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Optimization of Cellulase Production by *Aspergillus niger* and *Trichoderma viride* trough Water Hyacinth (*Eichornia crassipes*) as Substrate

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ABSTRACT

Water hyacinth (*Eichornia crassipes*) is one of many raw materials that can be used to produce bioethanol as renewable energy. One of critical process to produce bioethanol from water hyacinth is hydrolysis in order to convert cellulose to be glucose. Hydrolysis process which uses commercial cellulase is very expensive so that the product of bioethanol can not be accepted economically. The objective of this research is to optimize the production of crude cellulase enzyme produced by *Aspergillus niger* and *Trichoderma viride* in process of bioethanol production trough water hyacinth. This research showed that the production of crude cellulase enzyme by *Aspergillus niger* is optimum on 8 days of incubation, temperature 34°C and 8% of glucose concentration which obtain 5.84 U/g of Cellulase activity. In addition, the production of cride enzyme by *Trichoderma viride* is optimum on 6 days of incubation, temperature 30°C and 8% of glucose concentration which obtain 4.41 U/g of Cellulase activity. *Aspergillus niger* obtained hihger sellulase activity compare to *Trichoderma viride*. So that for the next study cellulase enzyme can be produced by using *Aspergillus niger*. Cellulase activity in this research was crude enzyme, for further study the experiment should be conducted by using pure enzyme for better result.

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INTRODUCTION

The need of energy is going to increase each year affect the decreasing of energy resources and creates it become expensive. Those because the resource of energy majorly from fossil energy which un-renewable and predicted will exhausted next 25 years [1, 2, 3]. Bioethanol is considered as alternative energy because it renewable and environment friendly [2, 4]. The production of bioethanol as alternative energy had been developed, but the production of bioethanol prior was not the solution because it used food-base material i.e. corn, seed and cassava [6, 8]. Water hyacinth (*Eichornia crassipes*) is one of non-food base material that can be used to produce bioethanol. It because water hyacinth contains cellulose (25%) that can be converted to be glucose which then can be used to produced bioethanol [2,4].

Hydrolysis in bioethanol production can be conducted by using cellulase enzyme [3, 5]. In the past process of cellulose hydrolysis in bioethanol production used commercial cellulase enzyme. The problem is cost of commercial enzyme is very expensive so that affect the bioethanol product trough water hyacinth cannot be accepted economically [2]. *Aspergillus niger* and *Trichoderma viride* had been reported as microorganisms that can produce cellulase enzyme [1]. Compare to other microorganism, *Aspergillus niger* and *Trichoderma viride* are better than other microorganism in producing cellulase enzyme. It due to the both fungus can produce three components of cellulase enzyme which are endoglukanase, eksoglucanase and glucosidase. So that this study is conducted to produce cellulase enzyme by *Aspergilus niger* and *Trichoderma viride* which will be used to hydrolyze cellulose on water hyacinth.

MATERIAL AND METHODS

Water hyacinth (*Eichornia crassipes*) was obtained from Selorejo bay, Malang district. Water hyacinth was separated from its root then washed with clean water to remove dirt and dash. Water hyacinth then was cut until it become ±2 cm in size. To decrease water content, water hyacinth was dried on oven temperature 100 ºC for 24 hours until the water content ±30%. After that water hyacinth was milled in disc mill and screened with 60 mesh of screener.

Aspergillus niger and *Trichoderma viride* was obtained from Indonesian Culture Collecton (InAcc) of Indonesian Institute of Science (LIPI). The both of microorganism then were cultured on Potato Dextrose Agar (PDA) which prepared by mixing 5.85 gr of PDA into 50 ml of aquades. PDA then was shrilled in autoclave temperature 121 ºC, 15 psi for 15 minutes. *Aspergillus niger* and *Trichoderma viride* cultured for 7 days in incubator temperature 30 ºC and then used to produce crude cellulase enzyme.

