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Lysine Production by Dry Biorefining of Wheat Straw and Cofermentation of *Corynebacterium glutamicum*

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ABSTRACT: A preliminary study shows that lysine production from lignocellulose feedstock is feasible, but the conversion of xylose in lignocellulose to lysine remains unsolved. Two technical barriers are responsible for the remaining xylose conversion: one is the xylose loss into the wastewater stream of the biorefinery processing chain, and the other is the lack of efficient lysine-producing strain with xylose utilization. Here, we conducted a new biorefinery approach of consequent dry acid pretreatment and biodetoxification, resulting in zero wastewater generation and then well-preserved xylose. To provide the lysine-producing strain with xylose utilization, we modified the *Corynebacterium glutamicum* by establishing the xylose assimilation pathway and improving the NADPH cofactor regeneration. The combinational modification of biorefinery processing and strain development led to 31.3 g/L of lysine production with a yield of 0.23 g lysine per gram of wheat straw derived sugars. This study provides a practical method for upgraded lysine production from lignocellulose for future industrial applications.

KEYWORDS: *lysine, wheat straw, fermentation, recombinant Corynebacterium glutamicum, lignocellulose-derived sugars*

■ INTRODUCTION

Lysine is a commercial amino acid used as a feed supplement with an annual production of more than 2 million tons.[1] Some cheap carbon sources have been already used, such as crude glycerol and silage juice, to extend its application potential as a commodity chemical.[2,3]

Among all the cheap carbon sources, lignocellulose is the most promising resource because of its abundance and availability. A preliminary lysine production has been tested in our previous study with a successful lysine synthesis from lignocellulose feedstock.[4] However, the fermenting strain *Corynebacterium glutamicum* used in the preliminary research was only capable of glucose utilization, and xylose from lignocellulose was left behind in the fermentation broth.

Xylose accounts for 30% of the total sugars in lignocellulose, but its practical utilization generally meets two technical barriers. The first barrier is the preservation of xylose during the biorefinery processing chain of pretreatment, detoxification (or conditioning), and saccharification. The regular pretreatment methods input large amounts of water and generate large quantities of wastewater streams, resulting in considerable xylose loss by washing into the pretreatment liquid stream.[5,6] The liquid stream also contains high levels of various inhibitors from pretreatment, blocking the further fermentations. The regular detoxification methods for inhibitor removal such as water-washing or over-liming further reduce the xylose content of the pretreated lignocellulose feedstock.[7,8] Even if the inhibitors are removed by detoxification, xylose content in the liquid stream is too low to be used as fermentation sugar. The second barrier is the lack of an available lysine-producing strain from both glucose and xylose. Although xylose utilization for producing some amino acid (glutamic acid and ornithine) and other fermentation products was successfully established in *C. glutamicum*, xylose assimilation for lysine synthesis has not reached a basic level of lysine accumulation.[9−21]

To overcome the two barriers, this study used a dry acid pretreatment method with the minimum use of acid catalyst solution to produce the pretreated lignocellulose in dry fine particle form with zero wastewater generation. A biodetoxification approach was followed to degrade the inhibitors in the pretreated lignocellulose feedstock completely. These dry refining steps led to the excellent preservation of xylose in the solid phase with cellulose.[22−25] Then, an industrial *C. glutamicum* was modified to assimilate both xylose and glucose with improved NADPH cofactor regeneration by metabolic modification approaches. Using the dry refined wheat straw with excellent preservation of xylose and the recombinant *C. glutamicum*, 31.3 g/L lysine was produced from the wheat straw hydrolysate with a high yield of 0.23 g/g sugar. This study offers a technical approach for improving the lysine accumulation level from lignocellulose feedstock with industrial application potential.

■ MATERIALS AND METHODS

Strains, Culture Conditions, and Medium. The storage and culture of *E. coli* DH5α, *E. coli* BL21, and *Pediococcus acidilactici* DSM 20284 are based on our previous study.[21] The *Amorphotheca resinae* ZN1 fungus was isolated by our lab previously, and the fungus was preserved in China General Microbiological Culture Collection

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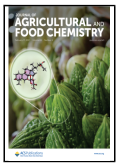

