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In-Situ Vacuum Distillation of Ethanol Helps To Recycle Cellulase and Yeast during SSF of Delignified Corncob Residues

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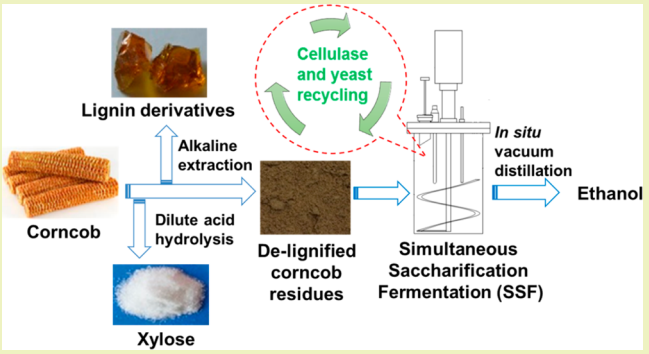
ABSTRACT: The high cost of the lignocellulose-degrading enzyme is one of the major bottlenecks for economically producing cellulosic ethanol. To overcome this bottleneck, we performed *in-situ* vacuum distillation (at 50 °C, 77 mmHg for 30 min) to remove ethanol after simultaneous saccharification and fermentation (SSF) of delignified corncob residues (DCCR). The cellulase and yeast cells left in the stillage were reused by adding fresh DCCR to start a new cycle. This process cycle was repeated five times. Both cellulase and yeast cells were reused five times with a significant cellulase reduction from 16.3 mg of enzyme protein (EP)/g cellulose to as low as 3.3 mg EP/g cellulose. Meanwhile the ethanol in the fermentation broth was maintained as high as 40 g/L before each vacuum distillation. The final ethanol yield after five cycles reached 72.7%. The process economic analysis based on the Aspen Plus model indicated that the cost of the saved cellulase was much higher than that of the additional energy needed to carry out the vacuum distillation. Almost 24% of the ethanol production cost could be reduced by using this new approach compared to batch SSF without cellulase recycling.



KEYWORDS: *Delignified corncob residues (DCCR), Enzyme recycling, Yeast recycling, Vacuum distillation, Ethanol, Microbial fermentation, Economic analysis, Biofuel*

INTRODUCTION

Although several cellulosic ethanol plants (2nd generation biorefineries) have been constructed in the past few years, the current low price of crude oil and cheap shale gas made it even more difficult to produce biofuels cost-competitive with petroleum fuels.[1] For this reason, construction of more biorefineries is postponed or has been canceled.[2] Biomass-degrading enzymes (cellulase and hemicellulase) that are used for hydrolyzing cellulose and hemicellulose present in lignocellulose into fermentable sugars are the most expensive components apart from feedstocks.[3] The cost of cellulase was estimated to range between $0.10 and $1.47 per gallon of ethanol, accounting for 15−50% of the total cellulosic ethanol production cost.[3−5]

A number of innovative methods were developed to reduce the cost of cellulase to make cellulosic ethanol cost-competitive.[6−12] Some of these studies focused on recycling cellulase from three process streams, including the following: (1) the hydrolysate slurry after enzymatic hydrolysis,[9−13] (2) the fermentation broth after simultaneous saccharification and fermentation (SSF),[6] and (3) the stillage from the distillation column.[14] Recovering cellulase present in hydrolysate (hydrolyzed sugar stream) using ultrafiltration was found to be too expensive, and only part of the enzymes that were originally loaded could be recovered, since some enzymes are irreversibly bound to the solid portion.[9] Partial enzymes reversibly bound to unhydrolyzed solid (UHS) residues could be recycled by removing the liquid hydrolysate and adding fresh substrates for a subsequent batch of hydrolysis.[10,11,13] Cellulase could also be recycled by reusing a small amount of SSF broth, but xylose and lignin accumulation will cause a negative effect on the next round of SSF.[6]

Recycling the cellulase present in the stillage requires vacuum distillation conducted at low temperatures (below 55 °C) to avoid denaturing the cellulase activity. This approach will help to reuse cellulase distributed both in solids and supernatants without laborious solid−liquid separation steps. Earlier studies on vacuum fermentation removed ethanol to alleviate toxicity to the fermenting microorganisms; thus continuous fermentation with yeast-cell reuse could be employed.[15,16] However, an

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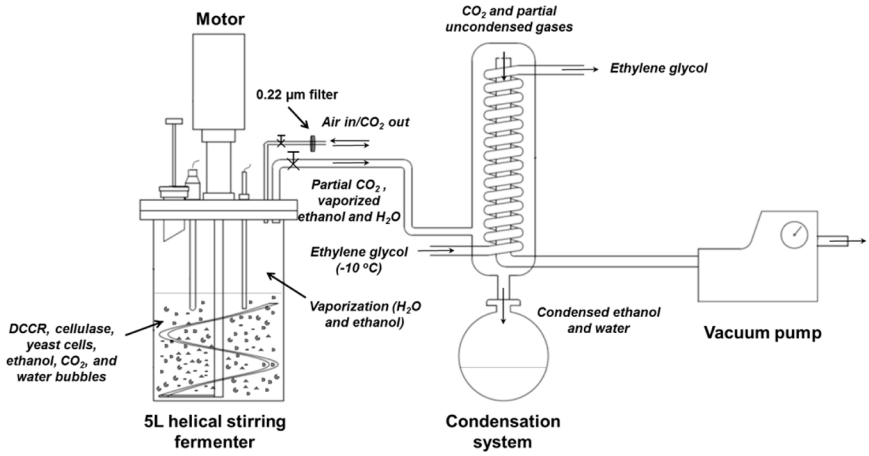
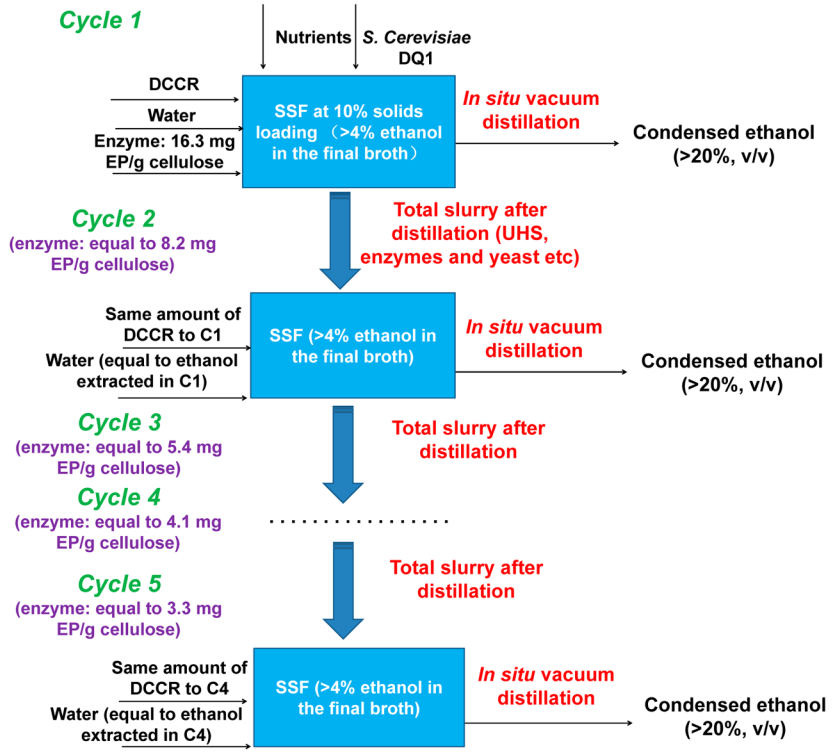


