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Efficient Corncob Biorefinery for Ethanol Initiated by a Novel Pretreatment of Densifying Lignocellulosic Biomass with Sulfuric Acid

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Abstract: Corncob is a potential feedstock for biorefineries to produce cellulosic ethanol and other chemicals. Densifying lignocellulosic biomass with chemicals followed by autoclave (DLCA) has been confirmed an efficient and economical pretreatment method, and it was applied in the present work for conversion of corncob to bioethanol. The dosage of sulfuric acid, solid loading of biomass, and autoclave time for pretreatment were investigated. Enzymatic hydrolysis at 25–35% solids loadings resulted in 91–97% sugar conversions. Fermentation of the resulted hydrolysates went well with the highest ethanol titer reaching 75.71 g/L at 35% solid loading. Simultaneous saccharification and co-fermentation was applied to further improve xylose consumption at high solids loadings and the ethanol titer was enhanced to 82.0 g/L at 35% solid loading with an ethanol yield of 21.67 kg/100 kg corncob. This study demonstrated DLCA provided a highly digestible and highly fermentable corncob for biorefinery.

Keywords: cellulosic ethanol; DLCA pretreatment; corncob; enzymatic hydrolysis; SSCF

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1. Introduction

Fossil energy has been the main energy source utilized by human beings since the industrial revolution, and it greatly contributes to the prosperity of human society. Meanwhile, the utilization of fossil energy has also caused climate change, global ecological deterioration, and species extinction. Therefore, clean and renewable alternative energy sources are critical for the future of the world [1]. The worldwide trend of reaching carbon neutrality is propelling the energy industry toward green energy sources, such as wind, solar, and biomass.

Biomass is a potential alternative resource to petroleum due to its abundance. Among various bio-products, bioethanol stands out as it is an excellent octane blending agent that can be added to gasoline to increase combustion performance, while it can also greatly reduce carbon emission in the transportation sector. Moreover, bioethanol also exhibits high potential as a feedstock to produce other chemicals, such as ethylene and hydrogen, rendering it more promising [2]. Bioethanol can be produced from a variety of feedstock, including food crops such as corn and sugarcane, as well as lignocellulosic biomass, such as agricultural wastes and forest leftovers [3]. Using food crops for ethanol production would reduce food supply and thus lignocellulosic biomass, which is more abundant, is a more promising feedstock for bioethanol production.

Corncob is an agricultural residue of maize (*Zea mays*) and about 164 million tons is produced globally each year. The contents of cellulose, hemicellulose, and lignin could be up to 36%, 38%, and 21%, respectively [4,5]. Large volume with low cost makes corncob a very promising renewable resource.

When employing lignocellulose for bioethanol production, the natural recalcitrance feature of the plant cell wall becomes a key hurdle. This is primarily due to the fact that lignin, hemicellulose, and cellulose are intricately connected to completing their biological function as the plant support, but this also makes them difficult to be degraded by enzymes or microbes [6]. Pretreatment is a critical step in reducing the recalcitrance of lignocellulose and improving its digestibility [7,8]. Various pretreatment techniques (, pretreatment, chemical pretreatment, combinatorial pretreatment, etc.) have been investigated for improving enzymatic digestibility of corncob.

Acid pretreatments (with H_2SO_4, HCl, HNO_3, H_3PO_4, etc., as pretreatment reagents) destroy the lignocellulosic structure by mainly hydrolyzing hemicellulose at 140–210 °C [9]. Dilute acid (DA) pretreatment was applied by Wang et al. [10] on corncob. They immersed corncob in a 0–0.7% (*v*/*v*) sulfuric acid solution with a liquid-to-solid (L/S) ratio of two, followed by treatment in an oil bath at around 160–190 °C. This method led to 97% and 75% of glucose yield and xylose recovery, respectively. However, this method, using high temperature, often causes further degradation of some sugars to inhibitors, which severely hinders subsequent bioconversions [11,12]. Washing or other detoxifying strategies can effectively relieve the inhibition, but they are usually accompanied by sugar loss and extra cost. In addition, it is worth noting that when the solid loading (in pretreatment, hydrolysis, and fermentation) is too low, the efficiencies of ethanol production and purification decrease [13].

Our laboratory recently invented a novel pretreatment technology: Densifying lignocellulosic biomass with chemicals (DLC) [14,15]. During the pretreatment processes, the bulk density of biomass is increased by 5–10 times, which facilitates biomass logistics and reduces costs for storage and transportation. Moreover, grinding and extrusion mix chemical reagents with biomass fully and have the potential to press chemical(s) into the plant cell wall to promote reactions with biomass components. As water content in the densified biomass is low, the chemical concentration in the biomass is high (up to hundreds of grams per liter), which reacts with biomass even at environmental temperature, greatly increasing biomass digestibility during storage and avoiding microbial contamination. Studies have shown that steam autoclave at 121 °C after DLC pretreatment with sulfuric acid (DLCA(sa)) is far superior to traditional dilute acid pretreatment in using less energy, generating fewer toxic degradation products, and delivering a higher ethanol yield. For instance, ethanol yields by DLCA(sa) pretreatment reached 202.4 g/kg corn stover [16] and 241.9 g/kg sugarcane bagasse [17] at 30% (*w*/*w*) solid loading without washing or detoxifying pretreated biomass.

In this study, in order to demonstrate the versatility of DLC pretreatment and realized the utilization of corncob efficiently and cost-effectively, we investigated the performance of DLCA(sa) on corncob for the first time. The effects of acid dosage, autoclaving time and solid loading during DLCA(sa) pretreatment were studied. The microstructure of pretreated biomass was characterized. Ethanol production on pretreated corncob was examined through separate hydrolysis and co-fermentation (SHCF) and simultaneous saccharification and co-fermentation (SSCF).

2. Materials and Methods

2.1. Biomass and Cellulase

The corncob used in this research was obtained from Lianyungang, Jiangsu Province and milled to 5–10 mm using a grinder. The corncob contained 30.11 ± 0.65% glucan, 29.41 ± 0.32% xylan, 20.25 ± 3.85% klason lignin, and 1.34 ± 0.37% ash (on a dry basis), which were measured according to the NREL method (NREL/TP-510-42618-42622-4218) [18]. The commercial cellulase Cellic® CTec2 (87.3 mg protein/mL) was used for hydrolyzing the pretreated corncob, which was purchased from Sigma-Aldrich (Shanghai, China).

