A Thi2p Regulatory Network Controls the Post-glucose Effect of Xylose Utilization in Saccharomyces cerevisiae

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The complete and efficient utilization of both glucose and xylose is necessary for the economically viable production of biofuels and chemicals using lignocellulosic feedstocks. Although recently obtained recombinant Saccharomyces cerevisiae strains metabolize xylose well when xylose is the sole carbon source in the medium (henceforth referred to as “X stage”), their xylose consumption rate is significantly reduced during the xylose-only consumption phase of glucose-xylose co-fermentation (“GX stage”). This post-glucose effect seriously decreases overall fermentation efficiency. We showed in previous work that THI2 deletion can alleviate this post-glucose effect, but the underlying mechanisms were ill-defined. In the present study, we profiled the transcriptome of a thi2Δ strain growing at the GX stage. Thi2p in GX stage cells regulates genes involved in the cell cycle, stress tolerance, and cell viability. Importantly, the regulation of Thi2p differs from a previous regulatory network that functions when glucose is the sole carbon source, which suggests that the function of Thi2p depends on the carbon source. Modeling research seeking to optimize metabolic engineering via TFs should account for this important carbon source difference. Building on our initial study, we confirmed that several identified factors did indeed increase fermentation efficiency. Specifically, overexpressing STT4, RGI2, and TFC3 increases specific xylose utilization rate of the strain by 36.9, 29.7, 42.8%, respectively, in the GX stage of anaerobic fermentation. Our study thus illustrates a promising strategy for the rational engineering of yeast for lignocellulosic ethanol production.

Keywords: Saccharomyces cerevisiae, xylose metabolism, regulation of carbon metabolism, Thi2p, anaerobic fermentation, the post-glucose effect

INTRODUCTION

The economic feasibility of producing biofuels and biochemicals via the industrial fermentation of lignocellulosic hydrolysates requires the full consumption of glucose and xylose, which are the most abundant sugars in this kind of material (Hou et al., 2017; Kwak et al., 2019; Li et al., 2019). Saccharomyces cerevisiae is a well-studied and robust cellular factory, but it cannot natively metabolize xylose. Engineering strategies have introduced the initial xylose metabolizing enzymes,
the xylose isomerase (XI), or xylose reductase and xylitol dehydrogenase. Strategies have also focused on altered transporters and modified expression of genes encoding xylulokinase and non-oxidative pentose phosphate pathway (PPP) in *S. cerevisiae*. However, such recombinant strains based on the strategies mentioned above only show limited xylose utilization capacity. Additionally, directed evolution with xylose as the sole carbon source in the growth medium has led to some substantial improvements (Hou et al., 2017; Kwak et al., 2019; Li et al., 2019). However, in many cases, the mechanistic details remain unclear, and this lack of understanding has hindered progress for realizing advanced strategies to rationally engineer further improvements (Myers et al., 2019).

Factors, including metabolic genes and transcription factors (TFs), that control xylose utilization in *S. cerevisiae* have been the focus of specific research in recent decades. For example, it has been confirmed that XI has significant effects on the capacity of *S. cerevisiae* to metabolize xylose. Efficient xylose utilization by an evolved strain has been partially attributed to elevated expression levels of XI, which was accomplished via multiple-copy chromosomal integration of the heterogenous *xylA* gene (Zhou et al., 2012). The enhancement of another evolved strain was attributed to the improvement of XI activity in *S. cerevisiae* by upregulating the expression molecular chaperones (Hou et al., 2016a). Additionally, improved xylose utilization capacity in some other evolved strains was attributed to the reprogramming of their carbon metabolism regulatory networks, such as the MAP Kinase (MAPK) signaling pathway and Protein Kinase A (PKA) signaling pathways, and several TFs in these signaling pathways, such as Hog1p and Tra2p, have showed their effects on xylose metabolism (Sato et al., 2016; Osiro et al., 2018; Myers et al., 2019). These findings confirmed that globally modifying the gene expression state by regulate the key transcription factors could be a way to optimize the xylose metabolism in yeast.

The carbon source conditions at the start of fermentation also significantly affect xylose metabolism. The evolved strain metabolize xylose well when xylose is the sole carbon source (referred to as the X stage). However, their specific xylose consumption rate is generally lower in the xylose consumption phase after glucose depletion in glucose-xylose co-fermentation (referred to as the GX stage), although there still remains more than half of the xylose when cells enter the GX stage (Michael et al., 2016; Wei et al., 2018). That is the yeast cells do not recognize xylose in the GX stage as they do in the X stage. It is industrially attractive to alleviate this post-glucose effect because it significantly decreases xylose utilization and prolongs fermentation times. To date, there are insufficient data regarding the mechanisms of control of the post-glucose effect and limited strategies to overcome it.

In our previous work, we revealed that deletion of the TF gene *THI2* improved the xylose consumption in the GX stage (Wei et al., 2018). *THI2* is a transcriptional activator of thiamine biosynthetic genes (Nosaka et al., 2005), but little information exists on how *THI2* affects carbon metabolism. Here, we demonstrated that deletion of *THI2* does not affect the activity of xylose isomerase, which catalyzes the first step of xylose metabolism and significantly affects the metabolic efficiency. We then compared the specific transcriptome differences during the GX stage between the thi2Δ strain and parental strain and examined the effects of *THI2* target genes on xylose metabolism. We thusly discovered a *THI2* regulatory network that improved xylose utilization in the GX stage. In addition, we revealed that deleting *THI2* or overexpressing its target genes *MID2*, *STT4*, and *CDC42* decreased the proportion of dead cells present in cultures. Finally, we showed that overexpressing *THI2* target genes *STT4* (Phosphatidylinositol-4-kinase), *RGI2* (respiratory growth induced, function unknown), and *TFC3* (subunit of RNA polymerase III transcription initiation factor complex) significantly enhanced xylose utilization in the GX stage of anaerobic fermentation, thereby illustrating a promising strategy for the rational engineering of yeast for lignocellulosic ethanol production. Moreover, our work illustrates the important point that yeast metabolic modeling, both in basic systems studies and in more applied efforts directed towards optimization and engineering, needs to account for the carbon-source-dependent regulatory functions of TFs like *THI2*.

**METHODS**

**Construction of Plasmids and Strains**

All plasmids and strains used in this study are listed in Table 1. The ORFs of all genes were amplified from the genomic DNA of the *S. cerevisiae* strain CEN.PK 113-5D (Entian and Kotter, 2007) using the primers listed in Additional file 1: **Supplementary Table S1**. The fragments of ORFs were digested by restriction enzymes and ligated into plasmid pUC20. The genes in the resultant recombinant plasmids were under the control of the *TEF1* promoter. The genes were overexpressed by transferring these recombinant plasmids into BSGX001.

Gene knockout was performed by homologous recombination using a *KanMX4* expression cassette, which was cloned from pUG6 (Guldener et al., 1996), to replace the target gene. The *KanMX4* marker was then discarded by transferring plasmid YEp-CH into the strains and inducing the expression of Cre recombinase (Li et al., 2016).

