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Effect of Isolate of Trichoderma Sp. And Incubation Period to Glucose Production

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ABSTRACT: *This study was carried out to determine the effect of isolates of Trichoderma sp. and the incubation period to glucose production. A glucose produced by the three isolates of Trichoderma sp. (TV.0209, TV.0305 and TV.0710) which were obtained from the collection of the Laboratory of Microbiology, Department of Chemical Engineering of The State Polytechnic of Malang. It was determined that the isolates had a significant correlation in producing glucose with incubation period for 10 days. Data were then analyzed using two-way anova with the alpha of 5% and using Microsoft Excel. Isolate TV.0209 was capable to produce the highest average of a total glucose production (3,616 ppm) when compared with TV.0305 (3,253 ppm) and TV.0710 (2,448 ppm). The optimum incubation period of treatment isolate variables was achieved during 6 days.*

Keywords: *Incubation Period, Trichoderma, Cellulase, Glucose*

I. INTRODUCTION

Various microbes utilize organic materials as an energy source. This process is usually followed by the formation of a bioactivator enzymes in decomposing organic compounds which change a complex polymer into simpler compounds. The addition of microbes into organic material can be regulated and controlled in such a way that the decomposition process can occur more quickly. Various ways of arrangements regarding the type and environmental conditions have been carried out in order to select organic materials which will be decomposed into simpler compounds.

Trichoderma is a fungus producing cellulolytic enzyme to degrade compound cellulose into glucose by enzyme called glucosidase. Each species of *Trichoderma* produces certain enzyme in decomposing cellulose material of the agricultural waste [1,2]. Fungus *Trichoderma* sp. is a potential agent to produce cellulase enzymes that degrade cellulose materials. In addition, these microbes can also act as an antagonist against phytopathogen [3]. Cellulase of these organisms have been isolated and studied from the genus *Trichoderma* [4]. The medium used for inoculation is usually carried out on PDA (Potato Dextrose Agar). Enzyme production close relation to a way of controlling microorganisms so that productivity can be improved and modified by this control. Cellulase produced depends on several factors such as pH, temperature, incubation period, source of carbon, and nitrogen [5].

Several previous researches have determined that many strains of *Trichoderma* sp. potential to produce cellulase enzymes in higher quantities than bacteria [6]. The enzymatic hydrolysis of cellulose is more advantageous than using some chemical acid. Processing of enzymatic hydrolysis is not causing corrotion, requiring less time, and resulting glucose levels higher in optimum conditions [7].

Several types of bacteria, such as actinomycetes and fungi can produce extracellular cellulase that indicated with glucose produced when grown on a substrate containing cellulose. Cellulose is an organic material of the abundant presence in the natural world around us. Generally, some agricultural waste contains cellulose 46.6% were able to be decomposed by *Trichoderma viride* into glucose [8].

Cellulase enzymes can be produced among others by fungi or bacteria. Normally, the production of commercial enzyme using fungi or bacteria. Fungi that can produce cellulase : species of *Trichoderma* (*Trichoderma viride, Trichoderma longibrachiatum*), *Aspergillus* (*Aspergillus fumigates, Aspergillus nidulans*), and *Penicillium* [9]. Cellulase is an enzyme that can hydrolyze the bond β-1,4 on cellulose which is composed of three types [10]; The first type endoglucanase or carboxymethyl cellulase (CMCase) which breaks the bonds β-1,4-D-glucoside to produce oligosaccharides and monosaccharides such as glucose. While the second and the third type is a β-glucosidase (β-1,4-D-glucoside glucohydrolase) and exoglucanase (exo-β-1,4-glucanase) [11].

II. MATERIALS AND METHODS

Material

The experiment was conducted to determine the ability of isolates of *Trichoderma* sp. in producing glucose. Three isolates of *Trichoderma* sp. (TV.0209, TV.0305 and TV.0710) were obtained from the collection of the Laboratory of Microbiology Department of Chemical Engineering Polytechnic of Malang. While the Standard Glucose material obtained from E-Merck and support materials purchased from local products.

Preparation Method of Culture

Experimental variables were three isolates of *Trichoderma* sp. and incubation period. The glucose concentration was determined spectrophotometrically by measuring the absorbance of the standard solutions. Three isolates of *Trichoderma* sp. TV.0209, TV.0305 and TV.0710 subcultured using scratch and incubated at 30°C for 5 days. Each isolate of *Trichoderma* sp. made conidia suspension with a density of 10^7 conidia / ml. 10 ml of the conidial suspension at each isolate was incubated for ten days. The beginning of each two-day incubation and the amount of glucose was analyzed by measuring the absorbance. Measurement potential of the *Trichoderma* sp. isolates in producing glucose was measured by a Perkin Elmer UV/Vis Spectrophotometer at a wavelength of 540 nm.

