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# Enhancing economic and environmental friendliness of xylonic acid bioproduction from corncob hydrolysate by the combined recycling-technology of detoxifying-resin and catalyzing-cell

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A B S T R A C T

The bioproduction of xylonic acid (XA) from acidic lignocellulosic hydrolysate presents a potential booming research area, that XA serves as an important building block of biomass-based chemicals for biomass valorization. However, the existence of inhibiting compounds such as acetic acid in hydrolysate greatly influences bacterial cell metabolism and catalysis, as well as the following electrodialysis separation for XA purification. Therefore, the removal of inhibitors including acetic acid is an essential issue in the industrial bioproduction of XA. In this study, five polymer resins were evaluated on their ability to remove acetic acid and other inhibitors from acidic hydrolysate of corncob (AHC). By comparison, anion exchange resin 335 performed the best on the removal of acetic acid in the model solution and AHC at 100 % and 80 %, respectively, while the sugar loss was also confined within 10 %. Finally, 208.6 g XA was obtained with a productivity of 5.8 g/L/h by 6 rounds of cell- recycling bioconversion. Moreover, recycled resin kept at least 80% toxic removal efficiency after 4 renewed rounds with hot water washing. The combined recycling-technology of detoxifying-resin and catalyzing-cell greatly facilitate economic and environmental bioproduction of XA from lignocellulosic biomass.

*Keywords:* Xylonic acid, Acidic lignocellulosic hydrolysate, Resin adsorption removal, Resin regeneration, Cell-recycling bioconversion

# 1. Introduction

Lignocellulosic biomass, as the second-generation biomass feedstock, is more promising and sustainable than edible-based first-generation biomass feedstock on meeting fuel provision requirements (Wright et al., 2010). It is composed of up to 24~40% C5 carbohydrates whose key building block is xylose (Kumar et al., 2009). The efficient utilization and transformation of xylose is a key technical bottleneck for the commercialization of lignocellulosic-based biorefinery systems. The platform chemical xylonic acid (XA), as the direct oxidation product of xylose, has a broad industrial application, such as concrete binder, cement water-reducing agent, and biopolymer materials, etc. Because of its similar properties to gluconic acid, XA can also be used as the substitute for gluconic acid and other polyol-carboxylic compounds (Hahn et al., 2020; Ma et al., 2016; Zhou et al., 2018). Compared to enzymatic oxidations, the whole-cell catalysis had presented a more promising pathway on industrial production of XA due to its advanced features such as environmentally friendly and low-cost.

*Gluconobacter oxydans* had been widely used in the industrial fermentation of gluconic acid, dihydroxyacetone, and vitamin C due to its excellent oxidation ability of sugars and alcohols (U. et al., 2002). It also showed a great xylose bio-oxidation performance in preparing 586.3 g/L XA within 125 h using a sealed oxygen supply system (Zhou et al., 2015). As a well-known fact, the cost of microbial cell culture is one of the critical issues for commercial fermentation because of costive nutrient consumption and sterilized operation (Rivas et al., 2004; Sharma et al., 2013; Wu et al., 2020). Unluckily, *G. oxydans* is really of hard-breezing microbe that needs high cost sorbitol and nitrogen sources for long periods of incubation, necessitating the cell-recycling procedure for commercial production. However, the toxicity of derived acids, furans, and phenolics in acidic lignocellulosic hydrolysate is a big obstacle to whole-cell catalysis and bioconversion of xylose (Guo and Lisbeth, 2015; Mhlongo et al., 2015; Soares et al., 2021). These degraded chemicals impose complex and serious inhibitory effects on cell metabolism and vitality of *G. oxydans*. For example, acetic acid like small organic acids can enter easily the cell and release $H^+$ into the cytoplasm, resulting in intracellular pH niche decrease and metabolic disorder, thus causing damages and inefficiency to microbial cells. Furthermore, these acids always act synergistically with furans and phenolic inhibitors to enhance toxicity (Shen et al., 2020). Another technical problem exists in the electrodialysis separation and purification steps (Cao and Xu, 2019; Liu et al., 2016). Here, acetic acid like ionizable chemicals usually not only raise processing loading but also impenetrate bipolar membranes with XA and impact end-product quality. To sum up, the effective separation and removal of acetic acid like inhibitors from acidic lignocellulosic hydrolysate is the critical issue to the techno-economic benefits of XA bioproduction, especially involving microbial cell-recycling bioconversion, purification step, and end-product quality.

Adsorption is a commonly used purification and detoxification technology in industrial production (Dong et al., 2018; Huang et al., 2020; Wu et al., 2016). An adsorbent with selective adsorption capacity is a good choice to remove acetic acid and other inhibitors from the hydrolysate (Castaldo et al., 2021). Polymeric resins are considered to be one of the best options for removing hydrolysate inhibitors (You et al., 2019; Yu and Christopher, 2017). In terms of industrial application, polymeric resins have strong adsorption capacity, can remove various substances, and can be regenerated under suitable conditions, so they have been widely used in wastewater treatment, food, and pharmaceutical industries (Coha et al., 2021; Nitzsche et al., 2019). Recently, with the development of bio-based high value-added chemicals, the use of polymeric resins for the detoxification and purification of hydrolysates has attracted increasing attention. For instance, Vinod Kumar et al. used ion-exchange resins to detoxify corncob hydrolysate for increased xylitol extraction (Kumar et al., 2018). Yang et al. used polymeric resins to treat hemicellulose-rich poplar hydrolysate and improved the fermentation performance of ethanol (Yu and Christopher, 2017). To date, there are few studies on enhancing XA production by detoxification hydrolysate with resin. This work aims to investigate polymeric resins for their ability to remove acetic acid and other inhibitors from acidic corncob hydrolysate and their

effect on cell-cycling in XA production, with the aim of providing theoretical support for industrial production of XA.