**Cultivation Conditions and Batch Fermentation**

*E. coli* recombinant cells were cultured at 37°C in Luria–Bertani (LB) medium (5 g L⁻¹ yeast extract, 10 g L⁻¹ tryptone, 10 g L⁻¹ NaCl, pH 7.0), and 100 mg L⁻¹ ampicillin was added for the selection of transformants. Yeast cells were cultured at 30°C in SC-Ura medium containing 1.7 g L⁻¹ yeast nitrogen base, 5 g L⁻¹ (NH₄)₂SO₄, 0.77 g L⁻¹ CSM-Ura (Sunrise Science Products, USA) and 20 g L⁻¹ glucose as the carbon source.

Fermentation was performed in shake flasks or 1 L bioreactors according to the experimental requirements. The fermentation medium was comprised of 1.7 g L⁻¹ yeast nitrogen base, 5 g L⁻¹...
TABLE 1 | S. cerevisiae strains and plasmids used in this study.

| S. cerevisiae strains and plasmids | Description | Sources |
|-----------------------------------|-------------|---------|
| PLASMIDS                          |             |         |
| pUG6                              | The plasmid with loxP-KanMX4-loxP cassette | Guldener et al., 1996 |
| YEp-CH                            | Shuttle plasmid for E. coli and S. cerevisiae, GAL2p-cre-CYC1t, HygR | Li et al., 2016 |
| pUC20                             | Yeast 2μ plasmid, KanMX4 | Wei et al., 2018 |
| pUC20-BOH2<sup>a</sup>            | pUC20, TEF1p-BOH2-ADHt | This study |
| S. cerevisiae STRAINS             |             |         |
| CEN.PK 113-5D                     | MATa; ura3-53 | Entian and Kotter, 2007 |
| BSGX001                           | CEN.PK 113-5D derivative; Ru-XI, XK, gaa3::PPP, cox4<sup>a</sup>, AE<sup>b</sup> | Hou et al., 2016b |
| BSGX001(Δ<sup>δ</sup>)             | BSGX001 derivative, Δ<sup>δ</sup>-TEF1p-BOH2-ADHt | This study |
| BSGX001(Δ<sup>δ</sup>)/BDH2<sup>c</sup> | BSGX001 derivative, Δ<sup>δ</sup>-TEF1p-BOH2-ADHt | This study |

<sup>a</sup>Other plasmids derived from pUC20 were named in the same way, and due to space limitations, they were not listed here.
<sup>b</sup>AE, adaptive evolution in medium using xylose as the sole carbon source.
<sup>c</sup>Other strains derived from BSGX001 with deleted genes were named in the same way, and due to space limitations, they were not listed here.
<sup>d</sup>Other strains derived from BSGX001 with overexpressed genes were named in the same way, and due to space limitations, they were not listed here.

(NH₄)₂SO₄, 20 g L⁻¹ glucose, and 20 g L⁻¹ xylose. Overnight cultures of a single colony were transferred into a 250 mL shake flask containing 50–60 mL fresh SC-Ura medium supplied with 20 g L⁻¹ glucose and an initial biomass of 0.23 g L⁻¹ dry cell weight (DCW) (OD₆₀₀ of 1) and cultured at 30 °C and 200 rpm for another 12–16 h. The cells were then collected and washed three times with sterile water and inoculated into the fermentation medium. Fermentation in shake flasks was performed at 30°C and 200 rpm. The initial biomass was 0.575 g L⁻¹ DCW. The anaerobic fermentation in bioreactors was performed with an initial biomass of 0.23 g L⁻¹ DCW at 30°C and pH 5.5, with 0.1vvm nitrogen and a stirring speed of 200 rpm for ~30 h. The pH was maintained by automatically pumping 5 mol L⁻¹ NaOH and 5 mol L⁻¹ H₃PO₄. All fermentations were carried out in triplicate.

Quantitative PCR (qPCR) Analysis
qPCR data were analyzed according to the 2⁻ΔΔCt method (Livak and Schmittgen, 2001). RNA was extracted from the cells collected from the 20 h glucose-xylose co-fermentation flasks using a UNIQ-10 Column Trizol Total RNA Isolation Kit (Sangon Biotech Co., Ltd., Shanghai, China). The cDNA was obtained using a PrimeScript<sup>TM</sup> RT reagent Kit (TaKaRa, Japan). The gene transcription levels were determined using the equation N = 2<sup>Ct(reference gene) - Ct(target gene)</sup>. ACT1 was used as the reference gene, and the t-test was applied to evaluate the differences between means.

Xylose Isomerase Activity Assay
The crude enzyme samples were prepared as previously described (Hou et al., 2016a). Yeast cells were collected at 20 h in glucose-xylose co-fermentation, then were broken by glass beads (Φ = 0.5 mm) using a FastPrep cell homogenizer (Thermo Savant, USA). The total cellular protein concentration was measured using a BCA protein assay reagent kit (Sangon Biotech Co., Ltd., Shanghai, China).

The XI activities were determined at 30°C by measuring the decrease in NADH concentration using a previously reported method (Hou et al., 2016a). Briefly, assays were performed in reaction mixtures containing 0.15 mmol L⁻¹ NADH, 10 mmol L⁻¹ MgCl₂, and 1 U of sorbitol dehydrogenase (Sigma-Aldrich, USA) in 100 mmol L⁻¹ Tris-HCl (pH 7.5) with appropriately diluted crude cell extracts. The reaction was initiated by adding 500 mmol L⁻¹ xylose. One unit of XI activity was defined as the amount of crude enzyme required to produce 1 mmol xylulose per min under the assay conditions.

Transcriptome Analysis
Samples from the batch fermentation shake flasks were taken at 20 h and subjected to transcriptome analysis. The cells in each sample were collected by centrifugation at 5,000 rpm and 4°C for 5 min and then frozen in liquid nitrogen. Total RNA was extracted using a UNIQ-10 Trizol RNA Purification Kit (Sangon Biotech, China) and then fragmented. DNA was digested with DNase I, and cDNA was synthesized by using short mRNA fragments as templates. Three independent RNA extractions were assayed for each strain. The resulting sample library was sequenced using an Illumina HiSeqTM 2000 (BGI Shenzhen, China).

Raw data from transcriptional analysis and processed data for genes exhibiting significant differences between BSGX001 (thi2Δ) and BSGX001 are available in the NCBI Gene Expression Omnibus database (GEO Accession Number: GSE119333). Significant differences were indicated by p-values of 0.001 or less, and an absolute fold-change threshold of 2.0 or greater. All annotations were derived from the Saccharomyces Genome Database (SGD) (http://www.yeastgenome.org/). Cluster analysis was performed using the Gene Ontology Slim Mapper tool supplied by the SGD (http://www.yeastgenome.org/).

