

Article

Circulating of In Situ Recovered Stream from Fermentation Broth as the Liquor for Lignocellulosic Biobutanol Production

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Abstract

Developing a more efficient, cleaner, and energy-saving pretreatment process is the primary goal for lignocellulosic biofuels production. This study demonstrated the feasibility of circulating high-concentration acetone–butanol–ethanol (ABE) obtained via in situ product recovery (ISPR) as a pretreatment liquor. Taking ABE solvent separated from pervaporation (PV) and gas stripping (GS) as examples, results indicated that under dilute alkaline (1% NaOH) catalysis, the highly recalcitrant lignocellulosic matrices can be efficiently depolymerized, thereby improving fermentable sugars recovery in saccharification stage and ABE yield in subsequent fermentation stage. Results also revealed delignification of 91.5% (stream from PV) and 94.3% (stream from GS), with total monosaccharides recovery rates of 56.5% and 57.1%, respectively, can be realized when using corn stover as feedstock. Coupled with ABE fermentation, mass balance indicated a maximal 106.6 g of ABE (65.8 g butanol) can be produced from 1 kg of dry corn stover by circulating the GS condensate in pretreatment (the optimized pretreatment conditions were 1% *w/v* alkali and 160 °C for 1 h). Additionally, technical lignin with low molecular weight and narrow distribution was isolated, which enabled further side-stream valorisation. Therefore, integrating ISPR product circulation with lignocellulosic biobutanol shows strong potential for application under the concept of biorefinery.

Keywords: in situ product recovery; lignocellulose; pretreatment; organosolv; acetone–butanol–ethanol fermentation



Academic Editor: Massimiliano Fabbicino

Received: 10 March 2025

Revised: 24 July 2025

Accepted: 30 July 2025

Published: 3 August 2025

Citation: Su, C.; Gao, Y.; Zhang, G.; Zhang, X.; Li, Y.; Zhang, H.; Wen, H.; Ren, W.; Zhang, C.; Cai, D. Circulating of In Situ Recovered Stream from Fermentation Broth as the Liquor for Lignocellulosic Biobutanol Production. *Fermentation* **2025**, *11*, 453. <https://doi.org/10.3390/fermentation11080453>

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1. Introduction

Lignocellulose pretreatment is one of the critical steps in the second-generation biofuels production, targeted to overcome the inherent recalcitrance of the complex structure of lignocellulose to improve the overall yield of final bioproducts [1]. Among the rapidly developed techniques involved in lignocellulose pretreatment, the organosolv processes have long been considered as a “clean” technology, which allowed the co-generation of high-quality technical lignin as co-production along with the generation of the main products with high yield and efficiency [2,3].

The commonly used solvents for organosolv pretreatment include ethanol, glycerol, tetrahydrofuran, acetone, butanol, ethylene glycol, etc. [4], in which acid or alkaline cat-

alysts always participate. More specifically, the acidic conditions in typical organosolv processes could accelerate hemicellulose and lignin–carbohydrate complexes hydrolysis [5]. In contrast, alkaline catalysts promoted the preferential depolymerization of lignin through ester bond saponification and β -O-4 bond cleavage and preserved hemicellulose integrity [6]. Moreover, because of the high delignification and the reduction of crystallinity of cellulose, the organosolv processes could significantly improve the accessibility of the pretreated solid, thus contributing to the adsorption of cellulase [7].

After the proper organosolv pretreatment of lignocelluloses, the highly polysaccharides content solid bagasse can be readily hydrolysed into pentoses and hexoses-enriched liquor and afforded to various biofuels production by fermentation [8], for instance, biobutanol (n-butanol), the superior biofuel candidate, which is primarily obtained by acetone–butanol–ethanol (ABE) fermentation [9]. In recent years, research has employed organosolv pretreatments in lignocellulosic biobutanol production, which generally showed high ABE productivity because of the high polysaccharides recovery and excellent fermentation performances [10,11]. One of the major concerns of previous research is to harness the relatively sustainable, effective, and low-toxic biobased organics, the by-products of the ABE production chain, including ethanol and acetone, as the liquor in the organosolv fractionation [11]. Meanwhile, several research also suggested that the lignin-rich stream and saccharides-rich stream can be easily separated in the biphasic reactant after butanol–water pretreatment, which further expanded the availability of organosolv pretreatment in lignocellulosic biobutanol production [12].

However, although it has been well revealed that using single ABE solvents in alkaline catalysis pretreatment exhibited high performance, when taking a comprehensive analysis of the whole process, these strategies are not matched well with the downstream separation systems [13]. For instance, it required an energy-intensive and complex downstream distillation sequence to separate these solvents solely [14]. In addition, in the organosolv process, it was revealed that an appropriate concentration of organics in an aqueous solution results in better lignocellulose pretreatment performance than that of the high-purity dehydrated solvents [15]. Therefore, the organic streams outputted in downstream processes must be diluted to fit the optimal liquor concentration for pretreatment. However, after pretreatment, the received liquid undergoes an energy-intensive process for solvent recovery. To this end, Yang and Wang et al. come up with the organosolv pretreatment based on model ABE mixture [16], which can be easily obtained from the in situ product recovery (ISPR) systems or a beer column by normal vacuum evaporation [17,18]. However, this research only evaluated the corresponding processes using an ideal ABE mixture with constant concentration and constitution [18], which did not match the output streams' composition in downstream processes.

In fact, ISPR is one of the most effective strategies to improve the ABE fermentation and separation [19]. It avoids the end-product inhibition and outputs high-concentration ABE solvents with low energy requirement [20]. Previous research indicated that pervaporation (PV) and gas stripping (GS) are the common ISPR techniques that fit well with ABE fermentation [21]. PV has the advantages of high selectivity and high-throughput solvent separation [22], while GS is famous for its easy operation [23].

In this study, we investigated the possibility of circulating the ISPR products in a typical ABE process for alkaline catalysis organosolv pretreatment (Figure 1). ABE solutions were collected from PV and GS [24], the typical ISPR technologies with high continuity and low influence on fermentation, and the performance of these solutions in organosolv pretreatment and the following ABE fermentation using lignocellulosic sugars are investigated. The lignin specimens isolated by the novel circulating ABE pretreatment process were also characterized to highlight co-products' benefit under the biorefinery concept.

