Pretreatment and enzymatic saccharification of Dioscorea zingiberensis C.H. Wright

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Abstract

The promising of sugars from Dioscorea zingiberensis C.H. Wright residues through pretreated with sulphuric acid and sodium hydroxide was investigated. The enzymatic hydrolysis process combined α- amylase, glucoamylase and Cellic® CTec2, shows higher sugar concentration and yield after sulphuric acid pretreatment, obtained 60.73 g/L and 0.73 g/g, respectively.

Keywords

Dioscorea zingiberensis C.H. Wright, pretreatment, Enzyme hydrolysis, Ethanol.

1. Introduction

Response to rising greenhouse gas emissions from petroleum fuels, it is particularly important to studying the sources of renewable fuels that reduce the net carbon output to the atmosphere. The conversion of biomass to bioethanol is an attractive renewable option and bioenergy currently[1-3]. Various biomass feedstocks are widely availabile and great effort has been made to find low-cost alternative materials to bioethanol production using different wastes such as the residue of agricultural bagasse, corncob, sawdust, and fruit kernels. What's more, there is a larger capacity for bioethanol production than what is currently being utilized. Nowadays, If ideal conversion and unlimited access to feedstock could be available, biofuels could satisfy large amount to the fuel requirements[4]. However, the economics of the bioethanol production are largely dependent on the cost of the fermentation substrate, and use of low price, non-food biomass is the key to lowering costs[4]. Improving fermentation efficiency will also enhance bioethanol competitiveness compared to petrochemical.

Dioscorea zingiberensis C.H. Wright（DZW）is one of the dioscorea plants, which is used to produce diosgenin in China[4-6]. The steroid saponin aglucon is one main form of diosgenin in DZW tubers and which can be widely used as raw materials producing steroids, contraceptives, cortisone, hormones and other steroids[5, 7]. Moreover, it can also precipitate apoptosis in colon cancer cell lines and osteosarcoma cells[7, 8]. DZW is in great demand because about 60% of steroidal drugs produced from diosgenin in pharmaceutical factory[9, 10]. DZW tubers are consisted of 38% starch, 45% cellulose and 2% saponin which is partly attached to cell walls or coated by cellulose and starch[10, 11]. With strong acid and high temperature conditions, diosgenin can be directly produced by hydrolyzing the total steroid saponins from DZW in traditional industrial production. However, the method extraction of diosgenin has brought serious environmental pollution problems. For example, a large numble of wastewater with high concentration of acids and chemical oxygen demand (COD) is discharged[6, 12], and producing per ton of diosgenin generated about 10 t of residues. The solid waste with low density will occupy a large area of land if dumped without disposal[4]. It has become one of the pivotal limiting factors in the development of saponin industry, which is urgent to solve the problem of DZW residues.

2. Experiment datels

2.1 Different reagents pretreatment DZW residues

The DZW residues was performed in a 100-mL serum bottle at 121 °C for 1 h,The 5 g DZWs and 50 mL 2% H_2SO_4 or 2% NaOH pretreatment liquid were added in the pretreatment system with solid-liquid ratio of 1:10, respectively. The samples were then cooled down to room temperature, solid - liquid separated and washed the solid.

2.2 Analysis DZW residues compositions

The starch, cellulose, himicellulose and lignin content of the DZW residues and pretreated DZW residues were determined according to the National Renewable Energy Laboratory Analytical Procedure and previous research[13, 14].

2.3 Enzymatic hydrolysis

And 880 uL 40%（w/v）NaOH or was added into the samples to adjust pH value at 5 after 2% H_2SO_4 pretreatment. And 440 uL 72%（w/v）$H_2SO_4$was added into the samples to adjust pH value at 5-6 after 2% NaOH pretreatment. Enzymatic hydrolysis was performed by adding an enzyme mixture of Cellic® CTec2, α-amylase and glucoamylase, and performed at 50 °C and 200 rpm for 72 h. Then the samples were collected at different time points and heated in boiling water for 10 min to terminate the reaction. Then the samples were centrifuged at 12,000 rpm for 10 min and the supernatant was collected.

2.4 Ethanol fermentation

After 72 h of enzymatic hydrolysis, ethanol fermentation were carried out by using *Saccharomyces cerevisiae* 1445 in orbital shaking incubator at 30 °C and 200 rpm anaerobically. Samples were collected throughout the fermentation process and then centrifuged at 12,000 rpm for 10 min. The supernatants was collected to analyze glucose and ethanol concentration by HPLC.

