PROCEEDING

ICOLIB

International Conference on Life Sciences and Biotechnology

EXPLORATION AND CONSERVATION OF BIODIVERSITY

The ICOLIB 2015 focuses on life sciences and biotechnology aspects to explore and conserve biodiversity by bringing together investigators from different fields such as health and medicine, agriculture, food technology and security, new and renewable energy, conservation and management including exploration of biodiversity

Aston Jember Hotel & Conference Center
Jember - Indonesia, 28-29 September 2015

DAAD Deutscher Akademischer Austauch Dienst
German Academic Exchange Service

ISBN : 978-602-9030-98-3

International Conference on Life Sciences and Biotechnology (ICOLIB)

Exploration and Conservation of Biodiversity

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Preface from the Editor

The explosive development of the sciences and its expansion into other disciplines such as the Life Sciences field is yielding groundbreaking discoveries from novel genes and bio-products to cutting-edge nanotechnology, resulting in a transformed science landscape with profound global applications in understanding life, eradicating diseases, securing a more equitable food and water supply distribution as well as creating novel bio-industries and products.

Based on these phenomena above, the ICOLIB 2015 with theme "**Exploration and Conservation of Biodiversity**", provide an interdisciplinary platform of life sciences for researchers, academics, students, professionals, industries, and policy makers. This meeting also proposed to among scientists and professionals to stay at the leading edge of recent advances in life sciences and sustainability, act as a catalyst for further research, improve international collaboration while bridging the scientific and technological differences among scientists, and foster global health security.In order to disseminate to community more broadest, the articles were published as a proceeding.

The conference was organized by the Department of Biology, Faculty of mathematic and natural sciences, The University of Jember collaboration with the Flensburg University of Applied Sciences, Deutscher Akademischer Austauch Dienst (DAAD), Indonesian-German Network for Teaching, Training and Research Collaboration (IGN-TTRC), University of Kassel and IndoBIC (Indonesian Biotechnology Information Centre) The Southeast Asian Regional Centre for Tropical Biology (SEAMEO BIOTROP). The conference participants from 5 countries and of which 9 lectures within the field health and medicine, agriculture, food technology and security, new and renewable energy, conservation and management including exploration of biodiversity. Presentation divided into plenary, oral and poster session. More than 150 researchers including students participated on this meeting.

On behalf of the organizing committee, i would like to thank all invited speakers and presenters for participating in the ICOLIB 2015 for giving valuable contribution to this conference. Also, acknowledgements are address to Rector University of Jember, Flensburg University of Applied Sciences, DAAD, Indonesian-German Network for Teaching, IGN-TTRC, University of Kassel and IndoBIC-SEAMO BIOTROP as well as all sponsors for the efforts. Finally, i would like to express deep appreciation to the member of the organizing committee for the good teamwork and the great effort to bring success to the conference.

Jember, September 2015

Kahar Muzakhar
Committee

CONTENTS

POSTER SESSION

DECOMPOSITION of COFFE PULP POLYSACCHARIDES by *Aspergillus niger* EXTRASELLULER ENZYME

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Abstract

Coffee pulp is agricultural waste which amount of annual production capacity of 336 tons in Indonesia at 2012. The coffee pulp containing a major component in the form of polysaccharides consisting of cellulose, hemicellulose, lignin and C / N ratio is relatively high which causes this agricultural waste takes longer to decompose naturally. *Aspergillus niger* strains B10 MCC-00136 is a fungus that can produce a wide variety of extracellular enzymes that can be utilized for the decomposition process. *A. niger* strain B10 MCC-00136 was grown on medium coffee pulp and crude enzyme to be extracted then carried out using a reducing sugars assay method Somogy Nelson. The optimum activity was obtained on day 5th with reduction sugars produced by 29.9 ug / ml. Testing results crude production of enzymes at pH 3.5 to 6 and a temperature of 30-50 °C showed optimum activity at pH 4, 5 and a temperature of 40 °C. The results of crude enzyme hydrolysis test reaches optimum at the 18th hour with an efficiency of 4,69 %.