2.2. Pretreatment of Corncob by Densifying Biomass with Sulfuric Acid

DLC(sa) was performed based on the procedure mentioned in our previous study [15]. The biomass densification was carried out using a flat die pellet machine (Zhengzhou Chibang Machinery Equipment Co., Ltd., Zhengzhou, Henan, China) whose compression capacity was about 70–120 kg/h. The flat die of the granulator has a diameter of 200 mm, a thickness of 38 mm and die holes of 6 mm. Biomass was densified with sulfuric acid (dosages: 0.025, 0.05 and 0.075 g/g corn cob, dissolving in water (0.5 g/g corn cob)), which was sprayed evenly onto the biomass before densification. After densification, the resulted biomass (DLC(sa)-CC) was autoclaved at 121 °C at different solids loadings (25%, 30%, 35%, and 40% (*w*/*w*)) for various times (15, 30, 45 and 60 min), resulting in DLCA(sa)-CC, which was then air-dried in a fume hood at room temperature.

2.3. Enzymatic Hydrolysis

To test the effects of different pretreatment conditions, DLCA(sa)-CC were hydrolyzed in 20 mL bottles at 3% (*w*/*w*) solid loading, 50 °C, 250 rpm, pH 4.8 for 24 h. Enzymatic hydrolysis of high solid loading (25–35%) were performed in 250 mL shake flasks for 72 h. The content of enzyme was 15 mg protein/g glucan. For 32% and 35% solids loadings enzymatic hydrolysis, 66.6% and 33.4% of DLCA(sa)-CC and cellulase were added at 0 h and 6 h, respectively, to avoid the high systematic viscosity and low free water caused by adding total biomass into the system at one time. Three parallel trials were conducted, and samples were taken at intervals to determine sugar concentrations by HPLC. The obtained hydrolysate was used for subsequent fermentation and analysis.

2.4. Separate Hydrolysis and Co-Fermentation

Addition of calcium hydroxide to the hydrolysates to raise the pH to 5.8, and nutrients of peptone and yeast extract were supplemented into the hydrolysate before fermentation at dosages of 10 g/L and 5 g/L, respectively. *S. cerevisiae* CRD5HS, a xylose-fermenting yeast constructed by our lab, was used for ethanol fermentation at an initial OD_{600} of 2.0. Fermentation was performed at 30 °C, 150 rpm for 72 h.

2.5. Simultaneous Saccharification and Co-Fermentation

SSCF were performed at 25%, 30%, 32%, and 35% solids loadings (*w*/*w*) in 250 mL shake flasks to further investigate the digestibility and fermentability of DLCA(sa)-CC. Using the same method of enzymatic hydrolysis described in Section 2.3 and fermentation condition described in Section 2.4, the SSCF was started by inoculating *S. cerevisiae* CRD5HS seeds after 12 h pre-hydrolysis and the pH was adjusted to 5.5.

SSCF at high solids loadings of 37% and 40% was performed with enzyme dosage of 20 mg protein/g glucan and the initial inoculum volume was increased to OD_{600} of 4.0. The time of pre-hydrolysis had an extension of 18 h and the DLCA(sa)-CC were added in batches at 0 h, 4 h, 8 h, and 12 h (50%, 20%, 15%, and 15%, respectively). The hemocytometer measurement was used to measure the total number of cells during fermentation and the live cells were measured by methylene blue staining method [19].

2.6. Analysis Methods

Glucose, xylose, and ethanol concentrations were measured with a HPLC system (Agilent 1260, Santa Clara, CA, USA) equipped with an Aminex HPX-87H column at 65 °C and a G7162A refractive index detection. Sulfuric acid (5 mM) was applied as the eluent, with a flow rate of 0.6 mL/min [20]. Oligosaccharide analysis was carried out by an autoclave-based acid hydrolysis method [21]. The mass balance regarding the SHCF and SSCF processes for DLCA(sa)-CC were calculated by a previously reported method [14].

Fourier transform infrared spectroscopy (FT-IR) was used to analyze the functional groups of corncob by with a resolution of 2 cm^{-1} and a range of 750 to 4000 cm^{-1} after crushing and freeze-drying the samples. The surficial morphology of biomass was observed by scanning electron microscopy (SEM). A small coating of gold was sputter-deposited on

the sample surface for imaging purposes. Depending on the characteristics to be traced, the photos were enlarged 1000 and 5000 times.

3. Results and Discussion

3.1. Effects of Sulfuric Acid Dosage, Solid Loading and Autoclave Time on Hydrolysis

Effects of sulfuric acid dosage, solid loading, and autoclave time on DLCA pretreatment were investigated. As a result, sugar conversion increased with the increasing dosage of sulfuric acid (Figure 1A). With an increase in the acid dosage from 0.025 g/g to 0.05 g/g, the total sugar conversion significantly improved from 62.5% to 90.7%. Further increasing acid dosage from 0.05 g/g to 0.075 g/g resulted in a slight increase of sugar conversion. When the dosage of sulfuric acid was 0.075 g/g, the glucan, xylan, and total sugar conversions were as high as 98.6%, 94.5%, and 96.7%, respectively. Therefore, 0.075 g/g was chosen as the dosage of sulfuric acid for further study.