Harvesting and Analyzing Cellulase Enzyme Activity

Harvesting of crude cellulase enzyme was conducted by taking 1 gram of fermented substrate then mixed with 5 mL of aquades sterill. After that, substrate was under a 15 minutes shaking and 10 minutes centrifuge at 8000 rpm. Then the solution of crude cellulase enzyme was tooked 125µl and mixed with 125 µl of CMC as assay substrate. The solution of crude enzyme and CMC was incubated for 30 minutes to allow the reaction between enzyme and substrate. After 30 minutes, solution was added with 250 µl of DNS to stop the reaction. Then the solution was heated on water temperature 100 ºC for 10 minutes. After that absorbance value was measured with spectrophotometer on 540 of wave length. Absorbance value then was putted on standard curve [3].

Optimization of Cellulase Enzyme Production by *Aspergillus niger* and *Trichoderma viride*

Optimization of Incubation Time: Optimization of incubation time was conducted for 2, 4, 6, 8 and 10 days. 3 gram of water hyacinth as substrate was prepared and sterilized with autoclave temperature 121 ºC, 15 psi for 15 minutes. The culture of *Aspergillus niger* and *Trichoderma viride* were inoculated on steriled water hyacinth by dissolving spore of the both fungus with 5 ml steriled aquades and mixed with skewers. The solution of spore then was poured to water hyacinth as substrate in the laminar air flow in sterile condition. Then sample was stored in 30ºC of incubator. Water hyacinth substrate which was not inoculated with *Aspergillus niger* and *Trichoderma viride* used as control.

Optimization of Incubation Temperature: Optimization of incubation temperature was conducted after obtaining the optimum incubation time. Incubation temperature were 30ºC, 34ºC and 38ºC. In optimization of incubation temperature, the optimum incubation time was used as incubation time. 3 gram of water hyacinth (substrate) was prepared and sterilized with autoclave temperature 121ºC, 15 psi for 15 minutes. The culture of *Aspergillus niger* and *Trichoderma viride* were inoculated on sterilized water hyacinth by dissolving spore of the both fungus with 5 ml sterliized aquades and mixed with skewers. The solution of spore then was poured to water hyacinth as substrate in the laminar air flow in sterile condition. Then sample was stored in incubator 30 ºC, 34 ºC and 38 ºC. Water hyacinth substrate which was not inoculated with *Aspergillus niger* and *Trichoderma viride* used as control.

Optimization of Carbon Source: Optimization of carbon source was conducted by adding 2% (w/w) of carbon source on the water hyacinth as substrate. Carbon source which used in this study were glucose, sucrose, xylose and maltose. 3 gram of water hyacinth as substrate was prepared and added with 2% of carbon source then steriled with autoclave temperature 121 ºC, 15 psi for 15 minutes. The culture of *Aspergillus niger* and *Trichoderma viride* were inoculated on sterilized water hyacinth by dissolving spore of the both fungus with 5 ml sterilized aquades and mixed with skewers. The solution of spore then was poured to water hyacinth as substrate in the laminar air flow in sterile condition. Then sample was stored in incubator with optimum temperature and optimum incubation time. Water hyacinth substrate which was not added with carbon source used as control.

Optimization of Carbon Concentration: Optimization of Carbon Concentration was conducted after obtaining the optimum carbon source. Optimization of carbon concentration used several carbon concentration which were 2%, 4%, 6%, 8% and 10%. 3 gram of water hyacinth as substrate was prepared and added with 2%, 4%,

6%, 8 and 10% of carbon then sterilized with autoclave temperature 121 ºC, 15 psi for 15 minutes. The culture of *Aspergillus niger* and *Trichoderma viride* were inoculated on sterilized water hyacinth by dissolving spore of the both fungus with 5 ml sterilized aquades and mixed with skewers. The solution of spore then was poured to water hyacinth as substrate in the laminar air flow in sterile condition. Then sample was stored in incubator with optimum temperature and optimum incubation time.

Figure 1. SSF Fermentation

Figure 2. Crude Enzyme

Figure 3. Enzyme Assay

RESULTS

Optimization of Incubation Time

Celluase activity produced by *Aspergillus niger* and *Trichoderma viride* increased by increasing the incubation time. The optimum incubation time for *Aspergillus niger* and *Trichoderma viride* was 8 days of incubation time where the activity of cellulase enzyme were 2.40 U/g and 3.38 U/g for *Aspergillus niger* and *Trichoderma viride* respectively. The activity of cellulase enzyme by *Aspergillus niger* and *Trichoderma viride* decreased on 10 days of incubation time which were 1.77 U/g and 2.12 U/g respectively.