**Table 1**

Physical and chemical properties of the resin.

| Resin | Functionality | Exchange capacity (mmol/g) | Particle diameter (mm) | Pearl size (%) | Moisture content (%) |
|---|---|---|---|---|---|
| D202 | -N + $(CH_3)_3$ | ≥ 3.5 | 0.315–1.2 | ≥ 95% | 47–57 |
| D303 | ---N | ≥ 6.0 | 0.35–1.0 | ≥ 95% | 50–60 |
| D315 | ---N | ≥ 6.5 | 0.315–1.2 | ≥ 95% | 47–57 |
| 335 | $-NH_2$,=N,$^{-}$ –$_{-}$ N | ≥ 9.0 | 0.315–1.25 | ≥ 90% | 60–70 |
| 711 | -N + $(CH_3)_3$ | ≥ 3.7 | 0.315–1.2 | ≥ 95% | 50–60 |

## 2. Materials and methods

### 2.1. *Raw materials and strains*

The biomass feedstock, raw corncob, was provided by Jiangsu Kangwei Co., Ltd. The raw corncob was air-dried followed by fine milling to 20–40 mesh. The composition of raw corncob was analyzed as 35.4 % cellulose, 29.5 % hemicellulose, and 19.2 % lignin by the procedure of the National Renewable Energy Laboratory (NREL) method (Sluiter et al., 2010).

The resins were obtained from Shanghai Huazhen Technology Co., Ltd. Three of them, D202, D303, and D315 are macroporous resins. 335 and 711 are weak and strong gel-type anion-exchange resins respectively. Table 1 lists the physical and chemical parameters of the resins.

*G. oxydans* was kept in sorbitol-agar medium (50 g/L sorbitol, 10 g/L yeast extract, and 20 g/L agar) at 4 ℃ before inoculation, and grown in a seed culture medium containing 100 g/L of sorbitol and 10 g/L of yeast extract at 30 ℃ under shaking at 220 rpm for 24 h. The proliferated cells were recovered by centrifugation at 5683 g of RCF (relative centrifugal force) for 5 min and then inoculated into acidic lignocellulose hydrolysate at a cell density of 6 g/L. All the chemicals were purchased from Sigma Co., Shanghai, China.

### 2.2. *Preparation of acidic lignocellulose hydrolysate*

The hydrolysis of corncob was carried out in a stainless steel autoclave. The corncob was combined with dilute sulfuric acid solution (1%, w/w) with a solid to liquid ratio of 1:5 for 30 min at 150 ℃ (Pedraza et al., 2016). The liquid part of the pretreatment mixture was collected using a vacuum filtration system with a filter paper pore size of 30–50 μm, and kept at 4 ℃ for subsequent treatment.

### 2.3. *Pretreatment of resins*

Certain pretreatments were carried out before the resins can be used in subsequent experiments. For macroporous resins, they were rinsed with ultrapure water to remove salts and other impurities, then soaked with 75 % ethanol for 2 h. For gel-type anion-exchange resins, the pretreatment process consists of soaking with 1 M NaOH, 1 M HCl, and 1 M NaOH, shaking at 150 rpm for 2 h at room temperature in a constant temperature water bath, and then they were washed with ultrapure water. The moisture content of the resins was measured by Infrared Moisture Determination Balance FD-720.

### 2.4. *Resin treatment of acidic hydrolysate of corncob*

Resins were used to adsorb the acetic acid and other inhibitors from the acidic hydrolysate of corncob (AHC). Before the resin treatment experiments, AHC was first neutralized with $CaCO_3$ to pH 5.5 and then centrifuged at 5683 g for 5 min. Thereafter, varying dosages of 2.5 %, 5 %, 10 %, and 15 % (w/v) of the resin were added into the AHC and neutralized AHC. The mixture placed in a 250 mL flask was covered with aluminum foil and incubated at 150 rpm for 4 h at 30 ℃ in a constant temperature bath oscillator (Yi et al., 2019). The resins were filtered out and the filtered hydrolysate was analyzed for sugars and inhibitors.

### 2.5. *Whole-cell catalysis and recycling of G. Oxydans cells*

The resin detoxified AHC was fermented to XA with whole-cell catalysis by *G. oxydans*. 0.5 g/L $MgSO_4$, 1 g/L $KH_2PO_4$, 2 g/L $K_2HPO_4$, 2 g/L $(NH_4)_2SO_4$, 10 g/L yeast extract were used in the fermentation media (Zhou et al., 2017). Whole-cell catalysis of XA was performed in a 250 mL shaken flask at 30 °C and 220 rpm.

The *G. oxydans* cells were recovered after each catalytic round by centrifugation at 5683 g for 5 min, and the catalytic time was 6 h for each round (Du et al., 2021). Subsequently, the recovered cells were then injected straight into the next batch of fermentation broth.

### 2.6. *Analytical methods*

The quantitative determination of inhibitors (acetic acid, levulinic acid, furfural, and 5-hydroxymethyl-furfural 5-HMF) and carbohydrate sugars (xylose, glucose, and arabinose) were determined using high-performance liquid chromatography (Agilent 1260, USA) equipped with Aminex HPX-87H column, and the column temperature was set at 55 ℃. The mobile phase was 5.0 mM $H_2SO_4$ solution at a flow rate of 0.6 mL/min.

