylose metabolism increased when PGI1p was replaced by weak promoters, SSA1p and PDA1p. *ZWF1* overexpression decreased while *PGI1* down-regulation increased the ethanol yield to some extent in the recombinant strains.

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Keywords Synthetic biology · Promoter replacement · Simultaneous utilization · Glucose · Xylose · *ZWF1* · *PGI1* · *Saccharomyces cerevisiae*

Abbreviations

GO488	Glucose6-phosphate dehydrogenase of *Gluconobacteroxydans*ATCC621
GO489	Glucose6-phosphate dehydrogenase of *Gluconobacteroxydans*DSM3054
H6PD	Hexose6-phosphate dehydrogenase of *Musmusculus*
KLSB lab	Key Laboratory of Systems Bioengineering (Ministry of Education of China), School of Chemical Engineering and Technology, Tianjin University
XYL1	Xylose reductase
XYL2	Xylitol dehydrogenase
XKS	Xylulokinase
RPE	Ribulose-5-phosphate 3-epimerase
RKI	Ribose-5-phosphate ketol-isomerase
TAL	Transaldolase
TKL	Transketolase
ZWF1	Glucose 6-phosphate dehydrogenase
SOL	6-Phosphogluconolactonase
GND	6-Phosphaogluconate dehydrogenase
PGI1	Phosphoglucoseisomerase
Ga-3-P	Glyceraldehyde 3-phosphate
G-6-P	Glucose 6-phosphate
DHAP	Dihydroxyacetone phosphate
S-7-P	Sedoheptulose 7-phosphate
F-6-P	Fructose 6-phosphate
F-1,6-BP	1,6 Fructose diphosphate
E-4-P	Erythrose 4-phosphate
Gl-6-P	Glucono-1,5-lactone 6-phosphate
Gn-6-P	Gluconate 6-phosphate

Ru-5-P	Ribulose 5-phosphate
Ri-5-P	Ribose 5-phosphate
X-5-P	Xylulose 5-phosphate

Introduction

Xylose is the second most abundant fermentable monosaccharide released from lignocelluloses biomass [1–3]. Efficient xylose utilization in the lignocellulosic hydrolysate is an important issue for economic bioconversion of lignocelluloses [4–7]. To achieve high product titer, solid-to-solid pretreatments have been preferred to facilitate fermentation with high solid loading of feedstock [8–10]. During such pretreatments, xylan (xylose) and glucan (glucose) are usually in the same pot during fermentation [1, 11–13]. Simultaneous xylose and glucose utilization would be an efficient approach for lignocellulose bioconversion.

Saccharomyces cerevisiae is the most widely used microorganism for industrial ethanol production, and heterogeneous xylose metabolic pathways have been engineered into *S. cerevisiae* [14, 15]. The following two pathways were extensively explored for xylose utilization: xylose reductase (XR) and xylitol dehydrogenase (XDH) pathway and xylose isomerase (XI) pathway [15]. For the XR-XDH pathway, previous research on co-factor balance and strain evolution significantly improved xylose utilization, although xylitol was always an unavoidable byproduct from xylose [15]. The XI pathway occurred in few eukaryotes and has been engineered and evolved to improve xylose utilization and ethanol yield [16, 17]. However, in most engineered strains, because of glucose repression, xylose is not utilized until glucose is exhausted. This leads to low fermentation efficiency [18–21].

Simultaneous xylose and glucose utilization is attracting increasing attention. Because of the limited understanding of glucose repression, it is difficult to rationally relieve glucose repression [18, 22]. Therefore, several strategies have been applied to achieve simultaneous xylose and glucose utilization [4]. Xylose-specific transporters and high-affinity transporters of xylose were developed by evolution of xylose-utilizing yeast, directed evolution or rational design of glucose transporters [23–27]. Except for transporter engineering, reconstitution of carbohydrate metabolic networks, such as the phosphoketolase pathway, is another approach to improve xylose utilization [15, 28]. Recently, it has been reported that disruption of some genes in glycolysis and pentose phosphate (PP) pathway resulted in simultaneous xylose and glucose utilization [5, 29]. Although simultaneous xylose and glucose utilization has been achieved in several studies, sugar consumption rate needs to be improved.

In our previous research, *RPE1* deletion achieved simultaneous glucose and xylose utilization by coupling glycolysis and PP pathway in the strain SyBE_Sc17004 [5]. Xylose utilization was closely related with the metabolic flux of glucose into the PP pathway. Metabolic flux analysis revealed the proportions of glucose that entered glycolysis and PP pathway in different culture types [30]. Here we regulated key gene (*ZWF1* and *PGI1*) expression for the metabolic flux of glucose into the PP pathway to increase xylose utilization during simultaneous xylose and glucose fermentation (Fig. 1).

Materials and Methods

Strains and Media

Yeast strains and plasmids used in this study are listed in Table 1. Primers used in this study are listed in Table 2.

