Study on Enzymatic Characteristics of Cellulose Produced by Aspergillus fumigatus and Optimization of Enzyme Production Medium

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Abstract. In addition to providing the necessary nutrient conditions for the growth of cells, the medium also provides a substrate for functional products. Fermentation medium optimization plays an important role in improving the fermentation level. At present, orthogonal design, response surface design, and uniform design are widely used to optimize the composition of the medium, and the effect is very significant. On the basis of exploring the enzyme-producing characteristics of Aspergillus fumigatus LY1 strain, the concentration of each component of the enzyme-producing medium was optimized by using the straw combination as the substrate and the central combination design.

1. Introduction
The current reserves of fossil energy are depleting at an alarming rate, and the grim situation of globalization has aroused widespread concern in the scientific community. Fluctuations in the price of oil and the production of a large number of harmful substances that destroy the ecological environment have led scientists to turn their attention to the development of renewable energy materials to produce biofuels instead of petroleum fuels. Using the inexhaustible natural cellulose biomass in nature as a raw material for the production of biofuels, the biotransformation of cellulosic polymers by cellulose into other high value-added products is considered to be the future processing of a large number of agricultural crops. The most promising and potential way of straw. Cellulose currently accounts for 8% of the global industrial enzymes, and the current market is expected to expand the cellulose market by $40 billion annually. Among them, microbial cellulose is widely used due to its short growth cycle and controllable factors. Although a large number of basic enzymes and industrial applications of cellulose are under study, the high production cost of cellulose is still the biggest obstacle, and the development of economically more cost-effective technical routes such as enzyme recycling and lowering production costs are still it is an important area of current research.

2. Cellulase enzymatic properties
(a). Cellulase optimum temperature
A 1% matrix solution was dispensed with a citric acid buffer having a pH of 4.8. The enzyme activity of the cellulose was determined at different temperatures (30°C, 40°C, 50°C, 60°C, 70°C, 80°C, 90°C), and the highest enzyme activity was defined as 100% at different temperatures. Residual
enzyme activity (expressed by relative enzyme activity, i.e. the remaining enzyme activity at different temperature conditions as a percentage of the highest value of enzyme activity).

(b). Cellulase optimum pH

Under the optimum temperature conditions determined, the crude enzyme solution was reacted in a buffer of different pH conditions (pH 4-9), and the highest enzyme activity was defined as 100%, and the residual enzyme activities at different pH were calculated respectively.

Different pH buffers required:
Sodium citrate monophosphate: PH 3-6;
Tris-HCL: PH 7-9

(c). Cellulase thermo stability

The crude enzyme solution was placed at 30°C, 40°C, 50°C, 60°C, 70°C, 80°C for 60 min, and samples were taken every 10 min. After the sample was returned to room temperature (CK was not treated at any time). The enzyme solution) measures the CMase activity at the optimum reaction temperature and the optimum pH, and the remaining enzyme activity is calculated as a percentage.

(d). Cellulase pH stability

The crude enzyme solution was incubated at different pH (3, 4, 5, 6, 7, 8) at room temperature for 60 min, and samples were taken every 10 min. The sample to be tested was adjusted to the optimum temperature at the optimum temperature. CMCase was measured at pH and the remaining enzyme activity was calculated as a percentage.

(e). Determination of cellulose kinetic constant

The activity of the enzyme was determined by using 1% CMC as the substrate, the optimum reaction temperature and pH value, and the \( \frac{1}{v} \)-1/s dynamic curve was drawn according to the Line weaver-Burk double reciprocal mapping method. The X-axis intercept negative reciprocal The \( \text{Km} \) value is reciprocal with the Y-axis and the \( \text{Vm} \) value is obtained.

3. A. fumigatusLY1 optimal carbon source and glucose concentration

The LY1 slant grown at 45°C for 72 h was scraped with 10 mL of distilled water to prepare a suspension of the stalk, and 2 mL was inoculated into 50 mL of 250 mL of fermentation medium containing different inducing substrates (straw straws, filter paper, CMC-Na, soluble starch). In a triangular bottle. The fermentation was continued for 144 h, and the cellulose activity was measured every 24 h to determine the optimal substrate and optimum incubation time.

After determining the optimum cellulose substrate, the glucose concentrations in the medium were set to 0, 2.5, 5, 7.5, and 10 g/L, respectively, and the cellulose activity was determined after the appropriate time to obtain the optimum glucose concentration.

4. Changes of pH, reducing sugar concentration and protein concentration during growth under different glucose concentrations

According to the method of spore suspension preparation, 2 mL was inoculated into a 500 mL flask containing 150 mL of liquid medium. The cells were continuously cultured for 120 hours, samples were taken every 24 hours, the pH of the sample was measured by a pH meter, and the reducing sugar concentration was determined by the DNS method. Protein content was determined by reference to Bradford's method using bovine serum albumin as the standard protein.