XA were analyzed by high-performance anion-exchange chromatography (Thermo, ICS-3000, USA) equipped with the CarboPac™ PA200 column, and the column temperature was set at 30 ℃. The mobile phase was 200 mM NaOH solution at a flow rate of 0.3 mL/min. The adsorptive capacity qe of the resin was calculated as the following equation: $qe\ (mg/g) = V \times (C0 - Ce)/m$.

C0 and Ce are the initial concentration and the equilibrium concentration; V represents the volume of solution; m represents the mass of dry adsorbent (Yu and Christopher, 2017). Three parallel assays were performed for each experiment to ensure the reliability of results due to the difference in the experimental data.

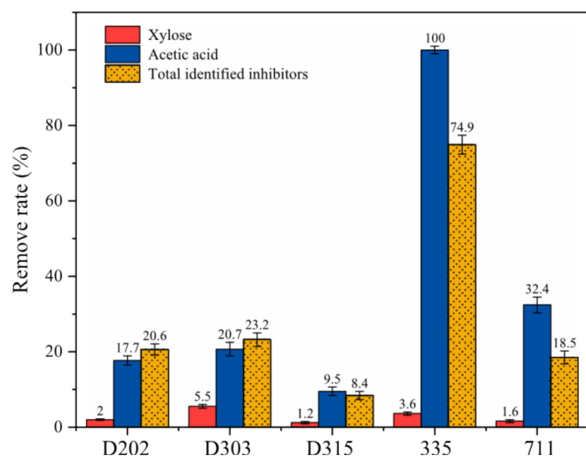

**Fig. 1.** Adsorption performance of resins on xylose, acetic acid, and total identified inhibitors in model solution.

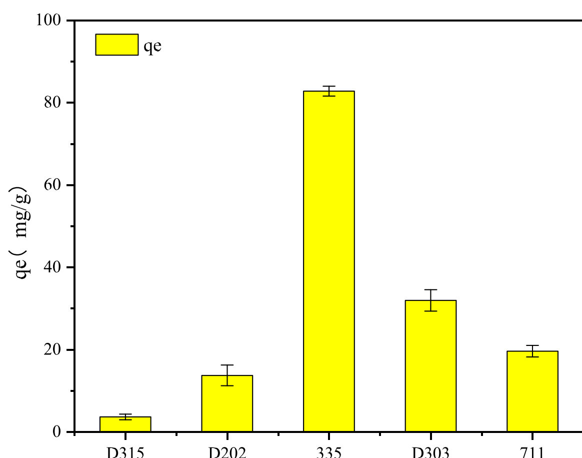

**Fig. 2.** Adsorption capacity (qe) of different resins for acetic acid.

## 3. Results and discussion

### 3.1. *Resin screening with model solution*

The adsorption performance of resins to acetic acid and other inhibitors in the model solution (MS) was first investigated to screen out suitable resin for the detoxification of real acidic hydrolysate (Demmelmayer et al., 2020). The five resins selected in this work are commonly used in organic acid refining, wastewater treatment, and sugar liquid decolorization in laboratory research and industrial production, and the industrial production of these resins is relatively mature and can be adapted to large-scale industrial XA production in the future. The MS was composed of 45.0 g/L xylose, 7.0 g/L acetic acid, 1.0 g/L levulinic acid, 1.0 g/L HMF, and 3.0 g/L furfural, which is consistent with the corresponding substance content in the real acidic lignocellulosic hydrolysate. The resin treatment was carried out at 150 rpm, 30 ℃, 1 h, and 5 % (w/v) dosage in a shaking water bath. The removal rate of xylose, acetic acid, and total identified inhibitors in MS was shown in Fig. 1. Obviously, it can be seen that 335 resin had the best removal effect on acetic acid, which can completely remove the acetic acid in the MS, and the removal rate of the total identified inhibitors was also close to 80%. Meanwhile, the xylose loss was held at about 5 %. After adsorption, the composition of the MS was 43.36 g/L xylose, 0 g/L acetic acid, 0.59 g/L levulinic acid, 0.68 g/L HMF and 1.98 g/L furfural. The performance of the three macroporous adsorption resins selected in this experiment was not satisfactory, which may be because they did not have strong interaction with inhibitors to adsorb inhibitor components from the MS. Macroporous resins D202, D303 and D315 have more internal voids and higher ion exchange rates, but are less selective than gel resins 335 and 711. 711 is a strong base anion resin, the positively charged groups of this resin can adsorb and bind to anions in solution, and the general order of inorganic acid adsorption is $SO^{2-}_4 > NO^-_3 > Cl^- > HCO^-_3 > OH^-$. 335 is a weak base type anionic resin, the general order of adsorption of inorganic acids is: $OH^- > SO^{2-}_4 > Cl^- > CH3COO^- > HCO^-_3$. Anyway, we have basically determined that the 335 resin has the strongest adsorption capacity for acetic acid. In the following sections, the performance of resins would be verified in the real acidic lignocellulose hydrolysate.

### 3.2. *Resin screening and optimization with AHC*

All five resins were evaluated again using AHC under the identical conditions to confirm the resin selection utilizing MS. Compared to MS, AHC are more complex in composition because they contain substances such

as phenolic compounds and pigments of different structures. The utilization of lignocellulose biomass largely relies on effective pretreatment process due to the strong interaction between the lignocellulose biomass components (Thiribhuvanamala et al., 2020). The most popular pretreatment method for converting hemicellulose into soluble sugars is dilute acid pretreatment. However, owing to the harsh conditions during the pretreatment process, various compounds are usually generated, such as weak aliphatic acids, furans and phenols compounds, which are poisonous and inhibitor chemicals to most bacteria (Xie et al., 2021). Therefore, polymer resins are used to detoxify the AHC for enhanced XA production. As shown in Fig. 2, 335 resins showed the best adsorption capacity for acetic acid in the AHC, this agrees with the results from adsorption in the MS. Moreover, the xylose loss rate was controlled within 10 % and the pigment or colored substances could be efficiently absorbed by 335 resin. However, acetic acid cannot be completely removed from the AHC as the MS, it is speculated that the complex components in AHC occupy the adsorption sites of resin, resulting in a small amount of acetic acid remaining in the solution. Although it is challenging to completely remove acetic acid from hydrolysate, we believe that the removal rate of acetic acid can be further improved by optimizing the processing conditions (Pang et al., 2020). Therefore, 335 resin was selected to further optimize the processing conditions to achieve the best adsorption effect.