Escherichia coli DH5α was used as a cloning host for plasmid replication and was grown in Luria–Bertani medium (10 g/L tryptone, 5 g/L yeast extract, and 5 g/L NaCl) containing 50 mg/L kanamycin at 37 °C and at 250 r/min. The yeast seed was grown in synthetic complete (SC) medium without uracil and tryptophan (SC-Ura-Trp) or SC medium without uracil, tryptophan, and leucine (SC-Ura-Trp-Leu) at 30 °C and at 200 r/min. Yeast fermentation was conducted in YP medium (10 g/L yeast extract, 20 g/L peptone) with desired concentrations of xylose and glucose.

DNA Manipulation and Plasmids Construction

Endogenous genes *ZWF1* was cloned from *S. cerevisiae* L2612 chromosomal DNA according to its sequence information published in *Saccharomyces* Genome Database (http://www.yeastgenome.org). *GO488* from *Gluconobacteroxydans* ATCC621, *GO489* from *Gluconobacteroxydans* DSM3054, and *H6PD* from *Mus-musculus* were synthesized artificially according to the codon-optimized sequences based on the amino acid sequences from Genebank (http://www.ncbi.nlm.nih.gov/genbank) (Table S1). PCR products of *ZWF1*, *GO488*, *GO489*, and *H6PD* were gel purified using a TIANgel Midi Purification Kit (TIANGEN Biotech, DP209) and digested with *Bsa*I, and the expression cassettes LD10 andLD12 were also digested with *Bsa*I. Then the digested gene segments and expression cassettes were ligated using T4 DNA ligase and transformed into DH5α. Selected clones were verified by colony PCR and restriction enzyme digestion. In this way, the gene expression cassettes LD10-*ZWF1*, LD10-*GO488*, LD10-*GO489*, LD10-*H6PD*, and LD12-*ZWF1* were constructed. The RADOM method was

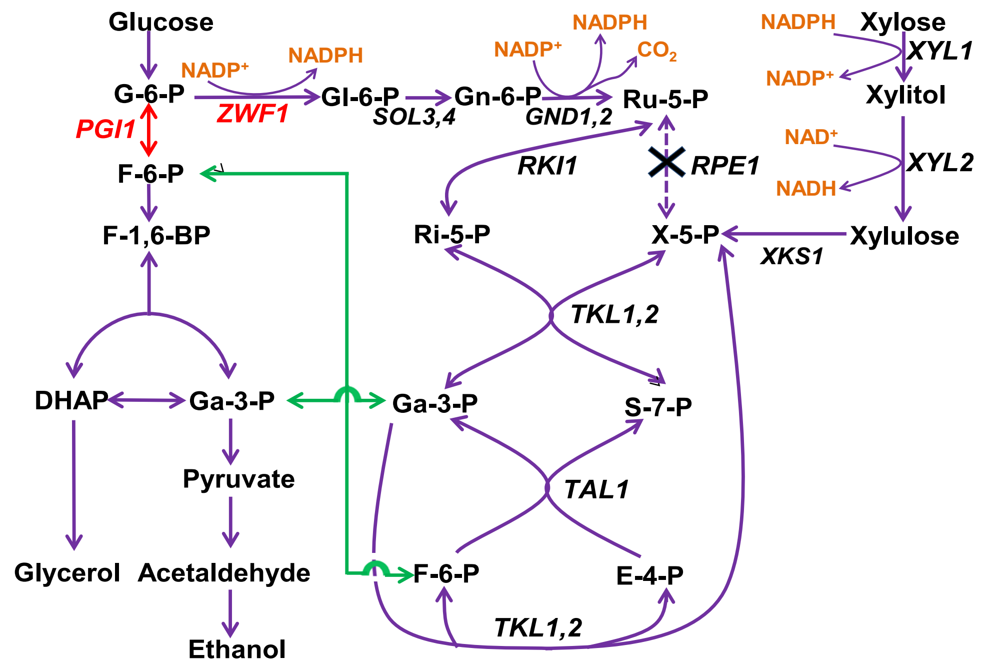

Fig. 1 Metabolic network for glucose and xylose metabolism in recombinant xylose-utilizing *Saccharomyces cerevisiae*