2.5 Analysis methods

The above experimental data were represented by mean±standard deviation（mean±SD）, and one-way anova was calculated by Minitab17.

Solid recovery using the following Eq.:

$$\text{Solid recovery（\%）} = \frac{\text{DZW residues before pretreated （g）}}{\text{DZW residues after pretreated （g）}} \times 100\%$$

Sugar yield using the following Eq.:

$$\text{Sugar yield（g/g）} = \frac{\text{Sugar concentration (g/L)}\times\text{V (L)}}{\text{Solid mass (g)}\times\left(\frac{\text{Starch (\%)}}{0.9}+\text{Cellulose (\%)}\times 1.11+\text{Xylan (\%)}\times 1.14\right)}$$

Ethanol yield using the following Eq.:

$$\text{Ethanol yield（\%）} = \frac{\text{Ethanol concentration （g/L）}}{\text{solid mass（g）}\times\left(\frac{\text{starch (\%)}}{0.9}\text{cellulose (\%)}\times 1.11\right)\times 0.51} \times 100\%$$

3. Results and discussion

3.1 Compositions and solid recovery of DZW residues

The content of the DZW residues reflects the relative changes of each content before and after pretreatment. Effects of pretreatment with sulfuric acid and sodium hydroxide on the percentage content of DZW residues. The main chemical composition of the DZW residues is shown in Table 1. The percentage contents of starch, cellulose, hemicellulose and lignin in untreated ginger residue were 30.31%, 38.86%, 8.93% and 8.53%, respectively. After sulfuric acid pretreatment, the starch content decreased sharply by 26.18%, and the percentage of cellulose and hemicellulose increased slightly. Lignin increased significantly by 12.43%. However, after sodium hydroxide pretreatment, except for the significant increase in cellulose content, other components were significantly reduced.

The content of cellulose increased by 38.23%, while the content of starch, hemicellulose and lignin decreased by 15.7%, 5.9% and 3.79%, respectively.

The solid recovery after sulfuric acid and sodium hydroxide pretreatment was 12.55% and 48.61%, respectively. The low solid recovery rate indicated that the more the content of ginger residue dissolved in the pretreatment solution, the solid recovery rate after sulfuric acid pretreatment was much lower than that after sodium hydroxide pretreatment. Show that in the acid pretreatment, turmeric slag structure easily damaged, sulfuric acid to starch, cellulose and hemicellulose acid hydrolysis, hydrolytic release sugar dissolved in water solution, because in sulfuric acid pretreatment, acid hydrolysis of starch in turmeric slag easily happened, generate monosaccharide dissolved in the fluid of the pretreatment, because the hemicellulose has branched chain, the low degree of polymerization, also easily by acid hydrolysis produce oligosaccharides or simple sugar dissolves in liquid pretreatment. After solid-liquid separation and washing, most of the sugar is lost.

Table 1 The main compositions and solid recovery were obtained before and after pretreatment with sulfuric acid and sodium hydroxide

DZWs	Composition (%)				Solid recovery rate （%）
	Starch	Cellulose	Hemicellulose	Lignin	
Untreated	30.31±0.37	38.86±0.01	8.93±0.41	8.53±0.02	100
2% H2SO4	4.13±1.43	39.14±1.29	10.34±0	20.96±3.38	12.55
2% NaOH	14.61±1.41	77.09±1.74	3.03±0.02	4.74±0.46	48.61

3.2 Enzymatic hydrolysis

The unpretreated and pretreated DZW residues were enzymatic hydrolysis for 72 h, and the sugar concentration and yield were shown in Fig. 1 and Fig. 2, respectively. The sugar concentration and yield of the unpretreated were 7.01 g/L and 0.08 g/g, respectively. The sugar concentration and yield of sulfuric acid pretreatment were 62.24 g/L and 0.74 g/g, respectively. After pretreatment with sodium hydroxide, the sugar concentration and yield were 49.35 g/L and 0.58 g/g, respectively. It can be directly seen from the data that the sugar concentration and yield after sulfuric acid pretreatment was significantly higher than sodium hydroxide pretreatment and unpretreatment. This is because during the acid pretreatment, hemicellulose is dissolved into the hydrolysate, thus improving the accessibility of cellulose and making the DZW residues release more monosaccharide in the subsequent during enzymatic hydrolysis process, thus improved the sugar concentration and yield. However, the whole system was very viscous after sodium hydroxide pretreatment, which was due to degradation and dissolution of lignin and other components. Because lignin degradation products in the hydrolysate become an obstacle to the contact between enzyme and cellulose, the enzymatic hydrolysis effect of alkali pretreatment is not as good as that of acid pretreatment.

