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# Effect of Acid-Base Pretreatment on Reducing Sugar Production by Wild Glutinous Rice

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**Abstract.** As a lignocellulosic biomass, wild colocasia can be efficiently hydrolyzed into reducing sugar after a certain degree of pretreatment. It is a high-quality raw material for the development and utilization of biomass energy such as fuel ethanol. The three factors of concentration, temperature, time, and solid-liquid ratio were selected as the three-level orthogonal experiment. The effects of acid-base pretreatment on the reduction of sugar in the wild carp were studied. The cell wall of the wild carp was treated by electron microscopy before and after pretreatment. The change. The experimental results show that the cell wall of the wild colocasia is thinner, the pores become larger, and the contact area is increased. The effect of acid pretreatment on the reduction of sugar by the wild colocasia is better than that of alkali pretreatment. When the acid concentration is 0.5% and the temperature is 100°C and solid-liquid ratio is 1:40, and the treatment time is 90min, the concentration of reducing sugar produced is up to 67.32mg/g.

## 1. Introduction

In order to reduce the excessive dependence on conventional fossil fuels, countries around the world are paying more and more attention to the research and development of biomass energy [1]-[2], including fuel ethanol and biogas. As a renewable energy source, lignocellulose is hydrolyzed to produce reducing sugars such as glucose and xylose, and fuel ethanol can be prepared after anaerobic fermentation of reducing sugars. However, the binding of lignin components to cellulose and hemicellulose leads to a decrease in enzymatic saccharification efficiency and a decrease in reducing sugar production, thereby limiting the production of fuel ethanol by cellulosic materials [3]-[6]. Therefore, lignocellulose needs to be pretreated before cellulase hydrolysis and anaerobic saccharification to remove lignin from the surface of cellulose and hemicellulose to achieve the effect of delignification [7]-[12]. Wild colocasia, also known as the big hoe, wild pheasant, etc., are distributed in the southern provinces of China, and their growth and reproduction speed is relatively fast [13]. At present, most researches on wild colocasia focus on the use of drugs, while the stems of



wild colocasia plants contain a large amount of starch, sugar and cellulose materials, which can be used as raw materials for the production of fuel ethanol. Therefore, this paper studies the effect of acid-base pretreatment on the amount of reducing sugar produced by wild colocasia, in order to obtain the best pretreatment method of wild colocasia, which can provide certain technical support for the production of ethanol from wild colocasia.

## 2. Experimental materials and methods

### 2.1. Experimental materials

The experimental wild colocasia was collected from a village in Xiamen.

### 2.2. Effect of acid pretreatment method on reducing sugar production in wild colocasia

The reagent concentration, temperature, time, and solid-liquid ratio were selected as three-level orthogonal experiments, in which the concentration was set to: 0.5%, 1%, 1.5%; the temperature was set to: 60 °C, 80 °C, 100 °C; time Set to: 30min, 60min, 90min; solid-liquid ratio is set to 1:20, 1:30, 1:40.

### 2.3. Effect of alkali pretreatment method on reducing sugar production in wild colocasia

The reagent concentration, temperature, time, and solid-liquid ratio were selected as three-level orthogonal experiments, in which the concentration was set to: 0.5%, 1%, 1.5%; the temperature was set to: 60 °C, 80 °C, 100 °C; time Set to: 30min, 60min, 90min; solid-liquid ratio is set to 1:20, 1:30, 1:40.

### 2.4. Determination of reducing sugar content

The pretreated sample was centrifuged in a centrifuge at 4000 r/min for 5 min, and then 0.5 mL of the supernatant, 1.5 mL of distilled water, and 1.5 mL of DNS reagent were shaken in a 25 mL stoppered colorimetric tube, and heated for 5 minutes in a water bath. Remove, and then cool to room temperature in an ice water bath, dilute to 25 mL, and shake. The absorbance value was measured by an ultraviolet spectrophotometer having a wavelength of 540 nm, and the reducing sugar content was calculated from the standard curve.

### 2.5. Effect of electron microscopy analysis on the cell wall of wild colocasia

The wild colocasia plants were ground into a powder and placed under a scanning electron microscope. The changes before and after pretreatment of the cell wall were analyzed by magnification.

## 3. Experimental results and analysis

### 3.1 Effect of acid pretreatment method on reducing sugar production in wild colocasia

The results of the orthogonal test using the acid treatment method on the yield of reducing sugar are shown in Table 1. It can be seen from the figure that the acid pretreatment method has the highest concentration of reducing sugar, which can reach 67.32 mg/g, when the acid concentration is 0.5%, the temperature is 100 °C, the solid-liquid ratio is 1:40, and the treatment time is 90 min.