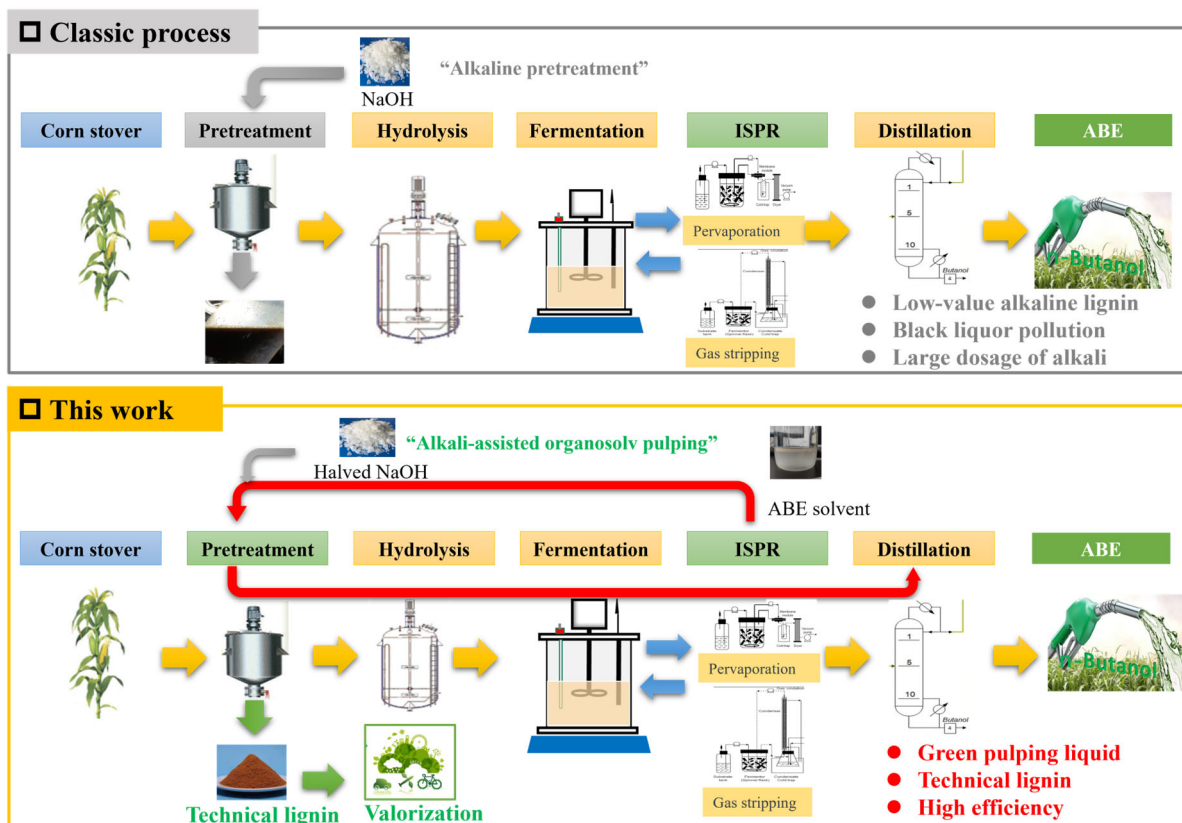


Figure 1. Diagram for circulation of ISPR stream as the liquor for lignocellulosic biobutanol production.

2. Materials and Methods

2.1. Materials

Corn stover was collected from a local farm in Qinhuangdao. After harvesting, the corn stover was dried out and ground into fine powder (~20 mesh). The corn stover bagasse mainly consisted of $32.5 \pm 1.2\%$ glucan, $18.2 \pm 0.9\%$ xylan, $18.6 \pm 0.5\%$ acid-insoluble lignin, and $3.4 \pm 0.2\%$ acid-soluble lignin (determined by NREL standard [25]).

All chemicals used in the experiments were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), and organic solvents were obtained from Beijing Chemical Works (Beijing, China). Cellulase was procured from KDN Biotechnology Co., Ltd. (Shandong, China). The activity of cellulase was determined at 65 ± 5 FPU/mL.

2.2. Organosolv Pretreatment

High-concentration ABE mixtures were used as the pretreatment liquor, according to the survey of our previous research on fermentation–PV coupling and fermentation–GS coupling processes [26]. Specifically, the PV liquid contained 15.69 g/L ethanol, 117.69 g/L acetone, and 180.00 g/L n-butanol (total solvent concentration of 313.38 g/L), while the GS liquid contained 11.39 g/L ethanol, 44.06 g/L acetone, and 112.79 g/L n-butanol (total solvent concentration of 168.24 g/L).

The alkaline catalysis ABE pretreatment was conducted at temperatures ranging from 120 °C to 220 °C for 1 h, with different NaOH catalysis dosages. The codes for experimental groups were as follows: type of ISPR + alkaline dosage + temperature. After cooling down to room temperature (~25 °C), the solid was subjected to solid-liquid separation. The solid was washed with fresh ABE solution and deionized water until the surface pH was approaching 7, followed by drying out at 105 °C for 12 h. The liquid fraction was collected and neutralized to pH 5.5 using 6M hydrochloric acid. Then, excessive dehydrated ethanol

was added to the liquor to remove the polysaccharides. After recovery of solvents by vacuum evaporation, pH of the concentrated lignin suspension was adjusted to 2.0 with 6M hydrochloric acid. The precipitates were collected and washed with deionized water and freeze-dried, and finally, the lignin specimens were analyzed. Solid recovery is the percentage of the solid after pretreatment and washing of the corresponding raw corn stover bagasse. The calculation formula is as follows:

$$\text{Solid recovery of pulp} = \frac{m_1}{m_2} \times 100\% \quad (1)$$

The calculation formula for the polysaccharides recovery after pretreatment is as follows:

$$\text{Recovery of glucan} = \frac{m_1 \times y_1}{m_2 \times y_2} \times 100\% \quad (2)$$

$$\text{Recovery of xylan} = \frac{m_1 \times y_3}{m_2 \times y_4} \times 100\% \quad (3)$$

$$\text{Recovery of total polysaccharides} = \frac{m_1 \times (y_1 + y_3)}{m_2 \times (y_2 + y_4)} \times 100\% \quad (4)$$

where m_1 represents the mass of the pretreated solid and y_1 represents the percentage of the glucan component in the pretreated solid. m_2 represents the mass of raw corn stover, and y_2 represents the percentage of the glucan component in corn stover. y_3 represents the percentage of the xylan component in the pretreated solid, and y_4 represents the percentage of the xylan component in the corn stover.