(a) Experimental setup of the SSF with *in situ* vacuum distillation.



(b) Flow diagram of the SSF with *in situ* vacuum distillation for cellulase and yeast recycling.

Figure 1. (a) Experimental setup of the SSF with *in-situ* vacuum distillation. (b) Flow diagram of the SSF with *in-situ* vacuum distillation for cellulase and yeast recycling.

ethanol criterion of 40 g/L required for economically separating ethanol from broth had never been reached using the lignocellulosic biomass as substrates,[15−17] which led to the incorrect conclusion that the vacuum distillation operation was uneconomical.[18] Lindedam et al. (2013) recently reported that almost 25% of the total cellulolytic enzyme activity could be recovered after vacuum distillation in a pilot-scale plant, and pointed out that the high temperature during enzymatic hydrolysis and vacuum distillation caused some deactivation to the cellulase.[14] However, the benefits from cellulase recycling over the additional energy needed in vacuum distillation is not reported.

The corncob biorefinery industry for production of xylose and its relative products has been firmly established in China.[19] The xylitol production capacity of China reached 50,000 t in 2009; meanwhile approximately 400,000 tons of corncob residues have been generated as byproducts.[20] Dealing with such a large amount of solid byproducts became a great challenge.[21] In 2012, Shandong Longlive Biotechnology Co. (Yucheng, Shandong, China), a corncob biorefinery company, was authorized by the Chinese government to produce 50,000 t/annum of cellulosic ethanol using corncob residues (www.longlive.cn). In this industrial scenario, corncob is subjected to acid hydrolysis to produce xylitol, xylo-oligosaccharide, and related products. The corncob residues are treated by alkali for lignin production. Then, the delignified corncob residues (DCCR) left behind are used for ethanol fermentation.

Since the cost of DCCR and pretreatment has been shared by the xylose-related and lignin-related products, the major costly unit operation is the enzymatic hydrolysis during ethanol

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production, especially the cellulase costs.[21] For reducing the cellulosic ethanol production cost using DCCR, this study aimed to reduce the cellulase dosage by recycling both cellulase and yeast via *in-situ* vacuum distillation, while maintaining an industrially relevant ethanol titer (more than 40 g/L) in the fermentation broth. The feasibility of recycling both cellulase and yeast via vacuum distillation was evaluated in this study using both model ethanol solution and DCCR. Furthermore, the Aspen Plus model was established on the basis of the obtained results to clarify whether or not the vacuum distillation was economical in terms of the saved cellulase and the additional energy needed.

MATERIALS AND METHODS

Raw Materials. Delignified corncob residues (DCCR) were kindly provided by Shandong Longlive Biotechnology Co. (Yucheng, Shandong, China). The corncobs were acid hydrolyzed to produce xylose-relative products, and then were extracted with alkaline to produce lignin-based resins.[22] The solids left behind mainly consisted of cellulose, and the composition was found to be cellulose (81.6%), hemicellulose (3.1%), and lignin (1.7%) on a dry weight basis analyzed using an ANKOM 200 fiber analyzer (ANKOM Technology, Macedon, NY).

Strains and Enzymes. The ethanol-fermenting microorganism was *Saccharomyces cerevisiae* DQ1 (CGMCC 2528). The cellulase enzyme Youtell 6 was purchased from Hunan Youtell Biochemical Co. (Yueyang, Hunan, China). The enzyme protein (EP) content of Youtell 6 was 90 mg/g cellulase cocktail. The filter paper activity and the cellobiase activity were 135 FPU/g and 344 CBU/g cellulase cocktail (FPU, filter paper unit; CBU, cellobiase unit).[23,24]

Yeast Seed Cultivation. A four-step yeast adaptation procedure was used to improve the inhibitor tolerance and fermentation performance of the yeast.[25] The synthetic media, 50%, 75%, and 100% DCCR hydrolysate, were used for adapting the yeast cells sequentially in flasks before inoculating into the SSF system. The culture conditions were 30 °C and 150 rpm in a shaking incubator for 15 h. The DCCR hydrolysate was prepared at 15% solids loading (w/w) and 12.3 mg EP/g cellulose (equal to 15 FPU/g DM; DM, dry matter). After hydrolysis at 50 °C for 48 h in a helical stirring bioreactor, the slurry was centrifuged at 10,000 rpm for 15 min to obtain liquid hydrolysate. The liquid hydrolysate contained 108.4 g/L of glucose and 5.6 g/L of xylose. The DCCR hydrolysate was mixed with a synthetic medium at the ratios of 0.5:0.5, 0.75:0.25, and 1:0 to prepare the 50%, 75%, and 100% DCCR hydrolysate, respectively. The synthetic medium contained 20 g/L of glucose, 2 g/L of KH_2PO_4, 1 g/L of $(NH_4)_2SO_4$, 1 g/L of $MgSO_4{\cdot}7H_2O$, and 1 g/L of yeast extract. All the media used were autoclaved at 115 °C for 30 min before use.

