



Short Communication

An alternative feedstock of corn meal for industrial fuel ethanol production: Delignified corncob residue



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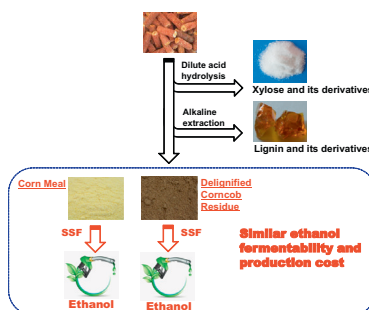
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HIGHLIGHTS

- Delignified corncob residue is competitive to corn meal in ethanol fermentation.
- Delignified corncob residue is cost effective as ethanol production feedstock.
- Single temperature profile is sufficient for whole SSF to cut cooling operation.

GRAPHICAL ABSTRACT



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ABSTRACT

Delignified corncob residue is an industrial solid waste from xylose production using corncob as feedstock. In this study, delignified corncob residue was used as the feedstock of ethanol production by simultaneous saccharification and fermentation (SSF) and the optimal fermentation performance was investigated under various operation conditions. The ethanol titer and yield reached 75.07 g/L and 89.38%, respectively, using a regular industrial yeast strain at moderate cellulase dosage and high solids loading. A uniform SSF temperature of 37 °C at both prehydrolysis and SSF stages was tested. The fermentation performance and cost of delignified corncob residue and corn meal was compared as feedstock of ethanol fermentation. The result shows that the delignified corncob residue is competitive to corn meal as ethanol production feedstock. The study gives a typical case to demonstrate the potential of intensively processed lignocellulose as the alternative feedstock of corn meal for industrial fuel ethanol production.

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1. Introduction

Among various lignocellulose feedstocks for cellulosic ethanol production, corncob residue shows advantages in its high cellulose content and no pretreatment requirement (Liu et al., 2010; Fang et al., 2010; Zhang et al., 2010a; Fan et al., 2013; Gu et al., 2014). Currently, the corncob biorefinery industry for productions of

xylose, xylo-oligomers, xylitol, or furfural has firmly established in China (Fang et al., 2010). In this industry scenario, hemicellulose is fully utilized and leaves behind the corncob residue as a solid waste. Recently, the industry tends to fractionate lignin from corncob residue using alkaline extraction as product used for resins, rubber additives and concrete additives. As the outcome, the delignified corncob residue is left and ready for further utilization. In the delignified corncob residue, the content of glucan in cellulose is higher than 70% on a dry basis and approximately equals to the starch content in corn meal (McAloon et al., 2000). Different

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from typical lignocellulose biomass treatment, no pretreatment operation is required before the enzymatic hydrolysis of delignified corncob residue.

Several studies have reported the ethanol production from lab-made delignified corncob residue. Liu et al. (2010) pretreated the corncobs with dilute acid and alkali respectively to obtain the delignified corncob residue, then obtained an ethanol concentration of 57.2 g/L and ethanol yield of 85.2% at 15% solids loading in a fed-batch mode. Zhang et al. (2010a) also prepared the delignified corncob residue in a lab scale condition and an ethanol concentration of 69.2 g/L and ethanol yield of 81.2% reached with 19% solids loading using batch SSF. Two major bottlenecks are still waiting for further solutions: (1) ethanol fermentation performance of industrial corncob product, instead of corncob residue prepared in the lab, should be evaluated; (2) well mixing of the high corncob residue solids loaded SSF should be solved by proper bioreactor design.

In this study, a direct simultaneous saccharification and ethanol fermentation (SSF) using the industrial byproduct of corncob processing after extraction of xylose and lignin, delignified corncob residue, as feedstock was investigated using a helical ribbon agitated bioreactors under various operation cases. The performance of delignified corncob residue was compared to corn meal as feedstock of ethanol fermentation. The study gives feasibility for potential of intensively processed lignocellulose as corn meal option for ethanol production.

2. Methods

2.1. Raw materials

Delignified corncob residue was kindly provided by Longlive Biotechnology Co., Yucheng, Shandong, China. The delignified corncob residue contained 72.90% of cellulose, 4.63% of hemicellulose and 7.18% of lignin on a dry basis analyzed by ANKOM 200 Cellulose Analyzer (ANKOM Technology, Macedon, NY, USA).

The corn meal was the milled powder of corn harvested in Tianjin, China. The corn meal contained 76.89% (w/w, dry base) of starch analyzed using the method described by Holm et al. (1986).

