Highly Efficient Production of D-xylonic Acid From Corn Stover Hydrolysate in Gluconobacter Oxydans By mGDH Overexpression

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Research

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Abstract

Background: D-xylonic acid is a versatile platform chemical with wide potential applications as the water reducer and disperser for cement and the precursor for 1,4-butenediol and 1,2,4-tributantriol. Microbial production of D-xylonic acid with bacteria like *Gluconobacter oxydans* from cheap lignocellulosic feedstock is generally regarded as one of the most promising and cost-effective methods to industrial production, but xylonic acid productivity is reduced by high substrate inhibition and hydrolysate inhibitors.

Results: D-xylonic acid productivity of *G. oxydans* DSM2003 was improved by overexpressing the mGDH gene, which encoded the membrane-bound glucose dehydrogenase. Using the mutated plasmids based on pBBR1MCS-5 in our previous work, the recombinant strain *G. oxydans*/pBBR-R3510-mGDH with the significant improvement in D-xylonic acid production and the strengthened tolerance to hydrolysate inhibitors was obtained. The fed-batch biotransformation of D-xylose by this recombinant strain reached the record high titer (588.7 g/L), yield (99.4%), and space-time yield (8.72 g/L/h). Moreover, up to 246.4 g/L D-xylonic acid was produced directly from corn stover hydroxylates without detoxification at a yield of 98.9% and a space-time yield of 11.2 g/L/h. In addition, *G. oxydans*/pBBR-R3510-mGDH performed strong tolerance to typical inhibitors, i.e., formic acid, furfural, and 5-hydroxymethylfurfural.

Conclusion: Through overexpression of mgdh in *G. oxydans*, we obtained a recombinant strain *G. oxydans*/pBBR-R3510-mGDH. It was capable to efficiently produce xylonic acid from the corn stover hydrolysate under high concentrations of inhibitors. The high D-xylonic acid productivity made *G. oxydans*/pBBR-R3510-mGDH an attractive choice for biotechnical production.

Background

D-xylonic acid (XA) is a versatile platform chemical with wide potential applications, such as the water reducer and disperser for cement[1, 2], the precursor for co-polyamides[3], 1,4-butenediol[4], 1,2,4-tributantriol[5], and 3,4-dihydroxybutyrate[6]. This bio-based chemical has been ranked into the top 30 high-value chemicals from biomass by the U.S. Department of Energy[7].

XA can be produced by enzymatic[8], electrochemical[9] or chemical oxidation[10]. By contrast, microbial conversion of D-xylose to XA has attracted widespread attention and is generally regarded as the most promising method because of its high efficiency. Native XA producers included *Pseudomonas fragi*[11], *Klebsiella pneumoniae*[12], *Enterobacter cloacae*[13], and *Gluconobacter oxydans*[14], while other species such as *Escherichia coli*[15], *Corynebacterium glutamicum*[16], *Pichia kudriavzevii*[17] and *Saccharomyces cerevisiae*[18] have been genetically modified to produce XA. The yield and productivity of XA varied significantly from different strains and their transformation conditions (Table S1). The highest performance of XA production was observed as yet with *G. oxydans* NL71 which was able to produce 586.3 g/L XA affording a productivity of 4.69 g/L/h in the fed-batch biotransformation with a compressed supply of oxygen[19].
D-xylose is a highly abundant monosaccharide in the nature. It could be generated from the hydrolysate of lignocellulose materials which are the most abundant renewable sources[20]. From the economic standpoint, oxidative production of XA from lignocellulose/ hemicellulose hydrolysates instead of pure D-xylose is cost-competitive and promising. *G. oxydans* is capable of sugar acids production directly from cheap lignocellulosic feedstock. Zhang et al reported that 132.46 g/L gluconic acid (GA) and 38.86 g/L XA were simultaneously produced by *G. oxydans* DSM2003 from corn stover hydrolysate (CSH) with biodetoxification[2]. A high-oxygen tension reactor was applied to enhance XA production from the corn stover pre-hydrolysate without a detoxification process by *G. oxydans* NL71, generating 143.9 g/L XA affording a space-time yield (STY) of 1.0 g/L/h[19].

