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# The Production and Activity Test Of Cellulases using Bagasse Substrate on *Aspergillus niger* Isolated from Clove field, Kare, Madiun

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**Abstract.** *Aspergillus sp* is a microorganism which has a high ability to produce cellulase enzymes. In producing Cellulase enzymes requires appropriate concentration and incubation time to obtain optimum enzyme activity. This study aimed to determine the effect of inoculum concentration and incubation time towards production and activity of cellulases from *Aspergillus sp* substrate bagasse. This research used experiments method; completely randomized design with 2 factorial repeated 2 times. The treatment study include differences inoculum (K) 5% (K1), 15% (K2) 25%, (K3) and incubation time (F) that is 3 days (F1), 6 days (F2), 9 days (F3), 12 days (F4). The data taken from the treatment are glucose reduction and protein levels of crude cellulase enzyme activity that use Nelson Somogyi and Biuret methods. Analysis of variance ANOVA data used two paths with significance level of 5% then continued with LSD test. The results showed that: Fhit>Ftab. Thus, there is effect of inoculum concentrations and incubation time toward activity of crude cellulases of *Aspergillus sp*. The highest glucose reduction of treatment is K3F4 (concentration of inoculum is 25% with 12 days incubation time) amount 12.834 g / ml and the highest protein content is K3F4 (concentration of inoculum is 25% with with 12 days incubation time) amount 0.740 g / ml.

**Keywords:** *Aspergillus sp*, bagasse, cellulase, incubation time

## INTRODUCTION

The enzyme has been widely used by the Indonesian in various fields of activities and occupies an important position in the industry, the use of enzymes to degrade the polymer is used for production cost efficiency. One of the enzymes used to degrade carbohydrate polymer is a cellulases. Cellulases are widely used in textiles, detergent, pulp, and paper. Seeing the many benefits and the high use of cellulases, it is necessary to cellulase production to meet those needs.

Cellulase enzyme is an enzyme that can hydrolyze the bond β (1-4) on the cellulose. The presence of cellulose in a substrate can induce the formation of cellulose enzymes by microorganisms selulotik (Nora Idiawati *et all*., 2014: 1). Fungi or bacteria are microorganisms that are often used to manufacture commercial cellulase enzymes. According U1-haq (in Nora Idiawati *et al*., 2014: 1) states that selulotik microorganisms that can be used to produce cellulase enzymes for example *Aspergillus niger*. Cellulase enzymes can be produced from lignocellulosic material substrate. The existence of cellulase enzymes is particularly important since can be used to overcome the environment from waste cellulose.

Potential cellulose enzymes are high on the *Aspergillus sp* encourage researchers to conduct research mold *Aspergillus sp* on the production and test enzyme activity of cellulase-treated fermentation time is different, that 3 days, 6 days, 9 days, and 12 days and inoculum concentration of 5 %, 15%, and 25%.

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Microorganisms have a growth period varies. Every microorganism has a growth curve that consists of several phases: a) Phase lag, the phase adjustment of the cells with an environmental formation of enzymes to break down the substrate; b) exponential phase, namely the phase of the multiplication of the number of cells in which the activity of the cells is very menigkat; c) stationary phase, the phase with the number of cells increases and the number of cells that die relatively balanced in which secondary metabolites can be harvested; d) the death phase, namely the phase of the number of cells that die more than the number of living cells.

Time/long fermentation can affect the activity of the enzyme cellulase. Time best fermentation to produce cellulase enzymes with the highest enzyme activity was 8 days. Cellulase enzyme activity will increase along with increasing fermentation time (Nora Idiawati *et all*., 2014: 7).

Microbial inoculum is inoculated into the culture medium at the time of the microbial culture in the growth phase. Activating the microbial decomposition through the fermentation process, thus speeding up the rate of decomposition. Inoculum is part pathogens that can initiate infection. Inoculum may be spores, sclerotia or mycelium parts.

Pujiati *et all*., (2014: 20) argues that the inoculum concentration is the number of microorganisms in the substrate and nutrients are added to the process of making cellulase enzymes. The number of microorganisms is very influential in the fermentation process. The fermentation process can be optimized by adding the amount of inoculum at appropriate concentrations, thus obtained cellulase enzyme with maximum activity of the enzyme. According to Widya *et all*., (2013): 36) states the size of the amount of inoculum affect the activity of the enzyme.

