Study on preparation of chito-oligosaccharide by cellulase hydrolysis

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Abstract. By using cellulase enzymatic hydrolysis preparation to obtain low molecular weight chito-oligosaccharide. The conditions for the hydrolysis of chitosan by cellulase were studied by single factor experiment. The effects of temperature, pH value, reaction time and enzyme dosage on the decomposition efficiency of chitosan were investigated. The conclusions from the experiment are as follows: the optimum conditions for the preparation of chito-oligosaccharide by cellulase hydrolysis are reaction temperature of 50 °C, pH of 4.8, enzymatic hydrolysis time of 3.5 h, the enzyme dosage of 50 U/mg, in this case its economic benefits is greatest.

1. Introduction

Chitosan is also known as polyurethane glucose, deacetylchitin, chemical name is β-(1,4)-2-amino-2-deoxy-D-glucose, molecular formula is \((C_6H_{11}NO)_n\) [1]. Chitin is also called as crustaceans — chitin; the chemical name is \(\beta-(1,4)-2\text{-acetylamino}-2\text{-deoxy}-D\text{-glucose}\), molecular formula is \((C_8H_{13}N_0_5)_n\), its relative molecular weight is between hundreds of thousands and millions, and it's a kind of straight chain polymer alkaline polysaccharide which is made of acetyl dextrose or glucosamine that connected by \(\beta\text{-l,4 glucoside bond}\) [2]. Generally speaking, after the removal of 55% of N-acetyl from chitin, the degree of N-acetyl above 55% can be called chitosan, and the degree of N-acetyl below 50% is chitin [3].

Chito-oligosaccharide is a low molecular weight product with good water solubility, high functional function and high biological activity, which is degraded by biotechnology to obtain and chitosan as raw material. The average molecular weight is about 2000. The molecular structure is N-acetyl-D-glucosamine (GLcNAc) and D-glucosamine dextrose (GLcN) connected by P-l,4-glucoside bond, heterozygous or homopolymerization oligosaccharide [4]. Compared with high molecular weight chitosan, chito-oligosaccharide has lower molecular weight, good water solubility, and it’s easy to be absorbed and used, and has special superior physiological activity and effect.

Compared with chitosan macromolecule, chito-oligosaccharide has unique physiological and biochemical characteristics, such as hygroscopicity, moisture retention and water solubility. Chitosan is a natural biological active substance of nitrogen-containing polysaccharides, which contains a large number of -NH₂ and -OH groups in its macromolecular chain structure. When chitosan is enzymatic hydrolyzed, a large number of strong polar radicals increased, which not only improves the water solubility of low molecular weight chitosan, but also significantly improves its moisture absorption and moisturizing function. Chito-oligosaccharide has stronger moisture absorption and moisturizing...
effect than hyaluronic acid and glycerol, and within a certain molecular weight range, with the
decrease of the average molecular weight, the moisturizing and humidifying performance gradually
increases, and with the increase of the molecular weight of oligosaccharide, the moisture absorption
rate also continuously decreases [5].

The purpose of this study is to prepare chito-oligosaccharide by enzymatic hydrolysis of cellulase
and to provide theoretical basis for industrial production.

2. Cellulase hydrolyzed chitosan

2.1. Enzymatic hydrolysis of chitosan

Using 1% acetic acid as solvent, 1% chitosan solution was prepared, proper amount of acetic acid
was added, sodium acetate buffer solution was used to adjust the pH value of the solution, and
appropriate amount of cellulase was added. Low molecular weight chitosan was prepared by
degradation in constant temperature water bath.

2.2. Determination of reductive sugar

DNS method was used to determine the content of reducing sugar by dinitrosalicylic acid method.
Under alkaline conditions, dinitrosalicylic acid redox with reducing sugar to form 3-amino-5-
nitrosalicylic acid. The product shows brown and red color under boiling condition, and the color
depth is proportional to the content of reducing sugar in a certain concentration range. The content of
reducing sugar is determined by colorimetric method. Because the depth of color development is only
related to the number of free reduction groups of carbohydrates, but there is no selectivity to the types
of reducing sugar, DNS method is suitable for a variety of reducing sugar systems produced by
hydrolysis of polysaccharides (such as cellulose, hemicellulose and starch, etc.). Standard curve
making: Taking nine drying tubes, and numbered according to the amount in Table 1, respectively,
added the concentration 0.1% accurate standard glucose solution and DNS reagent, covered with plugs,
shook each tube well, 5 minutes in a boiling water bath to heat, then cooled to room temperature by
cooling water immediately, adding distilled water to the tube 4mL, shook well, under the 540nm
wavelength, with tube 1 as the blank, determining absorbance values of the 1-9 pipe by
spectrophotometer. The standard curve was drawn with absorbance as the ordinate and glucose
concentration as the abscissa. See Table 1 for the standard curve of reducing sugar added by DNS
method. Seen from Figure 1 for the standard curve of glucose concentration and absorbance.

| Pipe No. | 0.1% Glucose Solution /mL | Distilled Water /mL | DNS Reagents /mL |
|---------|---------------------------|---------------------|-----------------|
| 1       | 0.00                      | 0.50                | 0.50            |
| 2       | 0.05                      | 0.45                | 0.50            |
| 3       | 0.10                      | 0.40                | 0.50            |