The commercial cellulase Youtell #6 was kindly supplied by Hunan Youtell Biochemical Co., Yueyang, Hunan, China. The filter paper activity and the cellobiase activity of the Youtell #6 were 135 FPU/g and 344 IU/g, respectively, assayed according to the protocol of NREL LAP-006 (Adney and Baker, 1996) and Ghose (1987).

The α -amylase HTAA and the glucoamylase GA-L NEW were purchased from Genencor International (Rochester, NY, USA). The activities of α -amylase HTAA and glucoamylase GA-L NEW were 22,000 U/mL and 100,000 U/mL, respectively.

2.2. Strain and short-term adaptation

The ethanol fermenting microorganism was the Angel instant dry yeast purchased from Angel Yeast Co., Yichang, Hubei, China. The hydrolysate used for yeast adaptation was prepared by enzymatic hydrolysis of delignified corncob residue at 15% (w/w) solids loading, 15 FPU/g DM (dry solid matter) cellulase, 50 °C, pH 4.8 and 150 rpm for 48 h in a helical stirring bioreactor described by Zhang et al. (2010b). Then the hydrolysate was centrifuged at 9000 g for 15 min to remove the solid residue and the supernatant was used for strain adaptation. The hydrolysate contained 98.16 ± 0.70 g/L of glucose and 6.49 ± 0.79 g/L of xylose. A four-step adaptation procedure of the angel yeast using the hydrolysate was conducted according to Gu et al. (2014) before the yeast culture was used as the seed of ethanol fermentation.

2.3. Simultaneous saccharification and ethanol fermentation (SSF) of delignified corncob residue

The SSF experiment was conducted in a helical stirring bioreactor at high feedstock solids loadings (Zhang et al., 2010b). The SSF started with a 12 h prehydrolysis at 50 °C, then followed by a 60 h SSF at 37 °C. The nutrients in the SSF system included 2.0 g/L of KH_2PO_4 , 1.0 g/L of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.0 g/L of $(\text{NH}_4)_2\text{SO}_4$, and 10 g/L of yeast extract. The cellulase dosage was 15 FPU/g DM and the pH was maintained at 4.8 by automatically regulation with 5 M NaOH. All the SSF experiments were duplicated except at different solids loadings, from which we could get a definite conclusion.

2.4. Simultaneous saccharification and ethanol fermentation of corn meal

Corn meal was converted to ethanol in a similar way to the industrial ethanol production processes (McAloon et al., 2000). Firstly, the dry corn meal slurry at 20% (w/w) solids loading was liquefied for 0.5 h at 90 °C with 5 U/g DM of α -amylase. Then the saccharification (pre-hydrolysis) was carried out for 5 h at 50 °C with 37.5 U/g DM of glucoamylase. Finally, the SSF stage started with the inoculation of non-adapted yeast seeds at 37 °C and the pH was maintained at 4.8 with 5 M NaOH. The SSF experiments were duplicated and the error ranges were given in tables and figures.

2.5. Analysis of sugars, ethanol, inhibitors and total phenolic content

Glucose, xylose, ethanol, and inhibitory compounds, such as furfural and HMF were determined using high-performance liquid chromatography (LC-20AD, refractive index detector RID-10A, Shimadzu, Japan) with a Bio-rad Aminex HPX-87H column at the column temperature of 65 °C. The mobile phase was 0.005 M H_2SO_4 at the rate of 0.6 mL/min. All samples were diluted properly and filtered through a 0.22 μm filter before analysis.

The total phenolic content in the whole slurries of the hydrolysate was measured with the modified Folin and Ciocalteu method (Ainsworth and Gillespie, 2007; Gu et al., 2014).

2.6. Calculation

The ethanol yield was calculated using the equation fitting for the high ethanol titer at high solids loading (Zhang and Bao, 2012):

$$\text{Ethanol yield}(\%) = \frac{[\text{Eth}] \times W}{976.9 - 0.804 \times [\text{Eth}]} \cdot \frac{1}{0.511 \times f \times [\text{Biomass}] \times m \times 1.111} \times 100\%$$

where [Eth] was the ethanol concentration in the fermentation broth (g/L); W was the total water usage during SSF process (g); f was the cellulose content in the delignified corncob residue (g/g); [Biomass] was the solids loading of delignified corncob residue in the SSF system (% w/w); m was the total weight of the SSF system (g).