Although *G. oxydans* exhibited more resistant to toxins in the hydrolysate of biomass compared with other XA-producing strains[2, 14], the yield and productivity were still reduced because of sensitivity to hydrolysate inhibitors, i.e., 5-hydroxymethyl furfural (HMF), furfural, 4-hydroxybenzaldehyde, acetic acid, levulinic acid, and vanillin[19–21]. These degraded chemicals generated from the lignocellulose pretreatment act as inhibitors that might inhibit the growth of microorganisms, protein synthesis, and enzyme activity in central metabolism and target product synthesis pathway[22–25]. Moreover, these inhibitors are probable to become more toxic to the microorganisms because of a cumulative or synergistic effect despite their very low content in the hydrolysates. The critical inhibitory impact of p-hydroxybenzaldehyde, formic acid, levulinic acid, and furfural on XA production by *G. oxydans* was reported[19, 26, 27].

Many efforts have been done to overcome the inhibitory effect in lignocellulose biomass utilization by microorganisms. Detoxification[28], screening of the inhibitor-resistant microbial strains[29], or adaptive evolution of microorganisms[30] is usually required for effective improvement of D-xylose conversion in hydrolysates. However, these methods increased the complexity of the production process. Thus, the approach of enhancing the microbe's resistance by genetic modification was attractive. For example, improvement of proline or myo-inositol synthesis via overexpression of PRO1 gene or INO1 gene in *S. cerevisiae* significantly increased the tolerance toward weak organic acid (acetic acid), furan compounds (furfural), and phenol[31]. Overexpression of the thioredoxin gene could enhance the tolerance of *G. oxydans* toward p-hydroxybenzaldehyde and formic acid in XA production[26].

In this study, Overexpression of the membrane-bound glucose dehydrogenase (mGDH), which was able to oxidize D-xylose to XA[32], in *G. oxydans* DSM2003 significantly improved XA production. We found that the typical inhibitors in CSH without detoxification did not have impact on the D-xylose oxidation efficiency of this recombinant strain. Thus, the effects of five typical lignocellulose-derived inhibitors, namely formic acid, acetic acid, HMF, furfural, and vanillin, on XA production were investigated, respectively. This study provided a potential strain for bioproduction of XA from cheap D-xylose feedstock, and the fed-batch biotransformation of this strain reached the record high XA production.

**Materials And Methods**
Organism and cultural conditions

The sorbitol medium consisting of 80 g/L sorbitol, 20 g/L yeast exact, 1 g/L KH2PO4, 0.3 g/L MgSO4, and 0.1 g/L glutamine was used for *G. oxydans* strains cultivation with initial pH 6.0 at 30°C in shaking flasks or in a 7 L fermenter. Luria–Bertani medium were used for the recombinant *E. coli* strains cultivation at 37°C and 200 rpm. The concentration of gentamicin or kanamycin in medium was 25µg/L for maintaining plasmids.

**Plasmids and the recombinant *G. oxydans* strains constructions**

Primers used in this work were showed in Table S2. To overexpress the mGDH in *G. oxydans* DSM2003, the fragments of the *mgdh* gene which encoded mGDH and promoter *tufb* were amplified from *G. oxydans* DSM2003 genome by using the primer pair gdh-f/gdh-r and tufb-f/tufb-r. The plasmid pBBR1MCS5 and the fragment of promoter *tufb* were digested by endonuclease *Xba*I and *Sac*I and then ligated by T4 ligase to construct the plasmid pBBR-P*tufb*. The fragment of *mgdh* and the plasmid pBBR-P*tufb* were digested by endonuclease *Xba*I and *Eco*RI, then ligated by T4 ligase to construct the recombinant plasmid pBBR-P*tufb*-mGDH used for overexpression of mGDH. The other six recombinant plasmids pBBR-35-P*tufb*-mGDH, pBBR-10-P*tufb*-mGDH, pBBR-R35-P*tufb*-mGDH, pBBR-R10-P*tufb*-mGDH, pBBR-3510-P*tufb*-mGDH, pBBR-R3510-P*tufb*-mGDH were constructed in the same way.