2.3. Enzymatic Hydrolysis

Enzymatic saccharification of the ABE pretreated solids was performed in a 250 mL conical flask with 100 mL working volume. The solid dosage was 6% (w/v), while cellulase loading was 20 FPU/g glucan. A total of 0.05 M phosphate buffer (pH = 4.8) was used as buffer. The enzymatic hydrolysis was performed at 50 °C and 200 rpm for 48 h. After hydrolysis, the solid fractions in slurry were separated by centrifugation, and the supernatants were collected, adjusted pH to ~6.8 with ammonium hydroxide, and stored at −20 °C before use.

The calculation formulas for the relevant enzymatic hydrolysis efficiency, sugars recovery, and yield were referred to previous work [27,28]. The specific equations are as follows:

$$\text{Enzymatic efficiency of glucan} = \frac{C_1 \times V \times 0.9}{m_1 \times y_1} \times 100\% \quad (5)$$

$$\text{Enzymatic efficiency of xylan} = \frac{C_2 \times V \times 0.88}{m_1 \times y_3} \times 100\% \quad (6)$$

$$\text{Enzymatic efficiency of total polysaccharides} = \frac{C_1 \times V \times 0.9 + C_2 \times V \times 0.88}{m_1 \times (y_1 + y_3)} \times 100\% \quad (7)$$

where c_1 represents the glucose concentration in hydrolysate, V represents the volume of the hydrolysate, and c_2 represents the xylose concentration in hydrolysate.

Furthermore, the sugars recovery after enzymatic hydrolysis is calculated as follows:

$$\text{Recovery of total monosaccharides} = \frac{C_1 \times V \times 0.9 + C_2 \times V \times 0.88}{m_2 \times (y_2 + y_4)} \times 100\% \quad (8)$$

2.4. ABE Fermentation

To verify whether the enzymatic hydrolysates of the ABE-pretreated solids can be used as a substrate for lignocellulosic ABE production, batch fermentation was conducted

in 250 mL serum bottles (with 150 mL of working volume) using *C. acetobutylicum* ABE 1401, a laboratory-stored, hyper biobutanol-producing, and inhibitor-tolerancing strain derived from *C. acetobutylicum* ATCC824 [23]. The seed medium contained glucose 40 g/L, ammonium acetate 2.2 g/L, KH_2PO_3 1.0 g/L, K_2HPO_3 1.0 g/L, MgSO_4 0.20 g/L, MnSO_4 0.01 g/L, FeSO_4 0.01 g/L, p-aminobenzoic acid 0.001 g/L, and biotin 0.0001 g/L. Before inoculation with a size of 10 vol%, the medium was autoclaved at 116 °C for 25 min. The anaerobic environment was constructed by purging sterilized N_2 (99.99%) into the medium for 15 min. ABE fermentations, either using the seed medium or the hydrolysate, were conducted at 37 °C and 50 rpm. For the ABE fermentation using lignocellulosic hydrolysate, nutrients in the seed medium were added to the crude hydrolysate, except for sugars.

2.5. Characterization and Analysis

The microstructural changes of raw corn stover bagasse and the pretreated solids were observed using a scanning electron microscope (SEM, S-3000N, Hitachi, Tokyo, Japan). The crystallinity was characterized by X-ray diffraction (XRD, 2500VB2, Rigaku Corporation, Osaka, Japan). Before testing, samples were scanned from 5° to 40° at a 2°/min rate. The crystallinity index (CrI) was calculated through the following equation:

$$\text{CrI} = (I_{002} - I_{am})/I_{002} \times 100\% \quad (9)$$

where I_{002} represents the intensity of the cellulose lattice diffraction peak at $2\theta = 22.5^\circ$ and I_{am} represents the intensity of the amorphous region diffraction peak at $2\theta = 15.5^\circ$.

The thermal stability of the pretreated solids and lignins was analyzed using a thermogravimetric analyzer (TGA-Q500, Taber Industries, New York, NY, USA). The Fourier transform infrared (FTIR) spectroscopy was conducted using the KBr pellet method using Thermo Scientific Nicolet 6700 (Thermo Fisher Scientific Inc., Waltham, MA, USA) with a scanning range of 400–4000/cm, 128 scans, and a resolution of 2/cm. The molecular weight distribution and polydispersity of lignin specimens were determined by gel permeation chromatography (GPC, Agilent 1200, Agilent Technologies Inc., Santa Clara, CA, USA). The heteronuclear single quantum correlation (HSQC) spectra were analyzed using a Bruker Magnet system 400 MHz NMR spectrometer (Bruker Technologies Co., Ltd., Billerica, MA, USA). Scans were performed in ^{13}C and ^1H dimensions, with scanning ranges of 18,000 Hz and 5000 Hz, respectively.

Glucose and xylose were analyzed by high-performance liquid chromatography (HPLC, U3000, Thermo Fisher Scientific Inc., Waltham, MA, USA) equipped with an organic acid column (Aminex HPX-87H, Bio-Rad Laboratories, Hercules, CA, USA). The column temperature was maintained at 65 °C. The mobile phase was 5 mM sulfuric acid at 0.6 mL/min flow rate, with an injection volume of 10 μL . Gas chromatography (GC) was used to detect organic solvents' concentration referred to previous work [27].