Incubation is a period between inoculation or infection to the colony growth characteristics or until the occurrence of typical symptoms of the disease caused by pathogenic microorganisms. Incubation or fermentation is a process of utilizing the ability of microbes to produce primary metabolites and secondary metabolites in a controlled environment. Incubation is the process of changing organic materials into another form that is more useful with the help of microorganisms involved are fungi, mold or fungus, bacteria, protozoa, and yeast or yeast (Suciningtyas, 2014: 18).

## Cellulase Enzyme

Enzymes are catalysts protein produced by the cells. Substances - have set the speed and specificity of thousands of chemical reactions taking place in cells. Although the enzyme is made in the cell, but to act as a catalyst does not have to be inside the cell. Some elements can not be extracted in the cell. Some elements can not be extracted from cells without damaging activities, which is then purified and used as crystal so that ability can be learned katalisisnya Walker, Barnes (1984: 70-71). Cellulase enzyme is a hydrolase enzyme that can catalyze bond reaksihidrolisa β-1,4 glucan 4 glukano hydrolase.

Cellulase enzymes is very important for cellulase enzymes can be used to overcome the environment from waste cellulose. Cellulase enzymes outlines cellulose into smaller groups that can then be elaborated further into glucose monomer. Cellulase enzymes including extracellular enzymes that have a great ability to degrade organic waste, mainly agricultural waste and industrial waste. Cordoba *et all* (in Kasmiran, Tarmizi, 2012: 1)

Inoculums concentration is the number of microorganisms in the substrate and nutrients are added to the process of making cellulases. The fermentation process (celluloses production) can be optimized by adding the amount of inoculum at appropriate concentrations, thus obtained celluloses with maximum activity.

## METHODS

Soil sampling conducted in Clove field, Kare, Madiun. The study was conducted in the Laboratory 2 Teachers' Training College PGRI Madiun March to July 2016 and the Chemical Laboratory of UMM in June 2016.

The research variables include independent and dependent variables. The independent variables were *Aspergillus* sp concentration (5%, 15%, 25%) incubation time (3, 6, 9, and 12 days). The dependent variable are the production and the activity of celluloses from *Aspergillus* sp.

**TABLE 1**. Treatment plan

| F (incubation time) | K (Inoculum concentration) | | |
|---|---|---|---|
| | $K_1$ (5%) | $K_2$ (15%) | $K_3$ (25%) |
| $F_1$ (3 days) | $K_1F_1$ | $K_2F_1$ | $K_3F_1$ |
| $F_2$ (6 days) | $K_1F_2$ | $K_2F_2$ | $K_3F_2$ |
| $F_3$ (9 days) | $K_1F_3$ | $K_2F_3$ | $K_3F_3$ |
| $F_4$ (12 days) | $K_1F_4$ | $K_2F_4$ | $K_3F_4$ |

Information:
K1F1: 5% of inoculum concentration , 3 days of incubation time
K2F1: 15% of inoculum concentration , 3 days of incubation time
K3F1: 25% of inoculum concentration , 3 days of incubation time
K1F2: 5% of inoculum concentration , 6 days of incubation time
K2F2: 15% of inoculum concentration , 6 days of incubation time
K3F2: 25% of inoculum concentration , 6 days of incubation time
K1F3: 5% of inoculum concentration , 9 days of incubation time
K2F3: 15% of inoculum concentration , 9 days of incubation time
K3F3: 25% of inoculum concentration , 9 days of incubation time
K1F4: 5% of inoculum concentration , 12 days of incubation time
K2F4: 15% of inoculum concentration , 12 days of incubation time
K3F4: 25% of inoculum concentration , 12 days of incubation time

## Data Collection

The data collection conducted in laboratory. Data collected included; (1) soil sampling. Clove field soil samples collected by taking a part in the land of 15 cm from the ground by means of combed the inside are then stored in jars and sealed. (2) Productivity test of celluloses. Testing productivity of celluloses from *Aspergillus* sp is to know the effect of the treatment of the celluloses production and to know the best treatment from the effect of incubation time and inoculum concentration. (3) The enzyme Activity Test. Test of enzyme activity examined from the treatment. Celluloses activity will increase along with increasing incubation time.