Analysis of Metabolites and Calculation
The concentrations of glucose, xylose, glycerol, acetate, and ethanol were measured using HPLC (Shimadzu, Japan) with an
Aminex HPX-87H ion exchange column (300 × 7.8 mm, Bio-Rad, Hercules, USA). The mobile phase was 5 mmol L⁻¹ H₂SO₄ with a flow rate of 0.6 ml/min, and the temperature of the column oven was 45°C. The specific xylose utilization rate ($r_{xylose}$) was calculated using the following equation, as previously described (Wei et al., 2018):

$$r = \frac{A_n - A_m}{\frac{1}{2} \sum_{i=m+1}^{n} (B_i + B_{i-1}) \times (t_i - t_{i-1})}$$

where $r$ is the specific utilization during the phase from sampling point $m$ to sampling point $n$; and $A$, $B$, and $t$ are the metabolite concentration, biomass concentration, and time, respectively, at sampling points $n$, $i$, and $m$.

Measurement of the Proportion of Dead Cells in Culture

Yeast cells were harvested at 20, 36, and 48 h, and diluted to a suitable multiple (≈ 6 × 10⁷ cells mL⁻¹). The cells were then incubated with 0.04% trypan blue for 3–10 min; dead cells were stained by trypan, while live cells were not (Bowie-Dellinger et al., 2017). The stained cells were counted manually using a hemocytometer. Samples were subjected to independent triplicate tests, each with more than 500 cells counted. For statistical analysis, the unpaired, two-tailed $t$-test was performed. Data with $p \leq 0.05$ were considered significantly different.

RESULTS

Increased Xylose Utilization of the THI2 Deletion Strain Is Not Related to Xylose Isomerase Activity

Xylose isomerase activity in recombinant yeast seriously affects the capacity of S. cerevisiae to utilize xylose (van Maris et al., 2007; Zhou et al., 2012; Hou et al., 2016a). The transcription level of $xylA$ and XI activity of the $\Delta$ strain and its control BSGX001 (Hou et al., 2016b) were detected to determine whether deletion of THI2 enhanced xylose utilization through increasing the $xylA$ gene expression or directly improving the XI activity. The results showed that the transcriptional level of $xylA$ in THI2 deletion strain is 54.2% of that in parent strain and the $p$-value is 0.147, which change is not significant. The xylose isomerase in THI2 deletion strain is 83.6% of parent strain ($p$-value is 0.048). These results suggested that neither the transcription level of $xylA$ nor XI activity increased in the $\Delta$ strain compared to BSGX001 in the GX stage (Figure 1), that is deletion of THI2 did not increase XI activity of strain.

The Transcriptional Profile of the THI2 Deletion Strain Suggested Engineering Strategies for Enhanced Xylose Utilization in S. cerevisiae

Transcriptional Profile of the THI2 Deletion Strain

To investigate how THI2 deletion improved the xylose utilization in the GX stage, we compared the transcriptome of THI2 deletion strain BSGX001($\Delta$) and its parent strain BSGX001 in the GX stage. The samples of both strains were taken at 2 h after glucose depletion (20 h) in glucose-xylose co-fermentation. At this time point, ~17–18 g L⁻¹ xylose remained in the medium. The transcriptome analysis results (Table 2) revealed that 93 and 16 genes were significantly up- and downregulated, respectively, in the THI2 deletion strain during the GX stage. The Gene Ontology (GO) cluster result showed that within the Molecular Functions category, the upregulated genes were primarily clustered (cluster frequency ≥ 10%) under the GO terms hydrolase activity and DNA binding; the downregulated genes were primarily clustered under the GO terms DNA binding, transferase activity and ligase activity; within the Biological Processes category, upregulated genes did not cluster to specific GO term, while the downregulated genes clustered to GO terms RNA polymerase II promoter, cellular response to DNA damage stimulus, vitamin metabolic process, and DNA repair.

The Effect of Differentially Expressed Genes (DEGs) on Xylose Utilization

According to the significant DEGs and previously reported factors that related to the xylose metabolism of S. cerevisiae (Salusjarvi et al., 2008; Cheng et al., 2018; Wei et al., 2018), 32 upregulated genes involved in ribosomal biosynthesis, signal transducer, generation of precursor metabolites and

![Image](https://www.frontiersin.org)
### TABLE 2 | Gene cluster analysis of transcriptome difference of BSGX001 (thi2Δ) vs. BSGX001 at the GX stage in the aspect of Molecular Function and Biological Processes.