Batch Simultaneous Saccharification and Fermentation (Batch SSF). Batch SSF was performed in a 5 L helical ribbon stirring bioreactor.[26] The solids loading of DCCR was 24% (w/w), and the cellulase dosage was 3.3 mg EP/g cellulose and 16.3 mg EP/g cellulose (equal to 5.0 and 24.5 FPU/g cellulose, respectively). The SSF started with inoculation of the adapted *S. cerevisiae* DQ1 at 10% (v/v), and was maintained at 37 °C, 150 rpm for 108 h. The pH was automatically regulated at 4.2 using 5 M NaOH. Samples were withdrawn at regular intervals for HPLC analysis and cell viability analysis using a colony forming unit (CFU) method reported before.[27] As the fermentation performance of SSF using DCCR at a uniform 37 °C (without prehydrolysis at 50 °C) was comparable to that with prehydrolysis (at 50 °C for a few hours before yeast inoculation), the temperature during the whole SSF process was controlled at 37 °C.[19]

SSF with Both Cellulase and Yeast Recycling via *in-Situ* Vacuum Distillation (SSF with Recycling). The experimental setup for SSF with both cellulase and yeast recycling via *in-situ* vacuum distillation (mentioned as SSF with recycling hereafter) is shown in Figure 1a. The 5 L fermenter was connected to a vacuum pump through a condensation system. During SSF, the check valve connected to the condenser (named V_c) was closed, but the check valve connected with a 0.22 μm filter (named V_f) was open to release the generated carbon dioxide. During vacuum distillation, the V_f was closed while V_c was open. The vacuum pump and the condensation system started to work and maintained the fermenter at 50 °C and 77 mmHg. The vaporized ethanol and water, as well as carbon dioxide, were pumped into the condensation system circulated with ethylene glycol at −10 °C, where the ethanol and water were condensed into the collector. The carbon dioxide and some uncondensed vapor were pumped out. After 30 min of vacuum distillation the V_c was closed, and V_f was opened slowly to restore the pressure inside the fermenter to atmospheric pressure. When one cycle was completed, the next cycle was started with the addition of a new batch of DCCR.

The flow diagram of SSF with recycling is shown in Figure 1b. Cycle 1 started with SSF at 10% solids loading (w/w), 10% (v/v) inoculation ratio of *S. cerevisiae* DQ1, and 16.3 mg EP/g cellulose at 37 °C, pH 4.2, and 150 rpm. Once the ethanol concentration reached 40 g/L in the fermenter, 30 min of vacuum distillation (50 °C and 77 mmHg) was conducted to remove the ethanol. After that, the same amount of DCCR in cycle 1 and an equal volume of fresh water to the distilled ethanol in cycle 1 were added into the fermenter; then, cycle 2 started without additional cellulase and yeast seed culture supplementation. The cycle was repeated five times. Samples were taken at regular intervals for HPLC analysis, cell viability analysis, and relative cellulase activity analysis.

Determination of the Relative Cellulase Activity during SSF with Recycling. The relative cellulase activity in the SSF with the recycling process was evaluated by digesting the fresh DCCR. Samples were taken at 36, 72, and 109 h time points after the completion of each cycle (cycle 1, cycle 2, and cycle 3), and fresh water was added into the system (before the new batch of DCCR was added). At these time points, glucose and ethanol in the system were low enough not to interfere with the enzymatic hydrolysis assays. The whole slurry, liquid, and solid portions were each analyzed. Samples of about 2 mL were taken during SSF and divided into three parts. Samples of about 1 mL were centrifuged at 4,000 rpm for 5 min to separate the solid and liquid portions, and then the citric acid buffer (50 mM, pH 4.8) was supplemented to a final volume of 1 mL for both portions. The other 1 mL samples without centrifugation were used as the whole slurry. About 0.5 mL of the whole slurry, diluted liquid, and solids portions were added into 50 mL flasks containing DCCR (2% solids loading, w/w). A 40 μL portion of tetracycline (10 mg/mL) was added to avoid microbial contamination. The total enzymatic hydrolysis volume was 10 mL supplemented with 50 mM citric acid buffer (pH 4.8). The enzymatic hydrolysis was conducted at 50 °C and 150 rpm in a water-bath shaking incubator for 48 h. The whole slurry, diluted liquid, and solids portions without fresh delignified corncob residues were used as controls. These enzymatic hydrolysis assays were conducted in duplicate. The glucan conversion was calculated and considered positively correlated to the cellulase activity.

Determination of Sugars, Ethanol, and Inhibitors. Glucose, ethanol, acetic acid, lactic acid, furfural, and HMF contained in samples were analyzed using HPLC (LC-20AD pump, RI detector RID-10A, Shimadzu, Kyoto, Japan) with a BioRad Aminex HPX-87H column operated at 65 °C and 0.6 mL/min of 5 mM H_2SO_4 as the mobile phase. The ethanol yield was calculated using the method described by Zhang and Bao (2012).[28]

Establishment of the Aspen Plus Model. Aspen Plus software (AspenTech Co., Cambridge, MA) was used for the flow-sheet simulation. All the components and the physical property data used in the model were either from the built-in database of the Aspen Plus system or from the published NREL (National Renewable Energy Laboratory) data.[3] The NRTL (nonrandom two-liquid) equation was selected as the base thermodynamic method, and the major assumptions included were as follows: (1) Process was operated at a continuous mode neglecting the plant start and shutdown, (2) Only glucose was converted into ethanol, although xylan was also hydrolyzed into xylose, (3) Components of lignin and extractives in DCCR were not hydrolyzed and did not contribute to ethanol fermentation, (4) Though SSF was carried out in one reactor during the experiments, two separate reactors (saccharification reactor and

