Effect of some factors on Production of cellulase by

*Trichoderma reesei HY07*

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Abstract

The effects of some factors including ammonium sulphate, inoculum, Tween 80, temperature and pH were assessed for the production of cellulase by *Trichoderma reesei HY07* isolated from decaying corn stalk. The results were as follows: the optimal concentrations of Ammonium sulphate, inoculum, Tween 80 for production of carboxymethyl cellulase (CMCase) were 1.5% (w/w), 2.5%(v/w) and 0.1% (v/w) and the optimal temperature for production of CMCase were 30℃. The optimal concentrations of ammonium sulphate, inoculum, Tween 80 for production of filter paper activity (FPA) were 0.5%(w/w),1.5%(v/w) and 0.1%(v/w) and the optimal temperature for production of FPA were 30℃.

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1. Introduction

Cellulose is the most abundant renewable resource in the world which is a linear polymer of glucose units and can be degraded into glucose by cellulase, which in turn can be converted to ethanol, single cell protein and other valuable chemicals [1-5]. A complete cellulase system consists of endoglucanase (EC 3.2.1.4, endo-1, 4-b-glucanase), exoglucanase (EC 3.2.1.91, 1, 4-b-cellobiohydrolase) and β-glucosidases (EC 3.2.1.21) [6]. A broad range of bacteria and filamentous fungi can produce cellulases, one of the most extensively studied cellulolytic microorganism is the soft rot fungus *Trichoderma reesei*, which is also used industrially for enzyme production [7-10].

The cost of the media has been estimated to be the major contribution to cellulase production cost;
especially carbon resource. Lignocellulose is a cheap raw material and several lignocellulosic substrates, such as wheat straw, bagasse, waste paper sludge, waste newsprint and aspen wood were used as main component of media for enzyme production [11-16]. However, the researches on corn stalk and wheat bran as carbon resource for cellulase production are few.

Environmental and nutritional factors are known to have marked effects on enzyme production by microorganisms. There are, therefore, variations in optimum conditions for cellulase production. In previous study, a cellulase-producing strain Trichoderma reesei HY07 was screened from decayed corn stalk [17]. The objectives of this study were to assess some factors including ammonium sulphate, Tween 80, inoculum and temperature for the production of cellulase by Trichoderma reesei HY07 in solid state fermentation using corn stalk and wheat bran as main component of media.

2. Materials and methods

2.1. Microorganism

The strain Trichoderma reesei HY07, isolated from decayed corn stalk, was used for cellulase production. was cultivated on potato dextrose agar containing 1.5 % agar and incubated at 28 °C for one week until complete sporulation. The spores from slants were suspended in sterile water containing 0.1% peptone. The suspension was used as inoculum ($10^7$ spores ml$^{-1}$).

2.2. Medium and Culture conditions

The initial cultivation medium was composed of corn stalk 4g, wheat bran 6g, tween80 20μL, (NH$_4$)$_2$SO$_4$ 0.01g, K$_2$HPO$_4$ 0.005g, MgSO$_4$·7H$_2$O 0.025g and 10mL distilled water in 250 ml Erlenmeyer flasks. pH 5.0 adjusted by 1N HCl and 1N NaOH. The flasks were plugged with cotton and autoclaved at 121°C for 20 min, cooled and inoculated spore suspension to $10^6$ spores g$^{-1}$ medium, 30°C for 5d. For the experiments of optimization, the cultivation medium was composed of different concentrations of corn stalk, bran, tween80, also varying the temperature of cultivation, according to the experimental design.

2.3. Extraction of cellulase

The mouldy substrates (koji) produced by solid state fermentation were mixed with 10 volumes of water to extract cellulase, stirred slowly at 30°C for 1 h and filtered. The liquid portion was then used for the measurement of cellulase activity.