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Table 1. Strains and Plasmids Used

strains	characteristics	sources
Escherichia coli DH5α	host for plasmid construction	lab stock
Escherichia coli BL21	genes *xylAB_BL21* and *araE* source	lab stock
Pediococcus acidilactici DSM 20284	genes *xylAB_2911* source	DSMZ[a]
Amorphotheca resinae ZN1 (CGMCC 7542)	biodetoxification fungus	Zhang et al.[22]
C. glutamicum B253	parental for lysine fermentation	SIIM[b]
B253-pPeftumob	*C. glutamicum* B253 harboring the plasmid Peftumob	this work
B253-pH 36-*xylAB_BL21*	*C. glutamicum* B253 harboring the plasmid pPH36-*xylAB_BL21*	this work
B253-pH 36-*xylAB_2911*	*C. glutamicum* B253 harboring the plasmid pPH36-*xylAB_2911*	this work
B253-pPsod-*xylAB_BL21*	*C. glutamicum* B253 harboring the plasmid pPsod-*xylAB_BL21*	this work
B253-pPsod-*xylAB_2911*	*C. glutamicum* B253 harboring the plasmid pPsod-*xylAB_2911*	this work
B253-pPeftu-*xylAB_BL21*	*C. glutamicum* B253 harboring the plasmid pPeftu-*xylAB_BL21*	this work
B253-pPeftu-*xylAB_2911*	*C. glutamicum* B253 harboring the plasmid pPeftu-*xylAB_2911*	this work
B253-Δ*ldhA*::*xylAB* (LJ01)	*ldhA* knockout and integration of the cassette Peftu_*xylAB_BL21* in *C. glutamicum* B253	this work
LJ01-pPeftumob	LJ01 harboring the empty plasmid Peftumob	this work
LJ01-pPeftu-*lysC-asd*	LJ01 harboring the plasmid Peftu-*lysC-asd*	this work
LJ01-pPeftu-*dapA*	LJ01 harboring the plasmid Peftu-*dapA*	this work
LJ01-pPeftu-*dapB*	LJ01 harboring the plasmid Peftu-*dapB*	this work
LJ01-pPeftu-*ddh*	LJ01 harboring the plasmid Peftu-*ddh*	this work
LJ01-pPeftu-*lysA*	LJ01 harboring the plasmid Peftu-*lysA*	this work
LJ01-pPeftu-*pyc*	LJ01 harboring the plasmid Peftu-*pyc*	this work
LJ01-pPeftu-*PntAB*	LJ01 harboring the plasmid Peftu-*PntAB*	this work
LJ01-pPeftu-*araE*	LJ01 harboring the plasmid Peftu-*araE*	this work
LJ01-pPeftu-*tkt-tal*	LJ01 harboring the plasmid Peftu-*tkt-tal*	this work
LJ01-Δ*pck*	*pck* knockout in LJ01	this work
LJ01-evolution	adaptive evolution of LJ01 in the xylose medium	this work
plasmids	characteristics	sources
pTRCmob	expression vector for *C. glutamicum*, TRC promoter, kanamycin resistance Km^R	Wang et al.[42]
pK18mobsacB	mobilizable vector for selection of double crossover in *C. glutamicum*, kanamycin resistance Km^R	Wang et al.[42]
pK18-Δ*ldhA*::*xylAB*	vector for replacement of *ldhA* by integrating the cassette Peftu_*xylAB_BL21* into the genome of *C. glutamicum* B253	this work
pK18-Δ*pck*	vector for truncation of *pck* in the genome of LJ01	this work
pPeftumob	expression vector for *C. glutamicum*, Peftu promoter, kanamycin resistance Km^R	this work
pPeftu-*lysC-asd*	vector for expression of *lysC* and *asd* by the Peftu promoter	this work
pPeftu-*dapA*	vector for expression of *dapA* by the Peftu promoter	this work
pPeftu-*dapB*	vector for expression of *dapB* by the Peftu promoter	this work
pPeftu-*ddh*	vector for expression of *ddh* by the Peftu promoter	this work
pPeftu-*lysA*	vector for expression of *lysA* by the Peftu promoter	this work
pPeftu-*pyc*	vector for expression of *pyc* by the Peftu promoter	this work
pPeftu-*PntAB*	vector for expression of *PntAB* by the Peftu promoter	this work
pPeftu-*araE*	vector for expression of *araE* by the Peftu promoter	this work
pPeftu-*tkt-tal*	vector for expression of *tkt* and *tal* by the Peftu promoter	this work

[a] DSMZ indicates the German Collection of Microorganisms and Cell Cultures (DSMZ), Braunschweig, Germany. [b] SIIM indicates the collection center of the Shanghai Industrial Institute of Microorganism, Shanghai, China.