The approach of triparental mating was applied to transform the recombinant plasmids to overexpress and the control plasmid pBBR-P*tufb* into *G. oxydans* DSM2003[42].

**Quantitative real-time PCR**

The real-time qPCR was applied to analyze the gene transcription level in *G. oxydans* strains. The reagents and enzymes were purchased from Transgene (China) which were used for the RNA isolation and cDNA synthesis. The GenScript Real-time PCR (TaqMan) Primer Design were applied to design the primers used in real-time qPCR. The cells were cultivated in the sorbitol medium for the late exponential phase and then harvested to do the RNA isolation. cDNA was synthesized and the transcription level of *mgdh* was measured in accordance with the manufacturer’s instructions. The 16sRNA of *G. oxydans* DSM2003 was applied as an internal control. All experiments were done in triplicate. Three parallel experiments were performed. The $2^{-\Delta\Delta\text{Ct}}$ method was used to analyze the data.

**Conversion of D-xylose by *G. oxydans* in shake flasks**

The level of XA production by *G. oxydans* was evaluated by the xylonate specific productivity. *G. oxydans* strains were cultivated for the late of exponential phase, then collected by centrifugation at 8000rpm for 8 min and washed by 50 mM citric acid buffer (pH 5.8) once to obtain the resting cells. The reaction system contained 40 g/L D-xylose, 50 mM citric acid buffer (pH 5.8), and 10 g wet wt./L resting cells in shake flasks at 30°C and 200rpm. All the experiments were done in triplicate.
Specific productivity of XA was determined by the XA production in reaction 2h and calculated by using Eq. (1).

\[
\text{Specific productivity of XA (g/g wet wt./h)} = \frac{[XA]}{[\text{resting cells}] * [time]}
\]

(1)
Where \([XA]\) (g/L) was the concentration of XA in reaction 2 h; \([\text{resting cells}]\) (g wet wt./L) was the concentration of resting cells in the reaction; \([time]\) (h) was the reaction time (2 h).

**Preparation of corn stover hydrolysate**

CSH without detoxication was provided by Prof. Jie Bao and Dr. Jian Zhang. Corn stover was preprocessed by dry diluted acid pretreatment and then hydrolyzed by cellulase to obtain the CSH without detoxification[43, 44]. The slurry of CSH was centrifuged at 8000rpm for 15 min for removing the solids. The supernatant of CSH was applied for bioconversion, which contained 83 g/L D-glucose, 45 g/L D-xylose, 2.9 g/L acetic acid, 0.48 g/L formic acid, 0.23 g/L furfural, 0.21 g/L HMF, and 0.1 g/L vanillin. The CSH containing more D-xylose and inhibitors was prepared via the addition of D-xylose and inhibitors to the CSH, which contained 225.3 g/L D-xylose, 77.6 g/L D-glucose, 10 g/L acetic acid, 1.5 g/L formic acid, 2.5 g/L furfural, 2.5 g/L HMF and 0.1 g/L vanillin.

**Whole cell transformation in the fermenter**

The whole cell transformation in the batch was performed in a 7 L fermenter with 4 vvm of aeration at 30°C and 600 rpm. A 4 M NaOH solution was used to control the pH in the reaction system at 5.8. The reaction system consisted of resting cells and D-xylose solution/hydrolysate/sugar solution. The concentration of resting cells in the reaction was 20 g wet wt./L.

The whole cell transformation in the fed-batch was also carried out in a 7 L fermenter. The working volume was 2 L. The conditions were consistent with those in batch transformation. The initial reaction system contained 380 g/L D-xylose and 20 g wet wt./L resting cells. In 22 h, 310 g of solid D-xylose was fed into the reaction system and the total loading concentration of D-xylose reached 535 g/L. Two parallel experiment groups were carried out.

