Waste-to-energy: biobutanol production from cellulosic residue of sweet potato by *Clostridia acetobutylicum*

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ABSTRACT

Biobutanol has been emerging as a renewable replacement to overcome fossil fuel limit due to its attractive qualities. The waste resources utilization is an economically feasible and eco-friendly way to produce biobutanol. In this work, the potential of butanol fermentation with sweet potato residue (SPR) obtained from ethanol fermentation was evaluated. To assist sugar release for butanol production by *Clostridia acetobutylicum*, a pretreatment approach with dilute sulfuric acid was established via response surface methodology. The maximum butanol concentration of 3.77 g/L was obtained at the optimal conditions of pretreatment at 1% (v/v) dilute sulfuric acid, and 115°C for 30 min. Detoxifying the hydrolysate with XAD-4 resin significantly enhanced butanol fermentation performance, while adding nutritional supplements had no significant influence. The stability of the fermentation process was verified by the 2 L-scale of fermentation, resulting in 7.96 g/L of butanol, 13.42 g/L h of Acetone-Butanol-Ethanol (ABE), and 0.34 g/g of ABE yield, respectively. This study demonstrates a bio-refinery strategy of conversion from waste biomass to energy, confirms the feasibility of utilizing the cost saving SPR as feedstock to produce butanol through fermentation, and provides a novel approach for minimization of environmental pollution.

Keywords: Biobutanol, *Clostridia acetobutylicum*, Sweet potato residue, Resources recovery

1. Introduction

Currently, oil, coal and gas still account for most of primary energy consumption [1]. However, due to the limited supply of fossil fuels, the increasing and fluctuating price of gasoline and environmental concerns brought by greenhouse gas emissions, there has been an increased interest in looking for green and sustainable energy [2]. Liquid fuel derived from biomass provides an opportunity for nations to replace fossil fuel in transportation sector, increase energy independency and reduce greenhouse gas emissions, because the employed raw material is renewable and widespread [3, 4].

Among the biomass derived liquid fuels, butanol is superior to ethanol, considering its more hydrophobic property, higher energy density, better anti-corrosive property, compatibility with existing transportation pipeline infrastructures, and can be mixed with gasoline at any ratio [5]. Global demand for butanol keeps on raising, and the market size of butanol is estimated at USD 4.0 billion by 2025 [6]. However, current commercial biobutanol is mainly

produced from starch or sugar based crops, which is called first-generation biobutanol. Considering the cost, first-generation biobutanol is not competitive with fossil fuels because of expensive feedstock [7, 8]. Therefore, availability of suitable and cheap raw materials is one of the key factors for the industrialization of biobutanol production. At the same time, there are huge quantities of biogenic waste and residue all around the world. Minimizing or eliminating waste is a common pressure faced by all countries. Therefore, biobutanol production from waste biomass can reduce production costs and has environmental benefits, which is also known as the second-generation biobutanol.

China is the biggest sweet potato producer, with an annual yield of about 100 million tons [9], and almost 50% of sweet potato is processed into varieties of products such as ethanol. Sweet potato is an attractive raw material for bioethanol production [10, 11]. After fermentation, most of starch and soluble sugars in sweet potato are converted to ethanol by *Saccharomyces cerevisiae*, leaving a large amount of residue as environmental pollutant. The main

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components in the sweet potato residue (SPR) are cellulose, residual sugar and protein. Although the carbohydrates in SPR cannot be used by *Saccharomyces cerevisiae* to keep on producing ethanol, they can be utilized via Acetone-Butanol-Ethanol (ABE) fermentation by clostridia to produce butanol. However, to the best of our knowledge, there is no research reported on biobutanol production from SPR. When SPR was used to produce biobutanol, sweet potato could be converted to bioethanol and biobutanol step by step in an integrated bio-refinery manner to maximize the economic value of the feedstock while reduce the waste streams produced.