3. Results and discussion

3.1. Fermentation performance of angel yeast using delignified corncob residue

The delignified corncob residue feedstock was used for ethanol fermentation by simultaneous saccharification and fermentation and the results are shown in Fig. 1. The necessity of yeast cell adaptation, the impact of feedstock solids loading, and the uniform SSF temperature were tested to obtain an optimal performance of ethanol production.

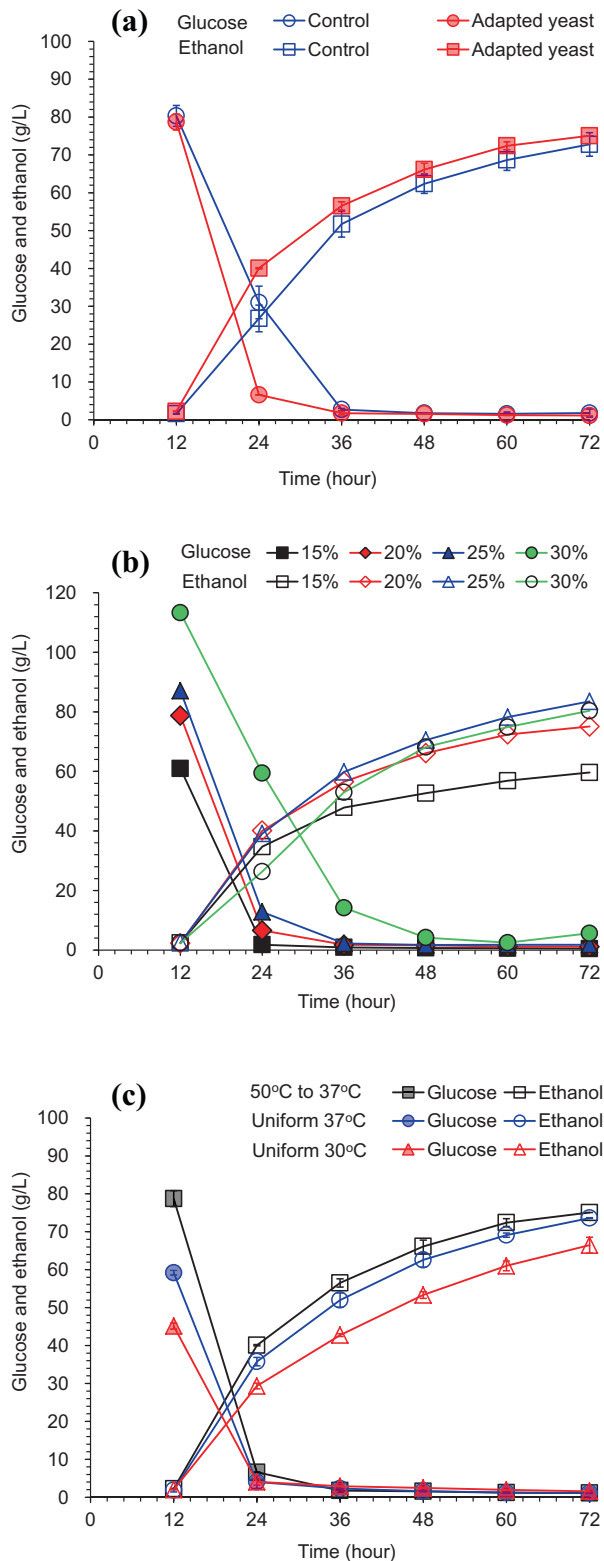


Fig. 1. Ethanol fermentation performance of angel yeast using delignified corncob residue. (a) Effect of short-term yeast adaptation; (b) effect of solids loadings; (c) effect of temperature profiles in SSF. SSF conditions for (a): solids loading 20%, cellulase dosage 15 FPU/g DM, 150 rpm, pH 4.8, yeast inoculation ratio of 10%, prehydrolysis for 12 h at 50 °C and SSF for 60 h at 37 °C; (b) all the conditions were the same as (a), except the solids loadings were 15%, 20%, 25% and 30%, respectively; (c): solids loading 20%, cellulase dosage 15 FPU/g DM, 150 rpm, pH 4.8, yeast inoculation ratio of 10%, prehydrolysis for 12 h at 50 °C and SSF for 60 h at 37 °C in the temperature shifting SSF process, while temperature in prehydrolysis and SSF stages was both kept at 37 °C or 30 °C in the uniform temperature profile, respectively. The yeast seeds were adapted as described in Section 2.

