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International
Journal
Of Modern Engineering Research (IJMER)

Study on Culture Conditions for A Cellulase Production From As Pergillus Unguis

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ABSTRACT: *Cellulase is a common name of enzymes which catalyze cellulolysis. Specially,cellulase is widely used in food processing, animal feed, chemicals, textile,fuel and pollution treatment.The objective of this research is to study on optimal conditions for the production of cellulase byAspergillus unguis. The study was designed as a comparative culture conditions such as carbon sources, moisture content, duration, nitrogen sources and citrate buffer content on cellulase production for Aspergillus unguis. Cellulase activity was determined by measuring the absorbance at λ = 540 nm with 3,5-DNS reagent. In optimized culture conditions, enzyme activity of Aspergillus unguis achieved 110.92U/ml in comparison with a commercial cellulase with 185.33U/ml in enzyme activity. The value of cellulase activity of Aspergillus unguis is 41% lower than commercial enzymes. However, enzyme in this study was raw enzyme and the cost of producing1 litter of this enzyme is just 1/8 that of purified ones. The enzyme activity would be increased by purification. That fact has proven the applicability of using the findings of this study to improve cellulase production.*

Keywords: *Aspergillus unguis, carbon sources, cellulase, culture conditions, duration, moisture content, and nitrogen sources.*

I. INTRODUCTION

Cellulolysis is the process of breaking down cellulose into smaller polysaccharides called cellodextrins or completely into glucose units. A cellulase system consists of three major components: endo-ß-glucanase (EC 3.2.1.4), exo-ß-glucanase(EC 3.2.1.91) and ß-glucosidase (EC 3.2.1.21).Exoglucanases (1,4-β-D-glucancellobiohydrolase, EC 3.2.1.9.1) are usually active on crystalline cellulose and cleave disaccharide units either from non – reducing or reducing end. Endoglucanases (endo-1,4-β-D-glucanase, EC 3.2.1.4) are more active against the amorphous regions of cellulose and they can also hydrolyze substituted celluloses, such as carboxymethyl cellulose (CMC) and hydroxyethyl cellulose (HEC). β-glucosidases(EC 3.2.1.21) hydrolyze cellobiose and short (soluble) cellooliogosaccharides to glucose. These enzyme is widely used in various industries such as animal feed production, starch processing malting and brewing, grain alcohol fermentation, extraction of fruit and vegetable juices as well as manufacture of pulp, paper and textiles [1].

The production of cellulase has been reported from a wide variety of bacteria [2] and fungi [3], [4]. However, fungi are preferred for commercial enzyme production because of high enzyme activity. *Aspergillus* and *Trichoderma* spp. are well known in efficient production of cellulases [5]. So, this study observe *Aspergillus unguis*for cellulase production.The cellulase production by filamentous fungi in solid state fermentation and submerged fermentation has been studied extensively [6], [7], [8]. From this point of view, the study used *Aspergillus unguis*for cellulase production. They were demonstrated for their improve efficiency in solid state fermentation for production of cellulase using byproducts ofagriculture(rice husk and rice bran) in Vietnam as raw material. The influence of various conditions wereevaluated inSSF. Besides, the optimal culture conditions for cellulase from *Aspergillusunguis*will also be investigated.

II. MATERIALS AND METHODS

2.1 Materials

The raw materials (rice husk and rice bran) was bought from Tien Giang province, Vietnam. Those materials are agricultural byproducts. They were chosen for this study due to their high quality, plenty of source from rice processing factories, and their convenience for preservation and transportation. The microorganism (*Aspergillus unguis*)was supported by Laboratory of Cell Biotechnology. The nitrogen sources, such as

$(NH_4)_2SO_4$, yeast extract, urea and tryptonewas supplied by Food Engineering Laboratory, International University – Vietnam National University in Ho Chi Minh City.

2.2Methods

2.2.1 Solid state fermentation (SSF)

Solid state fermentation was carried out in 250 ml Erlenmeyer flasks, the component nutrition was prepared followed Table 1. The flasks were sterilized at 121^{o}C for 20 minsbefore used.*A.unguis*was cultured on malt extract agar at 30^{o}C for 7 days until cell concentration reached around 5x10^8 spores/ml. The culture were mixed by gentle shaking.

2.2.2 Enzyme extraction

10g of byproducts from microorganisms as *A. unguis* was ground with 50 ml of distilled water within 5 mins. The suspension was filtered by cloth to eliminate solids and get liquid solution. After that, liquid solution were centrifuged at 6000rpm for 15 mins and clear supernatant was used as a source of extracellular enzyme.