The existed clostridia strains are not able to efficiently hydrolyze non-starch polysaccharides and cellulose-rich residues. Residues must be hydrolyzed to simple sugars for butanol fermentation using physical-chemical pretreatment and/or enzymatic hydrolysis [12]. Pretreatment is one of the most costly steps among the whole process of conversion from biomass into biobased products, so the lower-cost and effective pretreatment approaches are strongly encouraged [13]. Although a variety of methods has been developed for the pretreatment of cellulosic biomass, dilute sulphuric acid pretreatment is still the most widely used and profitable method [14]. Unfortunately, besides sugars, various microbial inhibitors are generated during acid hydrolysis such as furfural, 5-hydroxymethyl furfural, acetic acid, formic acid, ferulic acid, coumaric acid, syringaldehyde and vanillin [15, 16]. The toxicity of inhibitors in hydrolysate is a major obstacle to the following butanol fermentation. To ensure the vitality of the fermentation microorganisms and to have high-performance fermentation, removing the inhibitors by detoxification is necessary.

Therefore, the main objective of this work is to develop an novel approach for biobutanol production using the residue generated from bioethanol fermentation of sweet potato in an integrated bio-refinery concept to improve the overall efficiency of biomass utilization and ultimately reduce the waste stream. Specifically, this paper will optimize pretreatment process, overcome fermentation inhibition and investigate the effects of dilute sulphuric acid pretreatment, detoxification methods as well as nutrients supplementation on biobutanol fermentation performance of *Clostridia acetobutylicum* CICC 8012.

2. Material and Methods

2.1. Microorganism Strain and Inoculum Development

Clostridia acetobutylicum strain CICC 8012 was purchased from China Center of Industrial Culture Collection (CICC). The freeze-dried cultures were rejuvenated according to CICC's instructions. Activated spores which were ready for inoculum development were maintained in sterile corn medium (50 g corn powder per liter) and stored under anaerobic conditions at 4°C. Before fermentation, spores were heated at 100°C for 90 s, and vegetative cells were grown anaerobically in corn medium at 37°C for 24 h.

2.2. Characterization of the SPR

SPR were collected from an industrial plant with capacity of 10,000 tons ethanol per year [11]. Fresh SPR was dried and milled using a high-speed pulverizer (JP-300A; JIUPIN Industry and Trade Co.,

China). Cellulose content was determined by the acetic/nitric reagent extraction method [17]. Total residual sugar was determined by HPLC (ELSD 6000; All-tech. corp., CA) using an Aminex HPX-87-Pb column (Bio-Rad, Hercules, USA) at 80°C and flow rate of 0.6 ml/min with distilled water as eluent. Nitrogen gas was used as carrier at the pressure of 2.8 Bar and draft temperature was 105°C for the ELSD detector [11, 19]. Protein content was determined by the Kjeldahl method [18]. The ash content of lignin was determined gravimetrically in a muffle furnace at 550°C for 4 h. The sample was digested in a mixture of nitric and perchloric acids (5+1, v/v) and analyzed for Na, K, Ca, Mg, P, Cu, Mn, Zn and Fe using inductively coupled plasma atomic emission spectrometer (ICP-AES, IRIS 1000).

2.3. Pretreatment of the SPR

As pretreatment for enzymatic hydrolysis, every 100 g of SPR was suspended in 0.9 L of dilute sulfuric acid. The central composite design (CCD) with response surface methodology (RSM) was used to investigate the effects of pretreatment conditions on butanol production. Based on preliminary single factor experiments, dilute sulphuric acid concentration (A), thermal treatment temperature (B) and thermal treatment time (C) were chosen as factors (independent variables) and final butanol concentration as response variable (dependent variable). In this experimental design, there were 3 coded factor levels: -1, 0, +1; in which -1 corresponds to the low level of each factor, 0 to the mid-level, and +1 to the high level. The levels were settled down as A (0.5, 1.0, 1.5%, v/v), B (110, 115, 120°C), and C (15, 30, 45 min).