Statistical Method

Methods used for data analysis of the potential of the *Trichoderma* sp. isolates in producing glucose were graphical and statistical methods. Tests were conducted to determine the effect of incubation period of the isolates. Graphical method was used to determine the optimum incubation period which have a real impact. Statistical methods for testing was carried out by observing data. The first test based on the hypothesis by comparing the average value using anova. Meanwhile, the second test sample used by each group had considered that category of isolate and incubation period corresponding anova was done with a two-way anova with interactions.

At the two-way anova with interaction, three hypotheses were used as follow.

3.1 Hypothesis column

H0 : μ 1 = μ = μ * 2 * 3, there was no significant difference between the average value calculated incubation periods category.

H1 : μ * 1 $\neq$ μ $\neq$ μ * 2 * 3, there was a significant difference between the average value calculated from incubation periods category.

3.2 Hypothesis line

H0 : μ1 μ2 * = * = μ3 *, there was no significant difference between the average value calculated from isolates category.

H1 : μ1 $\neq$ μ2 * * $\neq$ μ3 *, there was a significant difference between the average value calculated from isolates category.

3.3 Hypothesis interaction

H0: (ab) 11 = (ab) 12 = ... = (ab) kj, there was no significant interaction between the incubation period and isolate.

H1: (ab) 11 $\neq$ (ab) $\neq$ 12 ... .. $\neq$ (ab) kj, there was a significant interaction between the incubation period and isolate.

III. RESULTS AND DISCUSSION

Glucose produced from the decomposition of organic matter is determined by means of spectrophotometry. A standard curve is plotted by observing the absorbance produced by each glucose concentration. Absorbance of each glucose concentrations are presented in Table 1. While the magnitude of absorbance resulting from the various incubation period is presented in Table 2.

Table 1. Results of the absorbance of various glucose concentrations

glucose concentrations (ppm)	absorbance
10	0,094
20	0,200
30	0,351
40	0,495
50	0,632
60	0,789

Data from the absorbance of various glucose concentrations is made a standard curve that is used to determine the amount of glucose through the absorbance produced by each isolate at various incubation periods. Results of calibration Table 1 to standard glucose shows a linear regression equation y = -0.0647 + 0.1404x. On this equation, y = absorbance while x = glucose concentration

Table 2. Results of isolates absorbance at various incubation periods

Isolate	incubation period (day)					
	0	2	4	6	8	10
TV.0209	0.028	0.100	0.488	0.697	0.605	0.502
	0.031	0.107	0.457	0.748	0.647	0.538
	0.035	0.109	0.780	0.815	0.706	0.580
TV.0305	0.025	0.090	0.382	0.635	0.557	0.465
	0.030	0.102	0.573	0.690	0.615	0.545
	0.028	0.096	0.469	0.668	0.588	0.502
TV.0710	0.020	0.062	0.375	0.416	0.388	0.360
	0.025	0.080	0.398	0.427	0.393	0.358
	0.017	0.095	0.414	0.435	0.402	0.364

The effect of isolates TV.0209 , TV.0305 and TV.0710 to incubation period can be described in Figure 1.

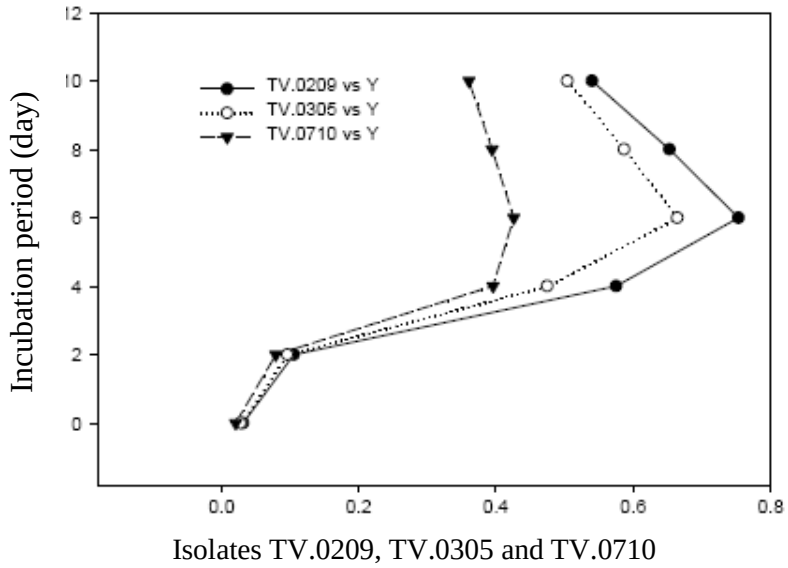

Figure 1. The Effect of Isolates to Incubation Periods

There is a correlation between isolates and incubation periods. The isolates TV.0209, TV.0305, and TV.0710 have showed a similar pattern. Optimum results of incubation period occur on the sixth day. Isolate of TV.0209 has a value with highest effect compared to isolate of TV.0305 and TV.0710. Furthermore, data on Table 2 are tested using two-way anova with the alpha 0.05 or 5%. The results of the analysis are processed using Microsoft Excel and presented on Table 3.