Keywords: *Aspergillus niger*, coffe pulp, decomposition, reducing sugar

Introduction

Coffee pulp is agricultural waste which amount of production capacity in Indonesia reached 336 tons in 2012. it contains the main components in the form of polysaccharides consisting of cellulose 18.65 %, hemicellulose 0.98 % and lignin 12 25 % [1] as well as the C / N ratio is relatively high, reaching 57,2 [2] which led to this agricultural waste takes longer to decompose naturally. In addition to the decomposition of a relatively long time, coffe pulp also are compounds such as caffeine, tannins and polyphenols which are compounds that could potentially cause contamination if it accumulates in nature.

Handling of organic wastes can be done by using microorganisms which aim to reduce pollution, reduce the amount of waste or increasing the economic value of waste [3]. Microorganisms such as bacteria and fungi capable of using organic compounds and inorganic compounds as a source of nutrients, carbon source and nitrogen source for the metabolism of primary [4]. Some mikroorganime decomposers have the ability to produce extracellular enzymes capable of hydrolyzing organic polymers. The use of microorganisms as enzyme producers has several advantages such as can be produced in a relatively short time, the production cost is relatively inexpensive, has a high growth rate as well as easy to control [5].

Aspergillus niger strain B10 MCC - 00136 is a filamentous fungus capable of producing a wide variety of extracellular enzymes that can be used for the decomposition process. Produced extracellular enzymes including proteases of *A. niger*, pectinase [6], sellulase, xylanase, invertase, amylase, and inulinase [4]. A variety of extracellular enzymes is what hopefully will be able to decomposing coffee pulp. So the goal was to determine the penilitian extracellular enzyme activity of *A. niger* in soft leather waste mendekompisisi cherries are based on the conditions of pH, temperature, and optimum incubation it..

In the determination of the optimum temperature, 500 mL of coffee fruit soft leather substrate 0.5% pH optimum of 60 mM were incubated at 37 ° C for 20 minutes and were divided into 2 groups: test and control. In the test group added 100 mL of crude enzyme in buffer 50 mM pH optimum and incubated at a temperature variation of 30 ° C - 70 ° C for 2 hours. Then added 0.5 ml of reagent Somogyi. While the control group, after incubated 20 minutes then passed back to the variation of incubation temperature of 30 ° C - 70 ° C for 2 hours. After it was added 0.5 ml of Somogyi reagent and 100 mL of crude enzyme in 50 mM pH optimum. Each then homogenized with vortex engine. Once homogeneous, the solution is heated for 15 minutes. Once cool, Nelson reagent solution was added 0.5 ml and 2.5 ml of distilled water and then dihomegenkan with vortex engine. Each of these is then taken into a 1 ml eppendorf and centrifuged at a speed of 800 rpm for 10 minutes. The supernatant is then taken and the reduction of sugar measured with a spectrophotometer wavelength of 500 nm as much as 2 repetitions. Reduction sugar test results are then compared with standard glucose curve that had been made previously.

Methods

Preparation of materials research

Substrate alkaline extract of coffe pulp

A total of 100 grams of powdered bark of the coffee pulp with water content of less than 1 % were mixed with 80 g NaOH 2 M and dissolved in 1000 ml distilled water. The resulting mixture is then homogenized using a magnetic stirrer for 24 hours. After 24 hours, the pH of the mixture was adjusted to reach 7 by adding a solution of acetic acid (CH_3COOH) little by little. After the pH to 7, followed by filtering the mixture with a filter paper to be taken filtrate. The filtrate obtained is then added 97 % ethanol with ethanol filtrate ratio is 6 : 4. The resulting mixture is then centrifuged and taken pelletnya to then dried at 50 °C.

Standard curves Glucose

Dilution series is made of 500 ug / ml stock glucose to 5 ug / ml , 10 ug / ml , 15 ug / ml , 20 ug / ml , 50 ug / ml and 75 ug / ml . A total of 0.5 ml of Somogyi Nelson reagent and 2.5 ml was added to each dilution series. Reducing sugar content measured by spectrophotometry using a wavelength of 500 nm absorbance value that will be used in the manufacture of a standard curve.