2.4. Enzymatic Hydrolysis of the SPR

The pretreated mixture was cooled to room temperature, and the pH was adjusted to 4.8 using $\text{Ca}(\text{OH})_2$. Subsequently, 1 mL of cellulase and 1 mL of xylanase were added to 1 L of the mixture. After this, the mixture was incubated at 50°C for 48 h. Finally, the mixture was centrifuged at 5,000 g for 5 min to remove sediment. The cellulase (GC220, DuPont™ Genencor® Science) had a declared activity of 89 FPU/mL, 184 mg protein/mL. The xylanase (Multifect, DuPont™ Genencor® Science) had a declared activity of 8,000 GXU/mL, 47 mg protein/mL. GXU is based on the release of Remazol Brilliant Blue-dyed birch wood xylan at pH 4.5 and 30°C in 10 min, using a xylanase reference standard [19].

2.5. Detoxification of the Hydrolysate

2.5.1. Over-liming

Over-liming was carried out by adding $\text{Ca}(\text{OH})_2$ to increase the pH of hydrolysate to 10. After 1 h, the hydrolysate was centrifuged to remove the sediment, and then H_2SO_4 was added to reach pH 6.8 ± 0.2 , suitable for biobutanol production. A precipitate formation was observed, which was eliminated by filtration [20-22].

2.5.2. Activated charcoal adsorption

Every 25 g of activated charcoal was added to 1 L hydrolysate, and the mixture was agitated at 150 rpm, 45°C for 60 min. Finally, the activated charcoal was removed by centrifugation at 1,100 g for 15 min [23].

2.5.3 XAD-4 Resin Adsorption

Water was first pumped through XAD-4 resin (Sigma–Aldrich, France) in a glass column (internal diameter, 2 cm; height, 20 cm), followed by the hydrolysate [24].

2.6. Batch Fermentation

The detoxified hydrolysate was used as a fermentation medium. Unless otherwise specified, no extra nutrients were added to the medium, and fermentation was performed in 200 mL screw-capped bottles with 100 mL fermentation medium. All the media were purged with N₂ to remove O₂. In all experiments, 5 mL of high motile cells of *C. acetobutylicum* CICC 8012 was inoculated and the temperature was maintained at 37°C. There was no agitation or pH control. During fermentation, 2 mL of samples were withdrawn for ABE and sugar analysis at 12 h intervals. Before analysis, the samples were centrifuged at 10,000 g for 2 min.

2.7. Determination of Sugars and ABE Concentrations

The carbohydrate compositions of SPR were also determined by HPLC (ELSD 6000; All-tech. corp., CA) as described in section 2.2. ABE concentration was determined by gas chromatography (FULI 9790; FULI Corp., China) fitted with a flame ionization detector (FID) under the following condition: a GDX-103 column (FULI Corp., China); temperature of injector and detector, 200°C; temperature of column oven, 180°C; nitrogen carrier gas flow rate, 30 ml/min. ABE yield was calculated using Eq. (1) [25]. Fermentation efficiency was calculated using Eq. (2) [26].

$$ABE\ yield\ (g/g) = \frac{Total\ ABE\ produced}{Total\ sugar\ utilized} \quad (1)$$

$$Fermentation\ efficiency\ (\%) = \frac{Actual\ yield}{Theoretical\ yield} \times 100\% \quad (2)$$

2.8. Morphological Observation of SPR Using Scanning Electron Microscopy (SEM)

The morphology of the SPR was investigated using a ZEISS Gemini SEM 300 microscope (Carl Zeiss NTS GmbH, Germany) equipped with a Schottky Field Emission Gun (FEG). All samples were deposited on adhesive carbon tape and sputtered by a DESK IVTM sputter unit (Denton Vacuum Inc., NJ, S.A.) equipped with Au/Pd target.