2.2.3 Enzyme assay

Cellulase activity was measured according to the method described by Ghose[9]. One unit of cellulase activity is defined as amount of enzyme that release 1µmole of reducing sugar per min with glucose standard. The value of enzyme activity were expressed as U/ml for SSF

2.2.4 Optimization of culture conditions for maximum cellulase production

Effect of supplements, such as carbon and nitrogen sources were supplied as individual components and added in flasks. The flasks were incubated and cooled at room temperature. 1ml of inoculum was added, mixed well and incubated at 30^{0}C incubator for 4 days.

- Effect of carbon source for Aspergillus unguis on cellulase production

The study fixed $(NH_4)_2SO_4$and changed carbon source from rice husk and rice bran. The fungi was cultured separately in same culture conditions. There were six treatments with different proportions of rice husk and rice bran. They were named X_1, X_2, X_3, X_4, X_5 and X_6 in order of increasing rice husk content and decreasing rice bran content. Oriented medium changed as following (Table 1).

Table 1: The component nutrition for Aspergillus unguis Unit: percent (%)

	X_1	X_2	X_3	X_4	X_5	X_6
Rice husk	20	25	30	35	40	45
Rice bran	79	74	69	64	59	54
$(NH_4)_2SO_4$	1	1	1	1	1	1

Moisture content is fixed at 60%. *Aspergillus unguis*(5x10^8 spores) was added in culture medium at room temperature (28-30^{o}C). Cellulase production of *Aspergillus unguis* was investigated for 4 days.

- Effect of various nitrogen sources on cellulase production

The study was fixed carbon source at optimal carbon source and changed nutrients source of nitrogen($(NH_4)_2SO_4$, yeast extract, urea and tryptone), and fixed at 60%.

2.2.5 Effect of moisture content on cellulase production

Water is essential for the metabolism of all cells. If it is reduced or removed, cellulase activity is decreased. Therefore, all optimal conditions were fixed, only moisture content was changing in a range as 48-52-56-60-64-68%.

2.2.6 Effect of duration on cellulase production

Duration plays an important role on cellulase production. Both too short and too long duration affect cellulase produced by microorganisms. Therefore, all optimal conditions were fixed, only duration was changing, follow in: 3 – 4 – 5 – 6 – 7 – 8 days, at 30^{o}C.

2.2.7Effect of citrate buffer content on extracted cellulase

The experiment observed citrate buffer content affect to enzyme recovery. So, adding citrate buffer (0.05M and pH 4.8) content is different following: 3/1 (30 ml citrate buffer/ 10g byproduct); 5/1 (50 ml citrate buffer / 10g byproduct); 7/1 (70ml citrate buffer/ 10g byproduct); 10/1 (100ml citrate buffer/ 10g byproduct).

III. RESULTS AND DISCUSSION

3.1 Effect of carbon sources on cellulase production

Influence of carbon source (rice bran) on cellulase production is showed in Figure 1. Carbon is the main source of nutrition for fungi. The moresubstrate concentration increased, the more cellulase was producedby microorganism because in an environment that was lack of monosaccharides, microorganism needed to excrete enzymes to break down polysaccharides into monosaccharides. In this case, *A. unguis* produced cellulase to convert cellulose in rice bran to monosaccharides for its usage. However, after a certain concentration, increasing of substrate will have no effect on cellulase activity because the enzymes are saturated, and not working at maximum possible rate. On the other hand, at low concentration of substrate, the catalytic site of enzyme is empty, so, product can be formed is limited. In the experiment, ammonium sulfate and moisture content were controlled while rice husk and rice bran proportions were changed in order to find the carbon source for the optimum growth of microorganisms. The percentage of these two substrates is varied in total of 99% content. In details, the rice husk content was increased while the bran content was decreased. The outcomes have been observed for 4 days for *A. unguis*.

Based on Figure 1, the highest cellulase activity was obtained at treatment X_4 of *A. unguis* for 4 days that carbon source of *A. unguis* was optimum at X_4 which cellulase activity gave a 48.86 U/ml