3. Results and Discussions

3.1. Acetone–Butanol–Ethanol Pretreatment Performances

Figure 2 shows the effect of alkaline-catalysed ABE pretreatment on solid recovery. As the reaction temperature increases from 120 °C to 220 °C, the solid recovery gradually decreased. More specifically, the solid recovery in the PV–ABE group dropped significantly from 72.8% to 32.5% obtained in the alkaline-free group (Figure 2a), mainly due to the rapid dissolution of lignin at high temperatures [29]. In addition, the removal of acetyl groups in hemicellulose at high temperatures would produce acetic acid, further exacerbating the loss of solid [30].

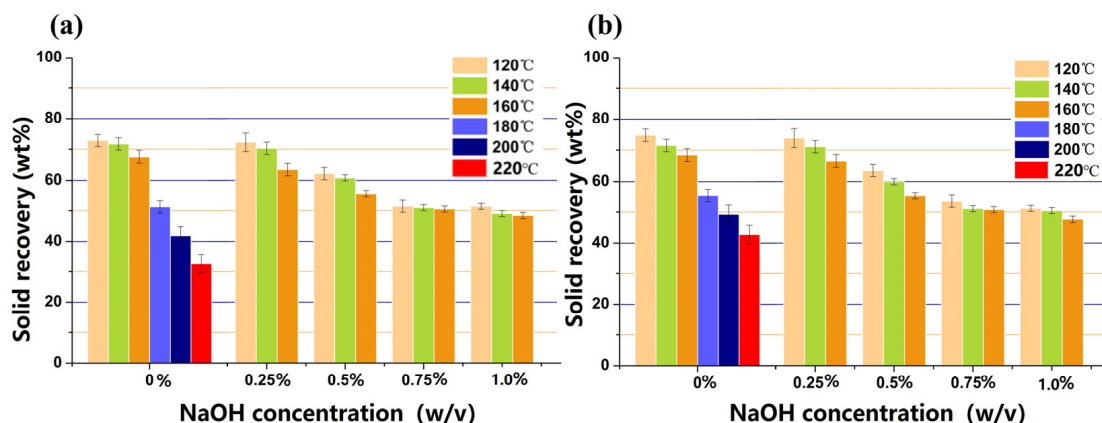


Figure 2. Effect of alkaline catalysis involved in ABE pretreatment. (a) PV-ABE groups and (b) GS-ABE groups.

The solid recoveries of the GS-ABE groups were slightly higher than the PV-ABE groups. This may be caused by different water content in initial liquid, resulting in higher lignin dissolution. Considering the impact of monosaccharides recovery in the saccharification process, a pretreatment temperature below 180 °C was more favorable in the subsequent alkaline-assisted ABE pretreatment.

The alkaline catalyst loading also influenced the solids' recovery. As the NaOH concentration gradually increased, the solid recovery rate continued to decrease. Interestingly, at a low concentration of NaOH (0.25%) loading, the temperature increase could significantly promote the delignification. Figure 2a shows that as the temperature increased from 120 °C to 160 °C, the solid recovery decreased from 72.2% to 63.5%. However, when loading high concentrations of alkaline (1%), the solid recovery remained between 51.2% and 47.7%, respectively, for GS-1%-120 °C and GS-1%-160 °C (Figure 2b).

The difference in solid recovery is fundamentally due to the difference in the main components of the solid after ABE pretreatment. Figure 3 shows that the lignin content gradually decreased with the increase of NaOH dosage [31]. In addition, the alkaline-catalysed saponification of the intermolecular ester bond further promoted the dissolution of hemicellulose and lignin [32].

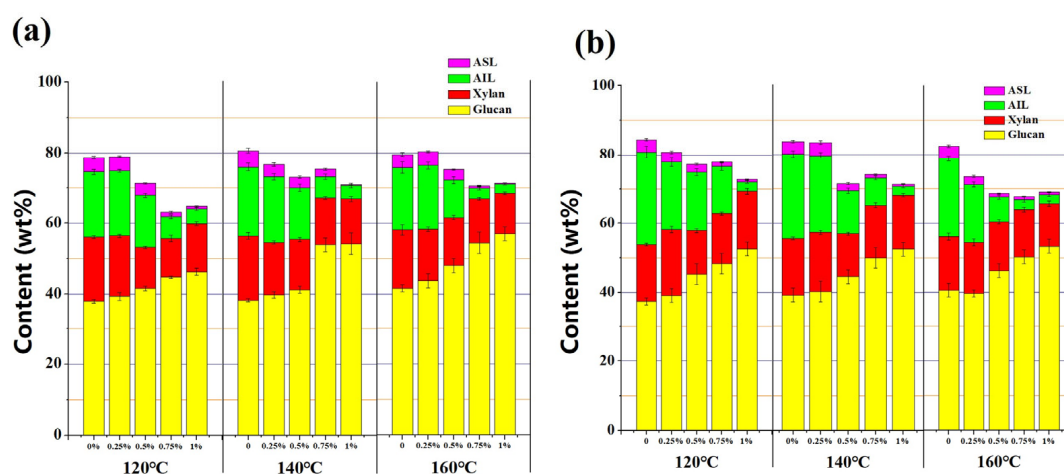


Figure 3. Chemical compositions of the solids after alkaline catalysis ABE pretreatment under various catalyst dosages and temperatures. (a) PV-ABE and (b) GS-ABE. AIL: acid-insoluble lignin; ASL: acid-soluble lignin.

The glucan recovery decreased slightly with the harsher organosolv pretreatment conditions (increased temperature and increased NaOH dosage) (see Figures S1a and S2a).

In contrast, the loss of xylan showed a similar and obvious increasing trend as the removal of lignin. The change of residual lignin content in solids also highlighted the importance of alkaline catalysis participating in ABE pretreatment.

3.2. Structural Characterization of the Pretreated Solids

SEM images (see Figure S3) show the changes in the micromorphology of the solid components after ABE pretreatment. The raw corn straw bagasse usually has a smooth and regular surface microstructure with neatly arranged fibers and no obvious wrinkles or porous structures. In contrast, the SEM images show that the surface morphology is significantly damaged and exhibits a large number of fragments and a rough surface due to the removal of hemicellulose and delignification after alkaline catalysis ABE pretreatment. We speculated that the rough surface of ABE pretreated solids can significantly improve the accessibility of cellulase, thereby improving the enzymatic hydrolysis efficiency in saccharification process [33].