Center (CGMCC) with the storage code of 7452.[22] The plasmids and strains used or constructed in this study are listed in Table S1. *C. glutamicum* B253 was purchased from the Shanghai Industrial Institute of Microorganism (Shanghai, China). The *C. glutamicum* B253 was cultured at 30 °C, 200 rpm, and initial pH 7.0 in 250 mL flasks. The seed medium consisted of 30 g/L glucose, 1.5 g/L monopotassium phosphate, 2.5 g/L urea, 0.6 g/L magnesium sulfate, and 25 g/L corn steep liquor. The fermentation medium consisted of 60 g/L glucose or xylose, 1.0 g/L monopotassium phosphate, 20 g/L ammonium sulfate, 0.6 g/L magnesium sulfate, 20 g/L corn steep liquor, 0.5 g/L threonine, and 0.5 g/L methionine.

Plasmid and Recombinant Construction. The primers are listed in Table S1. The plasmids and the recombinants are listed in Table 1. The Peftu promoter was obtained from the *C. glutamicum* S9114 by PCR, which substituted the TRC promoter on the pTRCmob plasmid to obtain the pPeftumob plasmid. The *dapA*, *dapB*, *ddh*, *lysA*, *PntAB*, and *araE* genes were ligated to SmaI/XbaI of pPeftumob to generate plasmids pPeftu-*dapA*, pPeftu-*dapB*, pPeftu-*ddh*,pPeftu-*lysA*, pPeftu-*PntAB*, and pPeftu-*araE*. The *lysC-asd*, *tkt-tal*, and *pyc* genes were ligated to XbaI/SalI of pPeftumob to generate plasmids pPeftu-*lysC-asd*, pPeftu-*tkt-tal*, and pPeftu-*pyc*.

The plasmid pK18-Δ*pck* was for deleting the *pck* gene. The upstream (*pck*-up) and downstream (*pck*-down) fragments of the *pck* gene of *C. glutamicum* B253 were overlapped to obtain the *pck* fragment, which was then ligated to SmaI/HindIII of pK18mobsacB to generate plasmid pK18-Δ*pck*. The upstream (*ldhA*-up) and downstream (*ldhA*-down) fragments of the *ldhA* gene of *C. glutamicum* B253 were overlapped with the cassette *Peftu-xylAB_BL21* by PCR. The PCR product, Δ*ldhA*::*xylAB*, was then ligated to SmaI/HindIII of pK18mobsacB to generate plasmid pK18-Δ*ldhA*::*xylAB* for genome integration of *xylAB* at the *ldhA* gene locus. Plasmids transformation, gene disruption, and correct mutant isolation were based on our previous procedures.[21]

Enzymes and Reagents. Cellulase Cellic CTec2, T4 ligase, DNA polymerase, Seamless cloning kits, restriction endonuclease, and other general chemicals were obtained according to the previous study.[21]

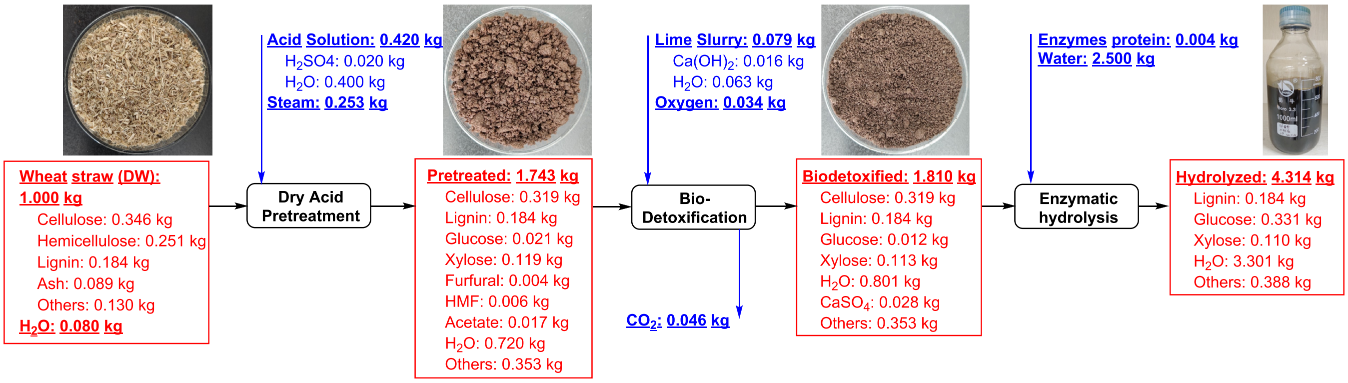

Figure 1. Mass balance of wheat straw in biorefinery processing.