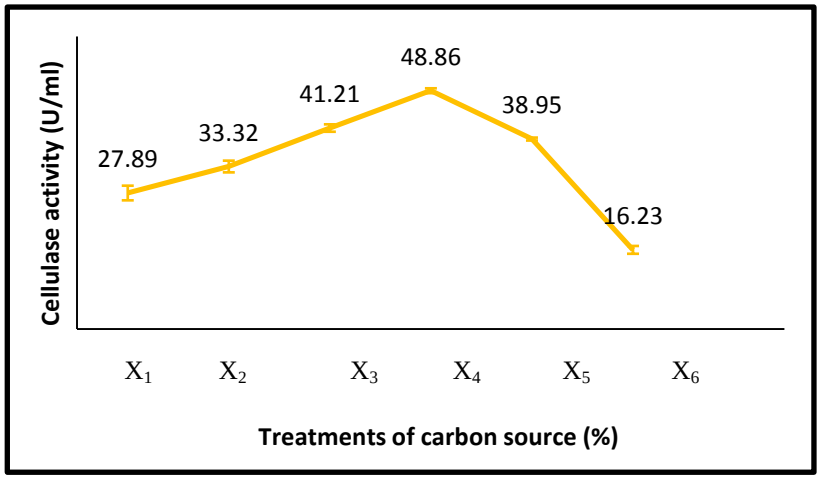


Figure 1: Effect of carbon source on cellulase production

3.2 Effect of various nitrogen sources on cellulase production

Significant variation in fungal growth among the one inorganic (ammonium sulfate) and three organic nitrogen (yeast extract, tryptone and urea) sources were observed in Figure 2. Yeast extract was the best nitrogen source to promote fungal growth rapidly. In addition, tryptone and ammonium sulfate supported fairly good for fungi growth. The activity of cellulase obtained were 87.43, 58.4, 48.86 and 9.83 U/ml, respectively. This study showed the highest cellulase activity was 87.43 U/ml and achieved in yeast extract culture. In other study, peptone is found as good nitrogen source for *A. niger* [11]. Therefore, the difference in optimal nitrogen source may due to the difference in species.

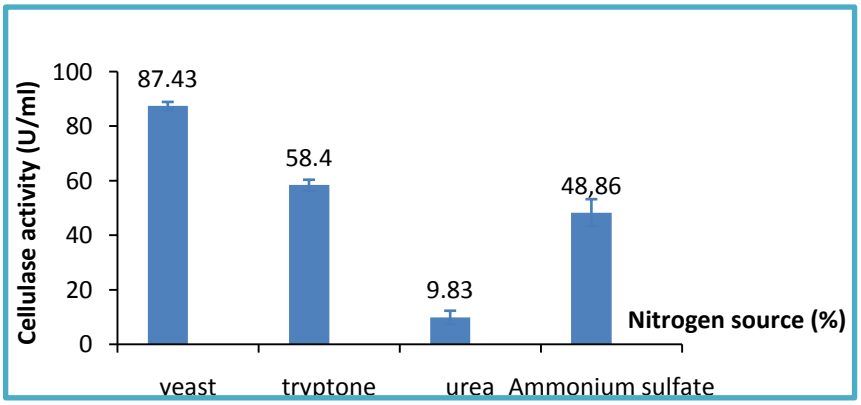
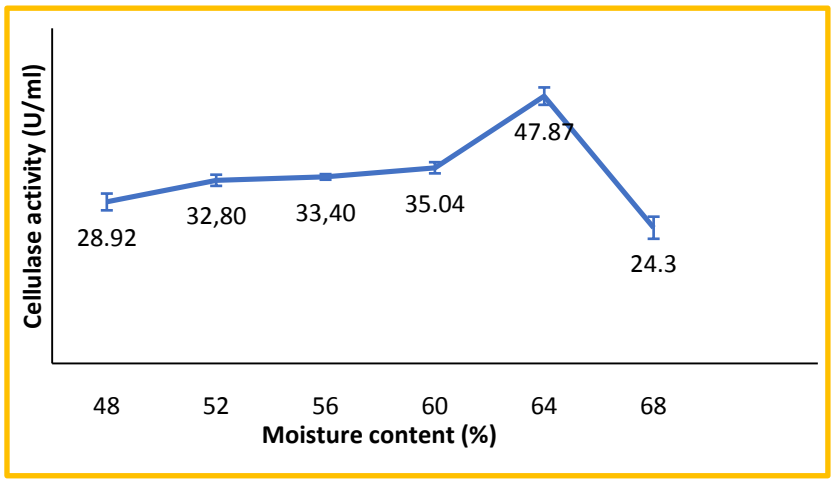


Figure 2: Effect of nitrogen source on cellulase production

3.3Effect of moisture content on cellulase production

Moisture content is an important factor in solid - state fermentation. It can promote microorganism's growth. Each species of microorganisms, for example *A. unguis* has their own specific requirement of moisture content to achieve the highest growth rate. In addition, the hydrolysis reaction between cellulose and cellulase demand the presence of water molecule, thus the determination of optimal moisture content for fungal activities is very necessary. In the study, the best moisture content for *A. unguis* is 64%.