The XRD spectra of ABE-fractionated solids are further analyzed (Figure S4). Generally, all samples showed weak diffraction peaks at 16.5° and strong diffraction peaks at 22.5° . Compared with untreated raw corn stover ($\text{CrI} = 43.2\%$, see Table 1), the crystallinity of the solids after pretreatment is reduced. In fact, the alkaline catalysis ABE pretreatment destroys the crystal structure of cellulose, resulting in a decrease in crystallinity [34]. However, as the pretreatment conditions become more severe, the delignification effect was enhanced, accompanied by enhanced hemicellulose hydrolysis, which increases the overall apparent crystallinity of the pretreated solids.

Table 1. Crystallinity of the pretreated solids after alkaline catalysis ABE pretreatment of corn stover.

Conditions	I_{002}	I_{am}	CrI (%)
PV-1%-120 °C	3.81×10^3	2.23×10^3	41.5
PV-1%-140 °C	3.46×10^3	2.16×10^3	37.6
PV-1%-160 °C	3.77×10^3	2.50×10^3	33.7
PV-0.25%-140 °C	3.59×10^3	2.17×10^3	39.6
PV-0.50%-140 °C	3.59×10^3	2.11×10^3	41.2
PV-0.75%-140 °C	3.60×10^3	2.25×10^3	37.5
GS-1%-120 °C	2.29×10^3	1.61×10^3	29.7
GS-1%-140 °C	2.23×10^3	1.49×10^3	33.2
GS-1%-160 °C	2.74×10^3	1.71×10^3	37.6
GS-0.25%-160 °C	2.77×10^3	1.88×10^3	32.1
GS-0.5%-160 °C	2.77×10^3	1.82×10^3	34.3
GS-0.75%-160 °C	2.73×10^3	1.75×10^3	35.9
Raw straw	2.36×10^3	1.34×10^3	43.2

3.3. Enzymatic Hydrolysis of the Pretreated Solids

Compared with raw corn stover, the enzymatic hydrolysis performances of ABE-pretreated solids were significantly improved. As shown in Figure 4a,b, the monosaccharides concentration in hydrolysate increased significantly, along with the severity of pretreatment conditions. For instance, the total sugars recovery was significantly affected by the increase of pretreatment temperature, while the delignification is promoted by alkaline catalysis [35] (Figure 4c,d). Specifically, for the PV-ABE groups, at 140 °C, with the increase in NaOH dosage, the total sugars concentration increased from 11.05 g/L (0% NaOH loading) to 39.1 g/L (1% NaOH loading, including 31.8 g/L glucose and 7.32 g/L xylose). For the GS-ABE group, due to the low ABE concentration in the initial liquor, the sugars yield was significantly increased, regardless of the increase of temperature or alkaline dosage. When the alkaline dosage was 1% and the temperature was 160 °C, a

maximum 40.67 g/L of mono-sugars (including 32.47 g/L glucose and 8.20 g/L xylose) was obtained.

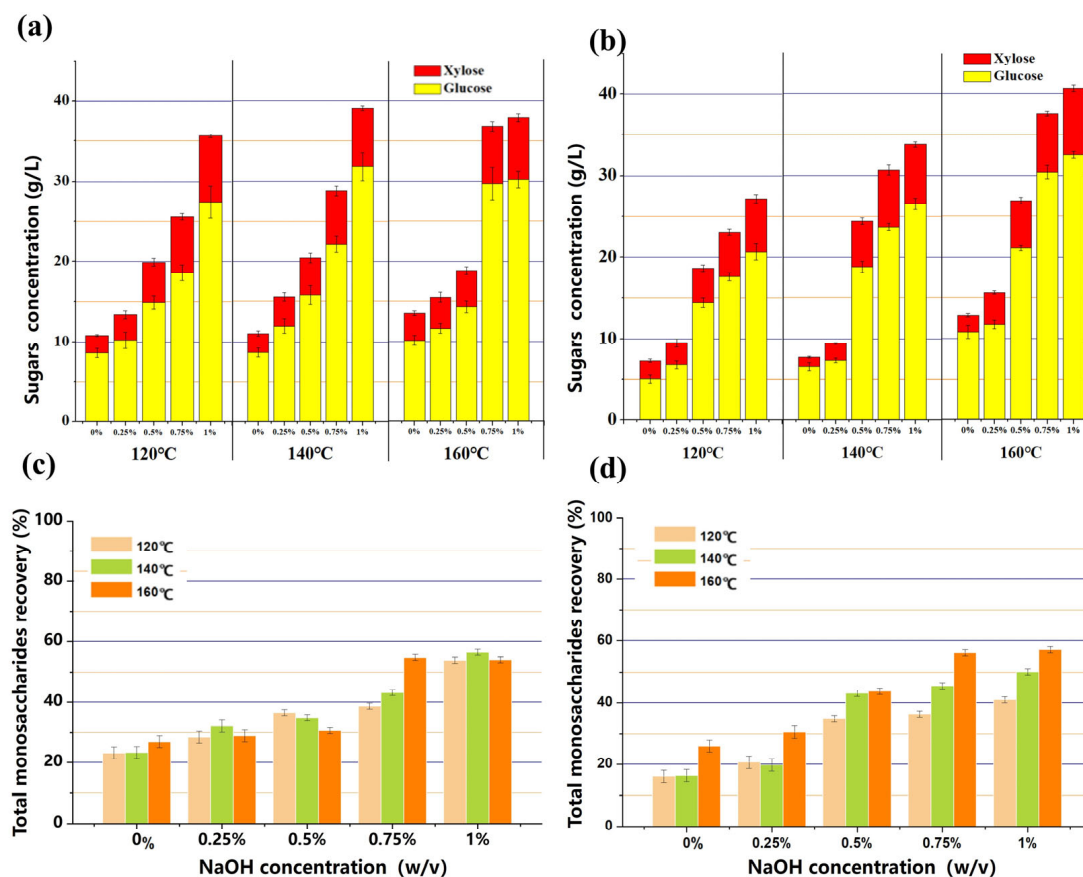


Figure 4. Sugars concentration in hydrolysate and the total monosaccharide recovery from the raw corn stover after enzymatic hydrolysis. (a,c) for PV-ABE groups and (b,d) for GS-ABE groups.