Lignocellulose Biomass and Biorefinery Engineering. The wheat straw used in this study was purchased from Nanyang, Henan Province, China, in autumn 2018. According to NREL protocols, the virgin wheat straw consisted of 25.1% hemicellulose, 34.6% cellulose, 8.9% ash, and 18.4% lignin.[23,24] For wheat straw hydrolysate preparation, dry acid pretreatment was conducted by simultaneously feeding sulfuric acid solution and wheat straw into a 20 L pretreatment reactor at a solid/liquid ratio of 2:1 (w/w) with a sulfuric acid usage of 20.0 mg/g dry wheat straw. The pretreatment process was conducted at 175 ± 1 °C for 5 min with helical mixing, and no wastewater was generated in the whole pretreated process. 20% (w/w) $Ca(OH)_2$ was used to neutralize the residual sulfuric acid in the pretreated wheat straw until the pH value reached 5.5.

The *A. resinae* ZN1 fungus, which consumed the inhibitors before the xylose and glucose, was used to remove the pretreated wheat straw's inhibitory compounds. For biodetoxification, the *A. resinae* ZN1 fungus spores stored at the PDA slant were inoculated onto 500 g of pretreated wheat straw with intensive mixing and stationarily cultured in a 2 L box with a lip at room temperature for 4−5 days as the solid seed. Then the solid seed (the wheat straw with the *A. resinae* ZN1 fungus) was added to 5 kg of fresh pretreated wheat straw at a 10% (w/w) inoculation rate in a 15 L bioreactor. The biodetoxification process was conducted at 28 °C and 0.8 vvm of aeration for 2−3 days until more than 90% of inhibitors were removed.

For enzymatic hydrolysis, the detoxified wheat straw, sterile water, and cellulase were added into a 5 L fermentor with a helical agitator, and the cellulase dosage was 4 mg protein/g dry wheat straw matter (DM). The hydrolysis process was conducted at 50 °C, 150 rpm for 48 h, and the pH was maintained at 5.0 by 5 M NaOH and 2 M H_2SO_4. The wheat straw hydrolysate slurry was centrifuged to remove the solid residues, then autoclaved, and filtered to obtain the clear hydrolysate. The components of the wheat straw hydrolysate were detected by HPLC, and it contained 33.3 g/L xylose and 100.2 g/L glucose, and 5-hydroxymethylfurfural (HMF) and furfural were not detected.

Lysine Production in a 3 L Fermentor. The *C. glutamicum* stored at −80 °C was inoculated at the LB plate and cultured for 48 h at 30 °C. A single colony was inoculated in a 250 mL flask containing 30 mL of seed medium and cultured at 30 °C, 200 rpm for 12 h. Then it was transferred to a 500 mL flask containing 80 mL of fresh seed medium for 8 h in the same culture conditions before being inoculated to the fermentation medium.

The 3 L fermentor with 800 mL of fermentation medium or wheat straw hydrolysate was applied to lysine production. The fermentation was conducted at 600 rpm, 1.4 vvm of aeration, 30 °C, and the pH was regulated automatically at 7.0 with ammonia and sulfuric acid.

Analytical Methods. The glucose, xylose, and inhibitors in the medium were detected by HPLC (Shimadzu, Kyoto, Japan) with an HPX-87P chromatographic column maintained at 80 °C. 5 mM H_2SO_4 was used as the mobile phase, and the flow rate was set at 0.6 mL/min. The lysine concentration was determined using a biosensor SBA-40 (Shandong Academy of Sciences, Shandong, China). The cell growth was detected using a UV spectrophotometer (BIOMATE 3S; Thermo, Waltham, MA, USA).

Lysine Yield Calculation. Lysine yield from xylose and glucose was defined as the ratio of the increased lysine production to the xylose and glucose at the beginning of the fermentation

$$\text{lysine yield} = \frac{[\text{Lys}] \times V - [\text{Lys}]_0 \times V_0}{[X + G]_0 \times V_0 - [X + G] \times V} \times 100$$

where $[\text{Lys}]$ and $[\text{Lys}]_0$ represent the final and initial concentrations of lysine during the fermentation, respectively, $[X + G]$ and $[X + G]_0$ represent the final and initial concentrations of xylose and glucose during the fermentation, respectively, V and V_0 represent the final and initial volumes of fermentation broth, respectively.