3.3 Ethanol fermentation

High sugar concentration produced was obtain by enzyme hydrolysis process, including glucose and xylose. glucose can be subjected to ethanol fermentation by *S. cerevisiae* 1445. The maximum glucose concentration was obtained at 72 h hydrolysis. Glucose and ethanol concentration were emerged versus time are illustrated. It can be shown in Fig. 3 that initially glucose concentration was 56.33 g/L, glucose was completely and ethanol increases to the maximum value of 19.71 g/L at 12 h, and the ethanol yield reached the maximum of 84.71%. Furthermore, with the extension of fermentation time, the concentration of ethanol decreased slightly, the ethanol concentration was reduced to 16.03 g/L at 36 h. Because the strain uses ethanol as a carbon source for its own growth after glucose used up. xylose concentrations always remained at lower level because of the lower hemicellulose content of DZW residues(date not shown), Because of *S. cerevisiae* can't used xylose to produced ethanol. In the fermentation process of unpretreated DZWs, glucose was not detected, and the highest ethanol concentration and yield were 1.61 g/L and 6.92%, respectively.

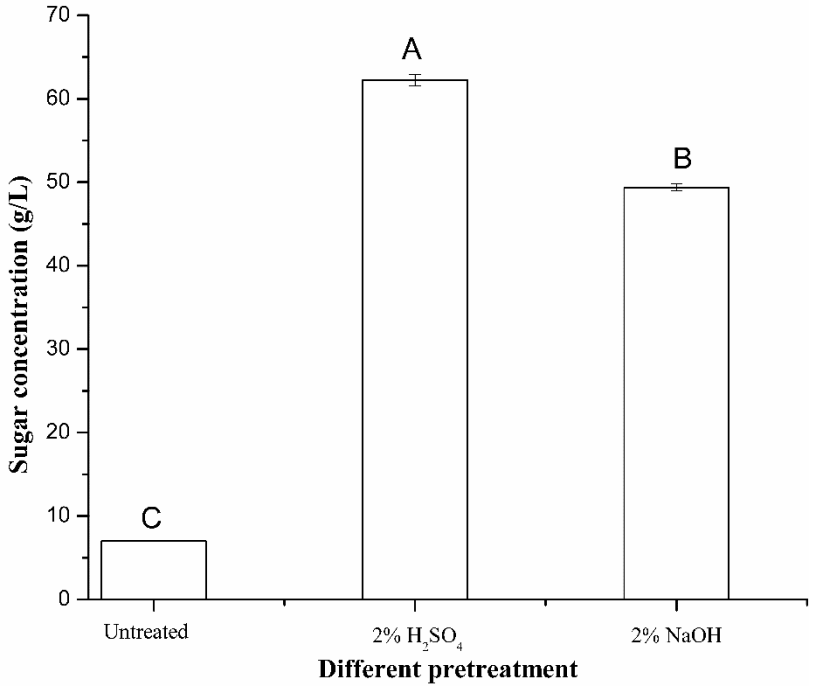

Fig 1 The enzymatic hydrolysis effect of different pretreatment methods. Values with different capital letters indicate significant differences ($p < 0.05$), analyzed by one-way ANOVA. n=2.