Taking into account the solid recovery, the recovery rate of monosaccharides by enzymatic hydrolysis also increases with the severity of the pretreatment conditions. For example, in the GS-1%-160 °C group, the total sugars recovery after enzymatic hydrolysis of the corresponding pretreated solid was 57.1%. The results in Table 2 show that the enzymatic hydrolysis efficiency of 0%, which is higher than that of the PV-ABE group under similar conditions. This may be due to the excessive solvent causing residual lignin to deposit on the surface of lignocellulose, resulting in the ineffective adsorption of cellulase [36].

Table 2. The enzymatic hydrolysis efficiency of total polysaccharides in saccharification.

Alkaline Dosage (%, w/v)	PV-ABE Pretreated Solid (%)			GS-ABE Pretreated Solid (%)		
	120 °C	140 °C	160 °C	120 °C	140 °C	160 °C
0.00	28.8	29.3	34.8	20.2	20.8	34.3
0.25	35.4	42.5	39.7	24.4	24.6	42.8
0.50	56.2	52.4	45.6	47.9	63.9	66.6
0.75	68.4	64.1	82.2	54.8	69.4	87.8
1.00	88.7	87.4	82.7	58.6	74.0	92.6

3.4. ABE Fermentation Using the Hydrolysates

The hydrolysates were used as substrates for ABE fermentation. The initial sugar concentrations in the fermentation medium were 32.1 ± 0.4 g/L glucose and 7.2 ± 0.2 g/L

xylose (PV-1%-140 °C) and 32.52 ± 0.06 g/L glucose and 8.3 ± 0.2 g/L xylose (GS-1%-160 °C), which were basically consistent with the enzymatic hydrolysis results described in Section 3.3. The synthetic medium with similar sugars constitution toward the actual hydrolysate was treated as the control.

As shown in Figure 5, after 120 h of batch fermentation of the PV-1%-140 °C hydrolysate, 7.86 g/L butanol (total 12.7 g/L ABE) was detected in the fermentation broth, with an overall 0.320 g/g (total sugars) of ABE yield. In contrast, 8.35 g/L butanol (total 13.5 g/L ABE) and 0.331 g/g yield were obtained using the GS-1%-160 °C hydrolysate as substrate under similar conditions. We ascribed the difference in ABE solvent production of the two groups to the difference in initial sugars concentration in the hydrolysate. The total sugars concentration of the GS-1%-160 °C hydrolysate was 3.99% higher than that of the PV-1%-140 °C hydrolysate.

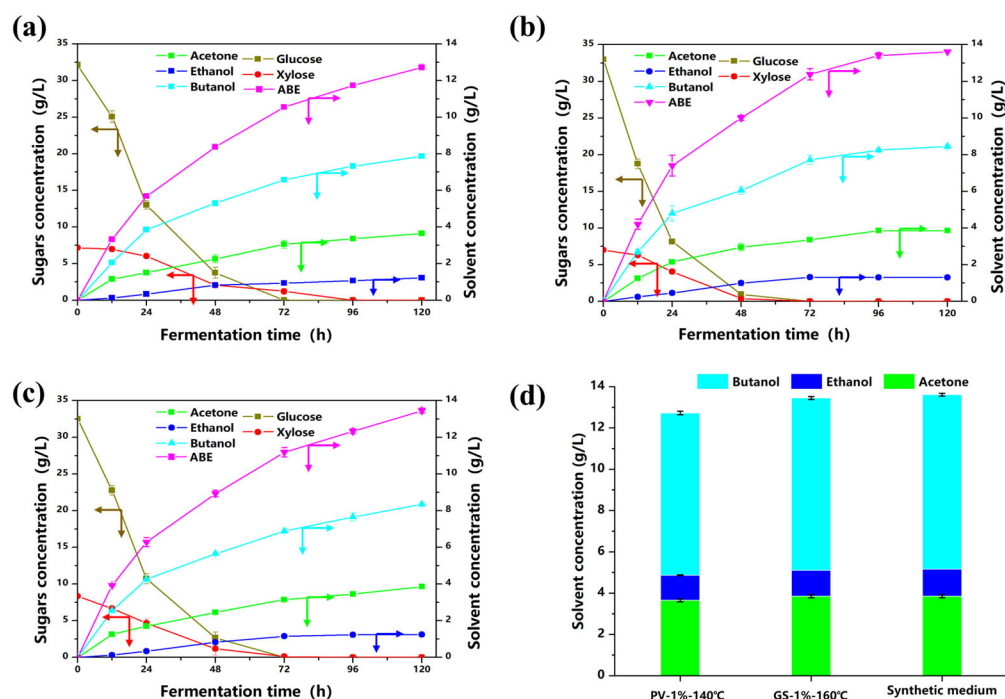


Figure 5. Time course of ABE fermentation using the hydrolysates. (a) PV-1%-140 °C, (b) GS-1%-160 °C, and (c) synthetic medium. (d) The specific ABE concentrations in final fermentation broth.

These results are comparable to the ABE fermentation using synthetic medium, except for a slight decrease in ABE yield and concentrations. In control group, 13.6 g/L ABE with a yield of 0.340 g/g can be obtained. The reductions of ABE yield and concentration in actual corn stover hydrolysates can be attributed to the residual inhibitors, such as the phenolic compounds involved in fermentation [37,38]. However, different with the previous literatures that the lignocellulosic biobutanol production suffered from severe by-production inhibition in hydrolysate, the toxic effect of the residual inhibitors exhibited very slight negative influence on the metabolism of clostridia cells and ABE production in current work, which could be attributed to the high tolerance of inhibitors by the mutant ABE 1401 strain [23] and the effective fractionation in the ABE pretreatment process. As shown in Figure 6, a maximum 106.6 g ABE (65.80 g butanol) can be obtained from 1 kg of dry corn stover using the ISPR condensate of GS as initial liquor (the pretreatment conditions were 1% NaOH catalyst and 160 °C for 1 h), based on the novel circulating ABE pretreatment strategy.