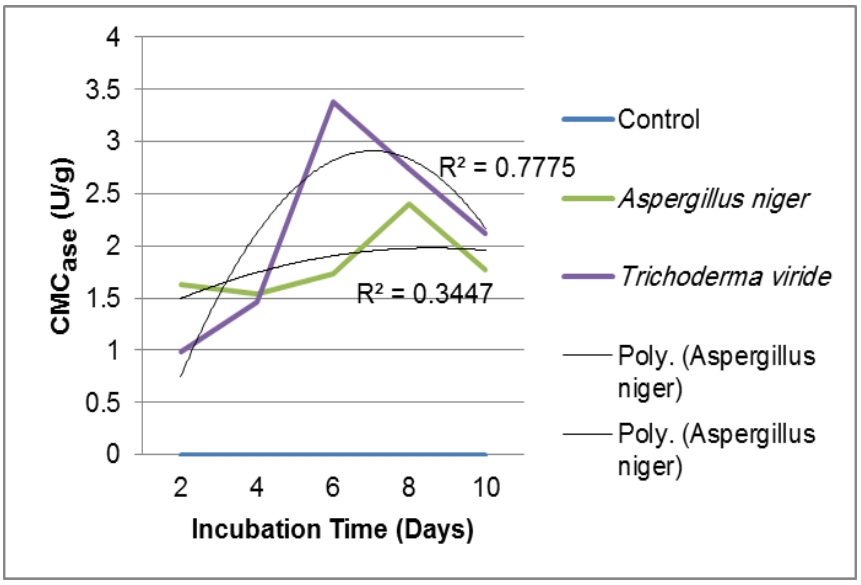

Figure 4. Effect of Differences Incubation Time for Cellulase Production

Optimization of Incubation Temperature

Cellulase activity produced by *Aspergillus niger* was optimum on 34 ºC of temperature which was 4.30 U/g. However cellulase activity produced by *Trichoderma viride* was optimum on 30 ºC which was 3.38 U/g.

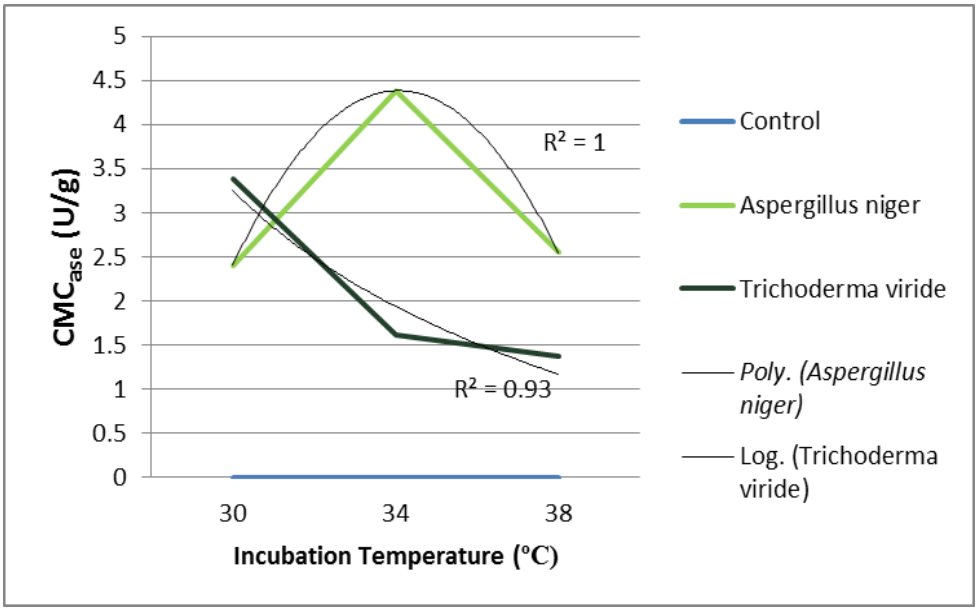

Figure 5. Effect of Differences Incubation Temperature for Cellulase Production

Optimization of Carbon Source

Carbon source which able to stimulate *Aspergillus niger* and *Trichoderma viride* to obtain the maximum cellulase activity was glucose. The maximum Cellulase activity by *Aspergillus niger* dan *Trichoderma viride* was obtained by glucose as carbon source were 5.62 U/g and 3.46 U/g respectively. The minimum sellulase activity by *Aspergillus niger* and *Trichoderma viride* was obtained with lactose as carbon source which were 4.28 U/g and 2.59 U/g respectively.