The effect of 335 resin dose, contact time, and pH value on acetic acid and total identified inhibitors removed in AHC was evaluated. Fig. 3 illustrates that with the increase in resin dosage, acetic acid, and total identified inhibitors concentration were reduced, indicating that inhibitors content was adsorbed into the resin. As shown in Fig. 3, the acetic acid and total identified inhibitors concentration dropped very fast within the first 1 h, and afterward, it gradually decreased. Here, two pH values (1 and 6) were selected because pH 1 is the initial pH of the AHC, and pH 6 is the proper pH for *G. oxydans* cell growth and catalysis. After being treated with resin, the pH of the AHC will also rise close to the optimal catalytic pH of *G. oxydans* cell, which can greatly save the cost of the neutralizer. The studies on the optimization of 335 resin have shown, that the most efficient acetic acid and total identified inhibitors uptake at a dosage of 15 % (w/v) and lowest xylose uptake at a dosage of 2.5 % (w/v). With 5 % and 10 % dosage of resin, the acetic acid concentration was reduced to below 4 g/L and 2 g/L, and the xylose loss rate was about 10 %. However, compared to the high cost of a 15 % dosage of resin and the unsatisfactory inhibitor removal of a 2.5 % dosage, the resin dosage of 5 % and 10 % was further considered to investigate the performance of whole-cell catalysis and cell-recycling in subsequent studies.

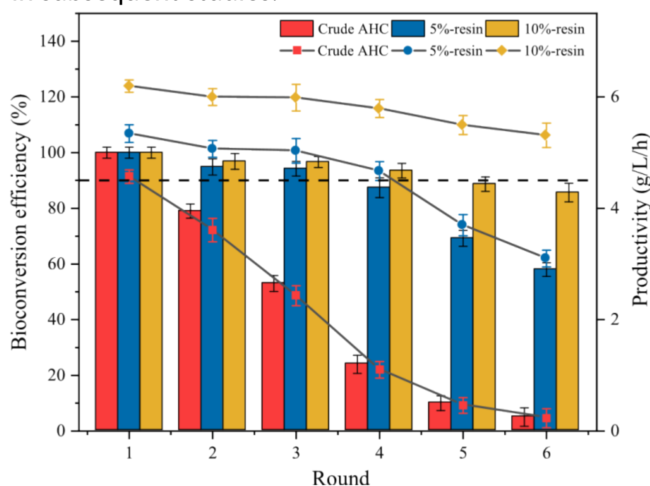


**Fig. 3.** Effect of the dosage of resin 335, contact time and pH value on the concentration of acetic acid and total identified inhibitors, and xylose loss rate (Ca represents acetic acid concentration, Ci represents inhibitor concentration and L represents xylose loss rate).

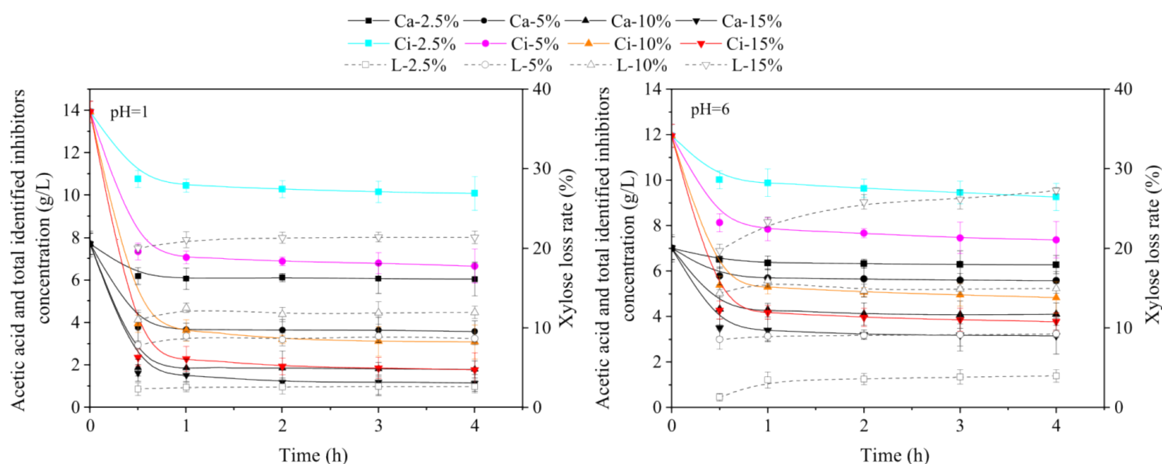


**Fig. 4.** Cell-recycling catalysis of 335 resin detoxified AHC (The column represents the bioconversion efficiency and the line represents the productivity. The dashed line represents the 90% bioconversion efficiency).