Table 1 Strains and plasmids used in this study

Strain or plasmid	Relevant genotype or phenotype	Source or references
Strain		
SyBE_Sc17004	L2612,delta::XYL1, XYL2, XYL3; *RPE1::TRP1*	[5]
SyBE_Sc122000	SyBE_Sc17004 with plasmid pRS425K	This study
SyBE_Sc122001	SyBE_Sc17004 with plasmid LD10-*ZWF1*	This study
SyBE_Sc122002	SyBE_Sc17004 with plasmid LD10-*GO488*	This study
SyBE_Sc122003	SyBE_Sc17004 with plasmid LD10-*GO489*	This study
SyBE_Sc122004	SyBE_Sc17004 with plasmid LD10-*H6PD*	This study
SyBE_Sc122006	SyBE_Sc17004 with plasmid LD12-*ZWF1*	This study
SyBE_Sc122009	SyBE_Sc17004 with plasmid LD46-*ZWF1*	This study
SyBE_Sc122037	SyBE_Sc17004, PGI1p:: YEF3p	This study
SyBE_Sc122038	SyBE_Sc17004,PGI1p:: RPL8Bp	This study
SyBE_Sc122039	SyBE_Sc17004, PGI1p:: SSB1p	This study
SyBE_Sc122040	SyBE_Sc17004, PGI1p:: SSA1p	This study
SyBE_Sc122041	SyBE_Sc17004, PGI1p:: PDA1p	This study
Plasmid		
pRS425K	*LEU2*, a multicopy plasmid	KLSB lab
LD10	FBA1t-TPI1p-PGK1t, in pRS425K	KLSB lab
LD12	FBA1t-TDH1p-PGK1t, in pRS425K	KLSB lab
LD46	FBA1t-TEF1p-PGK1t, in pRS425K	KLSB lab
LD10-*ZWF1*	FBA1t-TPI1p-*ZWF1*-PGK1t	This study
LD10-*GO488*	FBA1t-TPI1p-*GO488*-PGK1t	This study
LD10-*GO489*	FBA1t-TPI1p-*GO489*-PGK1t	This study
LD10-*H6PD*	FBA1t-TPI1p-*H6PD*-PGK1t	This study
LD12-*ZWF1*	FBA1t-TDH1p-*ZWF1*-PGK1t	This study
LD46-*ZWF1*	FBA1t-TEF1p-*ZWF1*-PGK1t	This study

Table 2 Primers used in this study

Oligo	Sequence (5′–3′)
LD10(12)-*ZWF1*-F	*ggtctc*caaaaATGAGTGAAGGCCCCGTC
LD10-*ZWF1*-R	*ggtctc*ccaatCTAATTATCCTTCGTATCTTCTGGC
LD12-*ZWF1*-R	*ggtctc*ccaatCTAATTATCCTTCGTATCTTCTGGC
LD46-*ZWF1*-F	tagcaatctaatctaagttttaattacaaaATGAGTGAAGGCCCCGTC
LD46- *ZWF1*-R	attgatctatcgatttcaattcaattcaatCTAATTATCCTTCGTATCTTCTGGC
LD10-*GO488(489)*-F	*ggtctc*caaaaATGGAACATTTCCAACAAGTTG
LD10-*GO488(489)*-R	*ggtctc*ccaatTTATTCACTAGCTTCGTGCCA
LD10-H6PD-F	*ggtctc*caaaaATGTTGTTAGCAGCCATGTG
LD10-H6PD-R	*ggtctc*ccaatTTAACCTAAGAATGCTTCGTAATC
PGI1p-replacement-F	CTGAAGAAGGCATACTACGCC
PGI1p-replacement-R	GGAGTTGATGTGTTCACCTTTG
kanMX-yan-F	CGTCAATCGTATGTGAATGC
PGI1p-yan-F	CAGCTTCCTGTAATTCCAGG
PGI1-yan-R	GAGTCGACTTCTGGAGCAAC
Act1-F	CGTTCCAATTTACGCTGGTT
Act1-R	GGCCAAATCGATTCTCAAAA
PGI1-F	CTAACGCTAACACTGCCAAGAAC
PGI1-R	GCCGACCAGACAGAGTAACG
ZWF1-F	GTCGCATCGGGTGTCTTC
ZWF1-R	CCTCGTAAGCCTCTGGAATC

Homology arms were underlined and the restriction site *Bsa*I was italic

applied to construct LD46-*ZWF1* [31]. The fragments used for PGI1p replacement, including YEF3p, RPL8Bp, SSA1p, SSB1p, PDA1p, and the screen label *kanMX* were synthesized artificially.

Yeast Transformation

Yeast transformation was performed using the lithium acetate/single-stranded carrier DNA/PEG method as described previously [32, 33]. Transformants were selected on SC-Ura-Trp-Leu or SC-Ura-Trp agar plates with neomycin analogue G418 (200 μg/mL).

Yeast Fermentation

The seed cultures were grown in SC-Ura-Trp-Leu or SC-Ura-Trp medium and harvested in the late growth phase. Cells were washed twice using sterile water and inoculated into different fermentation media. The seed cells were grown in 100 mL YPXG medium (yeast extract 10 g/L, peptone 20 g/L with different concentrations of D-xylose and glucose) with an initial optimal density at 600 nm (OD_{600}) of 1.0 in a 250-mL flask sealed using a rubber stopper with a needle to release CO_2 produced during the fermentation process. Fermentations were performed at 30 °C and at 150 r/min. All experiments were conducted in duplicates.

Sugar and Product Analyses

The supernatant was collected for analysis on a HPLC system consisting of Waters 1515 HPLC pump, a Bio-Rad HPX-87H column (Bio-Rad, Hercules, CA, USA) and Waters 2414 refractive index detector [12]. The column was eluted at 65 °C with 5 mmol/L sulfuric acid at a flow rate of 0.6 mL/min.