Samples were taken at 4 h intervals and centrifuged at 12000rpm for 10 min to obtain the supernatant used to analyze substrates and products.

**D-xylonic acid production in the synthetic medium with different inhibitor contents**

The influence of inhibitors on XA production was evaluated by the relative specific productivity of XA in the synthetic medium with different inhibitor contents. The synthetic medium contained 40 g/L D-xylose, 10 g wet wt./L resting cells, 50 mM citric acid buffer (pH 5.8) and different contents of acetic acid, formic
acid, furfural, HMF, and vanillin. All experiments have three parallel groups. The specific productivity from the synthetic medium without inhibitors was determined as 100%.

**Analysis of substrates, products, and inhibitors**

Before analysis, 0.22 µm membrane filters were used to filter samples. Substrates (D-xylose and D-glucose) were measured by HPLC (1260 Infinity, Agilent Technologies, US) equipped with an Agilent ZORBAX Carbohydrate Analysis column and detected by a refractive index detector (G1362A, Agilent Technologies, US). The temperature of oven was maintained at 30°C. The mobile phase was 70% (v/v) acetonitrile-water solution. The flow rate was 1 mL/min.

XA and GA were analyzed by HPLC with a column of Bio-Rad Aminex HPX-87H at 55°C. They were detected by UV-VIS detector (G7114A, Agilent Technologies, US) at 210 nm. The mobile phase was 5 mM H2SO4. The flow rate was 0.4 mL/min.

Vanillin was measured by HPLC with a column of YM-Pack ODS-A (YMC, Japan) and detected at 270 nm by UV-VIS detector. The oven temperature was 35°C. Acetonitrile and 0.1% (v/v) formic acid solution were used for mobile phase. The acetonitrile content in mobile phase was adjusted as described in Khoddami’s report [45]. The flow rate was 1.0 mL/min.

A Bio-Rad Aminex HPX-87H column was used to analyzed furfural, HMF, acetic acid, and formic acid by HPLC with a refractive index detector (G1362A, Agilent Technologies, US). The oven temperature was 65°C. The mobile phase was 5 mM H2SO4. The flow rate was 0.6 mL/min.

The yield was determined by the ratio between the amount of the substrate converted into the product to the amount of loaded substrate and calculated by using Eq. (2).

\[
\text{Yield (\%)} = \frac{[\text{Product}] - [\text{Product}]_0}{F \times [\text{Substrate}]_0} \times 100 \%
\]

(2)

\([\text{Product}] (\text{g/L})\) - the concentration of product at the end;

\([\text{Product}]_0 (\text{g/L})\) - the concentration of product in the initial;

\([\text{Substrate}]_0 (\text{g/L})\) - the concentration of substrate in the initial;

\(F\) - the factor of substrate converted to equal product depending on the stoichiometric balance. The value of \(F\) used in the yield of XA was 1.11. The value of \(F\) used in the yield of GA was 1.09.

The STY of XA was determined by the XA production at the end of the reaction and calculated by using Eq. (3).

\[
\text{STY of XA (g/L/h)} = \frac{[\text{XA}]}{\text{time}}
\]
Where \([XA] \text{ (g/L)}\) was the concentration of XA at the end of the reaction; \([time] \text{ (h)}\) was the reaction time in the end.

Results And Discussion

Overexpression of \(mgdh\) in \(G. oxydans\) DSM2003

The mGDH in \(G. oxydans\) was capable to catalyze D-glucose and D-xyloses the corresponding sugar acid [33]. Overexpression of the membrane-bound PQQ-dependent glucose dehydrogenase gene (\(mgdh\)) was proved to significantly improve the mGDH activity in \(G. oxydans\) [32]. The precondition for highly efficient overexpression of an enzyme is an optimized expression plasmid. In our previous study, six new expression plasmids derived from pBBR1MCS-5 (named as pBBR-35, pBBR-10, pBBR-R35, pBBR-R10, pBBR-3510, pBBR-R3510) were constructed with higher copy numbers via rational mutagenesis[34]. Herein, to obtain the optimum recombinant strain for XA production, these six mutated plasmids and the origin plasmid pBBR1MCS5 were used to overexpress the mGDH gene in \(G. oxydans\) DSM2003.