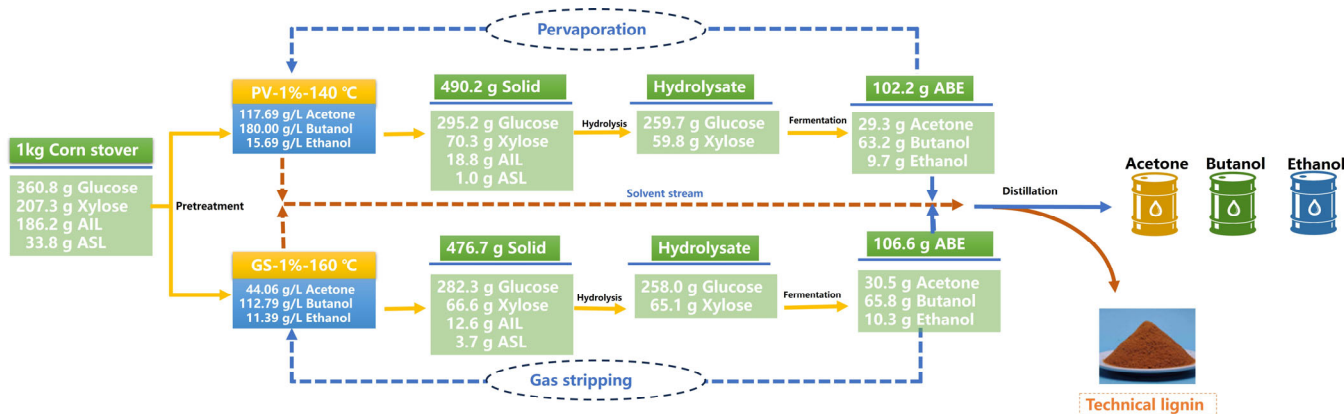


Figure 6. Mass balance of the circulating ABE pretreatment for lignocellulosic biobutanol production.

3.5. Characterization of the Isolated Lignin

Compared with other lignocellulose pretreatment techniques, one of the apparent advantages of organosolv pretreatment is the co-generation of the valuable technical lignin that is rich in active functional groups with relatively narrow molecular weight distributions [39]. For cases that solely used ABE solutions as initial liquor, various researchers have revealed that fractionated lignin can be further valorised into advanced fuels, chemicals, and materials [40]. However, because of the lack of research using ABE mixture for organosolv pretreatment, there is still little information on lignin obtained in such processes. This enables us to further characterize the isolated lignin specimens from PV-ABE and GS-ABE processes.

The chemical group variations of lignin specimens were analyzed by FTIR (Figure S5). The absorption peak at 3350 cm^{-1} is attributed to the stretching vibration of hydroxyl groups. In contrast, the 2910 cm^{-1} and 2870 cm^{-1} peaks correspond to the stretching vibrations of methyl and methylene C-H bonds, respectively [41]. Meanwhile, the intensity of the ester C=O stretching vibration peak at 1705 cm^{-1} is decreased with the increase of alkaline concentration in the initial liquor [42]. This peak is associated with the C=O stretching vibrations of acetyl groups in hemicellulose and ester bonds between lignin and hemicellulose, indicating more complete ester bond cleavage, reduced sugar content, and increased lignin purity [43]. The absorption peaks at 1168 cm^{-1} , 1605 cm^{-1} , 1510 cm^{-1} , and 1420 cm^{-1} are aromatic ring stretching vibrations in lignin [44]. The relatively weak peak at 1365 cm^{-1} corresponds to free phenolic hydroxyl groups [45]. Meanwhile, the weak peak at 920 cm^{-1} indicates the presence of impurities in the extracted lignin [46].

HSQC NMR spectroscopy further provides detailed structural information of lignin specimens (Figure S6). The β -O-4 ether linkage, as well as resinol (β - β) and phenylcoumaran (β -5) structures in lignin, were detected in the side-chain region of the two spectra. Compared to GS-1%-160 °C, signals for α - and β -aryl ether bonds in PV-1%-140 °C lignin were stronger, while the γ -aryl ether bond signal was more pronounced, indicating less cleavage of aryl ether bonds [47]. Besides, the alkaline dosage had a limited promotion to the cleavage of α -, β -, and γ -aryl ether bonds, whereas temperature played a more significant role in bond cleavage [41]. The β - β bond signal in PV-1%-140 °C lignin was weaker, while that in GS-1%-160 °C lignin was stronger. Additionally, both specimens exhibited signals for p-hydroxycinnamyl alcohol (I) at δ_C/δ_H 61.5–62.9/4.0–4.1 ppm. PV-1%-140 °C lignin retained a significant amount of β -O-4 bonds, while GS-1%-160 °C lignin showed a signal for spirodienone (D) at δ_C/δ_H 80.5–81.5/4.65–5.15 ppm, with no signal for xylan (X5). This is consistent with the information obtained from FTIR spectra, which shows that the ester bonds between lignin and acetyl groups in hemicellulose are completely cleavage.

In the aromatic region, PV-1%-140 °C and GS-1%-160 °C lignin specimens exhibited three signals for G-type units [48]. Meanwhile, S-type lignin units showed varying degrees of oxidation. H-type lignin signals were observed at δ_C/δ_H 127.1–131.2/6.9–7.3 ppm and δ_C/δ_H 125.5–131.5/6.6–7.3 ppm. Additionally, ferulate (FA) and p-coumarate (PCE) structures were also detected in both lignin specimens, indicating alkaline-facilitated ester bond cleavage, consistent with the side-chain region analysis.