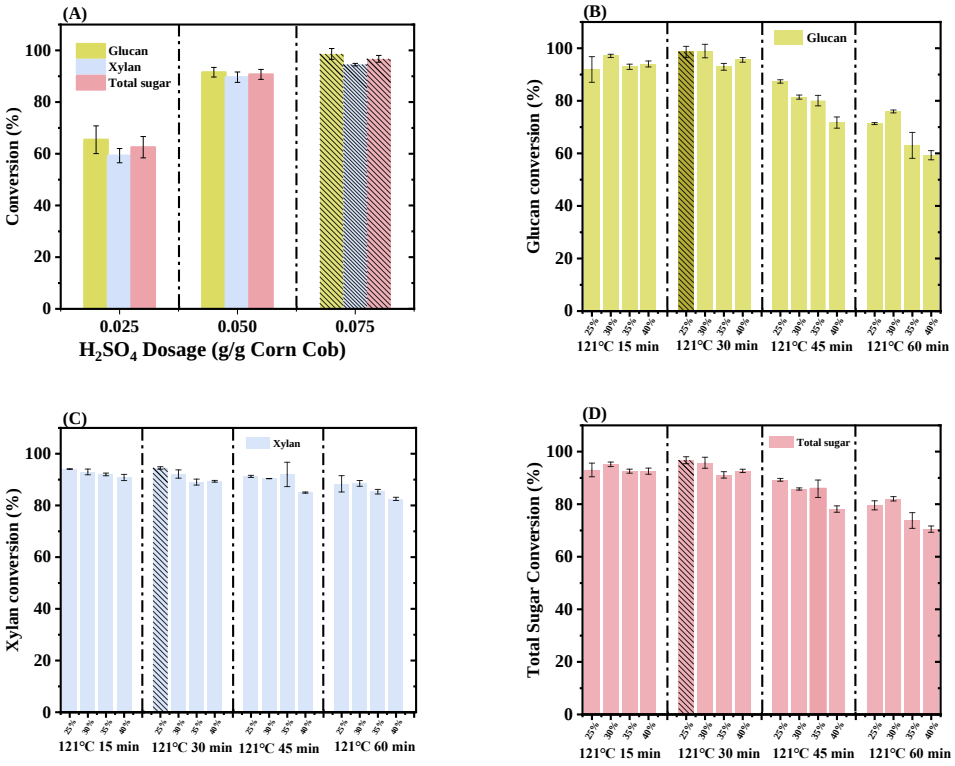

Figure 1. Effects of sulfuric acid dosage (**A**), autoclave time and solid loading (**B**–**D**) on DLCA pretreatment of corncob. Enzymatic hydrolysis on pretreated corncob at 3% (*w*/*w*) solid loading was performed for 24 h at 50 °C with enzyme dosage of 15 mg protein/g glucan to evaluate the sugar conversions for pretreated corncob. The effect of different sulfuric acid dosages was investigated after autoclaving for 30 min at 25% solid loading. DLCA: Densifying lignocellulosic biomass with sulfuric acid followed by autoclave.

Before autoclaving, different dosages of water were added to DLC(sa)-CC to obtain different solids loadings, which affected acid concentration during autoclave. As shown in Figure 1B–D, the highest glucan conversion was 98.9% at 30% solid loading (Figure 1B), the highest xylan conversion was 94.5% at 25% solid loading (Figure 1C), the highest total sugar conversion was 96.7% at 25% solid loading (Figure 1D), and all the highest conversions occurred with an autoclaving time of 30 min. In addition, for 30 min autoclaving, increasing solid loading to 30% did not significantly reduce sugar conversion and solid loading up to 40% still brought about a sugar conversion of over 90% (Figure 1D). High solid loading process plays a vital role in the cost-effective biorefining as it decreases equipment size, reduces water consumption and increases productivity [22]. The results demonstrated that DLCA pretreatment owned a superior advantage for high solid loading processing. For

autoclaving time, it seemed 15 min or 30 min was sufficient with longer time-reduced sugar conversions, especially glucan conversion (Figure 1B), which might be caused by sugar degradation during pretreatment with extended reaction time. Considering the total sugar conversion, pretreatment conditions were chosen as 0.075 g/g sulfuric acid dosage, 25% solid loading, and 30 min for further study.

3.2. Component Analysis and FT-IR/SEM

The effects of DLC pretreatment on corncob was further investigated by FT-IR and SEM analysis. FT-IR analysis (Figure 2A and Table 1) showed that the peak reduction at 1727 cm^{-1} was due to the carbonyl vibration of hemicellulose reduced after DLC(sa) and DLCA(sa) pretreatments. It was likely because of the removal of acetyl groups from hemicellulose or abscission of *p*-coumaric acid and ferulic acid compounds during pretreatments. In addition, the obviously weakened absorption peaks at 1053 cm^{-1}, 1245 cm^{-1}, and 3362 cm^{-1} (near the absorption peaks are flexural vibration of the C-O and O-H groups, which occur in cellulose) in DLC(sa)-CC and DLCA(sa)-CC samples further confirmed partial degradation of lignocellulose [23]. C-C and C-O vibrations in lignin were observed near 1332 cm^{-1} and the stretching and bending corresponded to the aromatic skeleton and benzene ring in lignin at about 1420 cm^{-1}, 1520 cm^{-1}, and 1608 cm^{-1} [24].