The seven engineered strains overexpressing \(mgdh\), the control strain \(G. oxydans\)/pBBR, and the parent \(G. oxydans\) DSM2003 were used to catalyze D-xylose in shaking flasks. The XA specific productivities of these strains were compared. All of the recombinant strains overexpressing \(mgdh\) produced higher titer of XA compared with the control \(G. oxydans\)/pBBR and the parental strain \(G. oxydans\) DSM2003 (Fig. 1). Among these recombinant strains, \(G. oxydans\)/pBBR-R3510-mGDH showed the highest XA specific productivity. The transcription level of \(mgdh\) in \(G. oxydans\)/pBBR-R3510-mGDH was significantly up-regulated analyzed via the quantitative real-time PCR (real-time qPCR), which exhibited 58.55 ± 5.72-fold higher compared with that in \(G. oxydans\) DSM2003.

To further assess the enhancement of XA production of \(G. oxydans\)/pBBR-R3510-mGDH, bioconversions of D-xylose were carried out in a 7 L fermenter with accurate control of aeration rate and pH value. The reaction system contained 350 g/L D-xylose and 20 g\(_{\text{wt}}\)/L the recombinant \(G. oxydans\)/pBBR-R3510-mGDH/ or the parent strain \(G. oxydans\) DSM2003. For the batch bioconversion by \(G. oxydans\)/pBBR-R3510-mGDH (Fig. 2), XA accumulation increased rapidly to the maximum titer of 377.8 ± 4.2 g/L affording a STY of 18.89 g/L/h in 20 h. In contrast, the parent strain \(G. oxydans\) DSM2003 needed more time (46 h) to complete convert 350g/L D-xylose, affording a STY of 8.18 g/L/h. Thus, overexpression of \(mgdh\) in \(G. oxydans\) with the modification plasmid pBBR-R3510 was able to obviously enhance D-xylose oxidation leading to improved XA STY, which was increased by approximately 131% compared with the parent strain \(G. oxydans\) DSM2003.

For exploring the potential of \(G. oxydans\)/pBBR-R3510-mGDH for XA production and achieving a higher titer of XA, the initial substrate concentration was increased to 535 g/L. In batch bioconversion, 20 g\(_{\text{wt}}\)/L \(G. oxydans\)/pBBR-R3510-mGDH could not completely exhaust the supplied D-xylose, although the convention time was extended to 65 h (Figure S2). It is likely that high D-xylose concentration might
perform a negative effect on the catalytic activity of \textit{G. oxydans/pBBR-R3510-mGDH}. Thus, the fed-batch operation was conducted with an initial 380 g/L D-xylose and 20 g\textsubscript{wet wt.}/L \textit{G. oxydans/pBBR-R3510-mGDH}. Solid D-xylose of 310 g was fed into the reaction system as the D-xylose concentration was below 40 g/L. The total 535 g/L D-xylose was exhausted completely and XA accumulated to its maximum after 68h (Fig. 3). The record high XA titer (588.7 g/L), the STY (8.72 g/L/h), and the yield (99.4%) were reached in the fed-batch conversion of D-xylose (Table S1).