| GOID   | GO terms (Molecular Function)                               | Frequency | Gene(s)                                                                 |
|--------|-------------------------------------------------------------|-----------|-------------------------------------------------------------------------|
|        | **UP-REGULATED**                                           |           |                                                                         |
| 3674   | Molecular function unknown                                  | 34/93, 36.6% | ATS1, MAK16, BOL1, BOL3, AIM2, ECM1, ERP1, PAU7, YAR023C, UIP3, MST28, YAR064W, YAR066W, YAR068W, RRT6, SPG1, RQ2, FAR10, ARV1, SYM1, YLR255C, TMA7, YLR264C-A, BOP2, CMG1, SMD2, YLR281C, YLR283W, YLR287C, COQ11, SPH1, NKP2, PEK30, YLR326W |
| 16787  | Hydrolase activity                                          | 14/93, 15.1% | CCR4, POP5, PHO11, YIL082W-A, CDC42, GPN3, CDD1, IRC20, MCM5, DBP9, CTS1, TAD3, SFH1, YRF1-6 |
| 3677   | DNA binding                                                 | 11/93, 11.8% | TFC3, SAW1, ECM22, EST1, RED1, NEJ1, PDR8, MCM5, YLR278C, MEC3, EST2 |
| 16740  | Transferase activity                                        | 9/93, 9.7%  | SMD1, YAT1, YIL082W-A, ERF2, IRC20, STT4, UBC12, EST2, GAS2             |
| 30234  | Enzyme regulator activity                                  | 8/93, 8.6%  | GIP4, CLN3, PEX22, GB2, BUD14, RFU1, PIG1, GCD7                        |
| 5198   | Structural molecule activity                                | 7/93, 7.5%  | NUP60, RED1, RPS28B, MRPL15, RPL38, RPS25B, RPP0                        |
| 3723   | RNA binding                                                 | 7/93, 7.5%  | POP5, YIL082W-A, EST1, DBP9, GCD7, YHC1, RPP0                         |
| 3735   | Structural constituent of ribosome                          | 5/93, 5.4%  | RPS28B, MRPL15, RPL38, RPS25B, RPP0                                    |
| 4386   | Helicase activity                                           | 4/93, 4.3%  | IRC20, MCM5, DBP9, YRF1-6                                              |
| 16887  | ATPase activity                                             | 3/93, 3.2%  | MCM5, DBP9, SFH1                                                        |
| 4518   | Nuclease activity                                           | 3/93, 3.2%  | CCR4, POP5, YIL082W-A                                                  |
| 1071   | Nucleic acid binding transcription factor activity           | 3/93, 3.2%  | TFC3, ECM22, PDR8                                                       |
| 3682   | Chromatin binding                                          | 3/93, 3.2%  | RED1, YCS4, MCM5                                                        |
| 5085   | Guanyl-nucleotide exchange factor activity                  | 3/93, 3.2%  | EFB1, LTE1, GCD7                                                       |
| 19899  | Enzyme binding                                              | 2/93, 2.2%  | VPS8, GIP4                                                              |
| 3729   | mRNA binding                                                | 2/93, 2.2%  | DBP9, YHC1                                                              |
| 16779  | Nucleotidytransferase activity                             | 2/93, 2.2%  | YIL082W-A, EST2                                                         |
| 4871   | Signal transducer activity                                  | 2/93, 2.2%  | GPB2, MID2                                                              |
| 43167  | Ion binding                                                | 2/93, 2.2%  | RFB1, NUP60                                                             |
| 3924   | GTPase activity                                             | 2/93, 2.2%  | CDC42, GPN3                                                             |
| 22857  | Transmembrane transporter activity                          | 2/93, 2.2%  | THI7, CSC1                                                              |
| 8135   | Translation factor activity, RNA binding                    | 1/93, 1.1%  | GCD7                                                                    |
| 16491  | Oxidoreductase activity                                    | 1/93, 1.1%  | BOD2                                                                    |
| 16798  | Hydrolyase activity, acting on glycosyl bonds               | 1/93, 1.1%  | CTS1                                                                    |
| 8233   | Peptidase activity                                          | 1/93, 1.1%  | YIL082W-A                                                               |
| 16853  | Isomerase activity                                          | 1/93, 1.1%  | ECII                                                                    |
| 16301  | Knase activity                                              | 1/93, 1.1%  | STT4                                                                    |
| 988    | Transcription factor activity, protein binding              | 1/93, 1.1%  | TFC3                                                                    |
| 30674  | Protein binding, bridging                                  | 1/93, 1.1%  | ATG39                                                                   |
| 19843  | rRNA binding                                               | 1/93, 1.1%  | RPP0                                                                    |
| 8166   | Methyltransferase activity                                 | 1/93, 1.1%  | SWD1                                                                    |
| 8289   | Lipid binding                                              | 1/93, 1.1%  | NUP60                                                                   |
| 16829  | Lyase activity                                             | 1/93, 1.1%  | CYC3                                                                    |
| 16874  | Ligase activity                                            | 1/93, 1.1%  | LIP2                                                                    |
| 51082  | Unfolded protein binding                                   | 1/93, 1.1%  | CNE1                                                                    |
| 16791  | Phosphatase activity                                       | 1/93, 1.1%  | PHO11                                                                   |
|        | **DOWN-REGULATED**                                         |           |                                                                         |
| 3674   | Molecular function unknown                                  | 6/16, 37.5% | BSC1, PRM7, YDR246W-A, RRT5, YGL015C, YOR338W                           |
| 3677   | DNA binding                                                 | 3/16, 18.8% | THl2, MQA1, IXR1                                                        |
| 16740  | Transferase activity                                       | 3/16, 18.8% | HOM3, TRA1, URA2                                                        |

*(Continued)*
TABLE 2 | Continued

| GOID   | GO terms (Molecular Function)                                      | Frequency | Gene(s)                  |
|--------|------------------------------------------------------------------|-----------|--------------------------|
| 16874  | Ligase activity                                                  | 2 out of 16 genes, 12.5% | SNZ3, URA2               |
| 30234  | Enzyme regulator activity                                        | 1 out of 16 genes, 6.3%  | CIP1                     |
| 1071   | Nucleic acid binding transcription factor activity                | 1 out of 16 genes, 6.3%  | MGA1                     |
| 16829  | Lyase activity                                                   | 1 out of 16 genes, 6.3%  | DAL3                     |
| 16301  | Kinase activity                                                  | 1 out of 16 genes, 6.3%  | HOM3                     |
| other  | Other                                                            | 1 out of 16 genes, 6.3%  | BTN2                     |

| GOID   | GO terms (Biological Processes)                                  | Frequency | Gene(s)                  |
|--------|------------------------------------------------------------------|-----------|--------------------------|
| 8150   | Biological process unknown                                       | 19 out of 93 genes, 20.4% | AIM2, BDH2, PAU7, YAR023C, UBP3, YAR064W, YAR066W, YAR068W, RRT6, SPO1, YLR255C, YLR264C-A, BOP2, CME1, YLR278C, YLR281C, YLR283W, YLR287C, YLR326W |
| 51726  | Regulation of cell cycle                                         | 9 out of 93 genes, 9.7%  | CCR4, LTE1, CLN3, BUD14, CDC42, RED1, YCS4, MEC3, SFH1 |
| 33043  | Regulation of organelle organization                             | 8 out of 93 genes, 8.6%  | EF1, LTE1, BUD14, CDC42, EST1, RED1, YCS4, MCM5 |
| 6974   | Cellular response to DNA damage stimulus                         | 7 out of 93 genes, 7.5%  | SAW1, NUP60, IRC20, NEJ1, MCM5, MEC3, SFH1 |
| 7059   | Chromosome segregation                                           | 7 out of 93 genes, 7.5%  | LTE1, GIP4, GPN3, RED1, YCS4, NK2P2, SFH1 |
| 278    | Mitotic cell cycle                                               | 7 out of 93 genes, 7.5%  | CCR4, LTE1, CLN3, CDC42, GPN3, YCS4, SFH1 |
| 42221  | Response to chemical                                             | 6 out of 93 genes, 6.5%  | GPB2, CNE1, CDC42, FAR10, SFH1, MID2 |
| 6281   | DNA repair                                                       | 6 out of 93 genes, 6.5%  | SAW1, NUP60, IRC20, NEJ1, MCM5, SFH1 |
| 6325   | Chromatin organization                                           | 6 out of 93 genes, 6.5%  | NUP60, SWD1, YCS4, MCM5, MEC3, SFH1 |
| 2181   | Cytoplasmic translation                                          | 6 out of 93 genes, 6.5%  | RBC1, TMA7, RPS28B, RPL38, RPS25B, RPP0 |
| 6605   | Protein targeting                                                | 5 out of 93 genes, 5.4%  | VPS8, PEX22, NUP60, GPN3, EF2 |
| 51052  | Regulation of DNA metabolic process                              | 5 out of 93 genes, 5.4%  | CCR4, SAW1, EST1, MCM5, SFH1 |
| 6366   | Transcription from RNA polymerase II promoter                    | 5 out of 93 genes, 5.4%  | CCR4, GPB2, ECM22, PDR8, SFH1 |
| 48285  | Organelle fission                                                | 5 out of 93 genes, 5.4%  | LTE1, CDC42, GPN3, RED1, YCS4 |
| 23052  | Signaling                                                       | 5 out of 93 genes, 5.4%  | PEX22, GPB2, CDC42, FAR10, MID2 |
| 32200  | Telomere organization                                           | 5 out of 93 genes, 5.4%  | SWD1, EST1, MEC3, EST2, YRF1-6 |
| 7010   | Cytoskeleton organization                                        | 4 out of 93 genes, 4.3%  | EF1, ATS1, BUD14, CDC42 |
| 746    | Conjugation                                                     | 4 out of 93 genes, 4.3%  | CDC42, FAR10, SFH1, MID2 |
| 31399  | Regulation of protein modification process                       | 4 out of 93 genes, 4.3%  | GPB4, CLN3, PEX22, NUP60 |
| 6629   | Lipid metabolic process                                          | 4 out of 93 genes, 4.3%  | ECM22, ARV1, EQ1, STT4 |
| 902    | Cell morphogenesis                                               | 4 out of 93 genes, 4.3%  | BUD14, CDC42, SPH1, MID2 |
| 6310   | DNA recombination                                               | 4 out of 93 genes, 4.3%  | IRC20, MCM5, MEC3, YRF1-6 |
| 51169  | Nuclear transport                                                | 4 out of 93 genes, 4.3%  | ECM1, NUP60, GPN3, RPS28B |
| 51321  | Meiotic cell cycle                                               | 4 out of 93 genes, 4.3%  | GPB2, RED1, YCS4, GAS2 |
| 6397   | mRNA processing                                                  | 3 out of 93 genes, 3.2%  | SMD2, YHC1, MID2 |
| 8380   | RNA splicing                                                     | 3 out of 93 genes, 3.2%  | SMD2, YHC1, MID2 |
| 6401   | RNA catabolic process                                            | 3 out of 93 genes, 3.2%  | CCR4, POP5, RPS28B |
| 6260   | DNA replication                                                  | 3 out of 93 genes, 3.2%  | CCR4, MCM5, SFH1 |
| 7114   | Cell budding                                                     | 3 out of 93 genes, 3.2%  | ATS1, CDC42, SPH1 |
| 8033   | tRNA processing                                                  | 3 out of 93 genes, 3.2%  | ATS1, POP5, TAD3 |
| 7124   | Pseudohypophilic growth                                          | 3 out of 93 genes, 3.2%  | GPB2, CDC42, SPH1 |
| 51049  | Regulation of transport                                          | 3 out of 93 genes, 3.2%  | BUD14, ECM22, CDC42 |
| 6364   | rRNA processing                                                  | 3 out of 93 genes, 3.2%  | MAK16, POP5, DBP9 |
| 70647  | Protein modification by small protein conjugation or removal      | 3 out of 93 genes, 3.2%  | PEX22, NUP60, UBC12 |
| 42273  | Ribosomal large subunit biogenesis                               | 3 out of 93 genes, 3.2%  | MAK16, DBP9, RPP0 |