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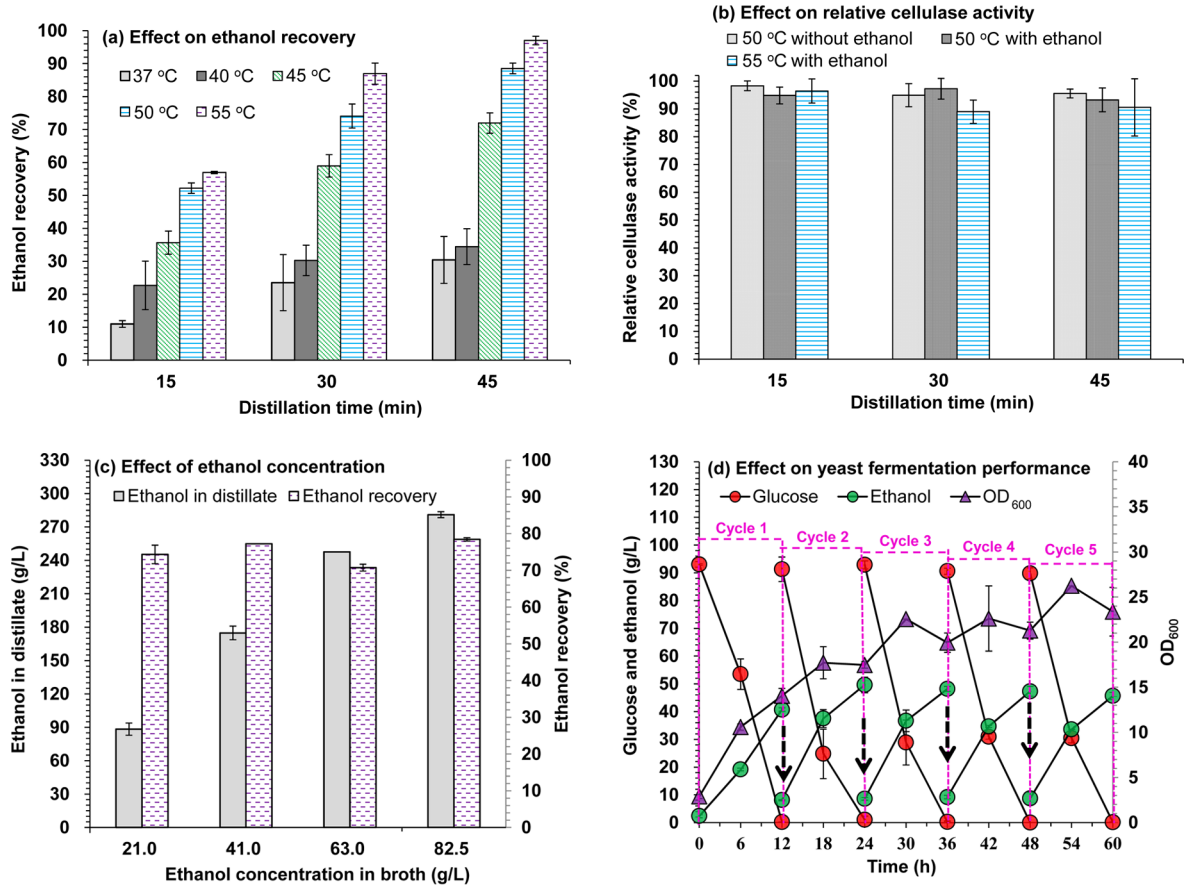

Figure 2. Effect of vacuum distillation using model ethanol solution. Here, (a) Effect of different vacuum distillation conditions on ethanol recovery, (b) Effect of cellulase activity as a function of vacuum distillation condition, (c) Effect of recovered ethanol concentration as a function of ethanol concentration in the fermentation broth and (d) Five cycles of fermentation with yeast-cell recycling using glucose as substrates. For experiments a and b, the model ethanol solution (56 g/L) and the model ethanol−cellulase solution (56 g/L ethanol supplement with 3.3 mg EP/mL, close to the cellulase concentration at 16.3 mg EP/g cellulose) were prepared using 0.1 M citric acid buffer (pH 4.8). After distillation, the cellulase activity was tested by digesting the DCCR as described in the Materials and Methods section. Vacuum distillation conditions for experiment c, 50 °C, 76 mmHg for 30 min. Fermentation conditions for experiment d, 37 °C, 150 rpm with 10% (v/v) yeast inoculation. The vacuum distillation was the same as that in experiment c. After distillation, the dry glucose powder was supplemented to a final concentration around 100 g/L, and the same amount of fresh water to distilled ethanol was added to the system. The black arrows refer to the four vacuum distillation cycles and the following glucose supplementation. The cycle including fermentation and vacuum distillation repeated five times.

fermentation reactor) were designed in the model to facilitate the different conversion ratio settings, (5) In the vacuum model, the cellulase recycle stream was not connected to the saccharification reactor to realize a calculation convergence and (6) Heat dissipation of the equipment was neglected.

RESULTS AND DISCUSSION

Evaluation of the Feasibility of Cellulase and Yeast Recycling via Vacuum Distillation Using the Model Ethanol Solution. To separate ethanol effectively and maintain more active cellulase components and viable yeast cells for reuse in the following cycles, we optimized the vacuum conditions and evaluated the effect on the yeast-cell viabilities using the model ethanol solution. Figure 2a shows that the ethanol recovery was dependent on both temperature and time. When the vacuum distillation operated at 50 °C for 45 min, or 55 °C for longer than 30 min, higher than 90% ethanol recovery was reached. These conditions were further used to evaluate their effects on cellulase activity (Figure 2b). Cellulase Youtell 6 was found to be highly stable in the tested conditions. About 90% of original cellulase activity could be retained after completing the vacuum distillation. Although Skovgaard et al. (2014) mentioned that formation of gas−liquid interfaces during distillation would be harmful to the cellulase activity, aside from temperature and ethanol, the shorter distillation time (within 45 min) helped to overcome this problem.[29]

Figure 2c shows that ethanol concentration in the distillate was positively correlated to the ethanol in the feed, but the ethanol recovery was almost the same (close to 80%) after distillation. This means that higher energy is required to rectify the distilled ethanol to a fuel grade if the ethanol concentration was lower in the broth.[17] Therefore, high-solids-loading enzymatic hydrolysis or SSF is always preferred to obtain high-concentration ethanol in the fermentation broth.[26,30]

Figure 2d shows that the fermentation performance was not affected when the yeast cells were reused in five cycles including fermentation following vacuum distillation (77 mmHg, 50 °C for 30 min). The yeast cells kept growing during the whole process. Glucose was consumed after 12 h of fermentation, and almost 40 g/L ethanol was generated (ethanol in the fermentation broth was about 50 g/L in the later 4 cycles, because around 10 g/L ethanol was left after each distillation) in each cycle with a similar ethanol productivity [3.19, 3.46, 3.30, 3.18, and 3.08 g/(L h), respectively, for the five cycles]

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and a similar ethanol metabolic yield (80.9%, 90.1%, 83.8%, 82.5%, and 80.8%, respectively, for the five cycles). The results here also indicated that *S. cerevisiae* DQ1 was a heat-tolerant yeast, which was originally evolved for high-temperature fermentation.[31] Considering the contribution of yeast preparation to the energy consumption and greenhouse gas emissions to cellulosic ethanol production, the yeast reuse might be another great merit of *in-situ* vacuum distillation besides cellulase recycling.[32]

To summarize, vacuum distillation operation at 50 °C and 77 mmHg for 30−45 min could obtain an ethanol recovery of 80% higher with a minor effect on the cellulase activity and yeast fermentation performance. We subsequently applied a similar vacuum distillation to recycle both the cellulase and yeast during the SSF of DCCR.