**D-xylonic acid production from corn stover hydrolysate**

To evaluate the potential application of the recombinant \textit{G. oxydans/pBBR-R3510-mGDH} in XA production from lignocellulose, the biotransformation performance of this strain was evaluated in CSH without biodetoxification. The experimental CSH contained 83 g/L D-glucose, 45 g/L D-xylose, and amounts of inhibitors. The bioconversion of pure sugar solution containing the same concentrations of D-xylose and D-glucose was carried out as the control. While carrying out mixed sugar conversion by the recombinant and parental strains, D-glucose and D-xylose were simultaneously transformed to GA and XA, and the conversion rate of D-glucose was higher than the D-xylose conversion rate (Fig. 4). As anticipated, both the conversion rates of D-glucose to GA and D-xylose to XA of \textit{G. oxydans/pBBR-R3510-mGDH} were significantly improved, compared with those of the parental \textit{G. oxydans DSM2003}. Hence, the total conversion time was decreased from 16h to 6h. It was noted that sugar conversion of CSH by \textit{G. oxydans/pBBR-R3510-mGDH} was essentially the same as that in the pure sugar solution (Fig. 4a), while both D-glucose conversion rate and D-xylose conversion rate of CSH by \textit{G. oxydans DSM2003} were reduced (Fig. 4b). These results showed that sugar (D-xylose and D-glucose) oxidation efficiency of the obtained recombinant strain performed nearly independent on inhibitors disturbance in the experimental CSH.

**The influence of inhibitors derived from lignocellulose on D-xylonic acid production**

To explore the potential tolerance of \textit{G. oxydans/pBBR-R3510-mGDH} to the lignocellulose-derived inhibitors, three types of compounds commonly found in lignocellulosic hydrolysates were selected to further assess their influence on XA productivity by the resting cells. The three types of compounds investigated included organic acids (formic acid and acetic acid), furan aldehydes (HMF and furfural), and an aromatic compound (vanillin). Impact of inhibitors on the parental strain \textit{G. oxydans DSM2003} was conducted as the control.

As an acetic acid bacterium, \textit{G. oxydans DSM 2003} was strongly tolerant to acetic acid[35, 36]. As shown in Fig. 5, XA productivity of \textit{G. oxydans/pBBR-R3510-mGDH} or \textit{G. oxydans DSM2003} was essentially not influenced by acetic acid in the concentration range studied (below 10 g/L). Furthermore, vanillin did not severely inhibit XA production of \textit{G. oxydans/pBBR-R3510-mGDH} or \textit{G. oxydans DSM2003} until the concentration exceeded 0.1 g/L. Figure 5 indicated that the mGDH-overexpressing strain \textit{G. oxydans/pBBR-R3510-mGDH} exhibited better resistance to formic acid, furfural, and HMF than the
parental strain *G. oxydans* DSM2003. For *G. oxydans/pBBR-R3510-mGDH*, formic acid within the concentration range of 0.2–0.5 g/L did not show a clear negative impact on XA production, but at higher formic acid concentrations XA productivity slightly decreased. In contrast, the XA productivity of *G. oxydans* was strongly affected by formic acid within the concentration range studied (0.2–2.5 g/L). Although XA productivity of *G. oxydans* DSM2003 declined as the concentration of furfural or HMF increased, XA productivity of *G. oxydans/pBBR-R3510-mGDH* remained almost constant at varying concentrations of furfural or HMF, even under the high concentration of 2.5 g/L which always reached the lethal inhibitory concentration to some microorganisms applied in lignocellulose bio-transformation[37–39].

These results clearly indicated that overexpression of mGDH responsible for D-glucose oxidation not only efficiently enhanced the XA productivity of *G. oxydans* DSM2003, but also strengthened the resistance to the inhibitors from the lignocellulose degradation. The high abundance of mGDH and the robust activity of mGDH toward typical lignocellulose-derived inhibitors should be one of the reasons to explain the improved XA productivity of *G. oxydans* from lignocellulose (i.e., corn stover) hydrolysate. According to the reports, *G. oxydans* exhibited strong tolerance to toxins in lignocellulose hydrolysate attributed to its rapid transformation of toxic inhibitors to the metabolites which was less toxic[2]. For example, HMF and furfural could be transformed to 5-hydroxymethyl-2-furoic acid and furoic acid by *G. oxydans*[40, 41]. We also noted that the conversion of furan compounds (furfural and HMF) by *G. oxydans/pBBR-R3510-mGDH* was enhanced compared with that by *G. oxydans* DSM2003 (Figure S3). This should be able to explain of the improved resistance of *G. oxydans/pBBR-R3510-mGDH* to furfural and HMF.