### 3.3. *Cell-recycling catalysis of resin detoxified AHC*

The cell-recycling catalysis of xylose to XA in resin detoxified AHC and undetoxified crude AHC by *G. oxydans* was investigated (Hua et al., 2019; Ke et al., 2019). As shown in Fig. 4, the bioconversion efficiency of xylose in undetoxified crude AHC presented an evident decrease, which falls 20 % in the second round and only performed 50 % bioconversion efficiency in the third round. Apparently, it was not practicable to directly recover *G. oxydans* cells from the crude ACH.

Since the cell-recycling operation is impractical from undetoxified crude ACH, we use the 335 resin to detoxify the crude ACH for improving XA bioproduction by *G. oxydans*. As can be seen from Fig. 4, resin detoxification significantly improved the fermentation performance of *G. oxydans* cells in AHC, owing to the less toxicity to the cells and the bioconversion efficiency is therefore higher. Here we set the biotransformation efficiency performance of the first round at 100 %.

With 5 % and 10 % (w/v) loading of 335 resin, the recycled cells all maintained a bioconversion efficiency of around 90% after 4 rounds and 6 rounds, respectively. 160.8 g/L XA was enriched and concentrated from 5 % resin detoxified ACH after four rounds of cell recoveries, with a productivity of 5.0 g/L/h. As 10 % resin detoxified ACH, 208.6 g/L XA was produced with a productivity of 5.8 g/L/h. Increases in the concentrations of acetic acid and other inhibitors severely affected the bioconversion performance, as shown by the results. Taking the entire cost of cell culture and operation into account, 10 % resin was selected as an appropriate and economical dosage for detoxification of AHC. In a latest study, Mao et al. overexpressed the mGDH in *Gluconobacter oxydans* and obtain 246.4 g/L XA from corn stover hydrolysate mixed with pure sugar, although the non-realistic hydrolysate environment and the complexity of gene overexpression remain issues to be considered (Mao et al., 2022). Dai et al. obtained 138.6 g/L of XA with a productivity of 4.48 g/L/h using activated carbon for detoxifying corncob hydrolysate (Dai et al., 2020), while the cost of activated carbon is considered to be higher and difficult to regenerate. Zhang et al. produced 66.42 g/L of XA with a productivity of 2.31 g/L/h from the cellulosic ethanol distillation stillage after bio-detoxified with *A. resinae* ZN1 (Zhang et al., 2017). And none of these studies involved the recovery of cells. In the microbiology industry, the cost of culturing cells often accounts for a large portion of the overall cost. Huang et al. obtained 44.4 g/L of XA within 48 h from black liquor detoxified by polystyrene divinylbenzene resin. However, the yield and productivity of XA in this study were not sufficient for industrial production (Huang et al., 2020). When it comes to other strains, the XA production was even more significantly reduced. According to a recent research, Herrera et al. obtained 11.1 g/L XA from sugarcane bagasse hydrolysate with a productivity of 0.32

g/L/h using an engineered *Zymomonas mobilis* (Herrera et al., 2021). Thus, 335 resin detoxification appears to be a feasible method for improving the recycling-cell catalysis and bioproduction of XA from AHC.

### 3.4. *Exploration on the reuse efficiency of regenerated resin*

On the basis that cell-recycling has brought the potential cost reduction, we carried on the exploration of resin reuse efficiency to further reduce the cost and provide a practical and economical process for industrial production of XA (Edgar and Boyer, 2021). For anion-exchange resins 335, the regeneration method provided by the manufacturer is repeated washing with several times the volume of acid and alkali. Moreover, after this operation, the reuse of the resin 335 still did not reach the adsorption performance of the first time, if the performance of the first time was set at 100 %, the second time was only about 90 %, which was not significantly different from the effect of the method used in this experiment. For industrial production, this will undoubtedly increase the cost of resin regeneration and wastewater treatment, resulting in a decrease in the utilization of the regenerated resin. The components in the hydrolysate are complex, and the resin after treating the hydrolysate adsorbs most of the inhibitors and some sugar compounds, as well as some unclear pigment molecules and colloidal substances. Therefore, various and abundant regenerating agents (acid liquor, alkali liquor, organic reagent, etc.) need to be put in for the complete regeneration of the resin. Considering the reuse efficiency and regeneration cost of the resin, we tried to regenerate the resin with water or ethanol in a 30 ℃ and 90 ℃ water bath, combined with low-concentration sodium hydroxide (1 mol/L), which can be obtained by self-supply in the electrodialysis step of separating XA. The resin regeneration process is carried out in a glass beaker sealed with tin foil. Fig. 5 displays the reuse efficiency of xylose, acetic acid, and total identified inhibitors compared with the first round under different four conditions, here we set the efficiency of the first round to 100 %. The recycled resin under high-temperature conditions had a greater recovery efficiency of more than 90 %. And the reuse efficiency of hot water treatment was not much different from that of hot ethanol. Therefore, we used hot water and sodium hydroxide to regenerate the resin and explore its reuse efficiency after multiple regenerations.

As shown in Table 2, it can be seen that after 4 rounds of reuse, the regenerated resin could still remove acetic acid and rest inhibitors, with reuse efficiencies of 74.7–75.82 % and 78.95–83.57 %, respectively. In addition, we were surprised to find that the loss rate of xylose decreased slightly with the increase of reuse times. The adsorbed substances (including acetic acid, levulinic acid, HMF, furfural and phenolic compounds) in the regenerating agent could be further extracted using filtration, distillation, and other combined techniques due to their differences in boiling point and molecular weight. These are all significant platform chemicals that may be used in other ways (Huang et al., 2020). In general, hot water and low-concentration sodium hydroxide, such a low-cost regenerating agent, is an economical and practical choice for the regeneration of 335 resin and has promising industrial applications for XA production.