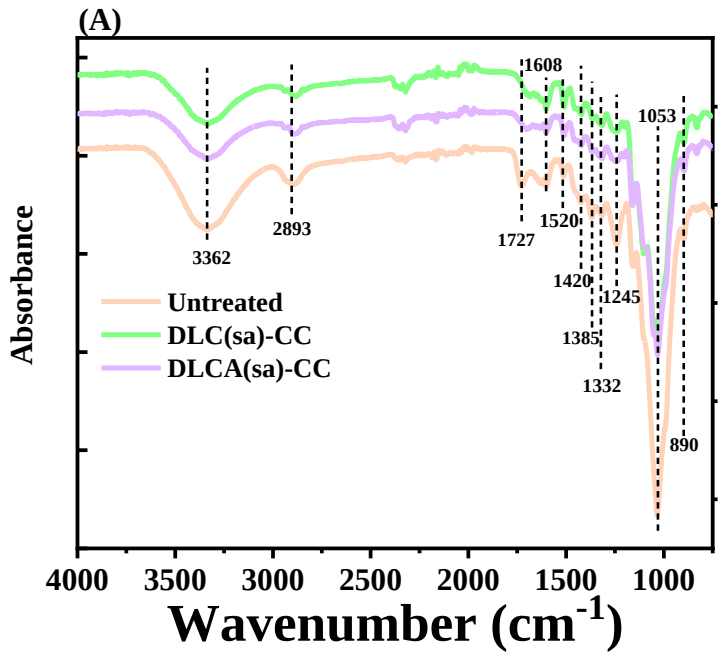

Figure 2. *Cont.*

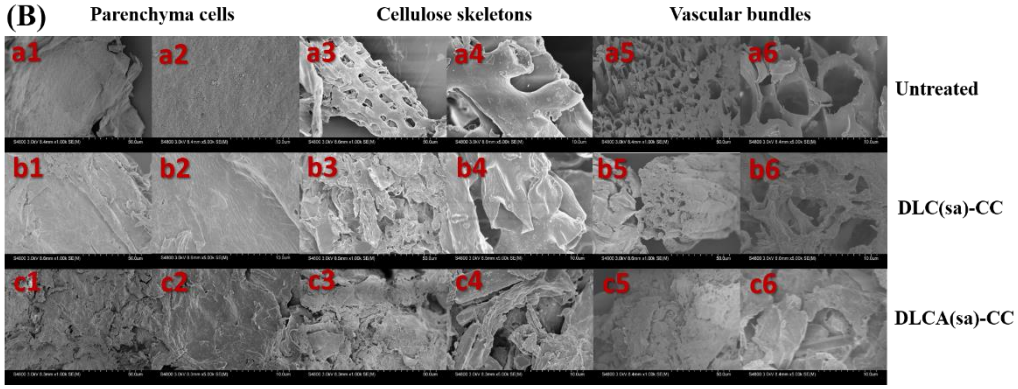

Figure 2. FT-IR spectra (**A**) and SEM images (**B**) of untreated and pretreated corncob. The SEM pictures of a1, b1, c1, a3, b3, c3, a5, b5, and c5 are at 1000×. Others are at 5000×. DLC(sa)-CC with 0.075 g sulfuric acid/g corncob was autoclave treated at 121 °C for 30 min and 25% solid loading, resulting in DLCA(sa)-CC. DLC(sa): densifying lignocellulosic biomass with sulfuric acid; DLC(sa)-CC: corncob pretreated by DLC(sa) method. DLCA(sa)-CC: corncob pretreated by DLCA(sa) method.

Table 1. Infrared spectrums for functional groups present in different components of corn cob [24,25].

Frequency Range (cm^{-1})	Functional Groups	Class of Compounds
890	C-H bending	Aromatic compounds in cellulose
1053	C-O stretching	Alcohol, phenols and esters in cellulose
1245	-OH bending	Phenolic and aliphatic structures in cellulose
1332	C-C and C-O stretching	Acetyl groups in lignin
1385	C-H bending	Aliphatic structures in cellulose and hemicellulose
1420	C-C stretching	Benzene rings in lignin
1520	C=C bending	Aromatic compounds in lignin
1608	C=O stretching	Carbonyl bonds in lignin
1727	C=O stretching	Carboxylic acids/ester groups in hemicellulose
2893	C-H stretching	Alkanes in cellulose
3362	-OH descending	Alcohol, phenols in cellulose

SEM images (Figure 2B) showed the parenchyma cells, cellulose skeletons, and vascular bundles of corncob. It can be seen that in the untreated corncob, the parenchyma cells had a smooth surface and were neatly arranged. Zou et al. reported that the cellulose skeletons of corncob had a dense structure, with the outer cellulose in a tubular hollow state and the inner part filled with lignin and hemicellulose [24]. The vascular bundle is a bundle structure consisting of xylem and bast, which are closely combined, with a hard texture [5]. SEM images showed that all these structures were damaged to a certain degree after pretreatment. What is striking from the SEM images is the change in DLCA(sa)-CC with numerous folds formed in the walls of the parenchyma cells, which would result in the increasing of specific surface area. This was presumably attributed to the disruption of lignocellulose structure. In addition, the dense structure of the plant was completely disrupted by the collapse of the cellulose skeleton after DLC and DLCA, which had been a tough three-dimensional network entity, resulting in the transformation of the compact vascular tissue into fragments. It was likely the mechanical action during densification of the pretreatment and sulfuric acid hydrolysis that leaded to the disruption of the cell wall structure of biomass and the dissolution of hemicellulose [25], which disintegrated the cellular structure, increased the enzymatic accessibility, and eventually enhanced the digestibility of corncob.

3.3. Enzymatic Hydrolysis of DLCA(sa)-CC at High Solids Loadings

By altering the physicochemical properties of the biomass and disrupting its structure, pretreatment boosts the efficiency of enzymatic hydrolysis. As mentioned in Section 3.1,

high loading is a prerequisite for an industrial process. Therefore, the enzymatic hydrolysis of DLCA(sa)-CC was investigated at 25%, 30%, 32%, and 35% solids loadings.

As shown in Figure 3, DLCA(sa)-CC enzymatic hydrolysis contributed to high sugar yields, and the concentration of sugars increased with the increasing solid loading. The highest glucose, xylose, and total sugar concentrations reached 115.23 g/L, 86.1 g/L, and 201.3 g/L, respectively, at 35% solid loading, which indicated that DLCA(sa)-CC had great digestibility. Moreover, the concentrations of xylose at 12 h were as high as 80–85% of those after 72 h hydrolysis in all scenarios, which is likely due to most xylan being already degraded into xylose during pretreatment [15]. For example, at 35% solid loading, the xylose concentration was 79.53 g/L at 12 h, which was 85.4% of that at 72 h (86.11 g/L) (Figure 3D). It is worth noting that the final conversions of glucan and xylan were both above 91% (Figure 3E) at tested high solids loadings, indicating that DLCA pretreatment is very efficient in pretreating corncob.