(Continued)
| GOID   | GO terms (Biological Processes)                                                                 | Frequency               | Gene(s)                      |
|--------|-------------------------------------------------------------------------------------------------|-------------------------|------------------------------|
| 9451   | RNA modification                                                                                 | 2 out of 93 genes, 2.2% | ATS1, TAD3                   |
| 6497   | Protein lipidation                                                                               | 2 out of 93 genes, 2.2% | ARV1, ERF2                   |
| 6869   | Lipid transport                                                                                 | 2 out of 93 genes, 2.2% | ECM22, ARV1                  |
| 55085  | Transmembrane transport                                                                          | 2 out of 93 genes, 2.2% | PEX22, THI7                  |
| 70271  | Protein complex biogenesis                                                                        | 2 out of 93 genes, 2.2% | CYC3, BUD14                  |
| 18193  | Peptidyl-amino acid modification                                                                | 2 out of 93 genes, 2.2% | NUP60, SWD1                  |
| 42594  | Response to starvation                                                                           | 2 out of 93 genes, 2.2% | RBG1, ECM22                  |
| 71554  | Cell wall organization or biogenesis                                                             | 2 out of 93 genes, 2.2% | MID2, GAS2                   |
| 910    | Cytokinesis                                                                                     | 2 out of 93 genes, 2.2% | BUD14, SPH1                  |
| 7033   | Vacuole organization                                                                             | 2 out of 93 genes, 2.2% | CLN3, CDC42                  |
| 6091   | Generation of precursor metabolites and energy                                                   | 2 out of 93 genes, 2.2% | RGI2, PIG1                   |
| 43934  | Sporulation                                                                                     | 2 out of 93 genes, 2.2% | GPB2, GAS2                   |
| 15931  | Nucleobase-containing compound transport                                                         | 2 out of 93 genes, 2.2% | NUP60, RPS28B                 |
| 51186  | Cofactor metabolic process                                                                        | 2 out of 93 genes, 2.2% | BOL1, COQ11                  |
| 6354   | DNA-templated transcription, elongation                                                          | 2 out of 93 genes, 2.2% | CCR4, SFH1                   |
| 51604  | Protein maturation                                                                              | 2 out of 93 genes, 2.2% | BOL1, BOL3                   |
| 1403   | Invasive growth in response to glucose limitation                                                | 2 out of 93 genes, 2.2% | GPB2, CDC42                  |
| 7031   | Peroxisome organization                                                                          | 2 out of 93 genes, 2.2% | PEX22, PEX30                  |
| 6417   | Regulation of translation                                                                        | 2 out of 93 genes, 2.2% | EFB1, GCD7                   |
| 48284  | Organelle fusion                                                                                 | 2 out of 93 genes, 2.2% | CLN3, CDC42                  |
| 61025  | Membrane fusion                                                                                 | 2 out of 93 genes, 2.2% | CLN3, CDC42                  |
| 5975   | Carbohydrate metabolic process                                                                   | 1 out of 93 genes, 1.1% | PIG1                         |
| 6413   | Translational initiation                                                                        | 1 out of 93 genes, 1.1% | GCD7                         |
| 16570  | Histone modification                                                                            | 1 out of 93 genes, 1.1% | SWD1                         |
| 6468   | Protein phosphorylation                                                                          | 1 out of 93 genes, 1.1% | CLN3                         |
| 16197  | Endosomal transport                                                                             | 1 out of 93 genes, 1.1% | VPS8                         |
| 70925  | Organelle assembly                                                                               | 1 out of 93 genes, 1.1% | RPP0                         |
| 32196  | Transposition                                                                                   | 1 out of 93 genes, 1.1% | YL082W-A                     |
| 6887   | Exocytosis                                                                                      | 1 out of 93 genes, 1.1% | CDC42                        |
| 6970   | Response to osmotic stress                                                                       | 1 out of 93 genes, 1.1% | MID2                         |
| 43144  | snoRNA processing                                                                               | 1 out of 93 genes, 1.1% | POP5                         |
| 54     | Ribosomal subunit export from nucleus                                                            | 1 out of 93 genes, 1.1% | ECM1                         |
| 8213   | Protein alkylation                                                                              | 1 out of 93 genes, 1.1% | SWD1                         |
| 6457   | Protein folding                                                                                 | 1 out of 93 genes, 1.1% | CNE1                         |
| 6383   | Transcription from RNA polymerase III promoter                                                   | 1 out of 93 genes, 1.1% | TFC3                         |
| 42255  | Ribosome assembly                                                                                | 1 out of 93 genes, 1.1% | RPP0                         |
| 6414   | Translational elongation                                                                        | 1 out of 93 genes, 1.1% | EFB1                         |
| 7005   | Mitochondrion organization                                                                       | 1 out of 93 genes, 1.1% | STT4                         |
| 32543  | Mitochondrial translation                                                                       | 1 out of 93 genes, 1.1% | MRPL15                       |
| 32787  | Monocarboxylic acid metabolic process                                                           | 1 out of 93 genes, 1.1% | EC1I                         |
| 16050  | Vesicle organization                                                                            | 1 out of 93 genes, 1.1% | MST28                        |
| 51603  | Proteolysis involved in cellular protein catabolic process                                       | 1 out of 93 genes, 1.1% | CNE1                         |
| 6811   | Ion transport                                                                                   | 1 out of 93 genes, 1.1% | CSC1                         |
| 6470   | Protein dephosphorylation                                                                       | 1 out of 93 genes, 1.1% | GIP4                         |
energy, starvation response, ATPase or GTPase activity, oxidoreductase activity, cofactor metabolic process, lipid metabolic process, transmembrane transporter activity, and protein modification, and 12 downregulated genes, were chosen for follow-up investigations.