SSF with Both Cellulase and Yeast Recycling via *in-Situ* Vacuum Distillation. To reach the 40 g/L ethanol in the fermentation broth, at least 10% solids loading (w/w) was required for SSF. (Assuming that ethanol yield was 80% based on the theoretical ethanol yield, then ethanol concentration should be 10% × 81.63% × 1.111 × 0.51 × 80%/90% × 1000 = 41.11 g/L.)[17] The SSF with recycling is shown in Figure 1b. Cycle 1 started with 10% solids loading (w/w) and a cellulase dosage of 16.3 mg EP/g cellulose at 37 °C and 150 rpm. Once the ethanol concentration reached 40 g/L in the SSF fermenter, vacuum distillation (50 °C, 77 mmHg) was conducted for 30 min to extract the ethanol in time. After that, the same amount of DCCR as in cycle 1 and fresh water (equivalent to the distilled ethanol) were added to the fermenter to start cycle 2 with total cellulase and yeast-cell recycling. The cycles were repeated five times without additional cellulase and yeast seed culture supplementation. The total solids loading was 24% (w/w), and the final cellulase dosage was 3.3 mg EP/g cellulose calculated on the basis of the total substrates and water added into the system.

Figure 3a shows the glucose consumption and ethanol generation during SSF with recycling. In the first three cycles, we could obtain an ethanol concentration of 40 g/L after 35 h of SSF (41.3 ± 2.2, 43.8 ± 3.2, and 41.5 ± 1.6 g/L, respectively), and glucose was kept below 1 g/L. For the fourth cycle, it took 47 h to obtain an ethanol concentration of 40.2 g/L. It took an even longer time (83 h) to obtain the ethanol concentration of 41.2 g/L in the fifth cycle. The decreasing cellulase dosage (from 16.3 to 8.2, 5.4, 4.1, and 3.3 mg EP/g cellulose) with corresponding cellulase activity was responsible for the decreasing ethanol productivities [from 1.12 to 0.91, 0.80, 0.62, and 0.37 g/(L h)] in these five cycles. The relative cellulase activity in the whole SSF slurry exhibited a continuous decreasing trend, from 90% at 0 h to 74.0% at 36 h, 65.3% at 72 h, and 50.9% at 109 h. The relative cellulase activity in the solid portion was stable (around 50%). However, there was almost no cellulase activity left in the liquid portion after 109 h. Even though the lignin content in DCCR was low, cellulase components like endoglucanases and exoglucanases were inclined to adsorb to the UHS reversibly, which was reported to be beneficial to the cellulase stability.[29] The adsorbed cellulase would desorb to the liquid and start to hydrolyze the cellulose again if the fresh substrates were added, but a substantial portion of cellulase activity was lost due to the heat, ethanol, and other factors during enzymatic hydrolysis and fermentation.[14,33] The results here also indicated that the solid-portion recycling during enzymatic hydrolysis or SSF should be the preferred option other than the liquid portion, neglecting

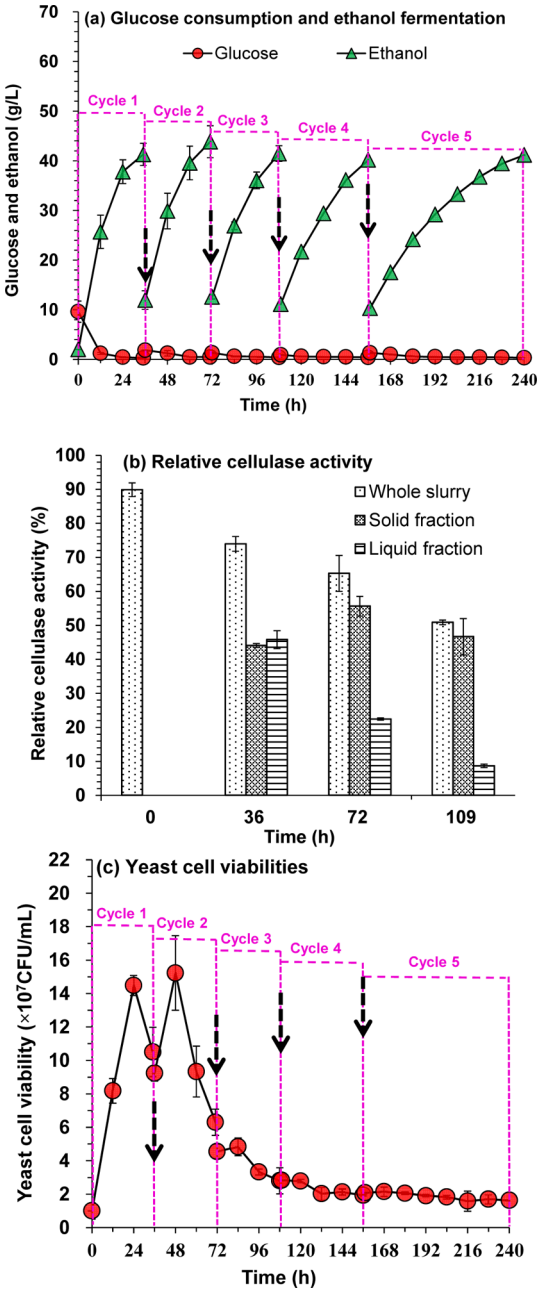

Figure 3. SSF with cellulase and yeast-cell recycling using DCCR. Here, (a) Glucose consumption and ethanol fermentation, (b) Relative cellulase activity and (c) Yeast-cell viabilities. The black arrows refer to the four cycles of vacuum distillation and the following DCCR supplementation.

the fact that the UHS accumulated after several cycles of solids recycling.

The yeast-cell viabilities during SSF with recycling were at a high level (about 1−2 × 10^8 CFU/mL) in the former two cycles, then decreased in the following three cycles and remained at 2−3 × 10^7 CFU/mL until the end. This trend was quite different from that in the glucose media in Figure 2d, possibly because of the inhibition of phenolic compounds present in DCCR discussed by Gu et al. (2014).[25] The glucose below 1 g/L through the whole process indicated that the viable yeast cells at this level were enough to ferment the generated glucose into ethanol. It is worthy to note that the operation of restoring the vacuum pressure in the fermenter to

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atmospheric pressure after vacuum distillation, accompanied by the filtered air coming in, provided a microaerobic condition at the beginning of the next SSF and facilitated the yeast growth and fermentation. This might be a good solution to overcome the problem associated with decreasing yeast viabilities due to oxygen deficiency encountered in the continuous vacuum fermentation.[34] In addition, *in-situ* vacuum distillation could recover 70−80% of the generated ethanol (ethanol concentration decreased from more than 40 to about 10 g/L after distillation), and the ethanol concentration in the distillate was higher than 20% (v/v), for which it was much easier to be rectified in the downstream processing.

Batch SSF as Controls. On the basis of the total DCCR added in SSF with recycling, two batch SSF experiments at the same solids loading with different cellulase dosages were designed as controls. One was 24% solids loading (w/w) at a cellulase dosage of 16.3 mg EP/g cellulose (equal to the starting cellulase dosage in SSF with recycling), and the other was 24% solids loading (w/w) at a cellulase dosage of 3.3 mg EP/g cellulose (equal to the final cellulase dosage in SSF with recycling). For batch SSF at 16.3 mg EP/g cellulose shown in Figure 4a, ethanol reached 82.5 g/L within 48 h, and no more ethanol was generated thereafter; however, glucose started to accumulate apparently from 36 h. The reason could be that the amount of viable yeast cells at this time was lower and cannot convert the released glucose into ethanol effectively (Figure 4).