Quantitative RT-PCR Analysis

Samples in the late exponential phase of flask cultivation were harvested by centrifugation for 5 min at 4000 r/min and at 4 °C, followed by two washes with ice-cold water. Total RNA of cell samples was extracted using a Mini RNA dropout kit (Tiandz Inc., Beijing, China). Reverse transcription was performed using the Reverse Transcription kit (Tiangen, Beijing, China). The cDNA products were then used for the quantitative PCR reaction using the RealMaster Mix Kit (Tiangen, Beijing, China). Primers were designed according to sequences in the *Saccharomyces* Genome Database (http://www.yeastgenome.org). Quantitative RT-PCR was run in an ABI7300 Thermo cycler (Applied Biosystems, Carlsbad, CA, USA). The threshold cycle value (C_T) for each sample was determined using the ABI7300 software. Three biological replicates were performed for each gene. Data were normalized using

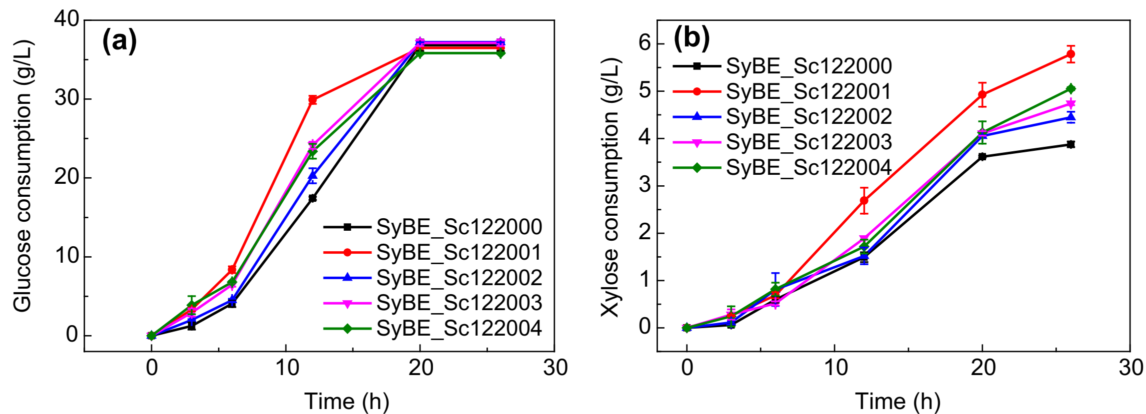

Fig. 2 Effects of glucose-6-phosphate dehydrogenase overexpression from different species on **a** glucose and **b** xylose utilization (The fermentation was conducted under anaerobic conditions in 100 mL YPXG medium containing 20 g/L D-xylose and 40 g/L glucose. The initial cell density was OD_{600} = 1.0. Data refer to the mean ± standard deviation of duplicate experiments.)

ACT1 as the internal standard and analyzed according to the $2^{-\Delta\Delta C_T}$ method [34].

Enzyme Assays

Cell samples in the exponential phase of flask cultivation were harvested by centrifugation for 5 min at 4000 r/min and at 4 °C. Cells were washed twice with ice-cold water and resuspended in the disintegration buffer. Crude protein was prepared by 20-min sonication at 4 °C. Protein concentration was determined using a Bradford protein assay kit (Tiangen, Beijing, China). The activities of glucose 6-phosphate dehydrogenase (G6PDH) and PGI were determined using the method described in previous studies with some modifications [35, 36]. For PGI assay, the standard assay (3 mL) contained 50 mmol/L triethanolamine hydrochloride (neutralized with KOH), 10 mmol/L $MgC1_2$ (pH 7.4), 0.4 mmol/L $NADP^+$, 10 mmol/L fructose 6-phosphate, and 3 units of G6PDH. For G6PDH assay, the same procedures as mentioned for PGI were used except that glucose 6-phosphate was used as substrate. One unit (U) of activity of the two enzymes was defined as the amount of enzyme that produces one micromole of NADPH per minute, and the specific activity was defined as units per milligram of total protein.

Results and Discussion

Glucose 6-Phosphate Dehydrogenase Overexpression to Improve Xylose and Glucose Utilization