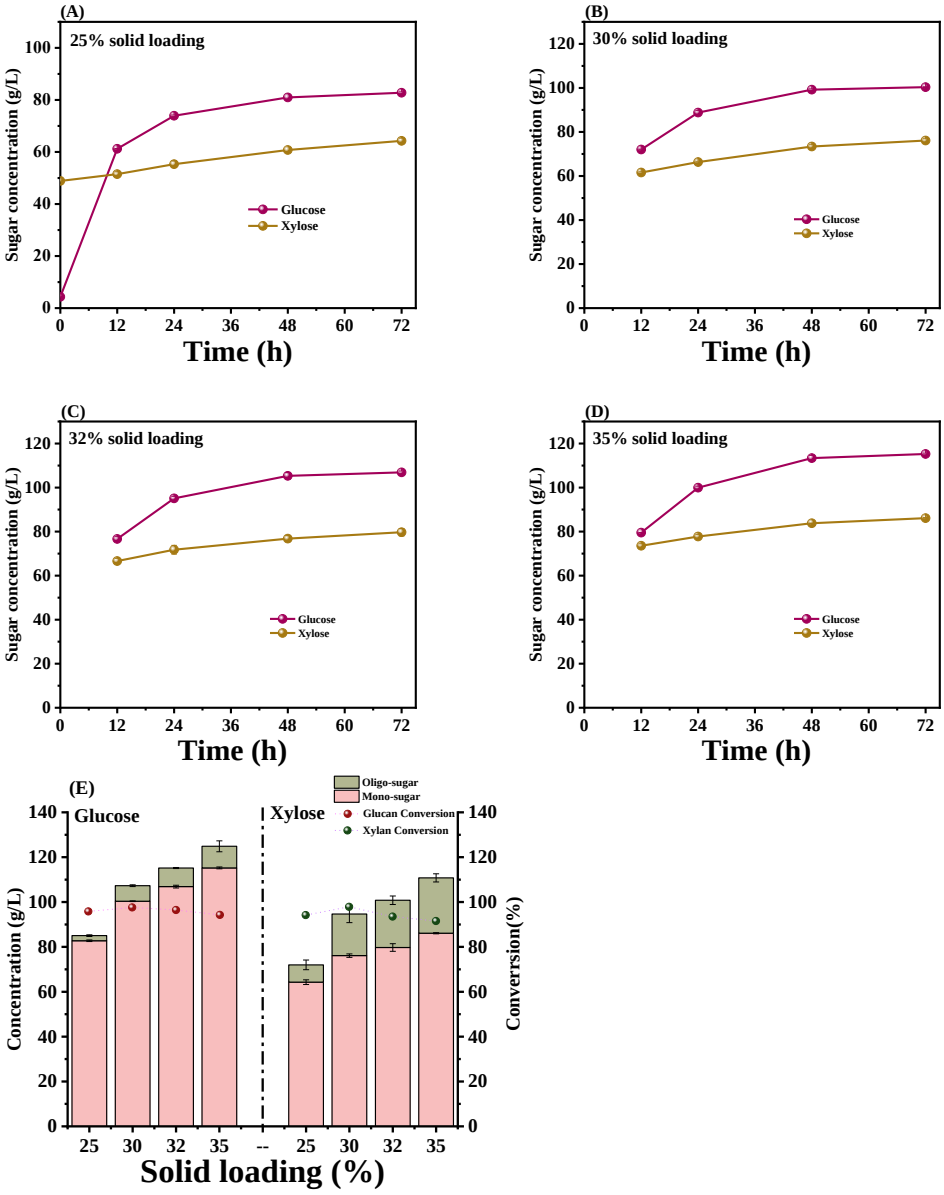

Figure 3. Enzymatic hydrolysis of DLCA(sa)-CC at 25% (**A**), 30% (**B**), 32% (**C**), and 35% (**D**) solid loading and sugar conversions at different solids loadings (**E**). Enzymatic hydrolyses were performed at 50 °C, 250 rpm, and pH 4.8 with an enzyme dosage of 15 mg protein/g glucan.

As can be seen from Figure 3E, glucan and xylan conversions were all higher than 90% and reached a maximum of 97.3% and 97.8%, respectively, at 30% solid loading. Sugar conversions decreased slightly as solid loading increased from 30% to 35%. Nonetheless, the yields for monomeric sugars (glucose and xylose) decreased significantly as more oligomeric sugars generated with increasing solid loading. For instance, the highest glucose and xylose yields were 93.3% and 82.3%, respectively, at 25% solid loading with production of small portion of gluco-oligosaccharides and xylo-oligosaccharides. In contrast, the glucose and xylose yields decreased to 82.83% and 70.23% when the solid loading increased to 35%, with more gluco-oligosaccharides and xylo-oligosaccharides presented in the hydrolysate. Meanwhile, compared to gluco-oligosaccharides, the hydrolysate contained more xylo-oligosaccharides. It was reported that the large amount of oligosaccharides in the hydrolysate affected the activity of CBH through competitive inhibition [26], which is a factor for the slow release of sugars in the late stages of hydrolysis. Another main reason for the decreased glucose and xylose yields is that more pretreatment degradation products/inhibitors presented as a higher solid loading, which strongly inhibits cellulases [27]. Meanwhile, the heat and mass transfers were also impaired by high solid loading [28]. As a consequence, the rate of enzymatic conversion decreased, and the oligosaccharide content increased. Furthermore, the feedback inhibition of glucose on cellulase [29–32] was also stronger at higher glucose concentration, which increased with increasing solid loading. In addition, unproductive binding of cellulase to lignin was also an adverse factor affecting enzymatic hydrolysis [33].

3.4. Separate Hydrolysis and Co-Fermentation for Ethanol Production

Fermentation performance is a key criterion for determining whether DLCA(sa)-CC can be used at a large scale for bioethanol production. The aforementioned hydrolysates with different solids loadings were fermented with *S. cerevisiae* CRD5HS. Hydrolysates at all solids loadings were fermented successfully with ethanol titer increased with increasing solid loading (Figure 4). Ethanol titer for 30% solid loading increased by 10.8% compared to 25% solid loading, while the ethanol titer increase was only 0.7% when solid loading increased from 32% to 35%. It is also worth noting that the production rate of ethanol was fast in the first 12 h due to the fast utilization of glucose by the strain. The production rate was reduced with increasing solids loadings. For example, at 12 h, the ethanol titer was 41.7 g/L at 25% solid loading as well, as it decreased to 35.1 g/L at 35% solid loading. This may be due to the increase of inhibitors, viscosity, and osmolality in the higher solids loadings, which resulted in the greater inhibition to the yeast and slower sugar utilization [34].

Glucose was completely consumed within 24 h by *S. cerevisiae* CRD5HS for all hydrolysates while xylose consumption decreased as solid loading increased probably due to the factors mentioned above and the increased ethanol titer. For instance, xylose was completely consumed at 25% solid loading while remained 27.1 g/L in the fermentation broth at 35% solid loading after 72 h fermentation. Nevertheless, all fermentations performed reasonably well and the ethanol concentration, at 35% solid loading, reached up to 75.7 g/L. It is rare to see successful fermentations in hydrolysates at high solids loadings on unwashed and undetoxified pretreated biomass. In the literature, most studies on ethanol production from corncob were carried out at relatively low solids loadings (3–20%) and resulted in relatively low ethanol titers [35–37]. For instance, Yu et al. obtained ethanol concentrations of 42.46 g/L and 53.24 g/L by SHCF and SSCF, respectively, on corncob by applying alkaline pretreatment [38].