# RESULTS AND DISCUSSION

## Data Description

The sugar test results examined with Nelson Somogyi. The data showed in Table 2.

**TABLE 2.** Sugar content analyse

| Treatment | Sugar content (mg/ml) | | Average |
|---|---|---|---|
| | Data 1 | Data 2 | |
| K1F1 | 2.802 | 2.769 | 2.786 |
| K2F1 | 3.124 | 3.189 | 3.157 |
| K3F1 | 3.608 | 3.656 | 3.632 |
| K1F2 | 4.092 | 4.189 | 4.141 |
| K2F2 | 4.834 | 4.915 | 4.875 |
| K3F2 | 5.269 | 5.318 | 5.294 |
| K1F3 | 6.366 | 6.398 | 6.382 |
| K2F3 | 7.931 | 7.995 | 7.963 |
| K3F3 | 8.673 | 8.737 | 8.705 |
| K1F4 | 10.237 | 10.285 | 10.261 |
| K2F4 | 11.850 | 11.915 | 11.883 |
| K3F4 | 12.818 | 12.850 | 12.834 |

The sugar content datas were obtained from the two repetitions are expressed in g/ml. The data in Table 2 describes that the highest sugar content is 12.834 with 25% of inoculum concentration and with 12 days (K3F4) of incubation time, whereas the lowest sugar lowest sugar content was 2.786 g/100 ml with 5% of inoculum concentration and with 3 days (K1F1) of incubation time. So the higher level of inoculum concentrations and the longer incubation time will make protein levels more high.

**TABLE 3**. Protein content of Crude celluloses from *Aspergillus* sp.

| Treatment | Sugar content (mg/ml) | | Average |
|---|---|---|---|
| | Data 1 | Data 2 | |
| K1F1 | 0.252 | 0.254 | 0.253 |
| K2F1 | 0.326 | 0.321 | 0.324 |
| K3F1 | 0.370 | 0.364 | 0.367 |
| K1F2 | 0.309 | 0.314 | 0.312 |
| K2F2 | 0.448 | 0.450 | 0.449 |
| K3F2 | 0.508 | 0.505 | 0.507 |
| K1F3 | 0.449 | 0.451 | 0.450 |
| K2F3 | 0.571 | 0.573 | 0.572 |
| K3F3 | 0.614 | 0.616 | 0.615 |
| K1F4 | 0.592 | 0.599 | 0.596 |
| K2F4 | 0.706 | 0.705 | 0.706 |
| K3F4 | 0.738 | 0.742 | 0.740 |

The research data shows that the highest protein content is 0.740 g/ml with 25% of inoculum concentration and with 12 days (K3F4) of incubation time, while the lowest protein content was 0,253 g/ml with 5% of inoculum concentration and with 3 days (K1F1) of incubation time. So the higher the level of inoculum concentrations and the longer of incubation time will make protein levels more high.

**TABLE 4**. SPPS analyse

Dependent Variable: content_protein

| Source | Type III Sum of Squares | Df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Corrected Model | .971[a] | 12 | .081 | 1.155E4 | .000 |
| Intercept | 3.680 | 1 | 3.680 | 5.257E5 | .000 |
| concentration_inoculum | .101 | 2 | .051 | 7.238E3 | .000 |
| time_fermentation | .448 | 3 | .149 | 2.134E4 | .000 |
| concentration_inoculum time_fermentation | .004 | 6 | .001 | 98.784 | .000 |
| Error | 9.100E-5 | 13 | 7.000E-6 | | |
| Total | 6.333 | 26 | | | |
| Corrected Total | .971 | 25 | | | |

a. R Squared = 1.000 (Adjusted R Squared = 1.000)

**TABLE 5.** SPSS Analyse cotent Protein *Crude* Enzyme Celulase Mold *Aspergillus* sp.

Dependent Variable:
content_sugar_reduction

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Corrected Model | 325.418[a] | 12 | 27.118 | 1.608E4 | .000 |
| Intercept | 750.647 | 1 | 750.647 | 4.451E5 | .000 |
| concetration_inoculum | 12.133 | 2 | 6.067 | 3.597E3 | .000 |
| time_fermentation | 249.207 | 3 | 83.069 | 4.926E4 | .000 |
| concetration_inoculum * time_fermentation | 2.350 | 6 | .392 | 232.271 | .000 |
| Error | .022 | 13 | .002 | | |
| Total | 1384.098 | 26 | | | |
| Corrected Total | 325.439 | 25 | | | |

a. R Squared = 1.000 (Adjusted R Squared =1.000)