**Table 1.** Treatment factors and levels of acid pretreatment methods

4 factors 3 level acid treatment					
Experiment No.	Acid concentration/%	Temperature/° C	Time/min	Solid-liquid ratio	Reducing sugar concentration mg/g
1	1	1	1	1	9.84
2	1	2	2	2	11.85

3	1	3	3	3	67.32
4	2	1	2	3	12.51
5	2	2	3	1	15.06
6	2	3	1	2	48.65
7	3	1	3	2	18.54
8	3	2	1	3	34.33
9	3	3	2	1	45.43

### 3.2 Effect of alkali pretreatment method on reducing sugar produced by wild colocasia

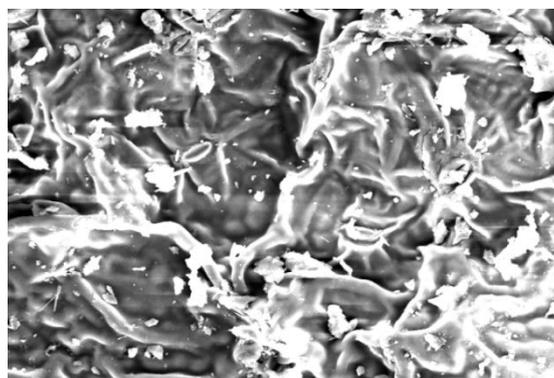
The results of the orthogonal test using the alkali treatment method on the yield of reducing sugar are shown in Table 2. It can be seen from the figure that the alkali pretreatment method has the highest concentration of reducing sugar, which can reach 6.93 mg/g, when the alkali concentration is 1.5%, the temperature is 100 °C, the solid-liquid ratio is 1:20, and the treatment time is 60 min.

**Table 2.** Treatment factors and levels of alkali pretreatment

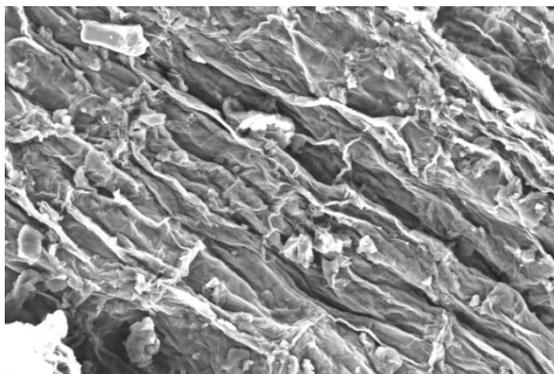
4 factors 3 level acid treatment						
Experiment No.	Acid concentration/%	Temperature/° C	Time/min	Solid-liquid ratio	Reducing sugar concentration mg/g	
1	1	1	1	1	5.21	
2	1	2	2	2	4.56	
3	1	3	3	3	4.05	
4	2	1	2	3	6.08	
5	2	2	3	1	5.96	
6	2	3	1	2	6.45	
7	3	1	3	2	6.12	
8	3	2	1	3	5.87	
9	3	3	2	1	6.93	

It can be seen from the comparison of experimental data that the effect of acid pretreatment on the yield of reducing sugar is much greater than that of alkali pretreatment.

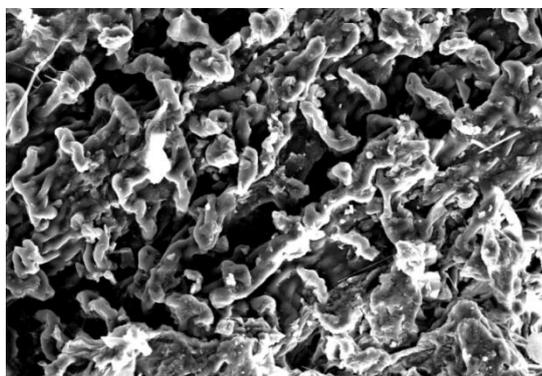
### 3.3 Effect of electron microscopy analysis on cell wall of wild colocasia



**Figure 1.** 500 times of unprocessed wild colocasia



**Figure. 2** Electron microscope images of wild colocasia at 500 times treated with alkali



**Figure. 3** 500 times electron microscope image of acid-treated wild colocasia

It can be seen from Figure. 1 that the cell wall of the untreated wild colocasia is intact and not destroyed. It can be seen from Figure. 2 that the alkali treated cell wall is thinned and the pores are slightly opened, so that the solvent can contact the cellulose and the like in the cell wall to produce reducing sugar in a large area. It can be seen from Figure. 3 that the cell wall of the wild colocasia is basically destroyed after the acid treatment, the pores are basically opened, and the contact area is larger, so that the solvent can contact the largest area of the material in the cell wall to produce the maximum amount of reducing sugar.

#### 4. Conclusion

(1) By orthogonal test, the highest yield of reducing sugar under acid pretreatment was 67.32mg/g, while the highest yield of reducing sugar under alkaline pretreatment was 6.93mg/g. The effect of reducing sugar produced by earthworms is far greater than that of alkali pretreatment.

(2) It was found by electron microscopy that the cell wall of the wild colocasia was basically destroyed, and the contact area between the solvent and the material in the cell wall was the largest. The effect of the acid pretreatment method on the reducing sugar produced by the wild colocasia was much greater than that of the alkali pretreatment method.

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