2.9. Statistical Analysis

Each experiment was conducted in triplicate, and the data were recorded as the mean. The CCD design and RSM values were processed using Design-Expert 12.0 software (Stat-Ease, USA). Significant differences between samples were evaluated using SPSS 19.0 software.

3. Results and Discussion

3.1. Composition of SPR

The main composition of SPR is cellulose (40.42%), which is not

able to be hydrolyzed by the gentle pretreatment and enzymatic hydrolysis in the previous ethanol production process. It should be noted that the residual sugar content in SPR was high (13.01%). The residual sugars mainly consisted of oligosaccharide or pentose, which are not able to be metabolized by *Saccharomyces cerevisiae* to keep on producing ethanol [11]. Interestingly, there was 16.49% of protein in SPR. That maybe due to high protein accumulation in *S. cerevisiae* [27]. *S. cerevisiae* is known as the most widely used eukaryotic microorganism for single cell protein production under aerobic condition. Although oxygen is an important factor in *S. cerevisiae* growth, the maximum specific growth rate of *S. cerevisiae* is similar under aerobic and anaerobic conditions [27, 28]. Therefore, the previous anaerobic fermentation of ethanol production also resulted in high content of protein in SPR. There was 14.54% of ashes in SPR, and Na, K, Ca, Mg, P, Cu, Mn, Zn and Fe in the SPR were 1274, 4321, 876, 586, 1489, 10.1, 15.2, 6.9 and 49.1 (mg/kg), respectively.

3.2. Effect of Dilute Sulfuric Acid Pretreatment on ABE Fermentation

3.2.1. Statistical analysis and model fitting using CCD-RSM

Although the microbial inhibitors are unavoidably generated during acid hydrolysis, dilute sulphuric acid pretreatment is still the most widely used approach for hydrolysis of lignocellulosic biomass [14, 29]. H⁺ concentration directly affects the hydrolysis efficiency of the carbohydrate polymers [30]. Acid pretreatment can be carried out with either a high acid concentration at low temperatures or a low acid concentration at high temperatures [13]. In addition, residence time for thermal treatment directly affects the production cost. Therefore, the effects of and thermal treatment time on ABE fermentation were investigated. The significant pretreatment parameters, including concentration of dilute sulphuric acid, thermal treatment temperature and thermal treatment time, were optimized using CCD. The experimental data (Table 1) were fitted to quadratic models, and the quadratic regression analysis was carried out using Design-Expert software [31]. The model equation obtained by Design-Expert 12.0 software in terms of code values is as follows:

$$Y = 3.64 - 0.5123A - 0.4061B - 0.0722C + 0.0200AB - 0.0503AC - 0.0435BC - 0.9319A^2 - 1.08B^2 - 0.3394C^2 \quad (3)$$

Where Y represents butanol concentration, A = concentration of dilute sulphuric acid, B = thermal treatment temperature, and C = thermal treatment time.

The model was analyzed by analysis of variance (ANOVA) and the results were shown in Table 2. F-test and P-value were used to check the statistical significance of each coefficient in the regression model. The F-value of the model was calculated by dividing the mean square of the model by the mean square of the residuals [32]. A large F value (79.92) with a small P value ($p < 0.0001$) indicated that the model was statistically significant. In Table 2, linear coefficients (A and B) and quadratic coefficients (A², B², and C²) were significant model terms ($p < 0.05$), indicating these factors were quite important to the butanol fermentation. The lack of fit is a measure of the failure of the model to fit the empirical data and the model should be discarded if the lack of fit was

Table 1. CCD Experimental Sets and Corresponding Response

Run	Factor A Concentration (%v/v)	Factor B Temperature (°C)	Factor C Time (min)	Response Butanol (g/L)
1	1.0	115	30	3.76
2	0.5	120	45	1.41
3	1.0	120	30	1.88
4	0.5	115	30	3.08
5	1.0	115	30	3.76
6	1.0	115	15	3.13
7	1.0	115	45	3.19
8	1.0	115	30	3.88
9	1.0	110	30	2.95
10	1.5	120	15	0.70
11	1.0	115	30	3.55
12	0.5	110	45	2.18
13	1.5	110	45	1.11
14	0.5	120	15	1.49
15	1.0	115	30	3.74
16	1.5	115	30	2.05
17	1.0	115	30	3.78
18	1.5	110	15	1.23
19	1.5	120	45	0.22
20	0.5	110	15	2.29