Data analyse on the number of sugar content and protein levels using statistical analyse of variance (ANOVA) two paths through SPSS 16. Statistical analysis was used to determine the effect of inoculum concentration and incubation time towards the production and the activity of cellulases of crude enzyme from *Aspergillus sp*. Based on the anava calculations, the result of significant analysis on sugar content showed F count ≥ F tables (232.271 ≥ 0,000), α = 0.005, and the protein content indicates F count ≥ F tables (98.784 ≥ 0,000), α = 0.005, it can be concluded that Ho rejected and Ha is accepted, then there is a significant effect between inoculum concentration and incubation time toward sugar and protein content.

## Discussion

Results of analysis showed that treatment with concentrations of inoculum and fermentation time on reducing sugar and crude protein content of the activity of the enzyme *Aspergillus sp* have a significant difference of 0.00 <0.05 significance level of 95%.

Table 3 Based on the test data reduction sugar *Aspergillus sp* showed that the concentration of inoculum and fermentation affect productivity crude celluloses produced by *Aspergillus sp* is measured by the percentage of protein content.

Here is presented a graph average reduction sugar in figure 1.

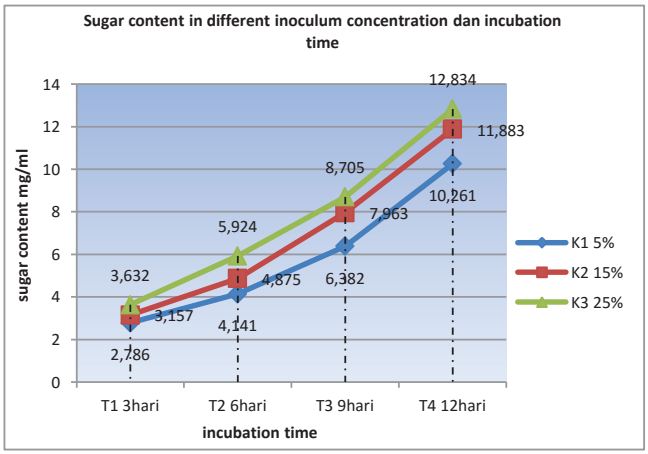


**FIGURE 1.** Sugar content average of crude cellulases from *Aspergillus* sp.

Based on the figure 1 it can be seen that the average sugar content highs on K3F4 (25% of inoculum concentration and 12 days incubation time) with an average of 12.834 g/ml and the lowest levels in K1F1 (concentration of 5% and fermentation time 3 days) with the average is equal to 2.786 g/ml.

Based on the data shows that inoculum concentration affects the sugar content, this is caused by inoculum concentration on fermentation process is less than 15%. It is not suitable for the production of cellulases so it cannot give the optimum data (Sesotyaningrum, 2014: 35 ). The data showed highest sugar content by treatment with 25% of inoculum concentration. More higher the concentration inoculum were inoculated on media, it means more *Aspergillus sp* that work to convert the substrate to the greater reduction sugar. So, it can be conclude that the sugar content correlate with inoculum concentration.

When associated with the table of variance fermentation influence reducing sugar crude cellulases, fermentation 12 days earned an average reduction is 12.834, which is in line with the statement of Abdul Aziz (in Kasmiran, 2012: 12) enzyme activity was obtained when the post-exponential (stationary) ie after four days of fermentation. In the fermentation time showed that crude cellulases work optimally in the substrates. The cellulases hydrolyze cellulose contained in the bagasse into glucose.