The up- and down-regulated genes were overexpressed and deleted, respectively, in strain BSGX001. The effect was then evaluated by determining the xylose-specific consumption rate of recombinant strains in shake flask fermentations at the GX stage. The results showed that all mutants had no significant effect on glucose metabolism. Overexpressing the cell wall integrity (CWI)-related genes MIDs2, STT4, and CDC42 increased the r\textsubscript{xylose} of the strain by 45.9, 49.2, and 13.1%, respectively. Overexpressing ECM22, CSCI, and BDH2 increased the r\textsubscript{xylose} of the strain by 11.5, 13.1, and 26.2%, respectively. Overexpressing GPN3 (encoding a putative GTPase) and TFC3 (encoding a subunit of the RNA polymerase III transcription initiation factor complex) increased the r\textsubscript{xylose} of the strain by 13.1 and 42.6%, respectively. Furthermore, overexpressing the function unknown genes BOP2 and RGI2 increased the r\textsubscript{xylose} of the strain by 11.5 and 41.0%, respectively. Deleting CIP1, IXR1, YDR246W-A, and YGLO15C increased the r\textsubscript{xylose} of the strain by 26.2, 36.1, 16.4, and 14.8%, respectively (Table 3). These results suggested that deleting THI2 enhanced xylose utilization through regulating these genes (Figure 2).

**Overexpressing Cell Wall Integrity Related Genes MIDs2, STT4, and CDC42 Decreased the Proportion of Dead Cells in the Culture**

Among the genes that positively enhanced xylose utilization, the genes MIDs2, STT4, and CDC42 belong to the CWI pathway,
### TABLE 3 | Genes regulated by Thi2p in GX stage and their effects on xylose utilization.

| Category | Strains | Gene annotation | \( \log_2(\text{fold changes})^b \) | \( r_b^{\text{xylose}} \) (g g\(^{-1}\) DCW h\(^{-1}\)) |
|----------|---------|-----------------|-------------------------------|------------------|
| **OVEREXPRESSION THE UP-REGULATED GENES** | | | | 0.061 ± 0.001 |
| RP-related genes | RPS25B | Protein component of the small (40S) ribosomal subunit | 1.379 | 0.053 ± 0.001* |
| | MRPL15 | Mitochondrial ribosomal protein of the large subunit | 1.157 | 0.035 ± 0.003* |
| | RPL38 | Ribosomal 60S subunit protein L38 | 1.048 | 0.022 ± 0.001* |
| | MAK16 | Constituent of 66S pre-ribosomal particles | 1.802 | 0.052 ± 0.001* |
| | RPP2 | Conserved ribosomal protein P0 of the ribosomal stalk | 1.345 | 0.054 ± 0.001* |
| | RPS28B | Protein component of the small (40S) ribosomal subunit | 1.113 | 0.060 ± 0.000* |
| | DBP9 | A putative ATP-dependent RNA helicase involved in 60S-ribosomal-subunit biogenesis | 1.380 | 0.060 ± 0.000* |
| | POP5 | Subunit of both RNase MRP and nuclear RNase P | 1.616 | 0.050 ± 0.001* |
| | RBB1 | Translating ribosomes | 1.275 | 0.060 ± 0.001* |
| Signal transducer genes | GPB2 | Multistep regulator of cAMP-PKA signaling | 1.146 | 0.039 ± 0.003* |
| | MID2 | Acts as a sensor for cell wall integrity signaling | 1.053 | 0.089 ± 0.003* |
| | CDC42 | Establishment and maintenance of cell polarity | 1.241 | 0.069 ± 0.002* |
| | PEX22 | Required for import of peroxisomal proteins | 1.422 | 0.049 ± 0.001* |
| | FAR10 | Protein involved in recovery from arrest in response to pheromone | 1.061 | 0.060 ± 0.001* |
| Generation of precursor | PIG1 | Glycogen synthesis | 1.412 | 0.045 ± 0.001* |
| Metabolites and energy | RGI2 | Involved in energy metabolism under respiratory conditions | 1.136 | 0.086 ± 0.001* |
| Response to starvation | ECM22 | Sterol regulatory element binding protein | 1.268 | 0.068 ± 0.002* |
| ATPase activity | MCM5 | An active ATP-dependent helicase | 1.334 | 0.022 ± 0.000* |
| | SFH1 | Component of the RSC chromatin remodeling complex | 1.075 | 0.000 ± 0.000* |
| GTPase activity | GPNP3 | Biogenesis of RNA pol II and polIII | 1.252 | 0.069 ± 0.001* |
| Oxidoreductase activity | BDH2 | Putative medium-chain alcohol dehydrogenase | 1.241 | 0.077 ± 0.001* |
| Transcription factor activity, protein binding | TFC3 | Subunit of RNA polymerase III Transcription initiation factor complex | 1.014 | 0.087 ± 0.000* |
| Cofactor metabolic process | COQ11 | Putative oxidoreductase, subunit of Coenzyme Q biosynthetic complexes | 1.069 | 0.064 ± 0.000 |
| | BOL1 | Mitochondrial matrix protein involved in Fe-S cluster biogenesis | 1.014 | 0.060 ± 0.002 |
| Lipid metabolic process | STT4 | Phosphatidylglycerol-4-kinase | 1.282 | 0.091 ± 0.001* |
| | ARV1 | Involved in intracellular sterol and sphingolipid transport | 1.374 | 0.055 ± 0.003* |
| | ECI1 | Essential for the beta-oxidation of unsaturated fatty acids | 1.306 | 0.057 ± 0.000* |
| Transmembrane | CSC1 | May be involved in detoxification | 1.071 | 0.069 ± 0.002* |
| Transporter activity | TH7 | Responsible for the uptake of thiamine | 1.233 | 0.020 ± 0.001* |
| Phosphatase activity | PHO11 | One of three repressible acid phosphatases | 1.492 | 0.034 ± 0.002* |
| Protein modification | UBC12 | Related to E2 ubiquitin-conjugating enzymes | 1.696 | 0.046 ± 0.002* |
| Function unknown | BOP2 | Protein of unknown function | 1.882 | 0.068 ± 0.001* |