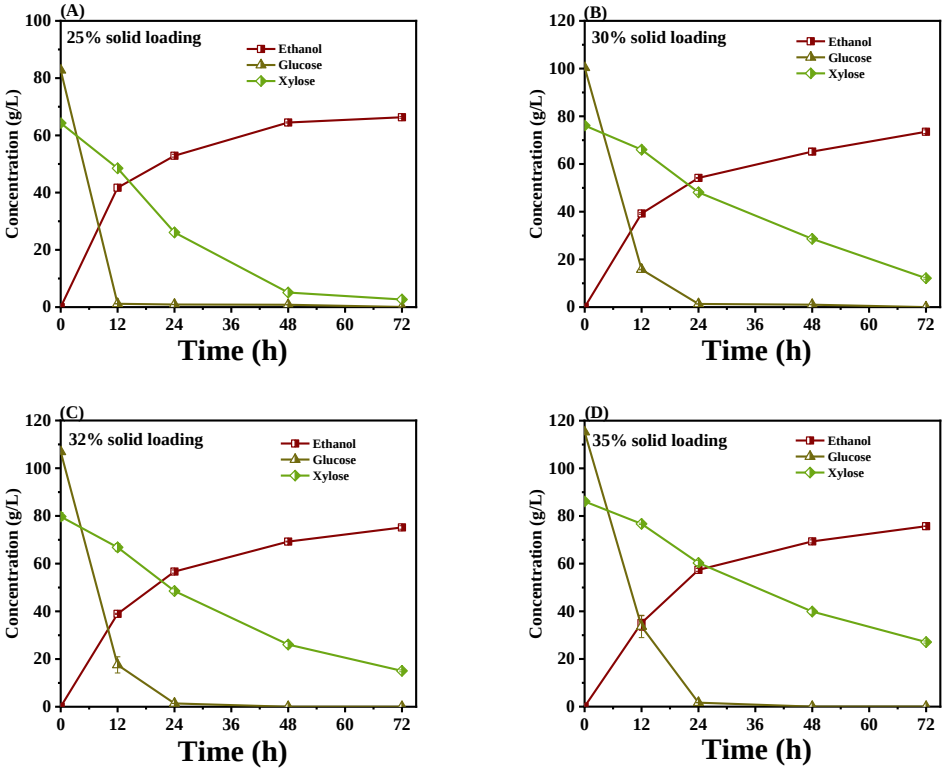

Figure 4. Ethanol fermentation in hydrolysates of DLCA(sa)-CC at 25% (**A**), 30% (**B**), 32% (**C**), and 35% (**D**) solid loading. *S. cerevisiae* CRD5HS (initial OD_{600} = 2) was used for fermentation at pH 5.5, 30 °C, 150 rpm for 72 h.

3.5. Simultaneous Saccharification and Co-Fermentation for Ethanol Production

As described above, despite high ethanol titers reached by SHCF of DLCA(sa)-CC, a considerable amount of xylose remained at high solids loadings. Compared to SHCF, SSCF offers advantages of shorter process time, less substrate inhibition, higher sugar conversion and lower risk of contamination [39]. Therefore, SSCF was applied by pre-hydrolyzing DLCA(sa)-CC for 12 h and then initiating fermentation by inoculating the yeast.

As shown in Figure 5, glucose concentration was reduced to and maintained at zero after 12 h of fermentation for all solids loadings, which indicated glucose was instantly consumed after its release in the middle and late fermentation phases. Xylose consumption during SSCF was improved compared to SHCF with xylose nearly completely consumed for solids loadings of 25%, 30%, and 32%. The residual xylose concentration for 35% solid loading was also reduced to 8.71 g/L. As a result, ethanol titers for 30%, 32%, and 35% solids loadings were enhanced from SHCFs 73.52 g/L, 75.18 g/L, and 75.71 g/L, to 74.03 g/L, 78.14 g/L, and 81.99 g/L, respectively.

With the increase in solid loading, SSCF sugar conversions gradually decreased, especially glucan conversion (Table 2). The reason for the decreased sugar conversion was likely caused by the inhibition of increased concentrations of ethanol and degradation products, as well as reduced enzyme activities (as SSCF was conducted under fermentation conditions). Sugar consumptions (94.7–98.3%) and ethanol yields (90.9–94.5%) were both higher during SSCF compared with those for SHCF (Table 2). The highest concentration of ethanol by SSCF reached 81.99 g/L at 35% solid loading. SSCF has been widely applied for enhancing ethanol yield. For instance, Su et al. applied a fed-batch SSCF strategy on corncob and increased the final ethanol concentration to 60.5 g/L at 25% solid loading [40]. Yuan et al. obtained a high ethanol titer of 74.6 g/L on corn stover by developing a SSCF strategy of "Fed-batch of DLC Biomass and Enzyme (FBE)" [16]. FBE-SSCF was also used for high solid loading saccharification and fermentation in the present study, which contributed to the high ethanol yields.

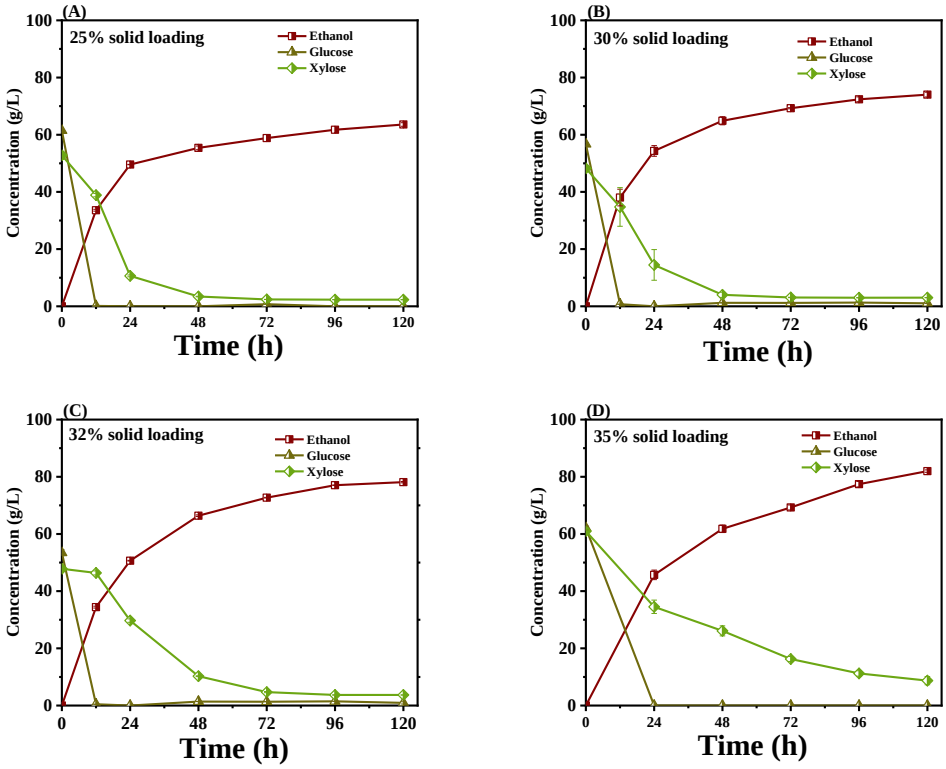

Figure 5. SSCF of DLCA(sa)-CC at different solids loadings. (**A**) 25% solid loading; (**B**) 30% solid loading; (**C**) 32% solid loading; (**D**) 35% solid loading. DLCA(sa)-CC was pre-hydrolyzed for 12 h at 50 °C, 250 rpm, and pH 4.8 with an enzyme dosage of 15 mg protein/g glucan. SSCF with *S. cerevisiae* CRD5HS (initial OD_{600} = 2) was conducted at 30 °C, 150 rpm, and pH 5.5 for 120 h.