2.4. Enzyme assays

Carboxymethyl cellulase (CMCase) and filter paper activity (FPA) assay were carried out by mixing 0.5 ml enzyme sample with 0.5 ml of 1% carboxymethylcellulose (CMC) in 0.05M sodium citrate buffer (pH 4.8) at 50°C for 30 min, or 50 mg of Whatman No. 1 filter paper suspended in 0.5 ml of the same buffer, and followed by incubation for 30 min by shaking at 50°C. Reducing sugar was determined using 3, 5-dinitrosalicylic acid (DNS) reagent with glucose as a standard [18]. The CMCase and FPA were both expressed as U/g of koji. One unit (U) of enzyme activity is defined as the amount of enzyme required to liberate 1μmol of product per 30min.
3. Results and discussion

3.1. Effect of ammonium sulphate on production of cellulase

According to the literature[19], ammonium sulfate is the best source of nitrogen for cellulose production by *Trichoderma reesei*, so ammonium sulfate was chosen as a nitrogen source. Ammonium sulfate were added to adjusted to the following final concentration 0.5%, 1.0%, 1.5%, 2.0% and 5%, 30 °C for 3 days. The results are shown in Figure 1.

![Fig.1 Effect of ammonium sulphate on production of cellulase](image1)

With concentration of ammonium sulfate increasing, the activity of CMCase first increased from 235.28U/g at 0.5% to maximum 271.97U/g at 1.5%, then decreased to 247.06U/g at 2.5%, but the activity of FPA gradually decreased from 80.87U/g at 0.5% to 46.68U/g at 2.5%, so the optimal concentrations of ammonium sulfate were 1.5% for CMCase and 0.5% for FPA, respectively.

3.2. Effect of inoculum on production of cellulase

With concentration of inoculum increasing, the activity of CMCase gradually increased from 355.06U/g at 0.5% to 386.47U/g at 2.5%, but the activity of FPA first increased from 81.28U/g at 0.5% to maximum 87.73U/g at 1.5%, then decreased to 84.71U/g at 2.5%, which showed that the inoculum had a great influence on the production of cellulose. The optimal concentrations of inoculums were 2.5% for CMCase and 1.5% for FPA, respectively.

3.3. Effect of tween 80 on production of cellulase

Surfactants affect the permeability of microbial cell membrane, thus contributing to make the enzyme secrete into the media, and lower the enzymes concentration of intracellular, thus increasing the enzyme production. Tween 80 was chosen to study effect of surfactant on the activity of CMCase and FPA. Tween 80 was added to the media and adjusted to the following final concentration 0, 0.05%, 0.10%, 0.15%, 0.20% and 0.25%, 30 °C for 3 days. The results are shown in Figure 3.

With concentration of tween 80 increasing, the activity of CMCase and FPA were first increased and
then decreased. The activity of CMCase gradually increased from 257.06U/g at 0.0% to 261.49U/g at 0.10% and then decreased to 213.02U/g at 0.25%, The activity of FPA gradually increased from 26.45U/g at 0.0% to 54.67U/g at 0.10% and then decreased to 20.35U/g at 0.25%, Which demonstrated that in the medium supplemented with tween 80 to promote cellulase production, especially for FPA. The optimal concentrations of tween 80 were all 0.10% for CMCase and FPA.

![Fig.3 Effect of tween 80 on production of cellulase](image1)

![Fig. 4 Effect of temperature on production of cellulase](image2)

3.4. Effect of temperature on production of cellulase

Temperature on microbial growth and enzyme production has a great influence. Culture temperature is too high, microorganism grows faster, but easy to aging and enzyme production is low, temperature too low, microbial growth is slow, resulting in long production cycle. Incubation temperature was adjusted to 26°C, 28°C, 30°C, 35°C and 40°C respectively. The result is shown in Figure 4.

With concentration of temperature increasing, the activity of CMCase and FPA were first increased and then decreased. The activity of CMCase gradually increased from 236.09U/g at 26°C to 377.20U/g at 30°C and then decreased to 21.61U/g at 40°C%, The activity of FPA gradually increased from 37.33U/g at 26°C to 92.16U/g at 30°C and then decreased to 5.48U/g at 40°C, Which demonstrated that temperature on the CMCase and FPA production have a great impact. The optimal incubation temperature were all 30°C for CMCase and FPA.

4. Conclusions

The optimal concentrations of Ammonium sulphate, inoculum, Tween 80 for production of carboxymethyl cellulase (CMCase) were 1.5% (w/w), 2.5%(v/w) and 0.1% (v/w) and the optimal temperature for production of CMCase were 30°C. The optimal concentrations of ammonium sulphate, inoculum, Tween 80 for production of filter paper activity (FPA) were 0.5%(w/w),1.5%(v/w) and 0.1%(v/w) and the optimal temperature for production of FPA were 30°C.

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