Production of crude extract enzyme

Optimization of the production of enzymes

1 ml suspension of *A. niger* strain B10 MCC - 00136 containing 5.775 x 10^9 spores/ml into 10 grams of coffee pulp substrate saturated with water, and then incubated at 30°C for 7 days and continued harvesting enzymes begin day 1 to day 7. Harvesting is done by adding 20 ml of distilled water containing 1 % NaCl and 0.1 % Sodium Azide and dishaker 10 rpm for 12 hours then filtered using filter paper. The filtrate centrifuged at a speed of 800 rpm for 10 minutes. Centrifugation supernatant was taken and tested the results of its activity using *Somogyi-Nelson* method.

Large -scale production of crude enzyme.

A total of 20 ml suspension of *A. niger* strain B10 MCC - 00136 containing 5.775 x 10^9 spores/ml into 200 grams of coffee pulp substrate saturated with water, and then incubated at 30 °C corresponding optimum incubation time of enzyme production. Harvesting is done by adding 20 ml of distilled water containing 1 % NaCl and 0.1 % Sodium Azide and dishaker 10 rpm for 12 hours then filtered using filter paper. The filtrate centrifuged at a speed of 800 rpm for 10 minutes. Centrifugation supernatant was taken and tested the results of its activity using *Somogyi - Nelson* method.

Characterization of crude enzyme ekstraseuler

Stability and optimum pH

PH stability test is divided into 2 test test test X and Y. A total of 500 mL to 500 mL using a mixed enzyme buffer 50 mM pH varisi (3 ; 3.5 ; 4 ; 4.5 ; 5 ; 5.5 ; 6 ; 6 , 5 ; 7 , 7.5, 8). While the Y test, enzyme treated using sterile distilled water of 500 mL of an enzyme that is mixed using 500 mL sterile distilled water. Each - each mixture is then incubated 4 hours at 37 ° C and reducing sugar testing methods Somogy nelson using soft leather substrate coffee fruit 0.5 % and 500 ul enzyme . After reducing sugar known test through X and Y, then the specified percent of the enzyme activity by comparing the results of reducing sugar X and Y.

In the determination of the optimum pH, 500 mL of coffee pulp substrate 0.5% pH 5 60 mM were incubated at 37 ° C for 20 minutes and were divided into 2 groups: test and control. In the test group added 100 mL of crude enzyme in 50 mM buffer pH variations and incubated at 37 ° C for 2 hours. Then added 0.5 ml of reagent Somogyi. While the control group, after incubated 20 minutes then passed back incubation at 37 ° C for 2 hours. After it was added 0.5 ml of Somogyi reagent and 100 mL of crude enzyme in 50 mM buffer pH variations. Each then homogenized with vortex engine.

Once homogeneous, the solution is heated in a water bath for 15 minutes. Once cool, Nelson reagent solution was added 0.5 ml and 2.5 ml of distilled water and then dihomegenkan with vortex engine. Each of these is then taken into a 1 ml eppendorf and centrifuged at a speed of 800 rpm for 10 minutes. The supernatant is then taken and the reduction of sugar measured with a spectrophotometer wavelength of 500 nm as much as 2 repetitions. Reduction sugar test results are then compared with standard glucose curve that had been made previously.

stability and optimum temperature

Temperature stability test is divided into 2 test, X and Y. 500 mL to 500 mL of enzyme in buffer in 50 mM buffer pH optimum at X incubated test using predetermined temperature variation that is a temperature of 30 °C , 35 °C , 40 °C , 45 °C , 50 °C , 55 °C , 60 °C , 65 °C and 70 °C, while the Y test, 500 mL to 500 mL of enzyme in buffer in 50 mM buffer pH optimum directly tested shortly after being taken from the place storage at -20 °C. Test X performed with reducing sugar analysis using Somogyi - Nelson method using soft leather substrate coffee fruit 0.5 % and 500 ul enzyme. After reducing sugar known test through X and Y, then the specified percent of the enzyme activity by comparing the results of reducing sugar X and Y.

