The Study on Pullulanase Used in Cassava Starch Degradation Technology

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Abstract: The aim of the research is to access the highest possible utilization of starch in the raw material through deep enzymatic degradation. It has been found that fulfilling the above was possible with the application of pullulanase. Adding pullulanase can make a further degree to accelerate starch hydrolysis. When the pullulanase was added before liquefaction, the final reducing sugar increased by8.36%. When the amount of pullulanase got to 20U/g-30U/g, the final reducing achieved highest point. Preliminary analysis of energy consumption of 1 t raw material indicated the possibility to save coal by 5% at least.

Keywords: Pullulanase; cassava starch; degradation

1 Introduction

Increasing needs for fuel ethanol push the interest in improvement of the ethanol production technology. Fuel ethanol producers are forced to seek solutions for maximum reduction of production costs. This can be achieved through, for instance, optimization in the usage of production potential and effective raw material utilization. Cassava as a raw material for producing ethanol is quiet potential and has been concerned. Cassava must be at first enzymatically hydrolyzed to simple sugars in order to be used by yeasts. In traditional technology, α-amylase and glucoamlyase are usually used in enzymatic preparations[1].

Cassava amylopectin content is high so that the degradation of raw materials is not complete, which directly impact on subsequent alcohol fermentation and alcohol production rate. Cassava starch has 4%~5% α-1,6-glycoside linkages that can inhibit saccharification. Pullulanase is a kind of debranching enzyme which hydrolyze α-1,6-glycoside linkages in amylopectin to obtain linear dextrins that are subject to speed up hydrolysis[2]. The way that pullulanase combined with the double-enzymatic degradation has been used in material hydrolysis process. This method accelerates starch enzymatic degradation and allows achieving deeper hydrolysis.

2 Materials and Methods

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2.1 Materials

Cassava(Yuanmou alcohol plant, Yunnan Province), α-amylase (Beijing Axing Biotechnology Co., Ltd. 4000U), glucoamylase(Hunan Hongyingxiang Biotechnology Co., Ltd.), pullulanase (Life and Science Department, Yunan Normal University).

2.2 Reagents

α-amylase, glucoamylase, pullulanase, DNS, 6mol/L HCl, 1mol/L HCl, 6mol/L NaOH, 1mol/L NaOH, phenolphthalein, distilled water.

2.3 Experiment Instruments

Electronic balance, thermostatic water bath, 800B centrifuge(Shanghai Anting Scientific Instrument Factory), oscillators, electric stove, 721 Spectrophotometer

2.4 Methods

2.4.1 The analysis of cassava ingredient

Analysis indexes: water, starch, cellulose, fat, reducing sugar, protein, ash.

2.4.2 Determination

Determination of reducing sugar: 3,5 - dinitrosalicylic acid (DNS) , weighing 1 gram sample, centrifuging twice, then setting to 50ml volumetric flask, diluted with distilled water get to suitable diluted value. Removing 1ml dilution into test tube, adding in 0.5ml DNS reagent, boiling water bath for 5min. Cool test tubes with cool water, then add distilled water 4.5ml into test tube. Under the condition of 540nm wavelength, testing OD values. According to the standard curve, reducing sugar can be

calculated.

Table 1 Ingredient contents of cassava %

Programmer	Index	Programmer	Index
Water	10.23	Protein	2.35
Starch	62.62	Reducing sugar	1.05
Cellulose	3.28	Ash	1.55
Fat	0.31		

Determination of oligosaccharides: removing 32ml from dilution into 50ml volumetric flask, adding 2.0ml 1mol/L HCl, keeping in 80 ℃ water bath for 30min. Then adding 2.0ml 1mol/L NaOH, keep volume constant after cooling. Using DNS method to measure the sugar content, which minus reducing sugar content, that is oligosaccharides value[3].

Determination of total sugar: weigh 1 gram sample, 10ml 6mol/L HCl and 15ml distilled water are added into conical flask, which must be kept in boiling water bath for 30min. After cooling, adjust pH to neuter. Then DNS method is used to measure total sugar.