| **DELETION THE DOWN-REGULATED GENES** | | | | |
| Cellular amino acid metabolic or transport | hom3Δ | Cytoplasmic enzyme that catalyzes the first step in the common pathway for methionine and threonine biosynthesis | −1.071 | 0.000 ± 0.000* |
| Amino acid transport | bbn2Δ | Modulates arginine uptake | −1.038 | 0.030 ± 0.001* |
| Cell cycle related genes | cip1Δ | Cyclin-dependent kinase inhibitor | −1.035 | 0.077 ± 0.002* |
| | yor338wΔ | Putative protein of unknown function | −1.455 | 0.039 ± 0.002* |
| Response to chemical | lkr1Δ | Transcriptional repressor that regulates hypoxic genes during normoxia | −1.044 | 0.083 ± 0.003* |
| DNA binding | mga1Δ | Protein similar to heat shock transcription factor | −1.055 | 0.039 ± 0.002* |
| | dal3Δ | Ureidoglycolate lyase | −1.108 | 0.037 ± 0.001* |
| Molecular function | pmr7Δ | Pheromone-regulated protein | −1.294 | 0.048 ± 0.001* |
| Unknown | ydr246w-AΔ | Unknown function | −1.070 | 0.071 ± 0.001* |
| | rmsΔ | Unknown function | −1.004 | 0.051 ± 0.002* |
| | ygl015cΔ | Null mutants accumulate cargo in the Golgi | −1.121 | 0.070 ± 0.005* |
| | Bsc1 | Null mutant has increased glycogen accumulation | −1.397 | 0.056 ± 0.004* |

Cells were cultured at 30°C in a shake flask and agitated at 200 rpm. All the data are the mean value ± standard deviation of independent triplicate tests. The bold gene names and values refers to the operations brought the positive effect in xylose utilization in the GX stage. *p<0.05.

The TH2 deletion strain compared to the parent strain BSGX001, up-regulate represents genes with higher expression in TH2 deletion strain compared to the parent strain BSGX001, down-regulate represents the reverse operation.

The specific consumption/production rates of xylose/ethanol \((r_{\text{xylose}}/r_{\text{ethanol}})\) were calculated from the data on the xylose consumption phase in the GX stage.
FIGURE 2 | Regulatory Network of Thi2p promoting xylose utilization in the GX stage. Green and red lines represent activation function and inhibition function, respectively; black lines represent unknown function. Genes with purple background frame encode transcription factors.

which may protect cells from environmental conditions that otherwise induced death (Mishra et al., 2017). We determined the proportion of dead cells in the culture of strains overexpressing these genes, as well as THI2 deletion strain, and their parent strain BSGX001. Samples were taken at 20, 36, and 48 h, respectively.

The results (Figure 3) provided information regarding three aspects of xylose utilization. First, in the phase that xylose was rapidly consumed (BSGX001, xylose fermentation, 20 h), the proportion of dead cells in the culture was low (<10%). After xylose was depleted (BSGX001, xylose fermentation, 36 and 48 h), the proportion of dead cells increased with time. The changes in nutritional condition apparently induced cell death. Second, the proportion of dead cells in the GX stage (BSGX001, glucose-xylose co-fermentation, 20, 36, 48 h) was much higher than after xylose was depleted (BSGX001, xylose fermentation, 36 and 48 h), which suggested that cell death was more significantly induced by glucose depletion than by xylose depletion. Third, overexpressing MID2, STT4, and CDC42 or deleting THI2 decreased the proportion of dead cells in the cultures. The decrease at all timepoints was significant (p-value < 0.05). Overexpression of MID2, STT4, and CDC42 or deleting THI2 enhanced xylose metabolism in the GX stage, in part, because overexpression of these genes promoted cell survival and continued metabolism.

Overexpressing STT4, RGI2, or TFC3 Enhanced Xylose Utilization in the GX Stage Under Anaerobic Conditions

The level of available oxygen has an impact on the xylose fermentation characteristics (Salusjarvi et al., 2008; Souto-Maior et al., 2009). The xylose utilization of strains overexpressing STT4, RGI2, TFC3, and MID2, or deleting IXR1, which showed the highest positive effect on xylose utilization in shake flask fermentations, were further evaluated in bioreactors under anaerobic conditions. Their glucose-xylose co-fermentation characteristics are shown in Figure 4 and Table 4. Overexpressing STT4 and RGI2 increased the specific consumption rate of xylose ($r_{xylose}$) of the strain by 36.9 and 29.7% in the GX stage, respectively. Although the specific production rate of ethanol ($r_{ethanol}$) and the ethanol yields ($Y_{ethanol}$) did not increase, the fermentation time was shortened (Figures 4A,B). Overexpressing TFC3 increased the $r_{xylose}$ and $r_{ethanol}$ by 42.8 and 32.5%, respectively, and this also shortened the fermentation time (Figure 4C). However, overexpression of MID2 or deletion of IXR1 did not yield positive effects on xylose utilization under anaerobic conditions, which indicated that oxygen levels played an important role in the strain utilization of xylose.

DISCUSSION

Despite the large amount of xylose present in many feedstocks that are commonly used in fermentation cultures in bio-industrial manufacturing, our basic understanding of xylose utilization by S. cerevisiae is limited. This lack of understanding has hindered rationally informed strategies for further improving recombinant S. cerevisiae strains to efficiently utilize this abundant carbon source. Understanding the regulatory networks controlling xylose metabolism will almost certainly inform and encourage rational engineering work focused on fully utilizing the mixed sugars in lignocellulosic hydrolysates. In the present study, we investigated the functional significance of how THI2 deletion promotes xylose utilization. We found that
**TABLE 4** The characteristics of anaerobic fermentation of strains overexpressing **STT4**, **RG12**, **TFC3**, **MID2**, and deleting **IXR1**.