(a). Drawing of standard curve

Take 6 tubes and prepare them separately according to the data in the table below. 0-100 lxg/mL BSA solution 1 ml each. Accurately absorb 1 mL of each tube solution prepared, and add 5 mL of Coomassie Brilliant Blue G-250 reagent. After 2 min, use No. 1 tube as CK, colorimetric at 595 nm, record the absorbance value, and obtain the absorbance value. For the ordinate, the standard protein content is the abscissa and a standard curve is drawn.
Table 1. Prepare 0-100 μg/mL BSA solution

| Number | 1   | 2   | 3   | 4   | 5   | 6   |
|--------|-----|-----|-----|-----|-----|-----|
| 0-100μg/mL BSA solution dosage (mL) | 0.0 | 0.2 | 0.4 | 0.6 | 0.8 | 1.0 |
| Distilled water consumption (mL)    | 1.0 | 0.8 | 0.6 | 0.4 | 0.2 | 0.0 |
| Protein content (mg/mL)             | 0.0 | 0.02| 0.04| 0.06| 0.08| 0.1 |

(b). Determination of protein concentration
After continuous incubation for 120 h, the samples were centrifuged every 24 h. 1 mL of the supernatant was added to 5 mL of Coomassie Brilliant Blue G-250 reagent, mixed well, and after 2 min, the absorbance values were recorded with blank medium as control, and the protein content of the sample solution was calculated by the standard curve drawn.

5. Optimum nitrogen source and nitrogen source concentration
LY1 scorpion was inoculated into the fermentation medium with soybean meal, yeast powder, corn flour, protein chen, corn syrup, etc. as organic nitrogen source. After 96 hours of culture, the enzyme activity of each component of cellulose was determined to determine the most. Good nitrogen source type. On this basis, the optimum nitrogen source concentrations were controlled at 0, 2.5, 5, 7.5, and 10 g/L, respectively, to determine the optimum concentration. Based on the composition of the medium, DPS software was used to design the quadratic regression rotation center combination experiment to determine the optimal concentration of each component. CMCase defaulted to cellulose activity as the response value. And finally through the DPS software for data analysis.

6. Enzymatic properties of cellulose produced by LY1
The enzyme-catalyzed reaction process also follows the general chemical reaction law: high temperature accelerates the reaction rate, and low temperature reduces the enzyme and substrate catalytic rate. Most of the enzymes belong to proteins. Excessive temperature may cause protein inactivation. Too low temperature will cause protein denaturation. Therefore, it is important to know the temperature range of the enzyme reaction and the optimum reaction temperature. In order to investigate the effect of temperature on the cellulose produced by LY1 strain, the enzyme activity was determined at a series of temperatures. In the range of 30-60°C, the activity of cellulose enzyme was positively correlated with temperature, and the enzyme activity was the highest at 60°C, which is the optimum reaction temperature. After that, the enzyme activity decreased with the further increase of temperature, and the curve was reduced. The slope shows that the enzyme activity drops sharply with increasing temperature, and the relative enzyme activity is less than 8% at 90°C, and most of the enzyme activity has been lost. According to the definition of high temperature enzyme, since the optimum temperature is between 60-80°C as a high temperature enzyme, it is preliminarily judged that the cellulose produced by A. fumigatus LY1 belongs to a high temperature enzyme.

The optimum pH is also an important characteristic parameter of the enzyme reaction, which can affect the surface structure of the enzyme, such as enzyme folding, chemical modification and post-translational modification. Under the optimum temperature (60°C) reaction conditions, the changes in enzyme activity under different pH conditions were investigated. The results showed that the cellulose activity of LY1I reached the highest in the pH 5.0 buffer system, followed by pH 6.0.

The enzyme solution was treated at different temperatures under the condition of pH 5.0, and the enzyme activity change was measured at 60°C after a certain time interval to analyse the thermal stability of the enzyme. The temperature-activity curve shows that when the treatment temperature is lower than 50°C, cellulose treatment can maintain higher activity for 30 min, even after 1 h of treatment, the relative enzyme activity remains 80%; after 60 h treatment for 1 h, its relative vigor remains. More than 50% did not reach half-life; when the temperature reached above 70°C, the enzyme activity entered the half-life within 10 min, and the enzyme activity decreased to less than 20% after 10 min.
The pH-activity curve indicated that the LY1 enzyme solution maintained a high enzyme activity in the range of pH 5.0-7.0. After 60 minutes of treatment, the enzyme activity was still more than 80%, especially after the treatment with pH 5.0. Highest. When the pH drops to 3.0-4.0, the enzyme activity enters a half-life within 10 min. The enzyme activity remained about 60% after 1 h of treatment at pH 8.0.