In the determination of the optimum temperature, 500 mL of coffee pulp substrate 0.5% pH optimum of 60 mM were incubated at 37 °C for 20 minutes and were divided into 2 groups: test and control. In the test group added 100 mL of crude

enzyme in buffer 50 mM pH optimum and incubated at a temperature variation of 30 °C - 70 °C for 2 hours. Then added 0.5 ml of reagent Somogyi. While the control group, after incubated 20 minutes then passed back to the variation of incubation temperature of 30 °C - 70 °C for 2 hours. After it was added 0.5 ml of Somogyi reagent and 100 mL of crude enzyme in 50 mM pH optimum. Each then homogenized with vortex engine. Once homogeneous, the solution is heated for 15 minutes. Once cool, Nelson reagent solution was added 0.5 ml and 2.5 ml of distilled water and then homogenized with vortex engine. Each of these is then taken into a 1 ml eppendorf and centrifuged at a speed of 800 rpm for 10 minutes. The supernatant is then taken and the reduction of sugar measured with a spectrophotometer wavelength of 500 nm as much as 2 repetitions. Reduction sugar test results are then compared with standard glucose curve that had been made previously.

Optimization of Coffee Fruit Soft Skin Hydrolysis by Enzyme Ekstraselules A. niger

The determination of the optimum time of hydrolysis was conducted to determine the highest activity of A. niger extracellular enzymes in decomposing waste coffee fruit soft skin over a certain period. A total of 2.5 grams of soft fruit skin smooth substrates coffee in 50 ml crude enzyme and added 500 ul Na Azide 1 %, incubated at 37 ° C for 72 hours. Sampling for the reduction of glucose testing is done by taking 1 ml campuaran substrate and enzyme incubation time stretcher at 0, 6, 12, 18, 24, 30, 36, 48, 60 and 72 hours . Each sample was then tested the reducing sugar by Somogyi - Nelson method. Efisisiensi hydrolysis was calculated using the formula :

$$\text{hydrolisis effiency} = \frac{\sum reduction\ sugars\ produced}{\sum \text{Substrate of polysaccharides}} \text{x100 \%}$$

Result

Optimization of enzyme production

Optimizations have been made known that the day-to - 5 is the optimum incubation time for enzyme production . Total reducing sugar produced on day 5 reached 29.9 ug / ml as seen on the curve 4.2

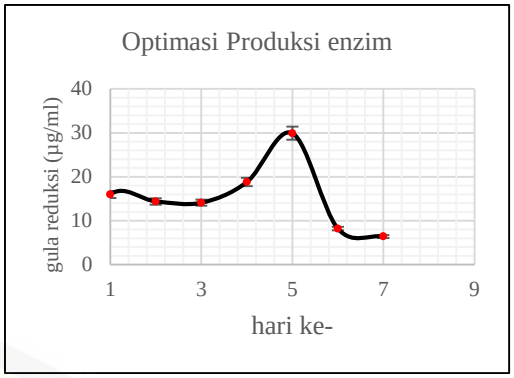

Figure 1. Curve optimization of the production of crude enzyme *A. niger* in media coffee pulp.

The amount of reducing sugar on the first day to the third day tends decreased from 16 pg / ml on day 1 to 14,1 ug/ml on the 3rd day. This happens because the isolates of *A. niger* are still utilizing the reducing sugar is in coffee pulp as a source of carbon. According to [7] on the skin of the cherries are soft sugar reduction of 12.4 % dry weight. A more modest reduction in sugar will tend to be used first by the *A. niger* for its metabolism before eventually break down more complex substrate which in this case is the polysaccharides in the coffee skin, so that the amount of enzyme breaker automatically polysaccharide produced is also relatively less. [8] in his research on the influence of several carbon sources on the growth of fungi, said fungi will tend to utilize a simpler carbon source first.

Stability and Optimum pH

Crude enzyme A. niger has a stable activity at pH has stabilized activity in the range pH 3.5 - 6, while the optimum activity is at pH 4.5 as depicted in the curve below.

