Research on the Cellulase Hydrolysis of Colocasia Antiquorumin in Producing Ethyl Alcohol

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Abstract: The cellulase hydrolysis experiment in the process of the ethyl alcohol generated by colocasia antiquorumin was studied in this paper. Adopting the single factor variable method, three influencing factors (the amount of cellulase, hydrolysis temperature and hydrolysis time) were selected to carry out enzymatic saccharification sugar production tests of pretreated colocasia antiquorums. The results demonstrate that the sugar yield amounts to the maximum (165mg/g) when the enzyme dosage is 0.04g/g, the enzymatic hydrolysis temperature is 45°C, and the reaction time is 48h.

1. Introduction
As one of the most abundant types of carbohydrates in nature, cellulose is also the most abundant biomass resource on the earth. The production of bioethanol is the most promising in terms of ethanol production. The use of modern biotechnology to develop bio-energy made up of fiber has become an important part of the energy strategies of developed countries in the world [1-4]. However, the main obstacle in the process of cellulose resource utilization is the low production efficiency and high cost of cellulase hydrolysis [5-7]. At present, only a small amount of cellulose in nature has been exploited, and most of the cellulose is wasted and even pollutes the environment [8-9]. There are plenty of cellulose and hemicellulose in the colocasia antiquorum, but little research has been carried out on the use of the cellulase hydrolysis of colocasia antiquorum to produce ethyl alcohol. Therefore, it is of great practical significance to study the factors affecting the cellulase hydrolysis of colocasia antiquorum to produce ethyl alcohol [10-11]. Taking advantage of the cellulase hydrolysis of pretreated colocasia antiquorum to obtain reducing sugar and its further fermentation to produce ethanol are of practical significance for the recycle of colocasia antiquorum and environmental protection [12-14].
2. Experiments

2.1 Experimental materials
Reagents: concentrated sulfuric acid (H_2SO_4) was purchased from Sinopharm Chemical Reagent Co., Ltd.; sodium hydroxide (NaOH) was purchased from Sinopharm Chemical Reagent Co., Ltd.; anhydrous calcium chloride (CaCl_2) was purchased from Sinopharm Chemical Reagent Co., Ltd.; The paste was purchased from Sinopharm Chemical Reagent Co., Ltd.

2.2 Experimental methods

(1) Pretreatment of colocasia antiquorum:A sample of colocasia antiquorum after comminution, concentrated sulfuric acid (H_2SO_4) concentration 0.50%, temperature 100℃, solid-liquid ratio 1:30, pretreatment for 60 min, then wash it with suction until the filtrate Neutral, then transfer the residue to the enzymatic system. The pretreated samples were washed to neutral and placed in 250mL stoppered conical flasks and numbered according to conditions. A hydrolyzed system (50mL) having a solid-liquid ratio of 1:50 was placed in an Erlenmeyer flask, sterilized at 121℃ for 20 min, pH was adjusted to 4.5, and 0.05g of CaCl_2 was added.

(2) Testing and determination of the optimal cellulase concentration: 0.01g, 0.02g, 0.03g, 0.04g, 0.05g according to the amount of enzyme added to the hydrolysis system, set the temperature to 45℃, the shaking tables speed to 120r·min-1, Then, the stoppered conical flask was placed in a shaker and hydrolyzed, and the reducing sugar content of the sample solution in the conical flask was determined, and the optimal enzyme dosage of cellulase hydrolysis and saccharification was compared [15].

(3) Testing and determination of the optimal Enzymatic Saccharification Temperature: set the shaking table temperature to 30℃, 35℃, 40℃, 45℃, 50℃, 120 r·min-1 after hydrolysis, then, The optimal temperature of cellulase hydrolysis glycosylation was obtained by measuring the content of reducing sugar in the sample solution in a tapered bottle.

(4) Testing and determination of the optimal enzymatic saccharification time: according to the optimal enzymatic hydrolysis temperature determined in (3), the temperature is set, 120r·min-1, after hydrolysis for 12h, 24h, 36h, 48h, 60h, respectively. The sample solution in the stopper flask was used to determine the reducing sugar content, and the optimal enzymatic saccharification time of the enzymatic saccharification was determined.

3. Experimental results and discussion

3.1 Effects of the amount of enzyme on the cellulose hydrolysis

Figures 1 Show Effects Of The Amount Of Cellulase Enzyme On The Production Of Reducing Sugar During Diastatic Fermentation Of Zantedeschia. It can be seen that when 0.01g of cellulase is added, the yield of reducing sugar is the lowest, only 124mg/g, and as the cellulase content increases, the yield of reducing sugar also increases. When the content of cellulase was 0.04g, the yield of reducing sugar reached the maximum 165mg/g, and the amount of cellulase continued to increase. The yield of reducing sugar was basically unchanged and tended to be stable. Therefore, the optimum dosage of the enzyme is 0.04g/g substrate.
3.2 Effects of enzymatic saccharification temperature on enzymatic saccharification

As shown in Figure 2, when the temperature is 30℃, the yield of reducing sugar is the lowest. With the increase of temperature, the yield of reducing sugar also increases. When the temperature rises to 45℃, the yield of reducing sugar is up to 165mg/g. When the temperature is higher than 45℃, the yield of reducing sugar will no longer increase with the increase of temperature, but will decrease, instead, the yield of reducing sugar is not increased. It can be seen that when the temperature is 45℃, Cellulase has the largest activity and highest efficiency, which can make the vitamin substances into reducing sugars at the maximum layer. When the temperature reaches the optimal temperature, As the temperature increases, the structure of cellulase will be destroyed, the activity and efficiency of cellulase will be affected, and the yield of reducing sugars will begin to decline. Therefore, this experiment adopts saccharifying enzyme solution treatment temperature 45℃
3.3 Effects of time on hydrolysis of cellulase enzyme

As shown in Figure 3, when the dosage of enzyme is 0.04mg/g, the temperature is 45℃, the time of enzymatic hydrolysis is 12h, the yield of reducing sugar is 130mg/g. With the increase of enzymatic hydrolysis time, the yield of reducing sugar is also increased. When the hydrolysis time is in 24-48h, the yield of reducing sugar increases faster in 14-36h with the increase of hydrolysis time, in 36-48h In the time period, the yield of reduction was also increasing, but the increase rate of reducing sugar began to slow down, and the maximum reducing sugar yield was 165mg/g at 48h. When the hydrolysis time continued to extend, the yield of reducing sugar began to decrease, which was too long with the enzymatic hydrolysis time, making partial reducing sugar unsteadily decomposed. Therefore, the optimum time for enzymatic saccharification is 48h.

4. Conclusions

With the single factor controlled variable method, this research took the yield of reducing sugar as the standard and investigated the optimal conditions of the enzyme dosage, hydrolysis temperature and hydrolysis time during the cellulase hydrolysis of colocasia antiquorum. Results show that the sugar yield reaches its maximum (165mg/g) when the enzyme dosage was 0.04g/g, the enzymatic hydrolysis temperature was 45℃, and the reaction time was 48h.

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5. References
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