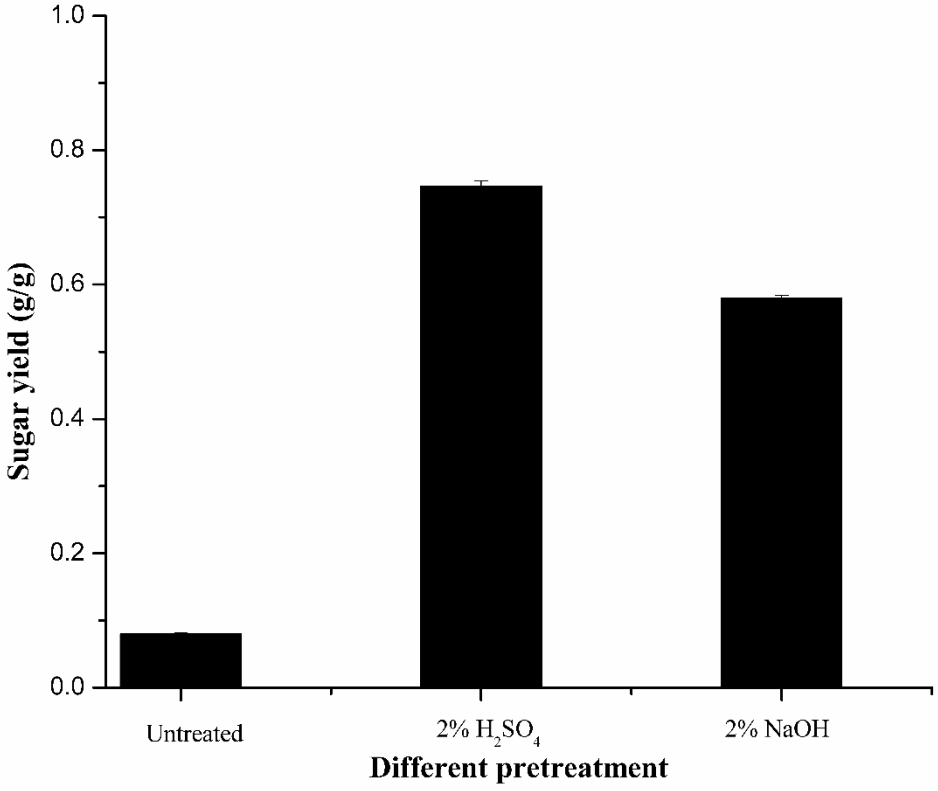

Fig 2 Sugar yield after different pretreatment methods.

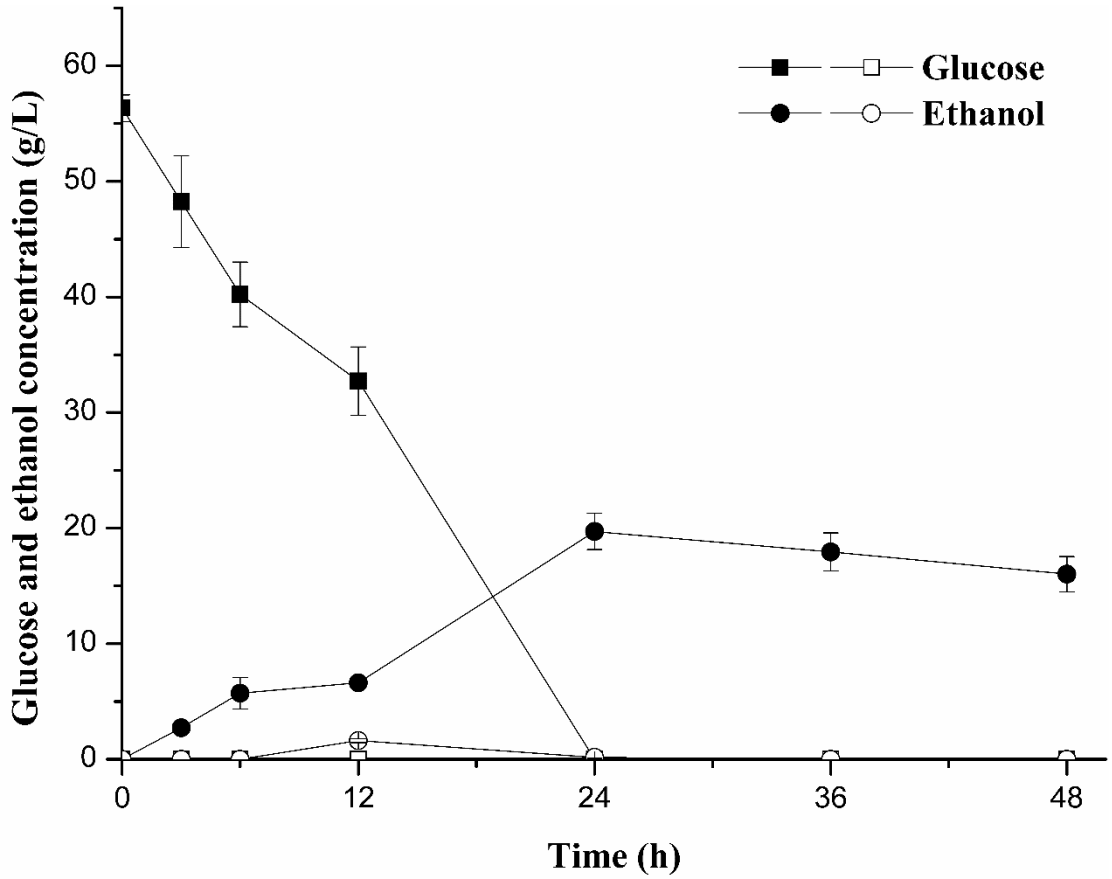

Fig 2 Glucose and ethanol concentrations during the ethanol fermentation of DZW residues after 2% H_2SO_4 pretreated.
Glucose concentration of pretreatment (■), ethanol concentration of pretreatment(●), glucose concentration of unpreated (□), ethanol concentration of unpretreated (○).