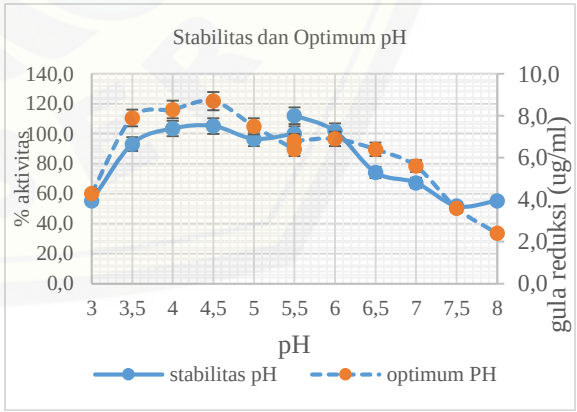

Figure 2. Curve stability and optimum enzyme activity of *A. niger* crude to pH.

On the curve above it can be seen that the enzyme activity is stable in the pH range from 3.5 to 6 and beyond the range of their activities tend to be lower. It can be seen from the % activity of the enzyme. At pH 3, % of enzyme activity reached

103.4 %. Then, at pH 3.5 the enzyme activity increased to 105.2%. Enzyme activity began to show its stability to pH 6 with activity 101.7% which also indicates that the enzyme is in the range of pH. However, At pH 6.5 to 8 relative enzyme activity began to decline with activity only amounted to 74.1 % at pH 6.5 and 55.2 % at pH 8. The enzyme has a structure that is very sensitive to changes in pH. pH that is too acidic or too alkaline will cause ionization of the active site of enzymes that will reduce or even inhibit the enzyme activity. Enzyme activity increases concurrently increase in the pH of the environment until it reaches its maximum activity or the so-called pH optimum, then fell back when the pH the more alkaline environment [9].

Decrease in enzyme activity due to the pH environment according to [10] due to the enzyme is charged molecules, changes in pH that is going to change the charge on the areas that govern the bonding between the substrate and the enzyme that causes the activity will be reduced. PH changes may also cause changes in the charge on the areas far from the active site of enzymes that may play a role in maintaining or tertiary quaternary structures of the enzyme active site. By changing this structure, the structure will change and inhibit enzyme substrate binding [10].

On the curve 4.3 can also be seen that the highest enzyme activity is at pH 4.5 with the amount of reducing sugar output reached 8.7 ug/ml. Its activity has continued to decline relative to pH 8 as seen from the amount of reducing sugar produced only by 2.4 ug/ml. In the study [1] Please also note that the fermentation activity of *A. niger* in coffee pulp also decline little by little towards the end pH of 4.5. [11] reported that the optimum activity sellulase *A. niger* is the range pH 4,5 - high as 7,5. Meanwhile, [12] saying that sellulase activity of *A. niger* showed high activity at pH 4 and then continued to decline until the activity close to 0 ug ml while the pH 9.

Stability and Optimum Temperature

Based on the analysis of the activity of the enzyme is known that the temperature of crude *A. niger* in media activities coffee pulp is stable at a temperature range of 30-50 °C, while the optimum activity is at a temperature of 40 °C, as it is seemingly on the curve below

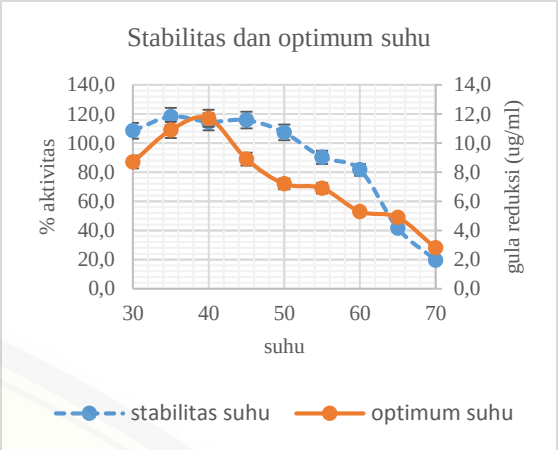

Figure 3. Curve stability and optimum enzyme activity of crude *A. niger* on media suede coffee fruit.