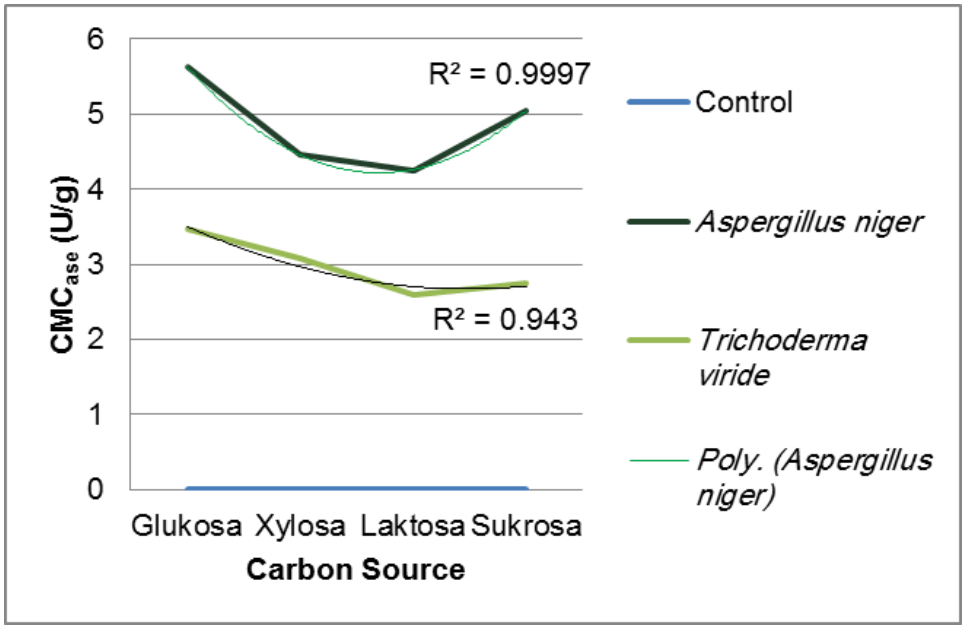

Figure 6. Effect of Differences Carbon Source for Cellulase Production

Optimization of Carbon Concentration

Optimum sellulase activity was obtained by adding 8% of carbon concentration both for *Aspergillus niger* and *Trichoderma viride*. Optimum cellulase activity by *Aspergillus niger* and *Trichoderma viride* were 5.83 U/g and 4.41 U/g respectively. However when carbon concentration was increased to be 10% cellulase activity both for *Aspergillus niger* and *Trichoderma viride* decreased to be 8.48 U/g and 2.21 U/g respectively.