Evolutionary adaption of yeast cells is an effective way to improve the fermentation performance of yeast cells in corncob residue (Gu et al., 2014). Fig. 1(a) shows the short-term yeast adaptation on the fermentation performance of delignified corncob residue at high solids loading. The ethanol titer, the yield and the productivity using the adapted yeast cells increased comparing to those of the non-adapted cells, but not significantly: 75.07 g/L, 89.38%, 1.04 g/L/h for the adapted yeast cells, while 72.79 g/L, 84.47%, 1.01 g/L/h for the non-adapted yeast, respectively. Acetic acid, furfural and 5-hydroxymethylfurfural (HMF) were not detectable, but the total phenolics concentration was still relatively high (1.95 ± 0.16 g/L) in the hydrolysate of delignified corncob residue, although it was lower than that of non-delignified corncob residue (5.31 ± 1.24 g/L) (Gu et al., 2014). The results implied that delignification by alkaline treatment removed considerable lignin-derived phenolic compounds.

Increasing solids loading is important for increase of ethanol titer, but it in turn decreases ethanol yield due to the poor mixing and mass transfer of high solids content slurry (Zhang et al., 2010b). Fig. 1(b) shows that the ethanol concentration increased with the increasing solids loadings from 15%, 20%, to 25% at 56.64, 75.07, and 83.53 g/L, respectively, and then decreased slightly from 25% to 30% at 80.36 g/L. The ethanol yield decreased gradually with the increasing solids loading from 15%, 20%, to 30% at 96.41%, 89.38%, 78.24%, and 57.37%, respectively. Balancing the final ethanol titer and yield, the solids loading of 20% (w/w) was selected as the optimum with the ethanol titer and yield of 75.07 g/L and 89.38%, respectively.

A typical SSF process includes a prehydrolysis stage at higher temperature (45–55 °C), followed by a SSF stage at the lower temperature (30–37 °C) (Mutturi and Liden, 2013, 2014). However, a uniform SSF temperature profile in both the prehydrolysis and SSF is preferred for large scale operations because the time-consuming (hours) cooling operation is required from 45–55 °C to 30–37 °C. The ethanol fermenting strain used in this study, Angel instant dry yeast, is a popular commercial *Saccharomyces cerevisiae* yeast product for its thermo-tolerance in the ethanol fermentation industry of China. 37 °C is recommended for ethanol fermentation because both the cell growth and fermentability show the best performance (www.angelyeast.com). The SSF at the low temperature, 30 °C, was tested as a negative control to show the high temperature preference. Fig. 1(c) shows the comparison between the typical temperature shifting SSF process and the uniform temperature SSF process. When the SSF started with 50 °C at prehydrolysis for 12 h and followed by 37 °C in SSF for 60 h, the final ethanol titer and yield were 75.07 g/L and 89.38%, respectively. When the whole SSF process was carried out at a uniform temperature of 37 °C for 72 h without prehydrolysis at 50 °C, the ethanol titer and yield were 73.62 g/L and 87.37%, respectively, and no essential difference with the typical temperature shifting process. However, if the uniform SSF temperature was decreased to 30 °C, the ethanol titer and yield decreased to 66.47 g/L and 79.78%, respectively. Balancing the difference in ethanol titer and yield with the time-consuming cooling operation from 50 °C to 37 °C, the uniform SSF temperature profile at 37 °C was apparently preferable in industrial operations.

For delignified corncob residue feedstock, the optimal performance suitable for large scale operations might be in the range of 73.62 g/L of ethanol titer, 87.37% of ethanol yield at a practical uniform SSF temperature profile at 37 °C using the adapted yeast cell as seeds at 20% solids loading.

3.2. Comparison of ethanol production between delignified corncob residue and corn meal

Corn meal is the major feedstock for commercial fuel ethanol production. In this study, the ethanol fermentation performance