Table 3. Test Results of Two Factors with Three Repetitions

incubation period (day)	0	2	4	6	8	10	Total
TV.0209							
Count	3	3	3	3	3	3	18
Sum	0.094	0.316	1.725	2.260	1.958	1.620	7.973
Average	0.031	0.105	0.575	0.753	0.653	0.540	0.443

Variance	0.000	0.000	0.032	0.004	0.003	0.002	0.084
TV.0305							
Count	3	3	3	3	3	3	18
Sum	0.083	0.288	1.424	1.993	1.760	1.512	7.060
Average	0.028	0.096	0.475	0.664	0.587	0.504	0.392
Variance	0.000	0.000	0.009	0.001	0.001	0.002	0.064
TV.0710							
Count	3	3	3	3	3	3	18
Sum	0.062	0.237	1.187	1.278	1.183	1.082	5.029
Average	0.021	0.079	0.396	0.426	0.394	0.361	0.279
Variance	0.000	0.000	0.000	0.000	0.000	0.000	0.029
Total							
Count	9	9	9	9	9	9	
Sum	0.239	0.841	4.336	5.531	4.901	4.214	
Average	0.027	0.093	0.482	0.615	0.545	0.468	
Variance	0.000	0.000	0.016	0.023	0.014	0.008	

Table 3 shows that the test is performed with three repetitions. The average total value of glucose for each isolate TV.0209 has 0443 (3,616 ppm) with variance 0084, TV.0305 has 0392 (3,253 ppm) with variance 0.064 and TV.0710 has 0279 (2,448 ppm) with variance 0.029.
The total average results show that isolates TV.0209 have a total average of the highest compared to TV.0305, while TV.0710 has a total average of the lowest. The same pattern occurs in which the variance TV.0209 has the highest value compared to TV.0305 and TV.0710. Analysis of total isolates TV.0209, TV.0305, and TV.0710 obtained total value of the average of 0.468 and 0.008 variance.

Table 4. Test Results of Two-Way Anova

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.252	2	0.126	43.160	0.000	3.259
Columns	2.762	5	0.552	188.944	0.000	2.477
Interaction	0.131	10	0.013	4.493	0.000	2.106
Within	0.105	36	0.003			
Total	3.250	53				

To determine the effect of incubation time isolates against statistically performed using two-way anova with the following results. Hypothesis column in Table 3, show the p value less than 0.05, then the decision to accept H_1 and reject H_0. Furthermore, it appears the value of F is higher than F_{crit}. In other words, $F_{calculated} > F_{table}$. It means that there is a significant difference between the average value calculated from categories of incubation period. Each isolate that is different glucose results in any period of incubation. Each incubation period applied for a 10 days, *Trichoderma* isolates give results significantly different values. This difference indicates that production of glucose produced by cellulase enzyme activity which produced by each isolate. The results of this study reinforce the research data conducted by Awojobi et al. [12] who did research some *Trichoderma* isolates within 5 to 11 days incubation. Research results mention that there are significant differences between the amount of cellulase with incubation period.

Hypotheses rows showed the p value less than 0.05, then the decision to accept H_1 and reject H_0. It appears the value of $F > F_{crit}$ means that there is a significant difference between the average value calculated from categories of isolates. Each isolate TV.0209, TV.0305, and TV.0710 have significantly different values in producing glucose at any incubation period. This data is confirmed by the results of research Singh et al. [13] which states that the ability of each species *Trichoderma* growth generally depend on a variety of organic materials applied in degrading complex compounds into simpler compounds. The large amount of degraded compounds associated with the incubation period applied in its growth. Therefore, each species *Trichoderma* can be determined the optimum conditions that exist in the resulting product.