Table 2. Analysis of Variance of Response Surface Quadratic Model

Source	Sum of Squares	df	Mean Square	F-value	p-value	Remarks
Model	25.29	9	2.81	79.92	< 0.0001	significant
A-Concentration	2.62	1	2.62	74.66	< 0.0001	
B-Temperature	1.65	1	1.65	46.92	< 0.0001	
C-Time	0.0521	1	0.0521	1.48	0.2513	
AB	0.0032	1	0.0032	0.091	0.769	
AC	0.0202	1	0.0202	0.5747	0.4659	
BC	0.0151	1	0.0151	0.4306	0.5265	
A ²	2.39	1	2.39	67.94	< 0.0001	
B ²	3.22	1	3.22	91.57	< 0.0001	
C ²	0.3168	1	0.3168	9.01	0.0133	
Residual	0.3515	10	0.0352			
Lack of Fit	0.2922	5	0.0584	4.92	0.0526	not significant
Pure Error	0.0594	5	0.0119			
Cor Total	25.64	19				
R ²	0.9863					
Adjusted R ²	0.9739					
Predicted R ²	0.9253					

significant [32]. In this case, the value of “the lack of fit” was not significant ($p = 0.0526 > 0.05$), representing that the lack of fit was not significant relative to the pure error and the regression model fitted the data properly. The determination coefficient ($R^2 = 0.9863$) was close to 1, which indicated that 98.63% of real pretreatment effects could be explained under the range of experimental variables. The predicted R^2 (0.9253) were in reasonable agreement with the adjusted R^2 (0.9739), which represented that

the observed experimental data had a high degree of correlation with the predicted values and the regression equation can be used to analyze and predict the experimental results.

3.2.2. Analysis of response surface plots

Adequacy of the model was further assessed by probability plot (Fig. 1(a)). All data points followed along straight line of regression indicating that a good correlation was given between the predicted

Fig. 1. Surface response plots of the factor's effect on butanol fermentation: (a) the correlation plot of predicted values vs. experimental measurements for butanol concentration, (b) interactive effects of dilute sulfuric acid concentration and thermal treatment temperature, (c) interactive effects of dilute sulfuric acid concentration and thermal treatment time, and (d) interactive effects of thermal treatment temperature and thermal treatment time.

and experimental butanol concentration under dilute sulfuric acid pretreatment. To elucidate the interactive effects between the various pretreatment parameters on the final butanol concentration, the three-dimensional (3D) surface plots were generated (Fig. 1(b)~(d)). Concentration of dilute sulfuric acid and thermal treatment temperature presented the most important factors ($p < 0.0001$), affecting the final butanol concentration effectively, which was consistent with the results of ANOVA analysis for the model. Interaction between dilute sulfuric acid concentration and thermal treatment temperature exhibited a steeper response surface than interactions between other independent factors (Fig. 1(b)). As shown in Fig. 1(b) and 1(c), the butanol concentration increased first and then decreased with increasing dilute sulfuric acid concentration when heat-treated was at constant temperature and time. The influence of thermal treatment temperature on the final butanol concentration showed a similar trend to dilute sulfuric acid concentration (Fig. 1(b) and 1(d)). These results indicate that the dilute sulfuric acid concentration and thermal treatment temperature are not the

higher the better, which maybe due to the inhibitors generated under the high H^+ concentration at high temperature. Fig. 1(c) and 1(d) showed that thermal treatment time had no significant influence on the final butanol concentration ($p = 0.2513 > 0.05$).