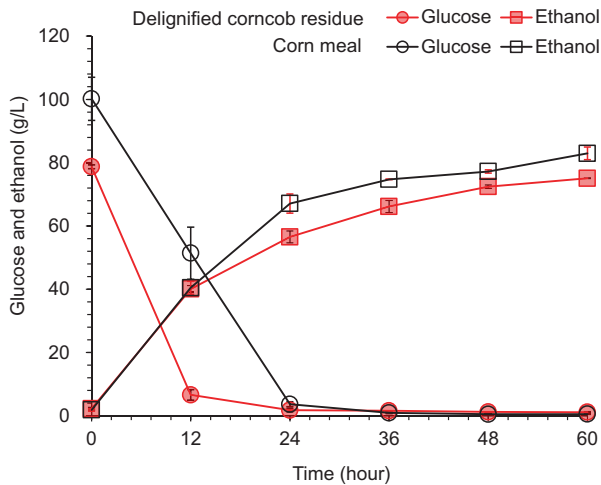


Fig. 2. Comparison of ethanol fermentation performance using delignified corncob residue and corn meal. For delignified corncob residue: 20% solids loading, cellulase 15 FPU/g DM, pH 4.8, 150 rpm, prehydrolysis at 50 °C for 12 h, and the followed SSF stage at 37 °C for 60 h, 10% inoculation ratio of the adapted yeast seeds. For corn meal: liquefaction stage, 20% solids loading, HTAA α -amylase 5 U/g DM, 90 °C, 150 rpm for 0.5 h; enzymatic hydrolysis stage, GA-L NEW glucoamylase 37.5 U/g DM, 50 °C, pH 4.6, 150 rpm for 5 h; SSF stage, 10% inoculation ratio of the non-adapted yeast seeds, 37 °C, pH 4.8, 150 rpm for 60 h. The starting point (0 h) in this figure refers to the inoculation of the yeast seeds.

using delignified corncob residue and corn meal as feedstocks was compared at the same solids loading (20%) of delignified corncob residue or corn meal in the practical range of operation procedures of each. In the corn meal case, 5 U/g dry corn meal (DM) of α -amylase and 37.5 U/g DM of glucoamylase were used for saccharification of corn meal because these enzyme dosages are commonly used in the existing bioethanol production plant (McAloon et al., 2000). Fig. 2 shows that 100.50 g/L of glucose was released from corn meal after half an hour liquefaction by α -amylase and 5 h hydrolysis by glucoamylase, which was approximately 21 g/L higher than the glucose released from delignified corncob residue after 12 h prehydrolysis (78.73 g/L) using 15 FPU/g DM (dry delignified corncob residue) of cellulase usage, but the difference was not significant. The 60 h fermentation for both feedstocks gave

the final ethanol titer and yield of 75.07 g/L and 89.38% for delignified corncob residue, and 82.95 g/L and 92.20% for corn meal feedstock, respectively. The results indicate that a similar fermentation performance were obtained for ethanol production using delignified corncob residue and corn meal feedstocks in the typical fermentation conditions for both. Corn meal showed advantages in ethanol titer and yield data over delignified corncob residue, but still not significant.

A preliminary cost estimation for ethanol production using delignified corncob residue and corn meal as feedstocks was calculated as shown in Table 1. Only substrates and enzymes were considered by assuming the operations of the two feedstocks processing were similar. If the delignified corncob residue feedstock is considered as a pure solid waste of the already profitable xylose production as in the present industry scenario (Case 1) (Zhou et al., 2013), it was more advantageous than corn meal in the cost estimation by saving 40% of the total cost (\$478.76 and \$796.81 per ton of ethanol, respectively). If the delignified corncob residue was assumed to be the same selling price with the original corncob feedstock (Case 2), the cost for the two feedstocks were almost the same (\$736.86 and \$796.81 per ton of ethanol, respectively). In addition, the cellulase cost accounted for nearly 65% of the ethanol production cost using delignified corncob residue (Case 1), while the ethanol production cost using corn meal was less 1% for enzyme.

Cellulase has been considered a major cost contributor to the lignocellulose biorefinery processes and has been demonstrated in this study. However, there will be a large space for reducing the cellulase cost with the improvement of the cellulase producing microorganisms. In Humbird et al. (2011), the enzyme cost was estimated as \$0.34/gal ethanol based on the assumption that at 20% total solids loading and 90% cellulose conversion ratio. In this study, the operational conditions meet the requirements of NREL's assumption (Humbird et al., 2010). If it comes true, the ethanol production cost will be decreased to $\frac{0.34}{1 \times 3.79 \times 0.789} \times 1000 = 113.70$ dollars per ton using delignified corncob residue (Case 1).