■ RESULTS AND DISCUSSION

Wheat Straw Hydrolysate Preparation with Xylose Reserved. Wheat straw was dry acid pretreated followed by biodetoxification as described in the Materials and Methods section, in which the xylose in wheat straw was well reserved as indicated in Figure 1. The dry acid pretreatment converted the hemicellulose in wheat straw into xylose in a yield of 0.119 ± 0.007 g/g dry wheat straw matter (DM). The water dosage is minimal; thus, the pretreated wheat straw was in the solid phase. The inhibitors were also accumulated in the pretreated wheat straw because no free water stream washed the inhibitors away from the wheat straw, including 4.5 mg furfural/g DM and 6.2 mg HMF/g DM. Solid-state biodetoxification was conducted on the pretreated wheat straw solids by *A. resinae* ZN1 until furfural and HMF were completely degraded (not detected by HPLC).[25−28] Xylose was still well retained in the biodetoxified wheat straw at 0.113 ± 0.005 g/g DM. The wheat straw hydrolysate was prepared by enzymatic hydrolysis to obtain 33.3 ± 1.7 g/L xylose and 100.2 ± 0.6 g/L glucose. The xylose content in the hydrolysate was translated to 0.110 ± 0.006 g/g DM. Only minor losses of xylose at 7.6% and glucose at 10.9% were detected in the whole dry biorefinery process.

The key to dry biorefinery is combined utilization of dry acid pretreatment and biodetoxification. The regular pretreatment methods include dilute-acid pretreatment, alkaline pretreatment, and hydrothermal processing, which use a large amount of water to immerse the biomass in the appropriate solvent.[29−34] Thus, a large quantity of xylose and inhibitors are dissolved in the liquid stream, and the high content of inhibitors blocks the further fermentation. This study used the dry acid pretreatment with extremely low water dosage to preserve xylose in the solid phase with cellulose, but the inhibitors also remained in the solid phase. The biodetox-