Glucose 6-phosphate dehydrogenase encoded by *ZWF1* catalyzes the rate-limiting NADPH-producing step in the PP pathway [37]. In the strain SyBE_Sc17004, xylose metabolism was coupled with the metabolic flux of glucose into the PP pathway by *RPE1* deletion [5]. G6PDH is the key enzyme to control the metabolic flux in the PP pathway from glucose. Therefore, we investigated the effect of G6PDH overexpression on xylose utilization. Several G6PDH encoding genes from different species were overexpressed, including *ZWF1* from *S. cerevisiae*, *GO488* from *Gluconobacteroxydans* ATCC621, *GO489* from *Gluconobacteroxydans* DSM3054, and *H6PD* from *Mus-musculus*. These G6PDH encoding genes *ZWF1*, *GO488*, *GO489*, and *H6PD* were separately expressed under the control of TPI1p promoter with a multi-copy plasmid pRS425K in the strain SyBE_Sc17004 to generate the strains SyBE_Sc122001, SyBE_Sc122002, SyBE_Sc122003, and SyBE_Sc122004, respectively. The strain SyBE_Sc122001 exhibited significant increase in simultaneous glucose and xylose utilization, while the other strains exhibited similar sugar utilization ability as the control strain (Fig. 2). The increase of glucose consumption in SyBE_Sc122001 was due to the increase of metabolic flux into the PP pathway from glucose, which drove the increase in xylose consumption due to the coupling by *RPE1* deletion. Besides, *ZWF1* overexpression also offered more NADPH as the co-factor for xylose reductase to accelerate xylose utilization [38, 39].

To further improve xylose utilization, we varied the promoters for *ZWF1* overexpression. *ZWF1* was overexpressed under the control of the promoters TDH1p and TEF1p in the strain SyBE_Sc17004 to generate SyBE_Sc122006 and SyBE_Sc122009, respectively. The engineered strains SyBE_Sc122001, SyBE_Sc122006, and SyBE_Sc122009 exhibited improved glucose and xylose utilization, but similar acceleration of sugar utilization was observed for three different promoters (Fig. 3a, b). We further quantified *ZWF1* transcription using qPCR, and significantly different transcriptions for the three promoters

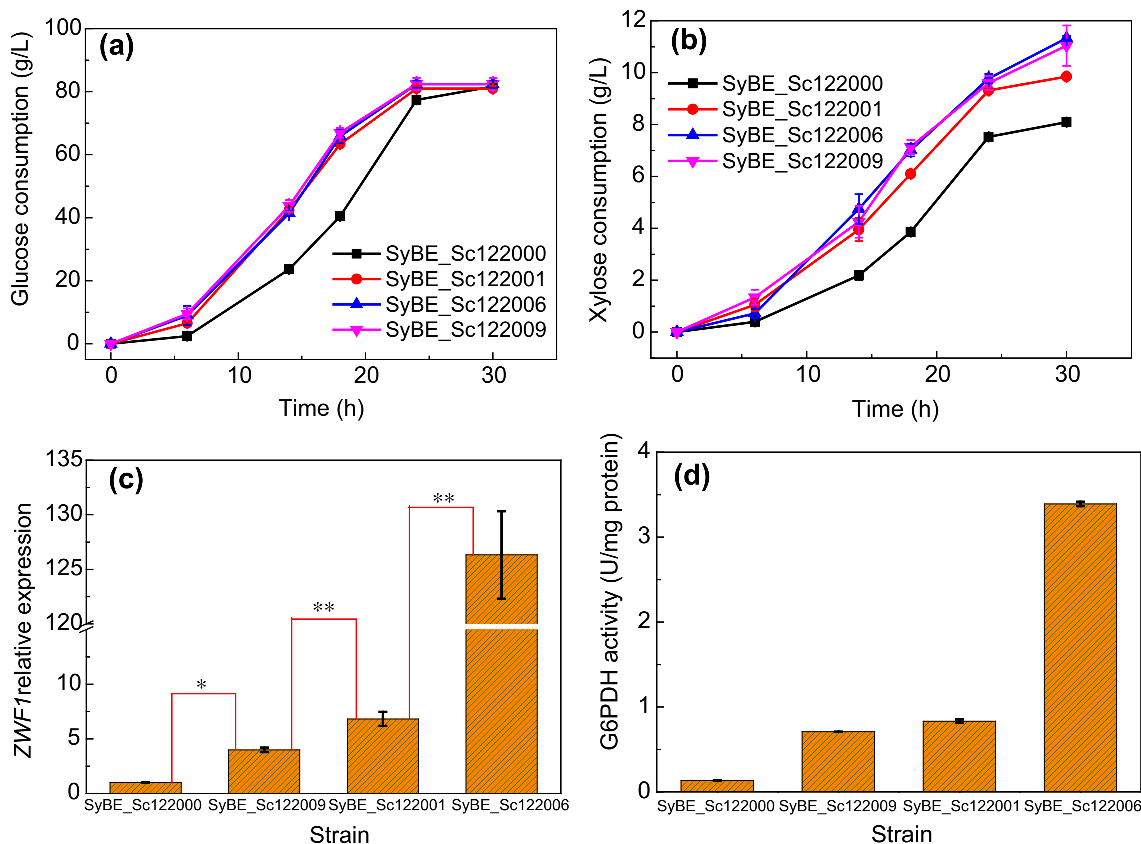

Fig. 3 Effect of *ZWF1* overexpression on the engineered strains. **a** Glucose, **b** xylose, **c** *ZWF1* relative expression in different strains, and **d** G6PDH activity in different strains (The fermentation was conducted under anaerobic conditions in 100 mL YPXG medium containing 20 g/L D-xylose and 80 g/L glucose. Significance levels of Student's *t* test: $*p < 0.05$, $**p < 0.01$.)