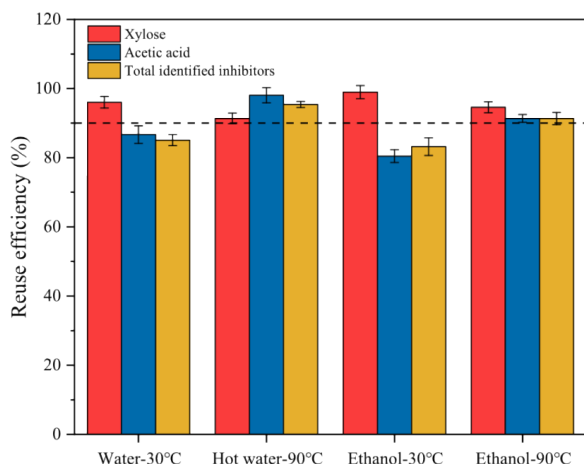


**Fig. 5.** Influence of different regeneration conditions on resin reuse efficiency (The experiment was conducted for the reuse round 1 and the dashed line represents the 90 % reuse efficiency).

### 3.5 Mass balance of the combined process for XA bioproduction

The mass of the whole combined process was balanced. As shown in Fig. 6, 1000 g corncob containing 308 g of xylan was pretreated by 1% sulfuric acid and resulted in 6.1 L of acidic corncob hydrolysate containing 248.1 g of xylose and 83.4 g of major inhibitors in the acidic hydrolysate. Then the acidic hydrolysate was detoxified by a 10 % dosage of resin 335 at 30 ℃ for 1 h. Most inhibitors were absorbed by resin 335 and the detoxified hydrolysate contained 222.5 g of xylose and 21.6 g of inhibitors was nearly the pH of 5.5 which is the most suitable pH environment for *G. oxydans* cells. The resin treatment process not only adsorbs most of the inhibitors in the hydrolysate to improve the catalytic performance of cells but also absorbs acidic substances such as acetic acid to stabilize the pH of the hydrolysate at an appropriate value, which facilitates the cost and steps of neutralizing the hydrolysate. Moreover, the resin 335 can be regenerated by simply soaking with hot water and low concentration sodium hydroxide. The low cost and high reuse efficiency of regenerated resin indicated that it has promising potential in industrial application. Finally, 208.6 g XA was obtained with a volumetric productivity of 5.8 g/L/h. The combined recycling- resin detoxification and recycling-cell catalysis technology greatly facilitate economic and environmental bioproduction of XA from lignocellulosic biomass.

**Table 2**
The reuse efficiency of regenerated resin after different round.

| | Crude AHC (g/L) | round 1 | | round 2 | | round 3 | | round 4 | |
|---|---|---|---|---|---|---|---|---|---|
| | | Concentration (g/L) | Efficiency (%) | Concentration (g/L) | Efficiency (%) | Concentration (g/L) | Efficiency (%) | Concentration (g/L) | Efficiency (%) |
| Xylose | 41.9 ± 1.2 | 36.3 ± 1.8 | 86.6 ± 5.2 | 36.6 ± 1.5 | 87.3 ± 4.5 | 37.1 ± 2.2 | 88.5 ± 3.8 | 37.5 ± 1.9 | 89.5 ± 2.6 |
| Glucose | 7.9 ± 0.6 | 6.8 ± 0.5 | 86.8 ± 4.8 | 6.9 ± 0.5 | 87.7 ± 5.3 | 6.9 ± 0.8 | 88.2 ± 2.9 | 7.1 ± 0.7 | 90.5 ± 3.2 |
| Arabinose | 7.0 ± 0.4 | 5.5 ± 0.6 | 78.2 ± 4.5 | 5.7 ± 0.7 | 80.2 ± 4.9 | 5.6 ± 0.5 | 79.3 ± 3.5 | 5.7 ± 0.4 | 80.7 ± 4.5 |
| Acetic acid | 7.7 ± 0.4 | 1.9 ± 0.3 | 75.8 ± 5.7 | 1.9 ± 0.2 | 75.5 ± 3.9 | 1.9 ± 0.1 | 74.9 ± 4.8 | 1.9 ± 0.3 | 74.7 ± 5.2 |
| levulinic acid | 1.8 ± 0.2 | 0.3 ± 0.08 | 81.5 ± 4.0 | 0.4 ± 0.05 | 80.4 ± 4.5 | 0.3 ± 0.07 | 80.9 ± 3.7 | 0.4 ± 0.05 | 80.4 ± 4.6 |
| HMF | 0.9 ± 0.2 | 0.2 ± 0.03 | 80.0 ± 3.5 | 0.2 ± 0.02 | 77.9 ± 4.5 | 0.2 ± 0.04 | 76.8 ± 4.5 | 0.2 ± 0.02 | 78.9 ± 3.8 |
| furfural | 3.7 ± 0.4 | 0.6 ± 0.05 | 83.6 ± 4.5 | 0.6 ± 0.08 | 82.9 ± 3.8 | 0.6 ± 0.12 | 82.7 ± 3.8 | 0.7 ± 0.06 | 82.1 ± 4.7 |