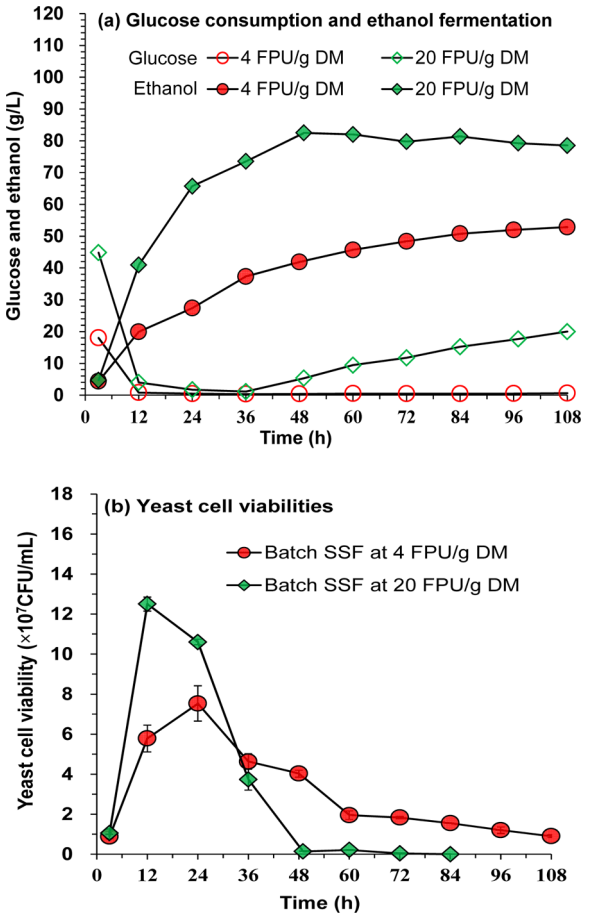

Figure 4. Batch SSF at 3.3 mg EP/g cellulose and 16.3 mg EP/g cellulose. Here, (a) Glucose consumption and ethanol fermentation and (b) Yeast-cell viabilities.

The higher ethanol concentration in combination with the high osmotic pressure at the high solids loading in batch SSF at 16.3 mg EP/g cellulose caused the death of most yeast cells. For batch SSF at 3.3 mg EP/g cellulose, ethanol was increased slowly to a final concentration of 52.9 g/L without glucose accumulation. The yeast-cell viabilities were a little lower than those in SSF with recycling, but maintained stability at the 1−2 × 10^7 CFU/mL level, which was capable of converting the released glucose into ethanol. However, the lower cellulase dosage (3.3 mg EP/g cellulose) led to a slow SSF process.

Comparison of Ethanol-Fermentation Performances between the SSF with Recycling and the Batch SSF. The results in Table 1 show that at the same cellulase dosage (3.3 mg EP/g cellulose) both the ethanol yield and productivity in SSF with recycling [72.7% and 0.67 g/(L h)] were higher than those in the batch SSF [43.2% and 0.45 g/(L h)]. When the cellulase dosage in batch SSF increased from 3.3 to 16.3 mg EP/g cellulose, the ethanol yield was still a little higher in SSF with recycling (72.7%) than that in batch SSF (66.4%), but the ethanol productivity was sacrificed [0.67 g/(L h) on average relative to 1.62 g/(L h) in batch SSF]. The ethanol produced on the basis of cellulase enzyme used in SSF with recycling was almost 2-fold higher when compared to that in the batch SSF at 3.3 mg EP/g cellulose, and five times higher when compared to that in the batch SSF at 16.3 mg EP/g cellulose. The removal of ethanol in time and the relatively low solids loading in the five cycles during SSF with recycling created a suitable environment for both cellulase hydrolysis and yeast-cell fermentation.

Table 1. Comparison of Ethanol-Fermentation Performances between SSF with Recycling and Batch SSF Using DCCR

SSF operations	ethanol yield[a] (%)	productivity[b] [g/(L h)]	ethanol yield[c] (g/g EP)
SSF with recycling[d]	72.7	0.67	125.3
batch SSF at 3.3 mg EP/g cellulose	43.2	0.45	69.9
batch SSF at 16.3 mg EP/g cellulose	66.4	1.62	22.6

[a]Ethanol yield was calculated on the basis of the theoretical ethanol yield produced from the cellulose in DCCR (Zhang and Bao, 2012). [b]Ethanol productivity here refers to the average ethanol productivity in total 240, 108, and 48 h for SSF with recycling, batch SSF at 3.3 mg EP/g cellulose, and batch SSF at 16.3 mg EP/g cellulose, respectively. The ethanol productivities in SSF with recycling were 1.12, 0.91, 0.80, 0.62, and 0.37 g/(L h) for the five cycles, respectively. [c]Ethanol yield refers to the ethanol produced per gram (g) and enzyme protein (EP) used. [d]Both cellulase and yeast cells were recycled 5 times via vacuum distillation. The cellulase protein dosage was 3.3 mg EP/g cellulose after the five cycles.

In comparison, Lindedam et al. (2013) reported that 25% of the cellulase could be recycled after SSF with an original cellulase dosage of 13 mg EP/g cellulose at industrially relevant conditions.[14] Jin et al. (2012) found that enzyme loading could be reduced from 36 to 25.8 mg EP/g cellulose using RaBIT (rapid bioconversion with integrated recycle technology) to convert the AFEX-treated corn stover into 40 g/L ethanol.[7] Currently they applied this technology to EA-pretreated (extractive-ammonia-pretreated) corn stover, and the enzyme loading was reduced by 30% to 8.4 mg EP/g cellulose while maintaining a 40 g/L ethanol concentration.[13] To our knowledge, the result obtained in this study was the lowest cellulase dosage (3.3 mg EP/g cellulose) that can reach an