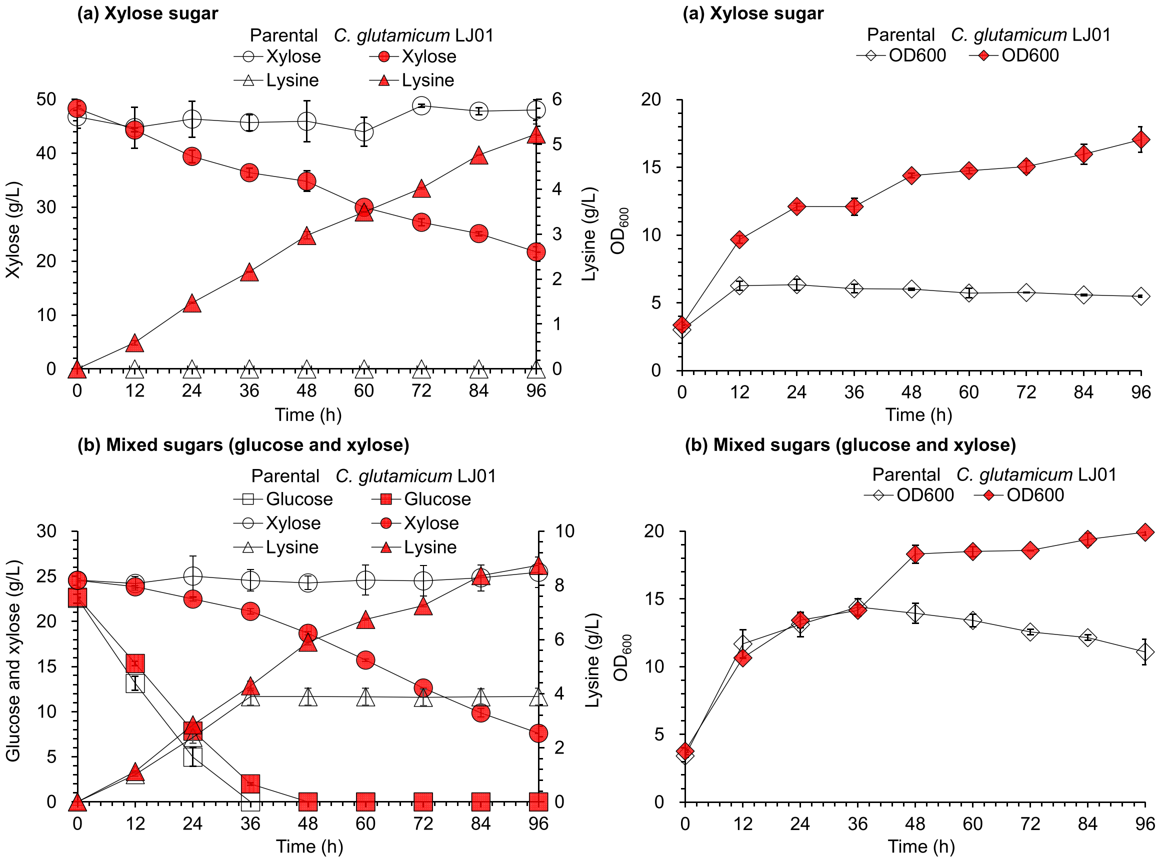

Figure 2. Lysine production from xylose or mixed sugars by *C. glutamicum* LJ01. (a) Lysine production in a medium containing xylose as the sole carbon source. (b) Lysine production in a medium containing glucose and xylose. Parental, the parental strain *C. glutamicum* B253; *C. glutamicum* LJ01, the recombinant strain with xylose utilization genes. The lysine production was conducted at 200 rpm, 30 °C, in flasks.

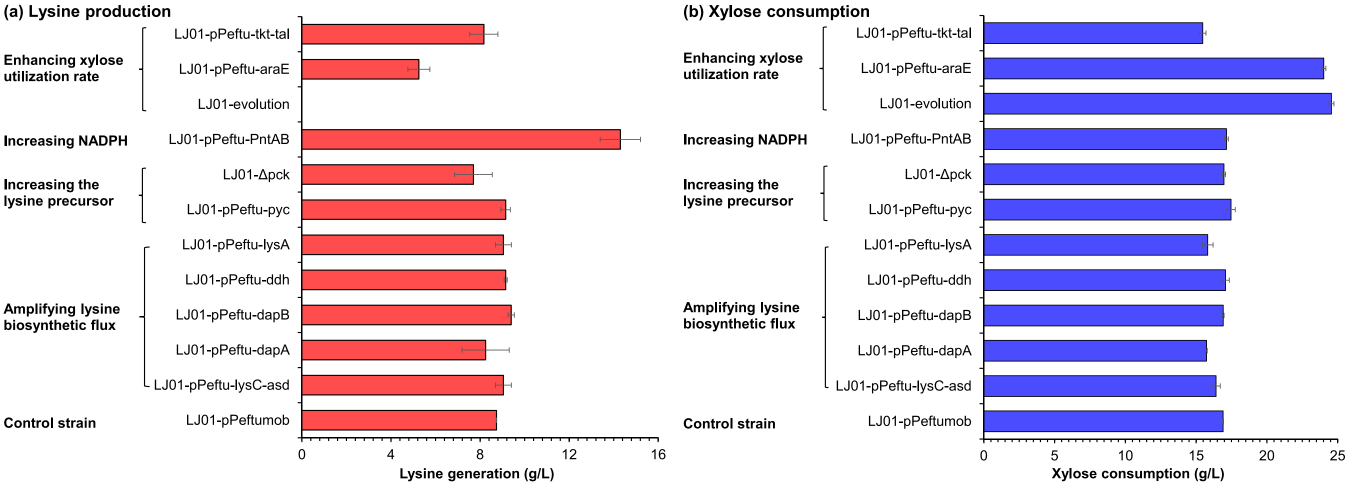

Figure 3. Increasing lysine production from xylose and glucose by metabolic engineering of *C. glutamicum* LJ01. (a) Lysine production. (b) Xylose consumption. LJ01-pPeftumob, the strain with empty plasmid pPeftumob in LJ01 as the control strain; LJ01-pPeftu-*lysC-asd*, the strain with expression plasmid pPeftu-*lysC-asd* in LJ01; LJ01-pPeftu-*dapA*, the strain with expression plasmid pPeftu-*dapA* in LJ01; LJ01-pPeftu-*dapB*, the strain with expression plasmid pPeftu-*dapB* in LJ01; LJ01-pPeftu-*ddh*, the strain with expression plasmid pPeftu-*ddh* in LJ01; LJ01-pPeftu-*lysA*, the strain with expression plasmid pPeftu-*lysA* in LJ01; LJ01-pPeftu-*pyc*, the strain with expression plasmid pPeftu-*pyc* in LJ01; LJ01-Δ*pck*, the strain with truncation of *pck* in LJ01; LJ01-pPeftu-*PntAB*, the strain with expression plasmid pPeftu-*PntAB* in LJ01; LJ01-evolution, the strain with adaptive evolution in a xylose medium by LJ01; LJ01-pPeftu-*araE*, the strain with expression plasmid pPeftu-*araE* in LJ01; LJ01-pPeftu-*tkt-tal*, the strain with expression plasmid pPeftu-*tkt-tal* in LJ01. The lysine production was carried out at 30 °C, 200 rpm in a 250 mL flask containing 30 mL of medium with 25 g/L xylose and 25 g/L glucose.