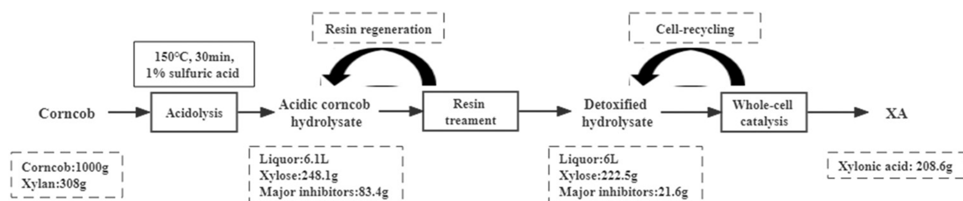


**Fig. 6.** Mass balance of XA bioproduction from corncob.

## 4. Conclusion

An economical and practical process for producing high value-added platform compound XA from lignocellulose biomass waste corncob was investigated. By using 335 resin with an optimal operating condition, acetic acid and other inhibitors in AHC were removed with minimal xylose loss, which broke through the toxic effect of inhibitors on *G. oxydans* cells, improved cell activity, and whole-cell catalytic efficiency. Finally, the cell-recycling could be carried out for 6 rounds, and 208.6 g/L XA was obtained with the productivity reaching 5.8 g/L/h. And the resin maintains 80% adsorption efficiency after 4 rounds of recycling, providing a solid basis for industrial utilization. The technology discussed in this work represents a feasible and promising future of industrial bioproduction of XA due to the high bioconversion efficiency and low cost of the feedstock, cell culture, and regenerating agent.

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## References

Cao, R., Xu, Y., 2019. Efficient preparation of xylonic acid from xylonate fermentation broth by bipolar membrane electrodialysis. Appl. Biochem. Biotechnol. 187 (1), 396–406.

Castaldo, R., Avolio, R., Cocca, M., Errico, M.E., Avella, M., Gentile, G., 2021. Amino- functionalized hyper-crosslinked resins for enhanced adsorption of carbon dioxide and polar dyes. Chem. Eng. J. 418, 129463.

Dai, L., Jiang, W., Zhou, X., Xu, Y., 2020. Enhancement in xylonate production from hemicellulose pre-hydrolysate by powdered activated carbon treatment. Bioresour. Technol. 316, 123944.

Demmelmayer, P., Hilgert, J.E., Wijaya, R., Kienberger, M., 2020. Screening of reactive extractants for the lignosulfonate extraction from lignosulfonate model solution and spent sulfite liquor. Ind. Eng. Chem. Res. 59 (37), 16420–16426.

Dong, J., Han, R., Xu, G., Gong, L., Xing, W., Ni, Y., 2018. Detoxification of furfural residues hydrolysate for butanol fermentation by Clostridium saccharobutylicum DSM 13864. Bioresour. Technol. 259, 40–45.

Du, G., Hua, X., Xu, B., Wang, H., Zhou, X., Xu, Y., 2021. The processing-module assembly strategy for continuous bio-oxidation of furan chemicals by integrated and coupled biotechnology. Green Chem. 23 (3), 1330–1336.

Edgar, M., Boyer, T.H., 2021. Removal of natural organic matter by ion exchange: comparing regenerated and non-regenerated columns. Water Res. 189, 116661.

Guo, Z., Lisbeth, O., 2015. Physiological response of Saccharomyces cerevisiae to weak acids present in lignocellulosic hydrolysate. FEMS Yeast Res. 8, 1234–1248.

Hahn, T., Torkler, S., van der Bolt, R., Gammel, N., Hesse, M., Moller, A., Preylowski, B.,¨

Hubracht, V., Patzsch, K., Zibek, S., 2020. Determining different impact factors on
the xylonic acid production using Gluconobacter oxydans DSM 2343. Process Biochem. 94, 172–179.

Herrera, C.R.J., Vieira, V.R., Benoliel, T., Carneiro, C.V.G.C., De Marco, J.L., de Moraes, L.M.P., de Almeida, J.R.M., Torres, F.A.G., 2021. Engineering zymomonas mobilis for the production of xylonic acid from sugarcane bagasse hydrolysate.
Microorganisms 9 (7), 1372.

Hua, X., Du, G., Xu, Y., 2019. Cost-practical of glycolic acid bioproduction by immobilized whole-cell catalysis accompanied with compressed oxygen supplied to enhance mass transfer. Bioresour. Technol. 283, 326–331.

Huang, C., Zheng, Y., Lin, W., Shi, Y., Huang, G., Yong, Q., 2020. Removal of fermentation inhibitors from pre-hydrolysis liquor using polystyrene divinylbenzene resin. Biotechnol. Biofuels 13 (1).

Ke, X., Lu, Y., Yu, P., Hu, Z., Chen, L., Sun, X., Zheng, Y., 2019. Glutamate addition improves the activity of membrane-bound sorbitol dehydrogenase in a pyrroloquinoline quinone-dependent manner: a feasible strategy for the cost- effective fermentation of Gluconobacter oxydans. Process Biochem. 84, 1–8.

Kumar, P., Barrett, D.M., Delwiche, M.J., et al., 2009. Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. Ind. Eng.
Chem. Res. 48 (8), 3713–3729.

Kumar, V., Krishania, M., Preet Sandhu, P., Ahluwalia, V., Gnansounou, E., Sangwan, R. S., 2018. Efficient detoxification of corn cob hydrolysate with ion-exchange resins for enhanced xylitol production by Candida tropicalis MTCC 6192. Bioresour. Technol. 251, 416–419.

Liu, J., Wu, S., Lu, Y., Liu, Q., Jiao, Q., Wang, X., Zhang, H., 2016. An integrated electrodialysis-biocatalysis-spray-drying process for efficient recycling of keratin acid hydrolysis industrial wastewater. Chem. Eng. J. 302, 146–154.

Ma, J., Zhong, L., Peng, X., Sun, R., 2016. d-Xylonic acid: a solvent and an effective biocatalyst for a three-component reaction. Green Chem. 18 (6), 1738–1750. Mao, X., Zhang, B., Zhao, C., Lin, J., Wei, D., 2022. Overexpression of mGDH in Gluconobacter oxydans to improve d-xylonic acid production from corn stover hydrolysate. Microb. Cell Fact. 21 (1).