were observed. The transcriptional strength order was TDH1p > TPI1p > TEF1p (Fig. 3c). Then the G6PDH activities were assayed in the *ZWF1* overexpressed *S. cerevisiae* strains grown to the exponential phase by flask cultivation. Significant amplification of the G6PDH activities in vitro can be observed for the *ZWF1* overexpressed strains. For the strain SyBE_Sc122006, it has been enhanced nearly 30 times compared with the control strain SyBE_Sc122000 (Fig. 3d). The inconsistence of *ZWF1* transcription and translation level and sugar utilization may be because of the tight regulation of G6PDH activity. G6PDH is a component of dynamic macromolecular depot structures in *S. cerevisiae*. The oligomeric state of G6PDH is affected by intracellular NADPH levels; G6PDH activity is under strong feedback inhibition by NADPH [40, 41]. In addition, NADPH is a product of G6PDH. Therefore, G6PDH is a self-control enzyme. Thus, the G6PDH activity in vivo and the gene expression strength are not necessarily in positive correlation [42]. Therefore, the observation in this study is reasonable: when we overexpressed *ZWF1*, G6PDH activity was improved in varying degrees compared with the control and led to increased sugar utilization. However, when *ZWF1* expression was further enhanced, G6PDH activity was inhibited, and no further increased sugar utilization was observed, although *ZWF1* transcriptional and translational levels were much higher. Ethanol and xylitol accumulation of these *ZWF1* overexpressed strains are shown in Fig. S1. The ethanol yields of *ZWF1* overexpressed strains are shown in Table 3. In SyBE_Sc122009, ethanol yield reached 0.423 g/g(sugar) and this was the optimal yield among the *ZWF1* overexpressed strains. In other strains the ethanol yields were slightly lower than those in the control strain SyBE_Sc122000. These results were consistent with a previous research that has shown that increasing the

Table 3 The ethanol yields of the strains with *ZWF1* overexpression

Strain	Ethanol yield (g/g sugar)
SyBE_Sc122000	0.424 ± 0.005
SyBE_Sc122001	0.417 ± 0.005
SyBE_Sc122006	0.418 ± 0.007
SyBE_Sc122009	0.423 ± 0.010

Values are given as the average and experimental deviation of two independent batch fermentations

G6PDH activity resulted in lower ethanol yield [43]. Thus *ZWF1* overexpression did improve the sugar consumption rate and simultaneous xylose and glucose utilization, but lowered ethanol yield.

Effect of *PGI1* Down-Regulationon Xylose and Glucose Utilization

As mentioned above, glucose 6-phosphate is the key node for the metabolic flux into the glycolysis and PP pathways. Thus, we hypothesized that the down-regulation of *PGI1*, encoding phosphoglucose isomerase, would be helpful to increase the metabolic flux into the PP pathway from glucose. However, phosphoglucose isomerase–deficient (*pgi1*) strains of *S. cerevisiae* exhibited growth defect on glucose [44]. Here, to knockdown *PGI1* expression, we designed the replacement of *PGI1* promoter in SyBE_Sc17004 with several weak promoters, including YEF3p, RPL8Bp, SSB1p, SSA1p, and PDA1p. In this order, the strains SyBE_Sc122037, SyBE_Sc122038, SyBE_Sc122039, SyBE_Sc122040, and SyBE_Sc122041 were obtained. Promoter replacements were confirmed by a diagnostic PCR. As shown in Fig. 4, the knockdown by YEF3p, RPL8Bp, and SSB1p resulted in the decrease of glucose and xylose utilization. However, the knockdown by SSA1p and PDA1p led to a slight increase of glucose and xylose utilization. The knockdown of *PGI1* in all the strains was verified using qPCR and the transcriptional strengths were in the following order: PGI1-p > YEF3p > RPL8Bp > SSB1p > SSA1p > PDA1p (Fig. 4c), which is consistent with data derived from GFP intensity [45]. At the same time, PGI activities were also assayed in these recombinant *S. cerevisiae* strains grown to the exponential phase in flask fermentation (Fig. 4d). Ethanol and xylitol accumulation were detected during cultivation of these recombinant strains (Fig. S2). The ethanol yields of these recombinant strains are listed in Table 4. All of the *PGI1* down-regulation strains showed higher ethanol yields than the control strain SyBE_Sc17004. These results indicated that glucose and xylose utilization can increase lightly when *PGI1* is knocked down significantly. This was consistent with the conclusion of a previous research that down-regulation of *PGI1* alone synergistically assisted xylitol production [36]. When the glycolysis pathway was blocked by *pgi* deletion in *E. coli*, xylose and glucose were consumed in the ratio of 1:1 at a relative slow rate [29]. In *pgi1* mutant *S. cerevisiae*, some extra cofactor transforming genes, such as *GDH2* coding for NAD(+)-specific glutamate dehydrogenase or *gapB* encoding NADPH-utilizing