The cost analysis of this study was rough and preliminary because only the cost of enzymes and substrates were considered, and neglected the other factors, such as the labor, supplies, overhead, variable operating costs, depreciation of capital, and the valuable co-products (McAloon et al., 2000). However, in light of

Table 1
Comparison of ethanol production cost from delignified corncob residue and corn meal in view of enzyme and feedstock.^a

Items	Delignified corncob residue (Case 1)	Delignified corncob residue (Case 2)	Corn meal (Case 3)
Ethanol titer (g/L) ^b	75.07	75.07	82.95 ± 2.01
Ethanol yield (% of theoretical) ^b	89.38 ± 1.90	89.38 ± 1.90	92.20 ± 2.40
Feedstock consumed (ton/ton ethanol) ^c	2.71	2.71	2.49
Enzyme consumed (kg/ton ethanol) ^d	301.11	301.11	1.50 ^e
Feedstock cost (\$/ton ethanol) ^f	0	258.10	790.58
Enzyme cost (\$/ton ethanol) ^g	478.76	478.76	6.23
Total production costs (\$/ton ethanol) ^h	478.76	736.86	796.81

^a The raw materials prices, including the feedstock and enzymes, were the up to date prices at the time of writing.

^b Results of this study.

^c Feedstock consumed = 1/([ethanol yield%] × [theoretical ethanol from 1 ton feedstock])/100; for delignified corncob residue: $1/(0.8938 \times 1 \times 0.729 \times 1.111 \times 0.51) = 2.71$ (ton) (Cases 1 and 2); for corn meal: $1/(0.922 \times 1 \times 0.7689 \times 1.111 \times 0.51) = 2.49$ (ton) (Case 3).

^d Enzyme consumed = enzyme dosage × feedstock consumed × 1000/enzyme activity; for delignified corncob residue: $15 \times 2.71 \times 1000/135 = 301.11$ (kg cellulase) (Cases 1 and 2); for corn meal: $5 \times 2.49 \times 1000/22,000 = 0.57$ (kg α -amylase HTAA) plus $37.5 \times 2.49 \times 1000/100,000 = 0.93$ (kg glucoamylase GA-L NEW) (Case 3).

^e 0.57 kg α -amylase HTAA and 0.93 kg glucoamylase GA-L NEW.

^f Feedstock cost = feedstock consumed × feedstock selling price; for delignified corncob residue in Case 1: $2.71 \times 0 = 0$ (\$) (delignified corncob residue is taken as the solid waste and has no practical value.); for delignified corncob residue in Case 2: $2.71 \times 95.24 = 258.10$ (\$) (the selling price of delignified corncob residue was from the Chinese eBay "Alibaba" website at www.1688.com); for corn meal: $2.49 \times 317.5 = 790.58$ (\$) (the selling price of the corn meal is from www.1688.com).

^g Enzyme cost = enzyme consumed × enzyme selling price; for delignified corncob residue in Cases 1 and 2: $301.11 \times 1.59 = 478.76$ (\$) (the selling price of cellulase Youtell #6 is \$1.59 per kg according to www.youtellbio.com); for corn meal: $0.57 \times 3.17 + 0.93 \times 4.76 = 6.23$ (\$) (the selling price of α -amylase HTAA and glucoamylase GA-L NEW were \$3.17 per kg and \$4.76 per kg, respectively according to Genencor International).

^h Total production cost = feedstock cost + enzyme cost; for delignified corncob residue in Case 1: $0 + 478.76 = 478.76$ (\$) ; for delignified corncob residue in Case 2: $258.10 + 478.76 = 736.86$ (\$) ; for corn meal: $790.58 + 6.23 = 796.81$ (\$) .

the similar glucan content in the substrates, the similar biochemical transformation pathway, and even the similar facilities, the other factors will contribute a similar extent to the final ethanol production cost using the two different feedstocks (the co-product DGGs produced from corn meal was less valuable than the xylose- and lignin-derived products produced from corncob). Therefore, delignified corncob residue released in the xylose-related industry as a solid waste could become a promising feedstock for ethanol production with a competitive performance with corn meal.

4. Conclusion

In this study, the ethanol fermentation performance from delignified corncob residue was investigated under various operation conditions. The high ethanol titer and yield of 75.07 g/L and 89.38%, respectively, were achieved using a regular yeast strain at a moderate cellulase dosage and a high solids loading. A uniform SSF temperature profile of 37 °C at both prehydrolysis and SSF stages was tested. Delignified corncob residue was compared to corn meal as feedstock of ethanol fermentation, and the result shows that delignified corncob residue is comparable to corn meal in both the fermentation performance and economic cost.

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