ification approach was developed to degrade the inhibitors prior to the xylose in the solid phase completely. Therefore, the combination of these two methods successfully avoids xylose loss in the biorefinery process.

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Development of the Recombinant *C. glutamicum* Strain. We developed a recombinant *C. glutamicum* for utilization of both xylose and glucose for lysine production. The genes and promoters related to the modification were screened (Table S2). The *xylAB* gene (B21_RS17860 and B21_RS17855) from *E. coli* BL21 with the P*eftu* promoter was integrated into the genome of *C. glutamicum* B253 (Figure S1) to obtain *C. glutamicum* LJ01. *C. glutamicum* LJ01 converted 26.6 g/L xylose and produced 5.2 g/L lysine when xylose was used as the sole carbon source (Figure 2a). When the mixed glucose and xylose were used as carbons sources, *C. glutamicum* LJ01 produced 8.7 g/L lysine, approximately 1.2-fold greater than the parent strain (3.9 g/L) (Figure 2b).

One mole of lysine generation requires 4 mol of NADPH supply, but no NADPH is generated when xylose is assimilated for lysine synthesis (Figure S1).[35−37] We expressed the *PntAB* gene (B21_RS08255 and B21_RS08250) encoding a membrane protein for converting NADP$^+$ to NADPH in *C. glutamicum* LJ01, and the lysine production increased by 64% (Figure 3).[38] The intracellular NADPH content was detected, and the results indicated that the concentration of NADPH decreased by 47% (23.3 to 12.4 nmol/mg prot) when the *C. glutamicum* LJ01 was cultured in the xylose medium compared to the glucose medium, and the NADPH content increased by 61% (12.4 to 20.0 nmol/mg prot) when the *PntAB* gene was overexpressed in the *C. glutamicum* LJ01 (Figure S2).

The lysine synthetic pathway involves the aspartokinase (*lysC*), aspartate-semialdehyde dehydrogenase (*asd*), dihydrodipicolinate synthase (*dapA*), dihydrodipicolinate reductase (*dapB*), diaminopimelate dehydrogenase (*ddh*), and diaminopimelate decarboxylase (*lysA*).[39] We overexpressed these genes to enhance lysine synthesis flux (Figure S1). Oxaloacetate is the important precursor for lysine production.[40,41] We overexpressed the pyruvate carboxylase (*pyc*) to convert more pyruvate to oxaloacetate and knocked out the phosphoenolpyruvate carboxykinase (*pck*) to reduce oxaloacetate loss (Figure S1). Increasing the xylose utilization rate is a direct way to enhance lysine synthesis. We conducted (i) the laboratory adaptive evolution of *C. glutamicum* LJ01 by successive transfer in the xylose medium (*C. glutamicum* LJ01-evolution obtained), (ii) the pentose transporter (*araE*) overexpression to increase the transmembrane transportation rate of xylose (*C. glutamicum* LJ01-pPeftu-*araE* obtained), and (iii) the transketolase (*tkt*) and transaldolase (*tal*) overexpression to enhance the phosphate pentose pathway (*C. glutamicum* LJ01-pPeftu-*tkt-tal* obtained). However, no significant changes were observed on lysine production by these methods (Figure 3).