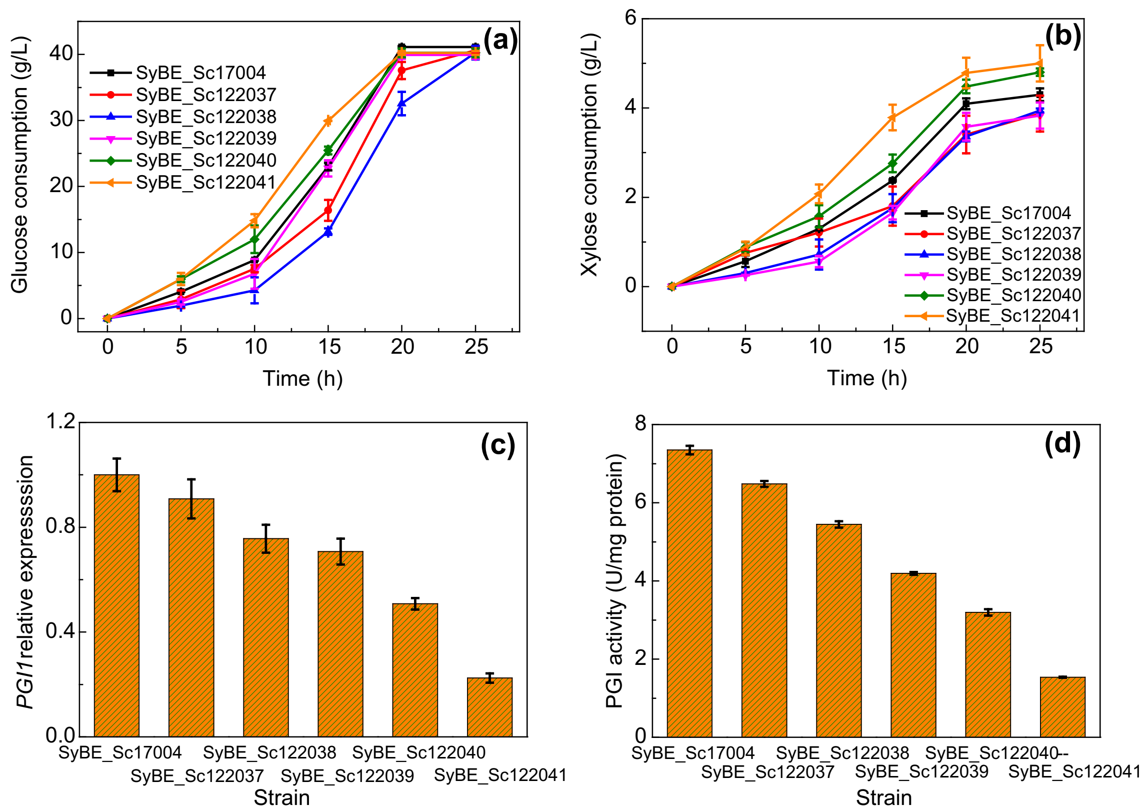

Fig. 4 Effect of *PGI1* down-regulation on the engineered strains. **a** Glucose, **b** xylose, **c** *PGI1* relative expression in different strains, **d** PGI activity in different strains (The fermentation was conducted under anaerobic conditions in 100 mL YPXG medium containing 20 g/L D-xylose and 40 g/L glucose.)

Table 4 Ethanol yields of the *PGI1* down-regulation strains

Strain	Ethanol yield (g/g sugar)
SyBE_Sc17004	0.428 ± 0.009
SyBE_Sc122037	0.431 ± 0.003
SyBE_Sc122038	0.443 ± 0.016
SyBE_Sc122039	0.436 ± 0.009
SyBE_Sc122040	0.439 ± 0.013
SyBE_Sc122041	0.441 ± 0.007

Values are given as the average and experimental deviation of two independent fermentations

glyceraldehyde-3-phosphate dehydrogenase, are essential for the growth on glucose [44]. But these strains often did not tolerate glucose very well. So, further improvements to these yeast strains for glucose and xylose co-fermentation are needed, such as up-regulation of the non-oxidative PP pathway flux and improvement of xylose metabolic capacity.

Effect of Sugar Concentration on Xylose and Glucose Utilization

In *S. cerevisiae* there are no specific xylose transporters, and xylose enters the cell through native hexose transporters. Transporter engineering for xylose significantly improved xylose utilization by increasing the affinity of the transporter for xylose [5]. If the transporter is the limiting step for xylose utilization in present biosystems, sugar concentration and the ratio of xylose and glucose would influence xylose utilization significantly. We designed several fermentation experiments with different sugar concentrations, and the strains SyBE_Sc122009 and SyBE_Sc122000 were used as examples. As shown in Fig. 5, the ratio of co-consumed xylose/glucose was almost stable for each strain at different sugar concentrations and ratios. Compared with SyBE_Sc122000, both xylose consumption and glucose metabolism were enhanced in SyBE_Sc122009 under all the conditions. Previous research has revealed that the increase in the concentrations of glucose decreased xylose consumption and that hexose transporter overexpression Hxt7 partially relieved the inhibitory effects of increasing glucose concentration [46]. Ishola et al. [47, 48] reported that glucose concentration affected xylose uptake in SSFF or in reverse membrane bioreactors. In recombinant flocculent *S. cerevisiae* the best ratio of glucose/xylose for the highest xylose consumption rate was 2:3 among different ratios [49]. But our results showed that the ratio of co-consumed xylose/glucose was independent on sugar ratio and concentration both in the chassis strain SyBE_Sc17004 [5] and in the *ZWF1*