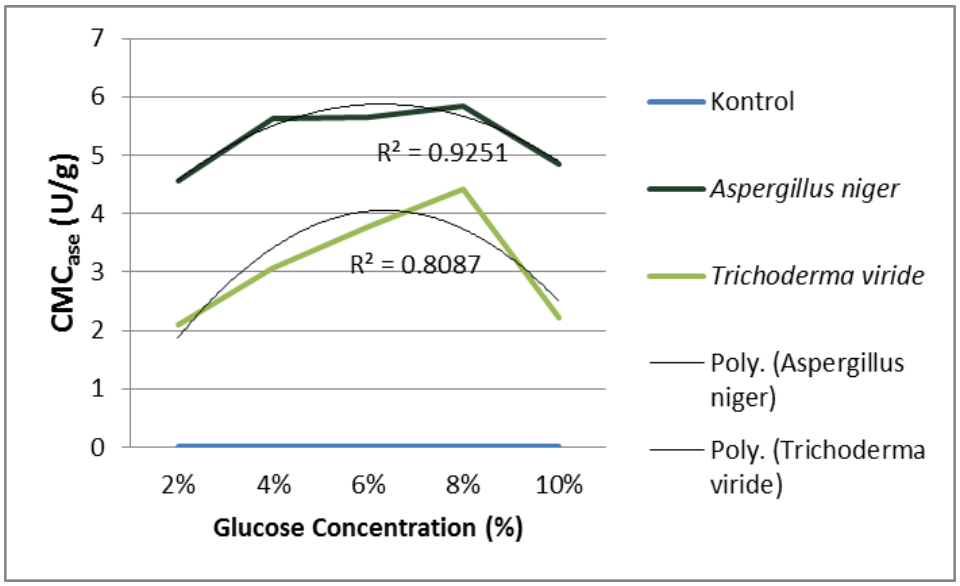

Figure 7. Effect of Differences Glucose Concentration for Cellulase Production

DISCUSSION

The decreasing of cellulase activity both by *Aspergillus niger* and *Trichoderma viride* on 10 days of incubation time suspected due to the effect of cellobiose accumulation which was the dimer of glucose. Cellobiose which was formed on 10 days of incubation time acted as agent which allow the mechanism of feedback inhibition which inhibit cellulase enzyme so that the activity of cellulase enzyme by *Aspergillus niger* and *Trichoderma viride* decreased [6, 7]. The differences of incubation temperature between *Aspergillus niger* and *Trichoderma viride* suspected due to the differences of living mechanism between the both microorganism. This result supported by Hammel et al. [8] and Mrudula [9] who reported that the optimum temperature of cellulase production by *Aspergillus niger* by using coconut peel was 34 ºC with the cellulase activity was 4.44 U/g. This result also supported by Murao [10] who reported that the optimum incubation temperature of cellulase production by *Trichoderma viride* by using corn cobs as substrate was 30 ºC with CMCase activity 1.88 U/g.

Glucose as carbon source which can stimulate *Aspergillus niger* and *Trichoderma viride* to produce the optimal sellulase activity suspected due to glucose was the carbon source which able to fill the carbon need for *Aspegillus niger* and *Trichoderma viride* without stimulate the effect of feedback inhibition [10]. Lactose as carbon source which produce the lowest selluase activity both for *Aspergillus niger* and *Trichoderma viride* suspected due to lactose was the most consumeable carbon affect the carbon supply for *Aspergillus niger* and *Trichoderma viride* higher than expected. The higher carbon than expected acted as agent which inhibit the function of cellulase enzyme or known as mechanism of feedback inhibition [1]. The addition of 2%, 4% and 6% of carbon concentration was not produce the optimum sellulase activity was suspected due to unable to fill the carbon need for *Aspergillus niger* and *Trichoderma viride*. The lack of carbon concentration for *Aspergillus niger* and *Trichoderma viride* could not stimulate the both microorganism to produce optimum cellulase enzyme [7, 14]. Furthermore the addition of10% of carbon concentration was higher than expected by both microorganism so that stimulate the effect of feedback inhibition. So that the optimum carbon source for *Aspergillus niger* and *Trichoderma viride* was 8%.

CONCLUSION

This research was conducted to determine the optimum condition of production cellulase enzyme by *Aspergillus niger* and *Trichoderma viride*. Optimum condition for cellulase production by *Aspergillus niger* was 8 days of incubation, 34ºC of incubation temperature and 8% of glucose concentration with 5.84 U/g of cellulase activity. However optimum condition for cellulase production by *Trichoderma viride* was 6 days of incubation, 30ºC of incubation temperature and 8% of glucose concentration with 4.41 U/g of cellulase activity. *Aspergillus niger* obtained higher sellulase activity compare to *Trichoderma viride*. So that for the next study cellulase enzyme can be

produced by using *Aspergillus niger*. Cellulase activity in this research was crude enzyme, for further study the experiment should be conducted by using pure enzyme for better result.

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Authors' Contributions

Conception and design of this research: Elwin, Joni Kusnadi, Yusuf Hendrawan, I Made Sudiana.
Data analysis, drafting and revising the manuscript: Elwin

Competing interests

We declare that we have no significant competing financial, professional or personal interest that might have influenced the performance of presentation of the work described in this manuscript.

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