Lysine Production in the Wheat Straw Hydrolysate. The maximum lysine-producing strain *C. glutamicum* LJ01-pPeftu-*PntAB* was used as the working strain to produce lysine from wheat straw. Figure 4 shows that *C. glutamicum* LJ01-pPeftu-*PntAB* simultaneously and completely consumed 33.3 g/L xylose and 100.2 g/L glucose in the wheat straw hydrolysate, and 31.3 g/L lysine was produced. The control strain *C. glutamicum* B253-pPeftumob only consumed the glucose in the hydrolysate, and no xylose consumption was observed, producing 24.9 g/L lysine. The lysine production by *C. glutamicum* LJ01-pPeftu-*PntAB* was approximately 25% greater than that of the control strain, though the lysine yield decreased slightly (approximately from 26 to 23%). One mole of lysine generation requires 4 mol of NADPH supply, but no NADPH is generated when xylose is assimilated for lysine synthesis. In this study, the *PntAB* gene was expressed to increased the NADPH regeneration, but the intracellular NADPH supply was still insufficient, leading to the lysine yield to decrease slightly.

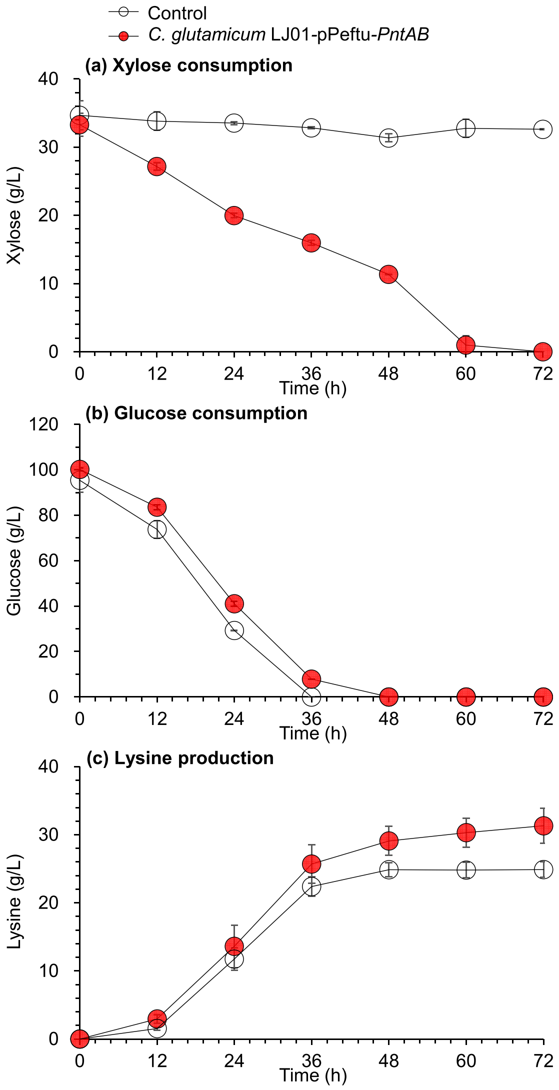

Figure 4. Lysine production using the wheat straw hydrolysate by *C. glutamicum* LJ01-pPeftu-*PntAB*. (a) Xylose consumption. (b) Glucose consumption. (c) Lysine production. Control, empty plasmid pPeftumob in *C. glutamicum* B253; *C. glutamicum* LJ01-pPeftu-*PntAB*, expression plasmid pPeftu-*PntAB* in LJ01.

In order to reduce the feedstock cost of lysine production, Meiswinkel et al. utilized crude glycerol to produce 3.4 g/L lysine with a lysine yield of 12% by *C. glutamicum* DM1729;[2] Neuner et al. obtained 1.7 g/L lysine from silage juices with a lysine yield of 24% by *C. glutamicum* SL;[3] Gopinath et al. produced 6.1 g/L lysine from a wheat bran hydrolysate with a lysine yield of 15% by *C. glutamicum* DM1729.[19] However, the concentration of lysine obtained from the above research is too low, and lignocellulose is the most promising lysine-producing feedstock because of its abundance and availability compared to those cheap feedstocks. In this study, the wheat straw was collected, dry acid pretreated, biodetoxified, and hydrolyzed to obtain the inhibitor-free hydrolysate containing 33.3 g/L xylose and 100.3 g/L glucose. The recombinant strain *C. glutamicum* LJ01-pPeftu-*PntAB* produced 31.3 g/L lysine with a yield of 23% from the wheat straw hydrolysate. Moreover, all glucose and xylose were consumed entirely and simultaneously. This study makes a breakthrough in the sufficient utilization of lignocellulose for lysine production.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.0c07902.

> Oligonucleotide primers used in this study (Table S1). Alternating promotors and xylose assimilation gene (Table S2). Metabolic engineering strategies for improving lysine production (Figure S1). Detection of the intracellular NADPH content (Figure S2) (PDF)

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Notes

The authors declare no competing financial interest.

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