2.5 experiment design (table2)

Table 2 Experiment Design

	Weight of cassava g	water ml	60℃ bath 30min	70℃ bath 30min （Liquefaction）	60℃ bath 30min （Saccharification）
Contrast route	25	75		α-amylase	Glucoamylase
Route 1	25	75	Pullulanase	α-amylase	Glucoamylase
Route 2	25	75		α-amylase + Pullulanase	Glucoamylase
Route 3	25	75		α-amylase	Glucoamylase + Pullulanase

Table 3 The comparison of reducing sugar content with different processing routes

	Weight of cassava g	Pullulanase u/g	60℃ bath 30min reducing sugar content %	70℃bath 30min reducing sugar content %	60℃bath 30min reducing sugar content %
Contrast route	25	0	1.49	9.32	12.98
Route 1	25	20	1.93	9.72	14.10
Route 2	25	20	1.49	9.82	13.53
Route 3	25	20	1.49	8.28	13.50

Table 4 The comparison of total sugar content with different processing routes

	Weight of cassava g	Pullulanase u/g	60℃ bath 30min total sugar %	70℃bath 30min total sugar %	60℃bath 30min total sugar %
Contrast route	25	0	10.77	13.77	16.29
Route 1	25	20	11.82	12.84	16.91
Route 2	25	20	12.15	13.91	18.30
Route 3	25	20	13.21	14.77	17.17

3 Results and Analysis

3.1Three kinds of processing routes on reducing sugar of cassava starch

Based on the experimental design routes, reducing sugars of different routes in different processing stages are determined. The experimental results are presented in the table 3. Table 3 shows in the degradation process of cassava starch adding pullulanase, the reducing sugar compared with the contrast are improved. Especially, it is obvious that the reducing sugar content in route 1 increased a lot, nearly increased by 8.63%. It indicates that adding pullulanase to pretreatment before the liquefaction, which is not only useful to hydrolyze the α-1,6-glycoside linkages of amylopectin in advance, but also contribute to saccharify.

As can be seen from table 4, in the cassava starch degradation process of adding pullulanase, the total sugar content significantly increases. In the first two stages total sugar of route 3 is highest, route 2 second. After saccharification stage, route 2 gets to highest total sugar content. Therefore it can be concluded that the pullulanase can improve the reducing sugar content, but the effect on total sugar content is not obvious.

Experimental data shows adding the pullulanase, reducing sugar and total sugar content have been increased to some degree. It has a great influence on reducing sugar content of the system, while the total sugar content hasn't been changed much. According to analyzing experimental data, the technology of double- enzymes combining with pulluanase used in starch degradation process has been established

3.2 The saccharification effect of adding different amount of pullulanase

To further determine that pullulanase affect starch degradation, the amount of pullulanase was experimentally explored, and the results have been presented in Figure1 to 3.

3.2.1 The change of reducing sugar content and oligosaccharides

Weigh flour 25g, according to liquid ratio 1:3 mixing, adding the pullulanase pretreatment 60℃30min, α-amylase 10U/g, liquefaction 30min, glucoamylase 200U/g, saccharification 30min. The amount of pullulanase are 0U/g, 12U/g, 20U/g, 28U/g, 36U/g, 40U/g, 44U/g. Measuring reducing sugar content, oligosaccharides content and total sugar content of different system. With different levels of pullulanase reducing sugar content and oligosaccharide content have been shown in Figure 1.

As can be seen from Figure 1 with the increase of the amount pullulanase, reducing sugar content has increased, while the amount of pullulanase from 0U/g to 10U/g, reducing sugar content slowly increases; when the amount increases from 10U/g to 20U/g, reducing sugar content increases obviously; when the amount increases from 20U/g to 30U/g, reducing sugar content of the system slowly increases, and in this stage reducing sugar content reaches the maximum; if more than 30U/g reducing sugar content decreases obviously. From the oligosaccharide content curve, we could see that reducing sugar content increases along with the oligosaccharide content decreasing. Oligosaccharide content at the stage 20U/g to 30U/g has achieved lowest point. From the Figure 1 indicates that when the amount of pullulanase is more than 30U/g, because concentration of reducing sugar produced in the system is so high that polymerize oligosaccharides bring down reducibility. The best range of pullulanase is from 20U/g to 30U/g.