3.2.3. Model Verification

From the model, the final optimal pretreatment parameters were predicted as: 1.0% (v/v) dilute sulfuric acid, and thermal treatment at 115°C for 30 min, under which the predicted butanol concentration was 3.64 g/L. Therefore, experiments were set up at the optimum predicted criteria for validating the optimization. Under the optimal pretreatment conditions, final butanol concentration was 3.77 g/L (Fig. 2(a)), which was close to the predicted value. The good correlation between the experimental data and predicted values confirmed that the predicted model was adequately accurate for predicting the final butanol concentration.

Under the optimal pretreatment conditions, the sugars obtained from acidically hydrolyzed SPR were as follows: 4.11 g/L of glucose,

Fig. 2. Butanol fermentation profiles under optimal pretreatment conditions: (a) butanol and residual sugars concentration and (b) butanol yield and fermentation efficiency.

Fig. 3. SEM images of SPR: (a) fresh SPR (2,000×), (b) fresh SPR (20,000×), (c) pre-treated SPR (2,000×), (d) pre-treated SPR (20,000×), (e) enzymatically hydrolysed SPR (2,000×), and (f) enzymatically hydrolysed SPR (20,000×)

10.30 g/L of xylose, 1.47 g/L of galactose, 1.32 g/L of arabinose, 1.67 g/L of mannose, and 0.36 g/L of cellobiose, resulting in the total sugar concentration of 19.23 g/L. After acidic hydrolysis, the sugars obtained from enzymatically hydrolyzed SPR were as follows: 22.86 g/L of glucose, 14.71 g/L of xylose, 2.07 g/L of galactose,

1.84 g/L of arabinose, 2.38 g/L of mannose, and 2.98 g/L of cellobiose, resulting in the total sugar concentration of 46.84 g/L, which increased to 2.44 times as compared with that in acidic hydrolysate. Furthermore, sugar release was consistent with the surface morphology of fresh SPR, pre-treated SPR and enzymatically hydrolysed

SPR. As shown in Fig. 3, both high and low magnifications showed the gradual degradation of SPR, and the effect of enzymatic hydrolysis on morphology was stronger than that of acidic hydrolysis. The fresh SPR showed a relatively compact and rigid surface before dilute sulphuric acid pretreatment. After acidic hydrolysis, it exhibited a corrugated and more porous surface. The enzymatically hydrolysed SPR showed a fragmented structure, revealing the disruption of tissue network and the degradation of SPR.

However, according to the fermentation profiles (Fig. 2), ABE fermentation almost stopped at 36 h, while the residual sugar was still as high as 24.02 g/L, indicating not all released sugars were fermented by *C. acetobutylicum* CICC 8012. In addition, butanol yield is only 0.16 g/g, and fermentation efficiency is only about 60%. The poor performance of *C. acetobutylicum* CICC 8012 during fermentation from SPR hydrolysate was attributed to by-products of hydrolysis generated by dilute sulfuric acid pretreatment [33]. Jonglertjunya *et al.* [34] found that only 0.27 g/L of butanol could be obtained by *Clostridium beijerinckii* (TISTR 1461) from un-detoxified sugarcane bagasse hydrolysate. Luo *et al.* [35] found that butanol yield is only 0.08 g/g in the ABE fermentation from un-detoxified corn cob hydrolysate. Although the dilute acid pretreatment commonly results in low concentrations of inhibitory compounds [36], we also have to consider the by-products produced in SPR from the last round of ethanol fermentation, which may inhibit the next round of ABE fermentation. Therefore, detoxification of hydrolysate is necessary.

3.3. Effect of Different Detoxification Approaches on ABE Fermentation

There are diverse of feedstock including wastes and residues of agriculture and industry have been reported for ABE production. Nearly all ABE fermentation from residues consists of following steps: pretreatment, hydrolysis and detoxification. In order to remove the inhibitory compounds in the SPR hydrolysate, effects of $\text{Ca}(\text{OH})_2$ over-liming, activated charcoal adsorption and XAD-4 resin adsorption on ABE fermentation were investigated.