**Xylonate production from corn stover hydrolysate containing more D-xylose and inhibitors by G. oxydans/pBBR-R3510-mGDH**

The performance of XA production by *G. oxydans/pBBR-R3510-mGDH* from CSH containing more D-xylose and inhibitors was also tested in the fermenter. This hydrolysate contained 225.3 g/L D-xylose, 77.6 g/L D-glucose, and five high concentrations of inhibitors. Figure 6a showed that both D-xylose and D-glucose in hydrolysate were capable to efficiently and completely converted by the recombinant strain *G. oxydans/pBBR-R3510-mGDH*. GA production maintained considerably high with the GA concentration of 81.60 g/L affording the GA yield of 96.5%. Meanwhile, 225.3 g/L D-xylose was converted to 246.4 g/L XA in 22h affording a STY of 11.2 g/L/h and a yield of 98.9%. A quite similar XA and GA production was found in the conversion of the pure sugar solution (Fig. 6b). All results indicated that *G. oxydans/pBBR-R3510-mGDH* possessed excellent bioconversion performance on CSH, even if the total amount of five inhibitors reached up to 11.6 g/L. To be sure, the recombinant strain *G. oxydans/pBBR-R3510-mGDH* was a competitive biocatalyst for high sugar acid productivity from lignocellulose feedstock.

**Conclusion**
In this work, by overexpression of mGDH in *G. oxydans* DSM2003, a significant improvement in XA production was successfully achieved. Fed-batch bioconversion of D-xylose by *G. oxydans*/*pBBR-R3510-mGDH* achieved the record highest XA production result, with the titer of 588.7 g/L, the STY of 8.72 g/L/h and the yield of 99.4%. Moreover, *G. oxydans*/*pBBR-R3510-mGDH* showed strengthened tolerance to the typical lignocellulose-derived inhibitors. 246.4 g/L XA was obtained directly from CSH without detoxification, at a STY of 11.2 g/L/h and a yield of 98.9%. These performances demonstrated that *G. oxydans*/*pBBR-R3510-mGDH* had a potential application in XA industry manufacture.

**Abbreviations**

XA: xylonic acid;  
GA: gluconic acid;  
HMF: 5-hydroxymethyl furfural;  
mGDH: membrane-bound glucose dehydrogenase enzyme;  
*mgdh*: membrane-bound glucose dehydrogenase gene;  
CSH: corn stover hydrolysate;  
STY: space-time yield;  
real-time qPCR: quantitative real-time PCR.

**Declarations**

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**Authors’ contributions**

JL conceived and designed the experiments, and contributed significantly to manuscript preparation. XM performed the experiments and contributed significantly to analysis and manuscript preparation. BZ and CZ helped perform the bioconversion experiments. DW contributed to the conception of the study. All authors read and approved the final manuscript.

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**Availability of data and materials**
The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no conflict of interests.

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Figures

![Bar chart showing specific productivities of different G. oxydans strains.](image)

**Figure 1**

Comparison of XA specific productivities by different G. oxydans strains.
Figure 2

Batch conversion of D-xylose to XA by the recombinant *G. oxydans/pBBR-R3510-mGDH* and the control *G. oxydans DSM2003.*
Figure 3

Fed-batch conversion of 535 g/L D-xylose by G. oxydans/pBBR-R3510-mGDH.
Figure 4

Comparison of biocatalysts performance between CSH and sugar solution. (a) D-xylose and D-glucose conversion by G. oxydans/pBBR-R3510-mGDH; (b) D-xylose and D-glucose conversion by G. oxydans DSM2003; (c) XA and GA production by G. oxydans/pBBR-R3510-mGDH; (d) XA and GA production by G. oxydans DSM2003.
Figure 5

Relative XA specific productivities in the synthetic medium with different inhibitor contents. (a) G. oxydans/pBBR-R3510-mGDH. (b) G. oxydans DSM2003.
Figure 6

XA and GA production under high concentration of inhibitors by G. oxydans/pBBR-R3510-mGDH in the fermenter. (a) conversion of CSH containing more D-xylose and inhibitors. (b) conversion of sugar solution.

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