As aforementioned, when considering treating the organosolv lignin as a valuable technical lignin, except for the functional group content and properties, another critical issue to be concerned about is the homogeneity [49]. Table 3 shows the weight-average molecular weight (M_w), number-average molecular weight (M_n), and polydispersity index (PDI) of lignin specimens. The information indicated that no matter the ABE composition, pretreatment temperature, and dosage of NaOH catalyst, lignin specimens obtained in the novel pretreatment process exhibited relatively low molecular weights and narrow distributions. Specifically, in the GS–ABE groups with 1% NaOH dosage, the PDI remained relatively stable when the pretreatment temperature increased from 140 °C to 180 °C, while the average molecular weight gradually increased. This phenomenon differs from those observed in lignin specimens obtained in PV–ABE groups. We speculate that higher solvent concentrations at higher pretreatment temperatures could facilitate the depolymerization of lignin, whereas low solvent concentrations promote lignin self-condensation.

Table 3. GPC results of the isolated lignin specimens from the ABE pretreatment processes.

Conditions	M_w (g/mol)	M_n (g/mol)	PDI
PV-1%-120 °C	1.54×10^3	1.29×10^3	1.19
PV-1%-140 °C	1.50×10^3	1.26×10^3	1.19
PV-1%-160 °C	1.38×10^3	1.22×10^3	1.13
PV-0.25%-140 °C	1.70×10^3	1.40×10^3	1.21
PV-0.50%-140 °C	1.65×10^3	1.36×10^3	1.21
PV-0.75%-140 °C	1.51×10^3	1.28×10^3	1.18
GS-1%-140 °C	1.20×10^3	1.11×10^3	1.08
GS-1%-160 °C	1.35×10^3	1.19×10^3	1.13
GS-1%-180 °C	1.45×10^3	1.24×10^3	1.17
GS-0.25%-160 °C	1.71×10^3	1.40×10^3	1.22
GS-0.50%-160 °C	1.71×10^3	1.38×10^3	1.24
GS-0.75%-160 °C	1.30×10^3	1.16×10^3	1.12

TGA-derivative thermogravimetric (DTG) analysis was further conducted to investigate the thermo-properties of the lignin specimens (Figure S7). The results revealed that the derivative DTG curves of PV-1%-140 °C and GS-1%-160 °C lignin specimens all exhibited single weight loss rate peaks at 348 °C and 352 °C. Additionally, the TGA curves indicated that moisture evaporation occurred in the temperature range from room temperature (~25 °C) to 200 °C. Between 200 °C and 500 °C, lignin undergoes significant degradation, producing gases such as CH₄ and CO, accompanied by the degradation of residual polysaccharides. Notably, the degradation rate of the GS-1%-160 °C lignin peaked at 352 °C, which was higher than that of the PV-1%-140 °C lignin (348 °C), suggesting that lignin undergoes self-condensation at higher pretreatment temperatures. This is in accordance with the GPC results (Table 2). Both lignin specimens reached the degradation endpoint at 600 °C. The residual weight of the PV-1%-140 °C lignin was 36.2%, while that of the GS-1%-160 °C lignin was 37.7%, which were consistent with the hypothesis of lignin condensation at higher temperatures.

4. Conclusions

This study highlights the potential of recycling high-concentration acetone–butanol–ethanol (ABE) stream after in situ product recovery (ISPR) as the initial liquor for alkaline catalysis organosolv pretreatment in upstream process. The results from high-concentration ISPR ABE from the well-established pervaporation (PV) and gas stripping (GS) show that the novel process could effectively decompose the complex lignocellulose matrices. Furthermore, the saccharification and fermentation processes showed competitive enzymatic hydrolysis efficiency and fermentation performance. Mass balance indicated under the optimized conditions of GS-1%-160 °C in organosolv pretreatment, maximal 65.8 g of butanol, and 106.6 g of ABE solvent were produced from 1 kg of dry corn stover, with high-purity technical lignin co-generated. The novel process in the current research provides a valuable technological advancement for the sustainable development of second-generation biofuels.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/fermentation11080453/s1>, Figure S1. Effect of alkali-assisted organosolv pretreatment on pulp composition in PV-ABE groups. (a) Glucan recovery, (b) xylan recovery, (c) total polysaccharides recovery, and (d) the delignification of corn stover. Figure S2. Effect of alkali-assisted organosolv pretreatment on pulp composition in GS-ABE groups. (a) Glucan recovery, (b) xylan recovery, (c) total polysaccharides recovery, and (d) the delignification of corn stover. Figure S3. SEM images of raw corn stover and pretreated solids. (a,b) The raw corn stover, and the solids pretreated by (c,d) PV-1%-140 °C and (e,f) GS-1%-160 °C. Figure S4. XRD spectra of raw corn stover, and (a) PV-ABE and (b) GS-ABE pretreated solids. Figure S5. FTIR spectra of isolated lignin specimens after ABE pretreatment. (a), (b) PV-ABE groups, and (c), (d) GS-ABE groups. Figure S6. HSQC HMR spectra of the isolated lignin specimens from the ABE pretreated liquors. (a) PV-1%-140 °C and (b) GS-1%-160 °C, and (c) structural formulas corresponding to the main groups. Figure S7. TGA/DTG curves for the isolated lignin specimens from the ABE pretreated liquors. (a) PV-1%-140 °C and (b) GS-1%-160 °C. Table S1. Functional group interpretation and spectral attribution in the FTIR spectra of the lignin specimens isolated from the ABE pretreated liquors. Table S2. Signal assignment of the lignin specimens in HSQC spectrums.

Author Contributions: Methodology, C.S., Y.G., H.W. and W.R.; Formal analysis, C.S. and H.Z.; Investigation, C.Z.; Data curation, C.S., G.Z., X.Z., Y.L. and H.W.; Writing—original draft, H.W. and D.C.; Writing—review & editing, D.C.; Funding acquisition, D.C. All authors have read and agreed to the published version of the manuscript.

Funding: This work is funded by the National Key R & D Program of China (Grant No. 2022YFC2106300), the National Natural Science Foundation of China (Grant No. 22478021), and the Guangxi Science and Technology Program (Grant No. AA24206052).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Conflicts of Interest: The authors declare no conflict of interest.

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