Table 2. Sugar conversions and fermentation performances for SSCF of DLCA(sa)-CC.

	Solid Loading	Sugar Conversion (%)			Sugar Consumption (%)			Ethanol Yield (%)	Ethanol Titer (g/L)
		Glucan	Xylan	Total Sugar	Glucan	Xylan	Total Sugar		
SHCF	25%	95.6 ± 0.4	94.1 ± 1.3	94.9 ± 0.9	100 ± 0.0	96.1 ± 0.05	98.3 ± 0.02	85.1 ± 0.4	66.3 ± 0.3
	30%	97.3 ± 0.1	97.8 ± 0.8	97.6 ± 0.5	100 ± 0.0	84.5 ± 0.4	93.3 ± 0.2	85.1 ± 0.8	73.5 ± 0.8
	32%	96.2 ± 0.5	93.5 ± 1.6	94.9 ± 1.1	100 ± 0.0	82.1 ± 0.4	92.3 ± 0.3	81.6 ± 0.6	75.2 ± 0.7
	35%	94.0 ± 0.3	91.5 ± 0.2	92.8 ± 0.3	100 ± 0.0	70.2 ± 0.4	87.2 ± 0.2	80.1 ± 0.04	75.7 ± 0.05
SSCF	25%	91.5 ± 0.02	97.0 ± 0.04	94.1 ± 0.03	100 ± 0.0	96.2 ± 0.03	98.3 ± 0.02	92.2 ± 0.8	63.6 ± 0.6
	30%	87.0 ± 1.2	96.2 ± 0.3	91.3 ± 0.7	98.8 ± 0.1	96.2 ± 0.04	97.5 ± 0.07	90.9 ± 0.8	74.0 ± 0.8
	32%	81.9 ± 4.5	94.9 ± 1.2	88.0 ± 3.0	98.7 ± 0.4	95.4 ± 0.04	96.9 ± 0.3	91.9 ± 0.2	78.1 ± 0.2
	35%	80.4 ± 0.9	94.4 ± 0.2	86.9 ± 0.6	99.9 ± 0.03	90.0 ± 1.3	94.7 ± 0.9	94.5 ± 0.01	82.0 ± 0.5

Total sugar = Glucan + Xylan; Sugar Conversion = $\frac{\text{sugar in DLCA(sa)}-\text{CC} - \text{sugar in solid residue after fermentation}}{\text{sugar in DLCA(sa)}-\text{CC}} \times 100\%$; Sugar Consumption = $\frac{\text{sugar in DLCA(sa)}-\text{CC} - \text{sugar in solid residue after fermentation} - \text{sugar in fermentation broth}}{\text{sugar in DLCA(sa)}-\text{CC} - \text{sugar in solid residue after fermentation} - \text{oligomeric sugar in fermentation broth}} \times 100\%$; ethanol yield (%) = $\frac{\text{Final ethanol concentration after fermentation}}{\text{Total mono} - \text{sugar consumption during fermentation} \times 0.51} \times 100\%$; DLCA(sa)-CC: corncob pretreated by DLCA(sa); SHCF: separate hydrolysis and co-fermentation. SSCF: simultaneous saccharification and co-fermentation.

To further explore the potential of DLCA(sa)-CC for ethanol production, higher solids loadings (37% and 40%) were investigated on SSCF. It was exciting to see that fermentations went well even at 40% solid loading (Figure 6), which further confirmed high fermentability/low toxicity of DLCA(sa)-CC. Glucose was consumed completely for all solids loadings. Most xylose was consumed at 37% solid loading. The number of cells was determined during SSCF (Figure 6C). The yeast cells grew until 72 h to a reasonably high level at 37% solid loading, while the live cell number started to decrease after 48 h. For 40% solid loading, the cell growth was reduced likely due to the inhibition from degradation products. After 72 h, a large number of cells died, and many dead cells were lysed (total

cell count decreased). It is worth noting that cells were mostly dead after 96 h, which was likely to be the reason for incomplete xylose consumption. The final ethanol concentration reached 86.96 g/L at 37% solid loading and 85.14 g/L at 40% solid loading with 20.66 g/L and 40.14 g/L xylose, respectively, remaining in the broth. Converting all these residual xyloses into ethanol would increase the concentration of ethanol to >100 g/L.

3.6. Mass Balance for SSCF

Figure 7 summarizes the overall mass balance of corncob conversion to ethanol by DLCA pretreatment and SSCF. The figure showed that more glucan remained in the solid residue and fermentation broth after SSCF at higher solids loadings. For example, processing 100 kg of corncob resulted in 2.99 kg and 6.89 kg of residual glucan unutilized at 25% and 35% solid loading, respectively. In contrast, xylan had a higher conversion, with only 0.91–1.72 kg unconverted in the solids although the initial xylan content was as high as 29.4 kg. Nevertheless, the consumption of xylose was not as efficient as glucose (Table 2), especially at higher solids loadings, and much xylan ended up as oligo-xylose that cannot be fermented by the yeast. Ethanol yield reached as high as 25.15 kg/100 kg corncob at 25% solid loading and 21.67 kg/100 kg corncob at 35% solid loading, which indicated the high potential of DLCA pretreatment for corncob conversion. This result is significantly superior to previous studies of corncob for ethanol production at high solids loadings [38,41–44].