As can be seen on the curve that the activity of the enzyme produced crude stable in the pH range of 30-50 °C. It is seen from the % activity of the enzyme that is relatively stable in the temperature range that the temperature of 30 °C activity reached 108.5%, at a temperature 35 °C reaches 118.3%, amounting to 114.5% of enzyme activity at a temperature of 40 °C, then at a temperature of 45 °C and 50 °C the enzyme showed activity amounted to 115.9 % and 107,3 %. Enzyme activity then decreased gradually as the temperature rises above 50 °C, the enzyme activity by 90,2% at a temperature of 55 °C were reduced to only 19,5% at 70 °C. The enzyme basically can be a pure protein, or is the result of a combination of proteins with other chemical cluster [13]. Such as proteins, enzymes also have a high sensitivity to temperature. The temperature is too low will reduce or even stop their activities, while the temperature is too high will denature or destroy most enzymes [13]. In the structure of enzymes which are part thermosensitive easily undergo denaturation when exposed to temperatures above an area of stability, even if any chemical reaction can be increased concurrently increase in temperature to some extent [9].

[6] said that the *A. niger* has a temperature range of approximately between 6 °C- 47 °C and automatically influence the temperature range in which the enzymes produced work. Point optimum enzyme activity is at a temperature of 40 °C with the amount of reducing sugar produced was 11,7 ug/ml. Then from the optimum point, reducing sugar produced become 8,9 ug/ml at a temperature of 45 °C to be only 2,8 ug/ml of reducing sugar produced at a temperature of 70 °C decrease in this activity. Based on the research [12] activity sellulase of *A. niger* are also reported to decrease after passing a temperature of 50 °C. Based on the above crude enzyme known that *A. niger* is a species that berisifat mesophilic [14].

Coffee pulp hydrolysis by A. niger strain B10 MCC – 00136.

The highest hydrolysis activity is on the clock to- 18 with a reducing sugar produced by 1039 ug/ml with an efficiency of 4.69 % of the total of 2.5 grams substrate. These values are relatively large when compared to the crude enzyme hydrolysis efficiency of *A. niger* on organic wastes such as oil palm empty fruit bunches (EFB) as in [15] which only reached 1,5 % with a reducing sugar of 756 ug/ml.

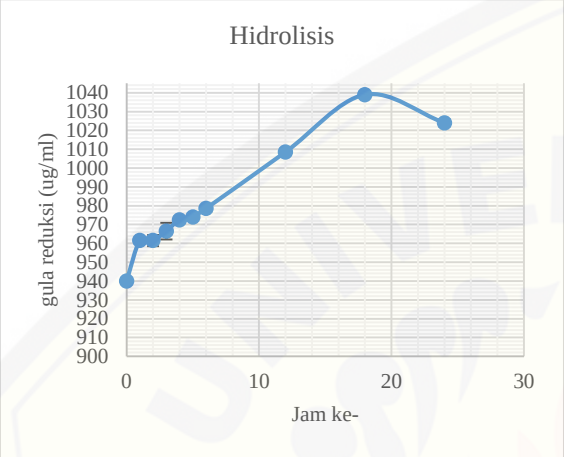

Figure 4. Curve hydrolysis crude *A. niger* extracellular enzymes on the coffee pulp substrate.

On the curve above it can be seen that the crude enzyme hydrolysis activity of extracellular *A. niger* against coffee pulp has increased along with the increase of incubation time to reach the peak at 18 hours incubation time. Then the activity began to decline in the hours to 24, this reduction may occur because due to the feedback mechanism inhibitor, which is the end product of a metabolic path which can stop its own synthesis by inhibiting the activity of one enzyme at the beginning of the track biosyntetic it [13]. According to [16] mentioned that the presence of metabolites such as glucose products will inhibit the enzyme activity lignoselulolitic particularly cellulose degrading manner allosterik attached to the side so that the active enzyme can no longer occupied by the substrate .

Conclusion

Results of the research that has been done can be seen that the *A. niger* strain B10 MCC - 00136 is able to decompose the coffee pulp. Production of enzyme with maximum activity on day 5th with the amount of reducing sugar produced by 29,9 ug/ml. Crude enzyme activity of *A. niger* strain B10 MCC - 00136 is stable at pH 3,5 to 6 with optimum activity at pH 4,5 and the enzyme was also stable at a temperature of 30-50 °C with optimum activity at a temperature of 40 °C. The test results *A. niger* crude enzyme hydrolysis showed that the hydrolysis process reaches optimum at the 18th hour with an efficiency of 4,69 %.

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