| Strains                  | \( \mu \text{a} \) | \( r_{xylose}^{b} \) (g g\(^{-1}\) DCW h\(^{-1}\)) | \( r_{ethanol}^{b} \) (g g\(^{-1}\) DCW h\(^{-1}\)) | \( Y_{ethanol}^{c} \) (g g\(^{-1}\) sugars) |
|-------------------------|-----------------|---------------------------------|-------------------------------|------------------------|
| BSGX001, xylose fermentation | 0.170 ± 0.001* | 0.407 ± 0.005                   | 0.191 ± 0.002                | 0.400 ± 0.002          |
| STT4                   | 0.185 ± 0.002*  | 0.557 ± 0.003                   | 0.191 ± 0.002*              | 0.296 ± 0.003          |
| RG12                   | 0.208 ± 0.001*  | 0.528 ± 0.000                   | 0.182 ± 0.004*              | 0.400 ± 0.005          |
| TFC3                   | 0.165 ± 0.000   | 0.581 ± 0.002                   | 0.253 ± 0.003               | 0.400 ± 0.006          |
| MID2                   | 0.191 ± 0.001*  | 0.486 ± 0.002                   | 0.185 ± 0.002*              | 0.360 ± 0.003          |
| br1Δ                   | 0.150 ± 0.002*  | 0.381 ± 0.003                   | 0.188 ± 0.001               | 0.402 ± 0.004          |

All the data are the mean ± standard deviation of independent triplicate tests. Cells were cultured in bioreactors at 30°C and pH 5.5, and a stirring speed of 200 rpm. The anaerobic condition was maintained by sparking nitrogen into the bioreactors with a speed of 0.1 vvm. *p < 0.05.

**a** The specific growth rates (\( \mu \)) were calculated from the data on the glucose consumption phase in the glucose and xylose co-fermentation.

**b** The specific consumption rates of xylose/ethanol (\( r_{xylose}^{b} \) or \( r_{ethanol}^{b} \)) were calculated from the data on the xylose consumption phase in the GX stage.

**c** The ethanol yields (\( Y_{ethanol}^{c} \)) of total sugars that strain consumed.

*THI2* positively affected xylose utilization by downregulating the cell cycle-related gene **CIP1** and the stress response-related gene **IXR1**; by upregulating the stress response-related genes **MID2**, **STT4**, **CDC42**, **ECM22**, **BDH2**, and **CSCI**; and by upregulating the cell viability-related genes **GPN3** and **TFC3**. These results reconfirm the findings of several previous studies that have shown xylose utilization is related to stress responses, and expression of stress-resistant genes affects the xylose metabolism in engineered *S. cerevisiae* (Cheng et al., 2018; Cunha et al., 2018).

Furthermore, we found that deletion of **THI2** increased xylose metabolism in the GX stage by regulating genes involved in the cell cycle, stress tolerance, and cell viability. Notably, these regulatory targets of Thi2p in the GX stage were apparently very different from its targets when cells are cultured in glucose (e.g., eponymous thiamine biosynthetic genes and some ribosomal protein genes) (Hu et al., 2007). A comparison of these results suggests that the function of Thi2p depends on the carbon source available, a phenomenon that has been observed for some other yeast TFs (Bergenholm et al., 2018). Further modeling investigations that are directed towards optimization and engineering through disruption of TFs, such as transcriptome engineering (Michael et al., 2016), should not ignore this important difference.

In addition, our work demonstrated that overexpression of **STT4**, **RG12**, and **TFC3** increased xylose consumption rates in both aerobic and anaerobic fermentation. Specifically, these genetically modified strains increased the \( r_{xylose}^{b} \) and shortened the overall fermentation time, a finding which has significance for production practices focused on improving economic efficiency. Additionally, we found that although overexpression of **MID2** or deletion of **IXR1** increased xylose consumption in aerobic fermentation, this effect was not seen in anaerobic fermentation. These results are not simply due to respiration, since the respiratory chain of our strains was blocked via deletion of **COX4**. The work of Myers et al. (2019) clearly showed large transcriptional changes to *S. cerevisiae* as it enters anaerobiosis in glucose or xylose. Future work resolving the apparent discrepancies between their transcriptome work and our findings for specific genetically manipulated strains will almost certainly reveal clues to deepen our understanding of how oxygen availability, beyond its role in respiration, impacts xylose metabolism in yeast and other organisms. Moreover, the stricter controlled pH condition in bioreactors than in shake flasks could also be a reason.

In summary, xylose has long been considered to be only a semi-fermentable carbon source for *S. cerevisiae* (Salusjarvi et al., 2008; Souto-Maior et al., 2009), and the post-glucose effect is obviously involved in shifting between carbon sources. However, this phenomenon has not been fully appreciated or understood to date. In this context, our molecular investigation of the global impacts of altering the regulatory networks controlling xylose utilization during the GX stage significantly advance our basic understanding of the mechanisms underlying such carbon source shift. Our results provide an initial proof-of-concept demonstration for new strategies to control and overcome inefficiencies for the exploitation of xylose as a carbon source in industrial biotechnology.

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**FIGURE 3** The proportion of dead cell in the culture. Cells were cultured at 30°C in a shake flask and agitated at 200 rpm. In xylose fermentation, xylose was depleted at about 30 h, while glucose-xylose co-fermentation, glucose was depleted before 20 h, while xylose was not depleted before 48 h. Samples were taken at 20, 36, and 48 h respectively. All data are the mean ± standard deviation of independent tests performed in triplicate. *p < 0.05.

BSGX001, xylose fermentation; BSGX001, glucose-xylose co-fermentation; the MID2 overexpression strain, glucose-xylose co-fermentation; the STT4 overexpression strain, glucose-xylose co-fermentation; the CDC42 overexpression strain, glucose-xylose co-fermentation; the THI2 deletion strain, glucose-xylose co-fermentation.
FIGURE 4 | Fermentation characteristics of strains overexpressing STT4, RGI2, TFC3, and MID2 or deleting IXR1 under anaerobic conditions. Cells were cultured in bioreactors at 30 °C, pH 5.5 with 0.1 vvm nitrogen and stirring at 200 rpm. (A) STT4 overexpression strain vs. BSGX001. (B) RGI2 overexpression strain vs. BSGX001. (C) TFC3 overexpression strain vs. BSGX001. (D) MID2 overexpression strain vs. BSGX001. (E) IXR1 deletion strain vs. BSGX001. Symbols: ■ glucose; • xylose; ▼ glycerol; ◄ acetate; ◄ ethanol; ▲ biomass. The red solid symbols are the test strains; the black hollow symbols are the control strain BSGX001. All the data represent the mean value of independent triplicate tests.

DATA AVAILABILITY

The raw data from transcriptional analysis and processed data of genes with significant differences between the thi2Δ strain and the parent strain in the GX stage are presented in the NCBI Gene Expression Omnibus database (GEO accession number: GSE119333).

AUTHOR CONTRIBUTIONS

YS and XB conceived the original research plan. SW, PB, YL, MY, and JM designed and performed the experiments. SW, YS, XB, and JH analyzed the data. SW, YS, WL, and XB wrote and revised the manuscript. All authors read and approved the final manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmib.2019.01649/full#supplementary-material