In the process of enzyme kinetics, the change of the enzymatic reaction rate with the substrate concentration is often determined under the optimum reaction conditions. Based on this, the Mie equation of the enzymatic reaction can be established, and the double reciprocal mapping is often used for the calculation. Also known as the Lineweaver-Burk curve. Using CMC as a substrate, the enzymatic reaction kinetic equation of cellulose produced by Aspergillus fumigatus LY1 is: 

$$y = 8.58x + 0.90$$

as shown in Figure 1. It can be inferred that $V_m = 1.11 \text{ umol} \cdot \text{mL}^{-1} \cdot \text{min}^{-1}$, $K_m = 9.52$.

![Cellulase Lineweaver-Burk Curve](image)

7. A. fumigatus LY1 optimum carbon source and glucose concentration

Induction of substrate species (straw straw, CMC-Na, filter paper, starch) on the fermentation of Aspergillus fumigatus LY1. With the increase of fermentation time, the enzyme activity induced by each substrate increased continuously, and it became stable after 120 hours of culture. The enzyme activities of each component of cellulose were highest in the medium with straw as the substrate for induction. The highest enzyme activities of endoglucanase, exoglucanase and glucosease were respectively 0.54 IU/mL, 0.52 IU/mL, 1.78 IU/mL. Complex substrates produce a strong induction of fungal cellulase. The plant cell wall has a "biomass anti-degradation barrier" that naturally evolves to resist the degradation of external factors. It has a multi-layered supramolecular structure. For example, cellulose in plant cell walls is often tightly formed with hemicellulose, lignin and other polymers. Cross-linking forms a network structure dominated by cell wall polysaccharides, and the crystallinity of cellulose itself leads to the diversity of enzymes required in the enzymatic hydrolysis process, the hierarchical and directionality of degradation. Therefore, it is presumed that CMC-Na, filter paper, etc. are relatively simple in terms of degree of polymerization, crystallinity, and cellulose structure stratification with respect to straw, and thus the straw induction activity is higher than that of other cellulose substrates.

The results showed that the enzyme activity of cellulose was significantly higher than that of other treatments when the glucose concentration was 2.5 g/L. Glucose is a fast-acting carbon source for microbial growth and an essential nutrient for microbial growth and synthesis of structural materials. Studies have shown that cellulose degradation of cellulose not only involves the adsorption and dissociation of multiple enzymes, but also the inhibition of the substrate and various products (mainly
cellulose, glucose). The available carbon source is beneficial to glucose. Bacterial growth, but cellulose as an inducing enzyme, excessive concentration of glucose will inhibit its enzyme activity or repress and induce expression.

Using the quadratic regression rotation center combined experimental design method of DPS software, the five main components in the original fermentation medium were optimized, and the CMCase activity in the bottle was taken as the experimental target. Based on the above single factor experiment results, the design experimental factors and levels are shown in Table 2.

| Table 2. Secondary rotation combined design factor level table and coded value |
|--------------------------|--------------------------|
| Factor | Level | |
| | -2 | -1 | 0 | 1 | 2 |
| X₁ | 0 | 1.25 | 2.5 | 3.75 | 5 |
| X₂ | 4.5 | 6 | 7.5 | 9 | 10.5 |
| X₃ | 0.5 | 0.75 | 1 | 1.25 | 1.5 |
| X₄ | 0.5 | 0.75 | 1 | 1.25 | 1.5 |
| X₅ | 0.1 | 0.3 | 0.5 | 0.7 | 0.9 |

8. Conclusion
Cellulase is an inducible enzyme whose synthesis is regulated by induction and decomposition products, and cellulose is the best inducer. The results of the research on the gene expression profile of Aspergillus fumigatus indicate that the gene expression levels of different induced carbon sources are different, so the purpose of increasing the gene expression and increasing the cellulose content in the fermentation broth can be achieved by optimizing the culture components.

In this paper, the enzymatic properties of cellulose produced by Aspergillus fumigatus LY1 were studied firstly. The single factor experimental design was used to screen the cellulosic substrate with the strongest enzyme activity induction ability, and the optimum addition amount of available carbon source. And the best nitrogen source species; on this basis, the concentration of glucose and other components in the fermentation culture of Aspergillus fumigatus was optimized using a powerful medium optimization software DPS data processing system. The results showed that the optimal reaction temperature of cellulose produced by Aspergillus fumigatus LY1 was 60 °C, the optimum pH range was 5.0-6.0, and it had certain thermal stability and PH stability.

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