Mhlongo, S.I., Haan, R.D., Viljoen-Bloom, M., Zyl, W.V., 2015. Lignocellulosic hydrolysate inhibitors selectively inhibit/deactivate cellulase performance. Enzym.
Micro Technol. 81, 16–22.

Nitzsche, R., Grong¨ roft, A., Kraume, M., 2019. Separation of lignin from beech wood¨ hydrolysate using polymeric resins and zeolites – Determination and application of adsorption isotherms. Sep. Purif. Technol. 209, 491–502.

Pang, H., Li, L., He, J., Yan, Z., Ma, Y., Nan, J., Liu, Y., 2020. New insight into enhanced production of short-chain fatty acids from waste activated sludge by cation exchange resin-induced hydrolysis. Chem. Eng. J. 388, 124235.

Pedraza, L., Flores, A., Toribio, H., Quintero, R., Borgne, S.L., Moss-Acosta, C., Martinez, A., 2016. Sequential thermochemical hydrolysis of corncobs and enzymatic saccharification of the whole slurry followed by fermentation of solubilized sugars to ethanol with the ethanologenic strain escherichia coli MS04. Bioenerg. Res. 9 (4), 1–7.

Rivas, B., Moldes, A.B., Domınguez, J.M., Pará jo, J.C., 2004. Development of culture´ media containing spent yeast cells of Debaryomyces hansenii and corn steep liquor for lactic acid production with Lactobacillus rhamnosus. Int. J. Food Microbiol. 97
(1), 93–98.

Sharma, N., Prasad, G.S., Choudhury, A.R., 2013. Utilization of corn steep liquor for biosynthesis of pullulan, an important exopolysaccharide. Carbohydr. Polym. 93 (1),
95–101.

Shen, Y., Zhou, X., Xu, Y., 2020. Enhancement of gluconobacter oxydans resistance to lignocellulosic-derived inhibitors in xylonic acid production by overexpressing thioredoxin. Appl. Biochem. Biotechnol. 191 (3), 1072–1083.

Sluiter A., Hames B., Ruiz R., Scarlata C., Sluiter J., Templeton D. et al., Determination of Structural Carbohydrates and Lignin in Biomass, Laboratory Analytical Procedure (LAP), Technical Report NREL/TP-510–42618. National Renewable Energy Laboratory (NREL), US Department of Energy, Golden, CO (2010).

Soares, C., Bergmann, J.C., Almeida, J., 2021. Variable and dose-dependent response of Saccharomyces and non-Saccharomyces yeasts toward lignocellulosic hydrolysate inhibitors. Braz. J. Microbiol. 1.

Thiribhuvanamala, G., Parthasarathy, S., Ahiladevi, P., 2020. Schizophyllum commune – a promising source of lignocellulose degrading enzymes suitable for biodegradation. Mushroom Res. 29 (1), 57.

Wright, M.M., Daugaard, D.E., Satrio, J.A., Brown, R.C., 2010. Techno-economic analysis of biomass fast pyrolysis to transportation fuels. Fuel 89, S2–S10.

Wu, W., Pang, B., Yang, R., Liu, G., Ai, C., Jiang, C., Shi, J., 2020. Improvement of the probiotic potential and yield of Lactobacillus rhamnosus cells using corn steep liquor. LWT 131, 109862.

Wu, X., Wang, J., Cao, L., 2016. Characterization and adsorption performance of chitosan/diatomite membranes for Orange G removal. E-Polym. 16 (2), 99–109.

Xie, J., Chen, J., Cheng, Z., Zhu, S., Xu, J., 2021. Pretreatment of pine lignocelluloses by recyclable deep eutectic solvent for elevated enzymatic saccharification and lignin nanoparticles extraction. Carbohydr. Polym. 269 (2), 118321.

Yi, X., Gao, Q., Zhang, L., Wang, X., Bao, J., 2019. Heterozygous diploid structure of Amorphotheca resinae ZN1 contributes efficient biodetoxification on solid pretreated corn stover. Biotechnol. Biofuels 12 (1).

You, X., Wang, X., Liang, C., Liu, X., Wang, S., 2019. Purification of hemicellulose from sugarcane bagasse alkaline hydrolysate using an aromatic-selective adsorption resin.
Carbohydr. Polym. 225, 115216.

Yu, Y., Christopher, L.P., 2017. Detoxification of hemicellulose-rich poplar hydrolysate by polymeric resins for improved ethanol fermentability. Fuel 203, 187–196.

Zhang, H., Han, X., Wei, C., Bao, J., 2017. Oxidative production of xylonic acid using xylose in distillation stillage of cellulosic ethanol fermentation broth by Gluconobacter oxydans. Bioresour. Technol. 224, 573–580.

Zhou, X., Lü, S., Xu, Y., Mo, Y., Yu, S., 2015. Improving the performance of cell compressed-oxygen-supplied sealed system and cell-recycle technique. Bioresour. biocatalysis and the productivity of xylonic acid using a compressed oxygen supply. Technol. 244 (2017), 1137–1141.

Biochem. Eng. J. 93, 196–199.

Zhou, X., Zhou, X., Tang, X., Xu, Y., 2018. Process for calcium xylonate production as a

Zhou, X., Zhou, X., Xu, Y., 2017. Improvement of fermentation performance of concrete admixture derived from in-situ fermentation of wheat straw pre- Gluconobacter oxydans by combination of enhanced oxygen mass transfer in hydrolysate. Bioresour. Technol. 261, 288–293.