As shown in Table 3, three detoxification approaches resulted in varied amount of ABE solvents. During 72 h of fermentation, 3.72 g/L of butanol and 6.21 g/L of ABE were produced in un-detoxified group, resulting in butanol yield and ABE yields were only 0.16 g/g and 0.26 g/g, respectively. As compared to un-detoxified hydrolysate, *C. acetobutylicum* CICC 8012 exhibited significantly better fermentation performance in detoxified hydrolysates, and ABE yield increased to 0.28 g/g ~ 0.35 g/g. However, detoxification treatment inevitably led to more or less sugar loss. After being detoxified by $\text{Ca}(\text{OH})_2$ over-liming, total sugar concentration in

the hydrolysate decreased to 33.03 g/L, resulting in 29.49% of sugar loss. The over-liming process has been reported as an effective method for detoxification of various hydrolysates such as: bagasse, wood and barley straw [37-39]. Sugar loss was recently reported as a major drawback of over-liming due to hydroxide-catalyzed degradation reactions and conversion of sugars to unfermentable compounds [40]. XAD-4 resin detoxification led to the lowest sugar loss with a total sugar concentration of 44.51 g/L, corresponding to 95.03% of the original concentration. In addition, only 51.22% of sugars were utilized in un-detoxified group, while 87.01% of sugars were consumed for ABE fermentation in the XAD-4 group, corresponding to the lowest final residual sugar concentration, which was in accordance with other papers [12, 41]. Therefore, *C. acetobutylicum* CICC 8012 showed the best fermentation performance in XAD-4 group, with a 115.59% improvement in final butanol concentration (8.02 g/L) as compared to the un-detoxified group. Furthermore, XAD-4 resin can be easily regenerated and reused to reduce production cost and waste generation. Detoxification strategy should be developed to attain the following objectives: (1) minimal loss of sugars; (2) low energy consumption; (3) limited wastes discharge; and (4) absence of residual metal salts that may inhibit fermentation [42]. XAD-4 resin detoxification can meet all the demands. Therefore, XAD-4 resin was considered as one of the excellent adsorbents for detoxification of small organic molecules such as furfural, hydroxymethyl furfural, and total phenolic compounds [43].

3.4. Effect of Additional Nutrients on ABE Fermentation

Nutrition supplement is necessary to many kinds of waste biomass for butanol fermentation. Yeast extract was supplied as an extra nitrogen source in corn fiber hydrolysate [12]. KH_2PO_4 and MgSO_4 were added to wheat straw hydrolysate to ensure the successful butanol fermentation [19]. In order to know whether nitrogen source and mineral were the restriction factor for cell growth and butanol production besides hydrolysis inhibitors, nutrition supplement experiments were carried out. According to the basic requirements of *C. acetobutylicum* CICC 8012 for ABE production, 3 g of soybean meal, 1 g of $\text{CH}_3\text{COONH}_4$, 0.6 g of KH_2PO_4 , and 0.2 g of MgSO_4 were add to 1L of medium [19]. Interestingly, the results obtained with the addition of nitrogen source and inorganic salts did not differ significantly from those in the non-addition group ($p > 0.05$). This could be due to the fact that the growth and metabolism requirements of *C. acetobutylicum* CICC 8012 for ABE production were satisfied with the nutrients contained in the SPR as described in section 3.1. The results demonstrated that additional nutrients are not necessary when utilizing SPR as a substrate for ABE pro-

Table 3. ABE Fermentation Parameters under Different Detoxification Processes

Parameters	XAD-4	Activated charcoal	Over-liming	Untreated
Total initial sugar (g/L)	44.51	38.03	33.03	46.84
Residual sugar (g/L)	5.78	17.11	14.71	22.85
Butanol concentration (g/L)	8.02	3.45	3.53	3.72
ABE concentration (g/L)	13.54	5.88	6.05	6.21
Butanol yield (g/g)	0.21	0.16	0.19	0.16
ABE yield (g/g)	0.35	0.28	0.33	0.26

duction, indicating SPR is an attractive option for ABE production as compared with other feedstocks that need nutrition supplement. This advantage makes SPR more economical.