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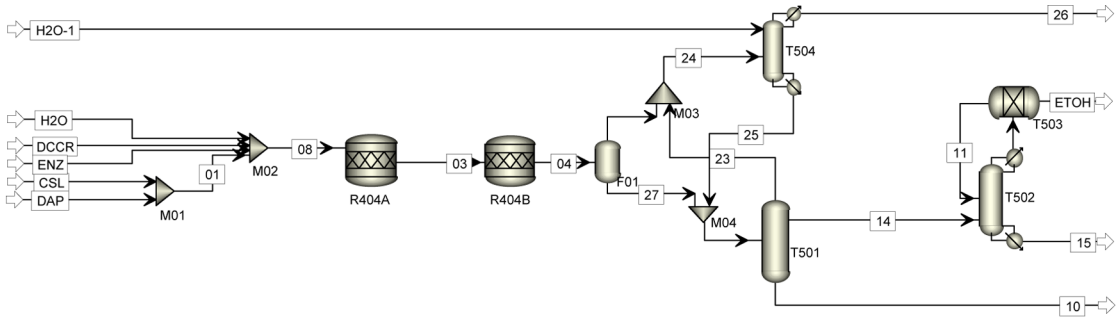
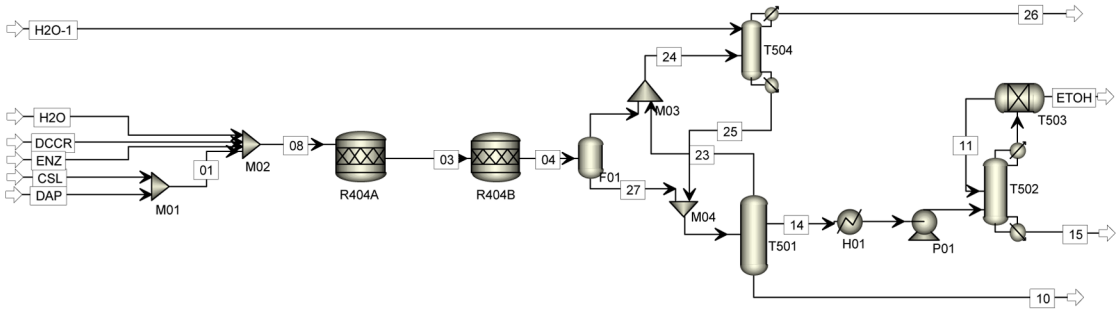


(a) Base model without cellulase recycling.



(b) Vacuum model with cellulase recycling.

Figure 5. Simulation flow sheet of the 50 000 t/annum ethanol production process using delignified corncob residues based on the Aspen Plus platform. Here, (a) base model without cellulase recycling and (b) vacuum model with cellulase recycling.

industrially relevant ethanol titer of 40 g/L. Such an improvement on the cellulase reduction could be attributed to the substrates of delignified corncob residues, which significantly reduced the dosage of nonproductive cellulase binding to lignin and enhanced the enzymatic hydrolysis efficiency.[35,36]

Process Economic Analysis of SSF with Recycling via Vacuum Distillation and SSF with Traditional Distillation. For clarification of the benefits of cellulase saving over the energy consumption in vacuum distillation, a process economic analysis was conducted on the basis of the Aspen Plus simulation model. The base model was built using SSF with traditional distillation method without cellulase recycling; the vacuum model was built using SSF with cellulase recycling via vacuum distillation. The production capacity of fuel ethanol (99.5%, w/w) was 50,000 t/annum using DCCR as substrates. The simulation flow-sheet chart and the operational parameters are shown in Figure 5 and Table 2.

Figure 5a shows the process flow sheet of the base model without cellulase recycling. The process started from enzymatic hydrolysis of DCCR in reactor R404A converting cellulose and hemicellulose into glucose and xylose by cellulase; then, the slurry was fed into the fermentation reactor R404B converting glucose into ethanol by the yeast cells. The CO_2 stream 22 was separated from the ethanol-fermentation stream 04 in a flash tank F01 and sent to a water scrubber T504 to remove the entrained ethanol. The slurry stream 27 left behind containing solid materials and ethanol was pumped into the stripping column T501 operated at 90 °C to elevate the ethanol from 4.0% to 35.0% (w/w). The distillate stream 14 was withdrawn from one side and fed into the rectification column T502 to distill to its azeotrope concentration of 92.5% (w/w). The stillage generated from column T501 containing cellulase and solid materials were dried and burned for heat and electricity generation (not shown in this flowchart). The final step was dehydration of ethanol to the fuel ethanol grade of 99.5% (w/w) in the molecule sieve column T503.

Figure 5b shows the process flow sheet of SSF with cellulase recycling via vacuum distillation. The front unit operations were similar to the base model, while the differences started from the stripping column T501 which was operated at 41.5 °C (reboiler

Table 2. Operational Conditions of Base Model without Cellulase Recycling and Vacuum Model with Cellulase Recycling in the Simulation Flow Sheet

parameter	base model	vacuum model
Stripping Column T501		
ethanol in feed (w/w, %)	4.0	4.0
ethanol in distillate (w/w, %)	35.0	35.0
operation pressure (MPa)	0.1	0.01
condenser temperature (°C)	50.0	15.0
first tray temperature (°C)	89.8	37.3
temperature of reboiler (°C)	100.1	41.5
gas flow rate at the top (m^3/h)	132.6	2,170.9
heat duty of condenser (MJ/h)	−881.7	−1,293.0
heat duty of reboiler (MJ/h)	91,576.3	58,361.1
Rectification Column T502		
heat duty of condenser (MJ/h)	−53,639.4	−53,400.3
heat duty of reboiler (MJ/h)	9,394.3	67,378.3
ethanol productivity (kg/h)	6,302.0	6,302.0
Additional Facilities		
power of vacuum pump[a] (kW h)	N/A	132.0
heat duty of condenser H01 (MJ/h)	N/A	−55,546.8
power of compressor P01 (kW h)	N/A	1.3

[a]The vacuum pump was present in the actual SSF with recycling process, but was not shown in the simulation flow sheet.

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temperature) at the vacuum condition created by a pump at the top of the stripping column T501. The ethanol distillate stream 14 from T501 with low pressure was first condensed to a liquid state in the heat exchanger H01 and pumped into the rectification column T502. The stillage stream 10 containing UHS, cellulase, and yeast cells from stripping column T501 was sent back into the SSF reactor for recycling (not shown in this flow sheet). The dehydration unit was the same as that of the base model.