Sugar content on K3F4 (25% of inoculum concentration and 12 days fermentation time) is the highest, it agrees with Frances (in Sesotyaningrum, 2014: 35). Frances describe if the inoculum concentration on fermentation process is less than 15% are not suitable for the production of cellulases because the amount of inoculum is not enough to work. A .niger showed maximum enzyme production in Czapek's medium. Vitamins like biotin, calcium pentothenate, riboflavin and thiamine- HCl could enhance the enzyme activity to a great extent but low activity was observed with nitrogen sources, like asparagine, and.sodium nitrate. All cellulase components of A.fumigatus gave maximum production on the 12$^{th}$ day of growth in basal medium containing cellulose as sole carbon source and a combination of ammonium sulphate and ammonium dihydrogen phosphate as nitrogen. Abdul Aziz (in Zulfatus, *et al*, 2010: 7) says that the enzyme activity will increase in line with increasing fermentation time and decreased on day 10. This is because the pattern of growth of microorganisms which experienced several phases of growth, namely adaptation phase, exponential phase, stationary phase and death phase. Spore forming organisms usually produce the enzyme in the post-exponential phase. So it can be presumed that at the time of the resulting high activity of the enzyme, the mold has been in this phase. The highest activity was obtained after four days of fermentation, but on day 6 decreased enzyme activity and on day 8 increased back. It can be concluded difference inoculum concentration and fermentation time affects the activity of crude cellulases from *Aspergillus sp.*

Here is presented a graph the average levels of the protein in Figure 2.

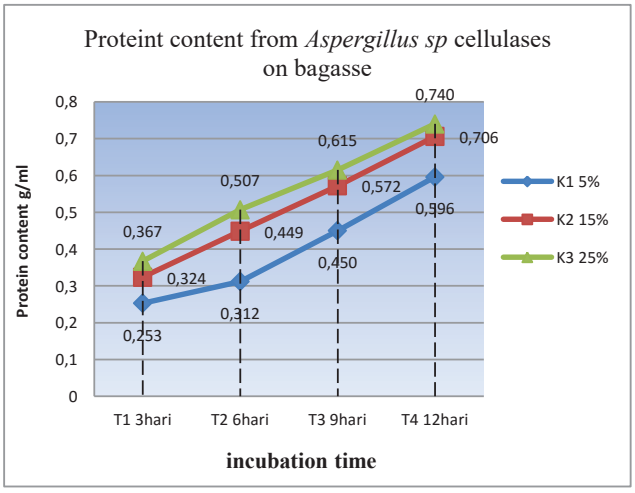


**FIGURE 2**. Proteint content from *Aspergillus sp* cellulases on bagasse

Based on Figure 2. it can be seen that the average protein content highs on K3F4 (25% of inoculum concentration and 12 days of incubation time) with 0.740 g/ ml and the lowest levels in K1F1 (5% of inoculum concentration and 3 days fermentation time) amount 0,253 g / ml.

The inoculum concentrations affect the levels of protein produced cellulolytic fungi using bagasse as substrate, with the highest levels of the K3 (concentration 25%). According Septiningrum (2011: 93) if the amount of inoculum is greater than 10%, there will be competition to get the nutrients of fungal fermentation process as a result the biomass formed is not the maximum so that the production of cellulase to be reduced. At a concentration of 15% has decreased, due to *Aspergillus sp* optimum period has reached the maximum value and also influenced by the substrate used is not homogenous in form so that microorganisms are difficult to perform hydrolysis.

The highest protein content in the F4 (fermentation 12 days). The results of this study are consistent with the Sa'adah (2010: 5) which states with increasing time into high protein concentrations, due to at the time of microbial growth has reached the maximum. Increased levels of the protein showed that the cellulases activity also increased. The longer the fermentation time, the levels of soluble protein that is produced tends to increase.

When associated with variance of inoculum concentration and incubation time effect the crude protein content of cellulases. K3F4 is the highest protein content (25% concentration and 12 days of incubation time). *Aspergillus sp* protein levels high enough on day 12 with an average of 0.740. The production of high levels of the protein is followed by a high specific activity. The results of this study are consistent with the research of Wayan *et.al*. XV (2): 32 which states on environmental conditions with high levels of a protein produced by the activity of the enzyme is also high, when the protein levels produced lower so that the enzyme activity tends to low.

## CONCLUSION

Based on research can be concluded that; (1) there is an effect of different incubation time to the activity of crude cellulases from *Aspergillus sp*, (2) there is an effect of different concentrations of inoculum to the activity of crude cellulases from *Aspergillus sp*. (3) the best sugar content and protein content showed on K3F4 (25% of inoculum concentration and 12 days incubation time) with 12.834 g/ml.

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