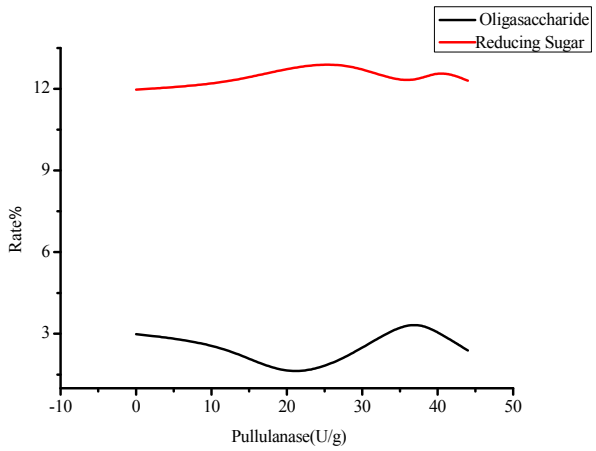

Figure 1 Different pullulanase levels reducing sugar and the residual oligosaccharides content

3.2.2 The change of total sugar and saccharification rate

Figure 2 shows that with the amount of pullulanase increased, the total sugar content gradually increases, when the amount of pullulanase gets to 40U/g, total sugar content of the system reaches to as much as 18.73%. As can be seen from Figure 3 with the amount of pullulanase increased, the saccharification rate increases and then decreases. The amount of pullulanase close to 30U/g obtaines the highest saccharification rate. When the amount is more than 30U/g, the occurring of polymerization lead to significantly decreasing in saccharification rate. Therefore, the conclusion: when the amount of pullulanase is 30U/g or so, reducing sugar content and various indexes obtain optimal effect.

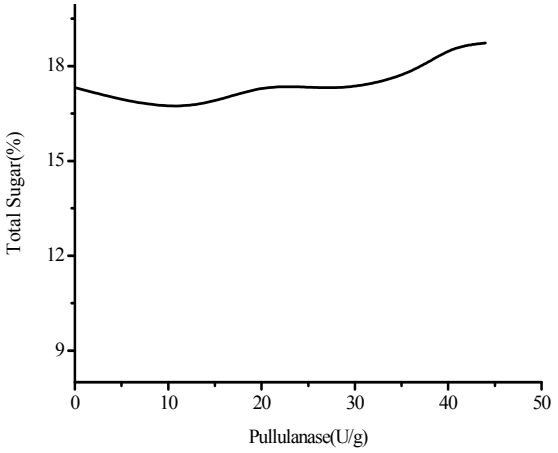

Figure 2 The change of total sugar content with different pullulanase levels

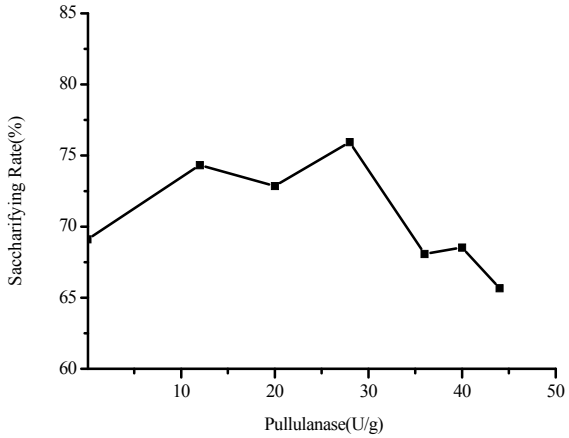

Figure3 The change of total saccharification rate with different pullulanase levels

4 Conclusions

4.1 Add the pullulanase combining with double- enzymatic degradation cassava starch, degradation rate of cassava starch is obviously increased. Efficiency of cassava starch degradation is optimal adding pullulanase before the double-enzymes.

4.2 Pretreat cassava starch by using pullulanase, and then take double-enzymes to degrade starch, in which optimum range for using pullulanase should be controlled between 20U/g and 30U/g.

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