**Table 1.** DNS Standard curve sampling table for reducing sugar measured by method.

**Figure 1.** Standard curve of glucose concentration and absorbance.
3. Data Analysis and conclusion

3.1. Effect of enzyme content on enzyme hydrolysis reaction

Table 2 and Figure 2 below show the effect of enzyme dosage on the reaction. When the added enzyme quantity was 10, 20, 30, 40, 50, 60 U/mg, the release value of reducing sugar in the enzymatic degradation reaction was shown in Figure 2. When the concentration of chitosan is constant, it can be seen from the Figure 2 that with the increase of enzyme dosage of cellulase at the beginning, the reducing sugar produced by degradation also increases. When the amount of enzyme added reaches 50 U/mg, the amount of enzyme added increases again, but the reducing sugar produced remains basically unchanged. In a word, 50 U/mg is the optimal amount for this enzymatic hydrolysis reaction.

Table 2. Effect of enzyme content on reaction.

| The amount of enzyme added ( U/mg ) | 10  | 20  | 30  | 40  | 50  | 60  |
|-----------------------------------|-----|-----|-----|-----|-----|-----|
| Suction luminosity                | 0.36| 0.42| 0.92| 1.34| 1.56| 1.58|
| Reduced sugar concentration ( mg/mL ) | 0.22| 0.32| 0.68| 1.12| 1.35| 1.36|

Figure 2. Effect of enzyme content on reaction

3.2. Effect of temperature on hydrolysis reaction of chitosan

The effect of temperature on the reaction is shown in Table 3 and Figure 3. The enzyme is highly sensitive to temperature when the temperature is too low, the enzyme activity decreases and the reaction rate increases with the increase of temperature, but when the reaction temperature is higher than the optimum temperature, the enzyme will gradually become inactivated and the reaction rate will decrease. Figure 3 shows that the hydrolysis of chitosan by cellulase reaches the highest value at about 50 °C, but if the temperature continues to rise, the concentration of reducing sugar will decrease, so it can be seen that the optimum temperature of degradation reaction is 50 °C.

Table 3. Effect of temperature on reaction.

| Temperature (°C) | 35  | 40  | 45  | 50  | 55  | 60  |
|------------------|-----|-----|-----|-----|-----|-----|
| Suction luminosity | 0.72| 0.98| 1.23| 1.65| 1.52| 0.96|
| Reductive sugar concentration (mg/mL) | 0.56| 0.85| 1.12| 1.56| 1.18| 0.78|
3.3. Effect of pH value on the degradation reaction of chitosan

The effect of pH value on the reaction is shown in Table 4 and Fig. 4. Fig. 4 shows that when pH is in the range of 3.2-4.8, the concentration of reducing sugar increases rapidly with the increase of pH value, when pH value is 4.8, the content of reducing sugar is the highest, and when pH is greater than 4.8, the concentration of reducing sugar begins to decrease. Therefore, under the condition of pH of 4.8, the degradation reaction was the best.

Table 4. Effect of pH value on reaction.

| pH   | 3.2 | 3.6 | 4.0 | 4.4 | 4.8 | 5.4 | 5.8 |
|------|-----|-----|-----|-----|-----|-----|-----|
| Suction luminosity | 0.92 | 1.36 | 1.42 | 1.58 | 1.60 | 1.36 | 1.21 |
| Reductive sugar     | 0.81 | 1.19 | 1.21 | 1.39 | 1.40 | 1.21 | 1.09 |

3.4. Effect of enzymatic time on hydrolysis reaction of chitosan

The effect of reaction time on the chitosan hydrolysis reaction is shown in Table 5 and Fig. 5. Under different reaction time, the release value of reducing sugar in enzyme degradation reaction is shown in Fig.5. Until 2 h before the reaction, the concentration of reducing sugar increased slowly. After 2 h of reaction, the content of reducing sugar tends to increase. After 3.5 h, the reaction basically reached
equilibrium. Therefore, 3.5 h was selected as the best time for chitosan degradation activity in cellulase.

Table 5. Effect of reaction time on reaction.

| Enzyme hydrolysis Time (h) | 1.5 | 2  | 2.5 | 3   | 3.5 | 4   | 4.5 |
|---------------------------|-----|----|-----|-----|-----|-----|-----|
| Suction luminosity        | 0.51| 0.78| 0.92| 1.38| 1.56| 1.58| 1.61|
| Reductive sugar concentration (mg/mL) | 0.43 | 0.59 | 0.82 | 1.23 | 1.38 | 1.48 | 1.51 |

Figure 5. Effect of reaction time on reaction.

4. Conclusion
(1) Enzyme hydrolysis reaction with the increase of enzyme dosage and the prolongation of reaction time were deepened.
(2) The optimum condition for the preparation of chito-oligosaccharide by cellulase hydrolysis is the reaction temperature of 50 °C, pH of 4.8, enzymatic hydrolysis time of 3.5h, added enzyme quantity of 50 U/mg. Under these conditions, its economic benefits is best.

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