The process economic analysis was calculated on the basis of two parts, including the energy consumption during ethanol separation and the cellulase used in SSF. The energy consumption consisted of the energy used in stripping column T501 and rectification column T502, as well as the vacuum pump, condenser H01, and pump P01 used in the vacuum model. The results in Table 3 show that the heat duty of reboilers (in hot steam) was 16,022.0 and 19,952.3 MJ/t ethanol, and the corresponding costs were $82.6 for the base model and $102.9 for the vacuum model. Lower heat duty was needed in the stripping column due to lower distillation temperature, but more energy was needed in the rectification column to heat the liquid to the boiling point of ethanol in the vacuum model. The steam consumed in both the base model and the vacuum model was a little higher when compared to the reported data, largely because of the steam reuse design in their models.[3,18,37] The heat duty of condensers (in cooling water) was 8,511.5 and 17,287.7 MJ/t ethanol, and the corresponding costs were $24.2 for the base model and $49.2 for the vacuum model. This difference was caused by an additional heat exchanger H01 needed in the vacuum model. The chilled water was required to cool down the vacuum-distilled ethanol; thus, the heat duty (in chilled water) was 205.2 MJ/t ethanol, and the corresponding cost was $3.0 for the vacuum model. The electricity consumed by the vacuum pump and compressor was 21.2 kW h/t ethanol, and the corresponding cost was $1.5 for the vacuum model. The total costs of the energy consumption were $106.8 per ton of ethanol production for the base model and $156.6 per ton of ethanol production for the vacuum model. Although the increment of 46.6% in energy consumption was a little higher, its net value was $49.8/t ethanol.

The cellulase cost per ton of ethanol production was calculated on the basis of the ethanol yield in SSF with recycling and batch SSF at 3.3 mg EP/g cellulose (125.3 and 69.9 g ethanol/g EP, respectively, listed in Table 1). According to the cellulase price discussed by Liu et al. (2016) ($21.6/kg EP for Youtell 6), the cellulase cost was calculated to be $172.1 and $308.5/t of ethanol, namely, $0.5 and $0.9/gal of ethanol.[38] The reduction in cellulase cost in the vacuum model was almost 44% (net value was $136/t of ethanol) relative to the base model, showing a great advantage of the current cellulase recycling method. Although the cellulase cost calculated here was still higher in comparison with those in some other reports, the calculation was based on the real experimental results without any other ideal assumptions.[3] In addition, the cellulase price is mainly dependent on the cellulase supply modes.[38] According to the simulated model established by Liu et al., in which the cellulase enzyme cost can be reduced to $0.08/gallon when the on-site production enzyme loading was as low as 5 mg/g cellulose,[38] there is still plenty of room to decrease the cellulase price by on-site cellulase production instead of purchasing from companies.

Table 3. Calculation of Ethanol Production Cost Based on the Distillation Energy Consumption and Cellulase Used in the Base Model and Vacuum Model

ethanol production cost[a] (per ton)	base model	vacuum model
Energy Consumption/Cost		
heat duty[b] (in steam, MJ)	16,022.0	19,952.3
heat duty[c] (in cooling water, MJ)	−8,511.5	−17,287.7
heat duty[d] (in chilled water, MJ)	0	−205.2
electricity power[e] (kW h)	0	21.2
steam cost[f] ($)	82.6	102.9
cooling water cost[g] ($)	24.2	49.2
chilled water cost[h] ($)	0	3.0
electricity cost[i] ($)	0	1.5
total energy cost ($)	106.8	156.6
Cellulase Used/Cost		
cellulase used[j] (kg protein)	14.3	8.0
cellulase enzyme cost[k] ($)	308.5	172.1
Energy and Enzyme Cost		
sum of energy and enzyme costs ($)	429.6	328.7

[a]The exchange rate of Chinese Yuan to U.S. dollars is 6.7. The calculation items were based on per ton ethanol produced. [b]Sum of heat duty of reboilers in columns T501 and T502. [c]Sum of heat duty of condensers in columns T501 and T502 for the base model. Sum of heat duty of condenser H01 and condenser in column T502 for the vacuum model. The temperature of cooling water was increased from 33 to 37 °C after passing through the condenser. [d]Heat duty of condenser in column T501 for the vacuum model. The temperature of the chilled water was increased from 4 to 15 °C after passing through the condenser according to Humbird et al. (2011). [e]Sum of electricity of vacuum pump and compressor P01. [f]The equation for steam cost is $\frac{[\text{heat duty}]}{Q} \times (\text{price})_{\text{steam}}$. [heat duty] here refers to the heat duty in the type of hot steam, MJ. Q is the heating value of the low-pressure hot steam used in industry; its average value is 3763 MJ/t. $(\text{price})_{\text{steam}}$ is the price of the low-pressure hot steam, its average price is about $19.40/t in north China. [g]The equation for cooling water cost is $\frac{[\text{heat duty}]_{(\text{cooling-water})}}{C \times \Delta t} \times (\text{price})_{(\text{cooling-water})}$. [heat duty] here refers to the heat duty in the type of cooling water, MJ. C is the heat capacity of water, 4.2 kJ/(kg °C). Δt is the temperature increment, and its value is 4 °C here. $(\text{price})_{(\text{cooling-water})}$ is the price of the cooling water used in industry, and its average price is about $0.0478/t. [h]The equation for chilled water cost is $\frac{[\text{heat duty}]_{(\text{chilled-water})}}{C \times \Delta t} \times (\text{price})_{(\text{chilled-water})}$. [heat duty] here refers to the heat duty in the type of chilled water, MJ. Δt is the temperature increment of the chilled water, its value is about 11 °C here. $(\text{price})_{(\text{chilled-water})}$ is the price of the chilled water used in industry, its average price is about $0.6716/t. [i]The equation for electricity cost is (electricity) × $(\text{price})_{\text{electricity}}$. (electricity) here refers to electricity that is needed in the vacuum pump and compressor, kW h. $(\text{price})_{\text{electricity}}$ is the price of the electricity used in industry, and its average price is about $0.0716/(kW h). [j]Cellulase used was based on ethanol yield listed in Table 1. [k]The equation for cellulase enzyme cost is $\frac{1000}{[\text{ethanol yield}]} \times (\text{price})_{(\text{cellulase})}$. [ethanol yield] here refers to the ethanol yield per gram enzyme protein used listed in Table 1, g/g EP. $(\text{price})_{(\text{cellulase})}$ is the price of enzyme protein. It is about $21.6/kg EP according to Liu et al. (2016).

Taking both the energy consumption costs and cellulase costs together, ethanol production costs were $429.6 and $328.7/t of ethanol for the base model without cellulase recycling and the vacuum model with cellulase recycling, respectively. The ethanol production cost can be lowered by up to 24% with cellulase recycling, which means that although vacuum distillation is an energy-consuming operation com-

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pared with the traditional distillation, the saved cost due to cellulase recycling can easily compensate for the energy cost. In addition, the benefits associated with yeast-cell recycling were not taken into consideration in the model. Taking a 50,000 t/annum cellulosic ethanol plant such as Shandong Longlive Biochem Co, Yucheng, China, as an example, the deployment of *in-situ* vacuum distillation for cellulase recycling technology would lead to a savings of around $5,000,000 per year, close to the annual government subsidies ($119 per ton of cellulose ethanol production).

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