The hypothesis interaction showed the p value less than 0.05, then the decision to accept H_1 and reject H_0. the value of $F > F_{crit}$. It means that there is a significant difference in the interaction between isolates TV.0209, TV.0305, and TV.0710 with any incubation period. It is also supported by the results of research conducted by Bhosle [14] and Maiko [15] which states that there are several types of microorganisms such as bacteria and fungi have a certain incubation period for the state or the substrate, temperature and growth media. The amount of time each microorganism incubation correlated with the rate of growth in organic medi. There is a significant correlation between some strains of *Pseudomonas* sp., *Rhizopus* sp,. and *Trichoderma* sp. to incubation period of 3, 7, 1, and 14 days in degrading organic matter. Besides the concentration of organic matter will also determine the level of degradation of organic compounds produced.

IV. CONCLUSION

This research results using graphical method and statistical tests show that there are significant TV.0209 isolates, TV.0305 and TV.0710 against incubation time. TV.0209 isolates have total influence of the highest average glucose levels of 3.253 ppm when compared to isolates TV.0305 (3.253 ppm) and TV.0710 (2.448 ppm) with the optimum period of 6 days. The test results of two-way anova with interactions also showed that the influence of the isolates had a significant influence on the incubation period. Isolates TV.0209, TV.0305, and TV.0710 as decomposers of organic material, capable of accelerating the decomposition of blotong in no more than six days of the incubation period. It can be concluded that fungus *Trichoderma* sp. is a high potential as a producer of cellulase enzymes.

REFERENCES

[1] Lee, R.L., Paul, J.W., Willem, H.V. and Isak, S.P. (2002) Microbial Cellulose Utilization: Fundamentals and Biotechnology. *Microbiology and Molecular Biology Reviews*, 66 (3), 506-577.

[2] Chauve, M. et al. (2010) Comparative kinetic analysis of two fungal β-glucosidases. *Biotechnology for Biofuels*, 3 (3), 97-112.

[3] Budiono, A., Suharjono, Santoso, I. and Soemarno, (2013) Application Compost of Mud Cake by *Trichoderma Viride* Apt01 to Apples to Increase Production in Bumiaji Batu. *International Journal Of Engineering And Science*, 3, 17-20.

[4] Yazdi, M.T., Woodland, J.R., Radford, A. (1990) The cellulase of *Neurospora crassa :* activity, stability and release. *Journal of General Microbiology*, 136, 1313-1319.

[5] Narasimha, G., Sridevi, A., Viswanath, B., Subbosh, C.M., Rajashekar, R.B. (2006) Nutrien Effects on Production of Cellulolytic Enzymes by *Aspergillus niger*. *African Journal of Biotechnology*, 5 (5), 472-476.

[6] Amouri, B. and Gargouri, A. (2006) Characterization of a novel β-glucosidase from a *Stachybotrys* strain. *Biochem. Eng. J*., 32, 191-197.

[7] Antony, (2012) Produksi Enzim Selulase. *Fakultas Teknologi Industri, Institut Teknologi Bandung*, hal. 11-33.

[8] Maheswari, UN. and Aiswarya, K. (2012) Conversion of Natural Wastes into Sugar by *Trichoderme viride. International Journal of Chemical and Pharmaceutical Sciences*, 3 (2), 8-12.

[9] Omojasola, P. et al., (2008) Cellulose Production by Some Fungi Cultured on Pineapple Waste. *Nature & Science*, 6 (2), 64-75.

[10] Ikram, U.H., Javed, M.M., Khan, T.S. and Siddiq, Z. (2005) Cotton Saccharifying Activity of Cellulases Produced by Co-culture of *Aspergillus niger* and *Trichoderma viride*. *Res. J. Agric & Biol. Sci*., 1(3), 241-245.

[11] Zhang, YH., Cui, J., Lynd, L.R. and Kuang, L.R. (2006) A transition from cellulose swelling to cellulose dissolution by o-phosphoric acid: evidence from enzymatic hydrolysis and supramolecular structure. *Biomacromolecules*, 7 (2), 644-648.

[12] Awojobi, K.O. et al. (2013) The screening and selection of *trichoderma* species capable of producing extracellular cellulolytic enzymes from soil of decaying plant materials. *Ife Journal of Science*, 15(2), 263-271.

[13] Singh, V. et al. (2014) Optimal Physical Parameters for Growth of Trichoderma Species at Varying pH, Temperature and Agitation. *Virol. And Mycol.*, 3, 1-7.

[14] Bhosle, N.P., Khan, Z.S. and Nasreen, S. (2013) *In-vitro* degradation of Cypermethrin through microorganisms by scale up technique. *International Journal of Advanced Research*, 1 (7), 229-238.

[15] Maiko, W. et al., (2014) Characteristics of bacterial and fungal growth in plastic bottled beverages under a consuming condition model. *Journal of Environmental Science and Health*, 49 (7), 819-826.