3: Effect of moisture content on cellulase production

3.3 Effect of duration on cellulase production

The growth curves of microorganisms describes an entire growth cycle. When fungi are added in culture medium, enzyme cannot be produced immediately at maximum level. They would firstly use monosaccharide and minerals in culture medium as the instant nutrition source for the lag phase. This trend would be kept remained until the early of exponential phase. During in the exponential (log) phase fungi is growing and dividing at the maximal rate.The enzyme emission only starts when most of these instant sources are used up. The kind of produced enzyme and its quantity would depend on the substrate. In lag phase and early exponential (log) phase, fungi would try to adapt and just a limited amount of enzyme was produced. The enzyme, as a consequence, might also be emitted in a rapidly increasing pace. And this pace would reach in stationary phase. After that, at the beginning of death phase, enzyme activity reduced due to some toxics presented by many other aspects of culture medium that affect microorganism growth and lead to cell death. As seen in Figure 4, the flasks were incubated at different time: 3, 4, 5,6, 7 and 8 days, and cellulase activities of 35.04, 46.51, 50.68, 57.36, 78.15 and 56.98 U/mL were obtained, respectively. Thus, at 7 day maximum degradation was observed.CMCase activity was achieved at the 4 day fermentation by *Trichoderma spp.* (Khan et al., 2007). Ojumu et al. (2003) found that the highest level of cellulase activity was occurred at the 12day fermentation by *A.flavus*.

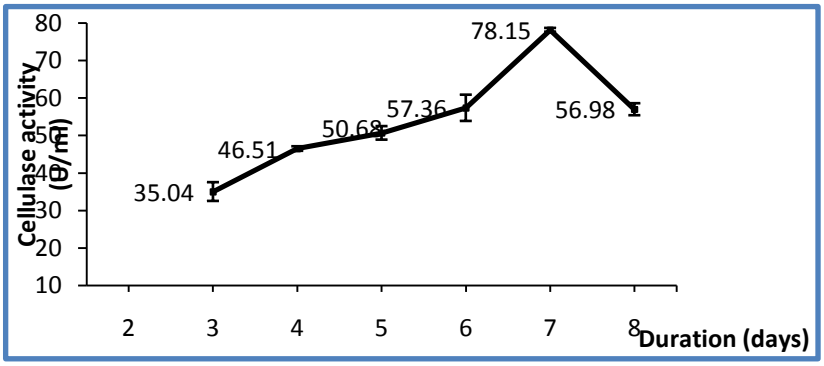


Figure 4: Effect of duration on cellulase production

3.5Effect of buffer content on cellulase production

Citrate buffer (0.05M, pH 4.8) was used to extract cellulase. As same as otherfactors, citrate buffer should be added in a suitable proportion in order to maximize enzyme extraction. Enzyme would not be fully

extracted if the citrate buffer is too low, on other hand, unnecessary high ratio of citrate buffer could also prevent the enzyme to be extracted completely. By undertaking 5 experiments for each fungus with the ratio of citrate buffer over byproduct are varied on the orders of 3/1, 5/1, 7/1 and 10/1, the observed outcome has proven that optimum citrate buffer ratio for *A. unguis* is 7/1 (vol/wt)

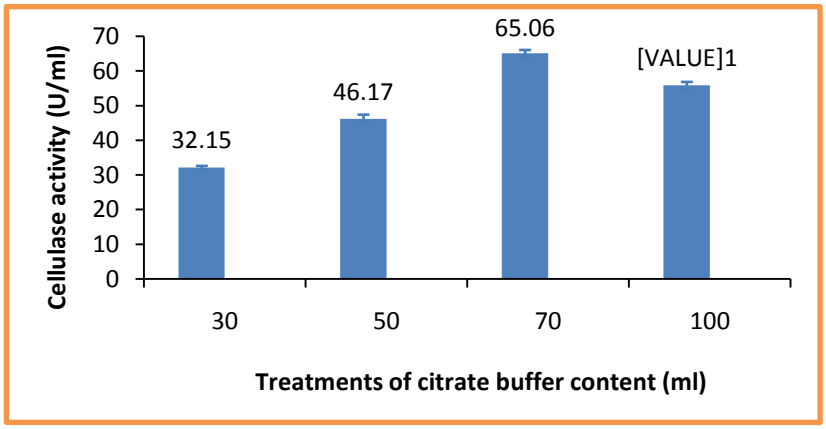


Figure 5: Effect of buffer content on cellulase production

IV. CONCLUSION

The study considered carbon source, nitrogen source, moisture content, duration, and citrate buffer content were significant influence to cellulase production. As the result, the optimum conditions for producing cellulase of Aspergillus unguisincluded: 7 days, 35% of rice husk and 64% of rice bran as carbon source, 64% of moisture content, 1% of yeast extract as nitrogen source and ratio of buffer content was 7/1 (vol/wt).

ACKNOWLEDGEMENTS

We would like to express our special thanks to International University – Vietnam National University in Ho Chi Minh City – Vietnam for all the supports. We also thank The Department of Science and Technology of Kon Tum province where granted the project "Study on the production of high quality coffee product by fermentation" at ĐắkHà District, Kon Tum Province, Vietnam.

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