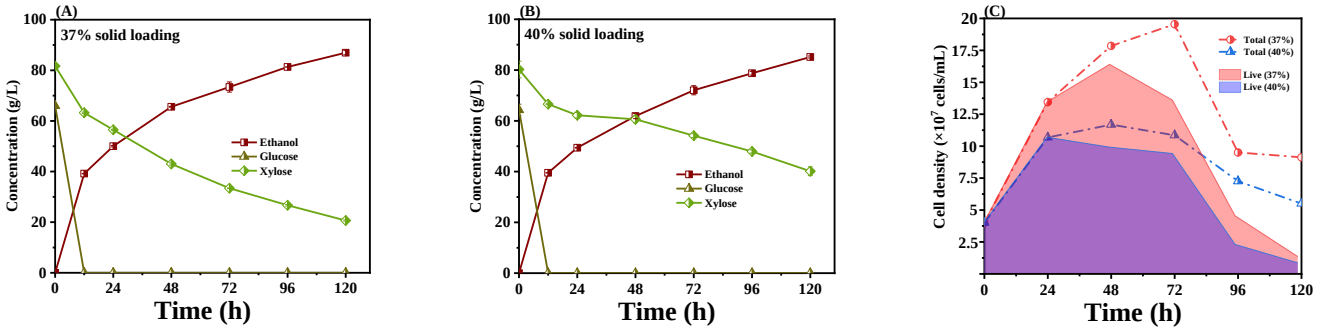

Figure 6. SSCF of DLCA(sa)-CC at ultra-high solids loadings; (**A**) 37% solid loading; (**B**) 40% solid loading; (**C**) cell density. DLCA(sa)-CC was pre-hydrolyzed for 18 h at 50 °C, 250 rpm, and pH 4.8 with an enzyme dosage of 20 mg protein/g glucan. SSCF with *S. cerevisiae* CRD5HS (initial OD_{600} = 4) was conducted at 30 °C, 150 rpm, and initial pH 5.5 for 120 h.

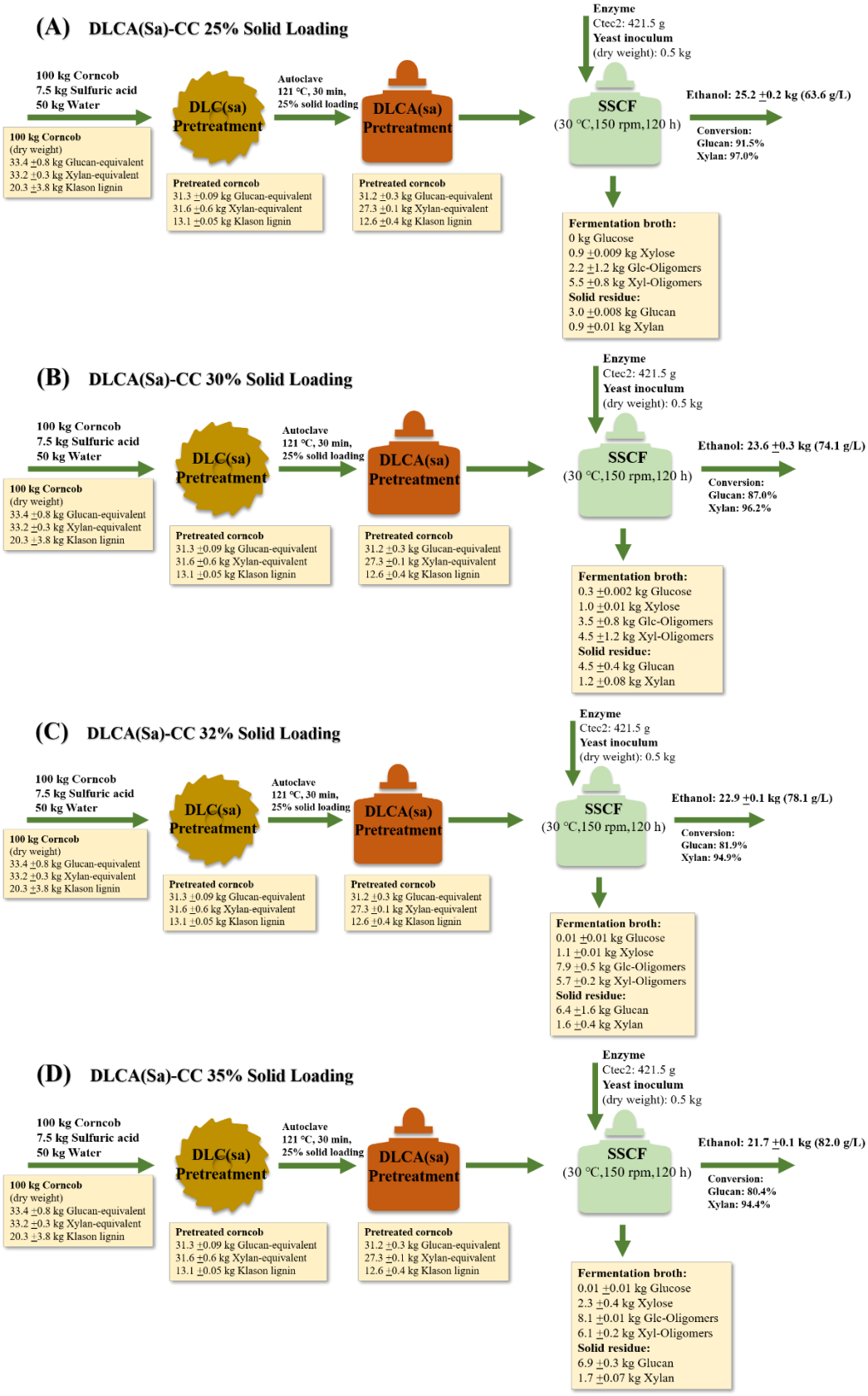

Figure 7. Mass balances of SSCF on DLCA(sa)-CC at different solids loadings. (**A**): 25% solid loading; (**B**): 30% solid loading; (**C**): 32% solid loading; (**D**): 35% solid loading.

4. Conclusions

DLCA(sa) pretreatment with mild conditions greatly enhanced the efficiency of enzymatic hydrolysis on corncob. Enzymatic hydrolysis and fermentation of DLCA(sa)-CC were conducted successfully at high solids loadings with no washing or detoxification required. The total sugar concentration reached up to 201.3 g/L after enzymatic hydrolysis at 35% solid loading, which led to 75.7 g/L ethanol after fermentation by *S. cerevisiae* CRD5HS. SSCF further enhanced xylose consumption and ethanol titer. As a result, an ethanol yield of 21.67 kg/100 kg corn cob was achieved with an ethanol titer of 81.99 g/L at 35% solid loading. Further exploration of higher solid loading SSCF resulted in an ethanol titer up to 86.96 g/L at 37% solid loading.

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