3.5. Large-scale Fermentation

Larger-scale fermentation was conducted in a 2-L screw-capped Pyrex bottle with 1 L XAD-4 resin treated hydrolysate. After fermentation finished, residual sugar concentration, butanol concentration, ABE concentration, ABE productivity, and ABE yield were 6.28 g/L, 7.96 g/L, 13.42 g/L, 0.19 g/L h, and 0.35 g/g, respectively. The results of large-scale fermentation had no statistical significance compared with that of 100 ml-scale fermentation ($p > 0.05$). It is reported that 9.3 g/L of total ABE solvents were produced by *C. berjerinckii* BA101 from corn fiber hydrolysate when treated by sulfuric acid combined with XAD-4 [12], and 3.59 g/L of butanol was produced from the XAD-4 treated palm kernel cake [41], which are all lower than those in SPR hydrolysate.

Notably, at the end of fermentation, 38.75 g/L of sugars were fermented by *C. acetobutylicum* CICC 8012, and un-fermented sugars remained in the medium were mainly pentose (Table 4). Almost all glucose and cellobiose were consumed, while 1.94 g/L of xylose, 1.36 g/L of galactose, 0.87 g/L of arabinose and 1.25 g/L of mannose couldn't be utilized by *C. acetobutylicum* CICC 8012 during the fermentation. Although wild clostridia could metabolize pentose by the pentose phosphate pathway, its efficiency is undesired [44]. The recombinant clostridia strains with increased pentose-utilizing ability are to be developed in future work.

Table 4. Sugar Composition in the Hydrolysate at Initial and Final Stages of Fermentation

Sugars (g/L)	Initial stage	Final stage
Glucose	22.45	0.57
Xylose	13.96	1.94
Galactose	1.98	1.36
Arabinose	1.71	0.87
Mannose	2.29	1.25
Cellobiose	2.64	0.29
Total sugars	45.03	6.28

4. Conclusions

The key issue of the second-generation biobutanol production is to find low-cost feedstocks. This research found a novel way of using SPR as an economical feedstock for biobutanol production. The optimal hydrolysis condition of pretreating SPR with 1% (v/v) sulfuric acid at 115°C for 30 min was obtained by CCD-RSM. The key procedure of the overall processes was the further detoxification with XAD-4 resin, which significantly enhanced butanol fermentation performance with a 115.59% improvement in final butanol concentration as compared to the un-detoxified SPR hydrolysate. Extra nutrients were not necessary when utilizing SPR as a substrate for butanol production. This is the prominent advantage of SPR over other feedstocks that need nutrition supplement. At a 2 L scale of fermentation, the butanol concentration, ABE concen-

tration, ABE yield, and ABE productivity were 7.96 g/L, 13.42 g/L, 0.35 g/g and 0.19 g/L h, respectively.

The major highlights in the current study include: (1) This study demonstrates a bio-refinery strategy of conversion from waste biomass to energy. (2) The fermentation results verify the feasibility of utilizing the cost saving SPR as feedstock to produce butanol. (3) Butanol production from SPR provides a novel approach for reducing production cost as well as eliminating environmental pollution brought by waste discharge from bioethanol industry.

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Author Contributions

Y.J. (Professor) conceived and designed the study, performed the experiments, analyzed the data, and wrote the paper. L.Z. (Ph.D. student) designed and performed the partial experiments. Z.Y. (Associate professor) and Y.F. (Associate professor) reviewed and edited the manuscript. H.Z. (Professor) conceived and designed the study, and acquired funding. All authors read and approved the manuscript.

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