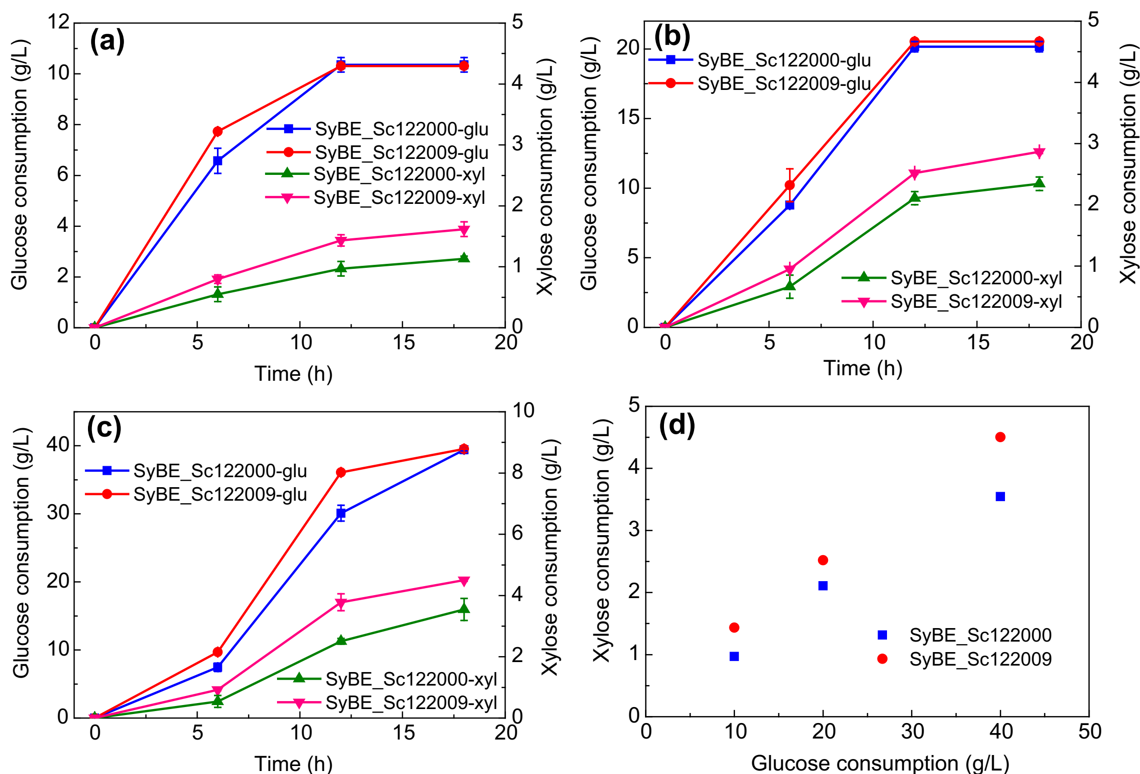

Fig. 5 Effect of glucose concentration on sugar utilization. The fermentation was conducted under anaerobic conditions in 100 mL YPXG medium containing 10 g/L D-xylose and **a** 10 g/L glucose, **b** 20 g/L glucose and **c** 40 g/L glucose, and **d** the ratio of simultaneously consumed xylose/glucose in different concentrations of glucose

overexpressed strain SyBE_Sc122009. Hence, we inferred that sugar transporters may not be the limiting factor for xylose utilization in our strains. On the other hand, these results were just the consequence of the coupling of glycolysis and PP pathway by *RPE1* deletion, as the PP pathway is the major source of NADPH that is necessary in *S.cerevisiae* [50]. The PP pathway network needs xylulose 5-phosphate as substrate and in our strains xylose is the only source of it. Thus, it is the metabolism request that drives the xylose utilization.

Conclusions

We developed several approaches to increase simultaneous xylose and glucose utilization in the xylose-utilizing *S. cerevisiae* with the deletion of *RPE1*. We made the following conclusions according to our results: (1) *ZWF1* overexpression can increase glucose and xylose utilization, but G6PDH is under tight regulation when *ZWF1* transcription is too high; (2) down-regulation of *PGI1* is not efficient to increase xylose utilization; (3) *ZWF1* overexpression decreased the ethanol yield while *PGI1* down-regulation can increase it slightly; (4) the ratio of simultaneously consumed xylose/glucose is independent on the concentration and ratio of xylose and glucose in the medium.

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