4. Conclusion

The DZW residues represent a prefect source of sugars for microbial conversion to ethanol. The effect pretreatment of 2% H_2SO_4 was better than 2% NaOH, and higher sugar concentration and yield were obtained during the enzymatic hydrolysis, 62.24 g/L and 0.74 g/g, respectively. In addition, a higher ethanol concentration and yield achieved 19.71 g/L and 84.71%, respectively. Hence, we provided a new technology of produced ethanol from DZW residues through sulfuric acid pretreatment.

Acknowledgements

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References

[1] Littlejohns J, Rehmann L, Murdy R, Oo A, Neill S. Current State and Future Prospects for Liquid Biofuels in Canada, Biofuel Research Journal, Vol. 5 (2018) No.1, p.759-79.
[2] Zhao X, Zhang L, Liu D. Biomass Recalcitrance. Part I: the chemical compositions and physical structures affecting the enzymatic hydrolysis of lignocellulose Biofuels, Bioproducts and Biorefining, Vol. 6 (2012) No.4, p.465-82.
[3] Bai Z, Gao Z, Sun J, Wu B, He B. D-Lactic acid production by Sporolactobacillus inulinus YBS1-5 with simultaneous utilization of cottonseed meal and corncob residue, Bioresource technology, Vol. 207 (2016), p.346-52.

[4] Xiao C, Fan W, Du S, Liu L, Wang C, Guo M, et al: A novel glycosylated solution from Dioscorea zingiberensis C.H. Wright significantly improves the solvent productivity of Clostridium beijerinckii, Bioresource technology, Vol. 241 (2017), p.317-24.
[5] Wei M, Bai Y, Ao M, Jin W, Yu P, Zhu M, et al. Novel method utilizing microbial treatment for cleaner production of diosgenin from Dioscorea zingiberensis C.H. Wright (DZW), Bioresource technology, Vol.146 (2013), p.549-55.
[6] Bai Y, Zhang L, Jin W, Wei M, Zhou P, Zheng G, et al. In situ high-valued utilization and transformation of sugars from Dioscorea zingiberensis C.H. Wright for clean production of diosgenin, Bioresource technology, Vol. 196 (2015), p.642-7.
[7] Corbiere C, Liagre B, Bianchi A, Bordji K, Dauca M, Netter P, et al. Different contribution of apoptosis to the antiproliferative effects of diosgenin and other plant steroids, hecogenin and tigogenin, on human 1547 osteosarcoma cells, Int J Oncol, Vol. 22 (2003) No. 4, p.899-905.
[8] Raju J, Bird RP: Diosgenin, a naturally occurring steroid [corrected] saponin suppresses 3-hydroxy-3-methylglutaryl CoA reductase expression and induces apoptosis in HCT-116 human colon carcinoma cells, Cancer letters, Vol. 255 (2007) No. 2, p.194-204.
[9] Fernandes P, Cruz A, Angelova B, Pinheiro HM, Cabral JMS. Microbial conversion of steroid compounds: recent development, Enzyme and Microbial Technology, Vol. 32 (2003) No. 6, p.688-705.
[10] Wang Y, Liu H, Bao J, Hong Y, Yang Z, Zhang C. The saccharification–membrane retrieval–hydrolysis (SMRH) process: a novel approach for cleaner production of diosgenin derived from Dioscorea zingiberensis, Journal of Cleaner Production, Vol. 16 (2008) No. 10, p.1133-1137.
[11] Ren Y, Chen Y, Hu B, Wu H, Lai F, Li X. Microwave-assisted extraction and a new determination method for total steroid saponins from Dioscorea zingiberensis C.H. Wright, Steroids, Vol. 104 (2015), p.145-152.
[12] Zhu Y, Huang W, Ni J. A promising clean process for production of diosgenin from Dioscorea zingiberensis C. H. Wright, Journal of Cleaner Production, Vol. 18 (2010) No. 3, p.242-247.
[13] Sluiter A, Hames B, R. Ruiz C, Scarlata C, Sluiter J, Templeton D, et al. Determination of Structural Carbohydrates and Lignin in Biomass, NREL, Gold. 2008.
[14] Z.F. Zhou, Z.A. Li : *Plant Physiology Experiment Instructio*, (www.bbioo.com, China 2008), p.82-86.