The 2nd ICOLIB

International Conference on Life Sciences and Biotechnology

Biology Department, Faculty of Mathematics and Natural Sciences, University of Jember

(ICOLIB BIO-UNEJ 2017)

Integrated Biological Sciences for Human Welfare

PROCEEDINGS

The Panorama Hotel and Resort Jember
East Java, Indonesia
August 7 - 8, 2017

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THE 2nd INTERNATIONAL CONFERENCE ON LIFE SCIENCES AND BIOTECHNOLOGY (ICOLIB): INTEGRATED BIOLOGICAL SCIENCES FOR HUMAN WELFARE

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WELCOMING ADDRESS (CONFERENCES)

The International Conference of Life Science and Biotechnology (ICOLIB) was organise by Biology department Faculty Mathematic and Basic Sciences, The University of Jember, Indonesia. This conference has been held biannually at different venues. The last one, (First ICOLIB) held in Aston Hotel Jember 2015, Indonesia. Now, we are held the 2nd ICOLIB at Panorama Hotel and Resort Jember, Indonesia. The ICOLIB is a forum for students, researchers, educators, observers and practitioners from university, research institutions, industry and general public, policy maker to exchange ideas and latest information in the field of life science and its application. The theme of the 2nd ICOLIB 2017 '**Integrated Biological Sciences for Human Welfare**' will underpin the need for collaboration and cooperation of individuals from a wide range of professional backgrounds. The scope of the 2nd ICOLIB covers several fields of studies, namely life sciences, environmental sciences, medical and pharmaceutical sciences, science of renewable energy, agricultural science and food security. This conference will also offer opportunities for discussion and sharing as well as encouraging for international research collaboration. Furthermore, the scientific articles will be peer-reviewed and published in Serial book volume publish with Cambridge Scholar Publishing UK. The selected scientific articles in the 2nd ICOLIB will be further reviewed and will also be published in Scopus-indexed Journal.

The 2nd ICOLIB have been fortunate to have Prof. Harald zur Hausen, 2008 Nobel Laureate in Physiology or Medicine for his discovery of human papilloma viruses causing cervical cancer. Prof. zurHausen and his team has made a breakthrough in 1982 and 1983 when they were able to isolate HPV 16 and HPV 18 as the virus types responsible for cervical cancer. Based on these findings, vaccines have been developed against cervical cancer, one of the most common forms of cancer among women. This work led to improved methods for predicting which women are in the risk zone. We are very honoured to present Prof. Harald zur Hausen, as a keynote speaker, and 6 distinguished scientists as invited speakers.

I sincerely hope that the results of this conference will enable all participating scientists from all over the world to have the opportunity to exchange knowledge through lectures and posters.

Purwatiningsih

Chairwoman of The 2nd ICOLIB 2017

PREFACE FROM EDITOR

Welcome to the The 2nd ICOLIB Proceedings, **Integrated Biological Sciences for Human Welfare,** The theme of this conference reflects our attention Biological science to support the human welfare across of different of fields and contexts. Indeed, the 17 contributions in these proceedings—including keynotes, invited and contributed papers—with authors from at least 6 different countries showed the great results of Biology.

All the manuscript have been peer reviewed with at least 2 people who competence with their subjects. It has been a great previlege for being Editor of the 2nd ICOLIB. Hope these output had a great support for human welfare needs in many levels. Thus enable all participating scientists from all over the world to have the opportunity to exchange knowledge through the conferencere.

Sincerely,

Editorial team of The 2nd ICOLIB

Contents

General Information for the Participant

Registration Information

Conference Venue

- The venue for the conference is the Panorama Hotel, Jember, East Java, Indonesia

Registration

- Registration includes:
  - 2nd ICOLIB Abstract Book
  - ID Card
  - Document Bag
  - Refreshment (coffee & tea) during the conference day
  - Buffet Lunch

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- Participants are requested to display their ID Card during the conference for entry to scientific sessions, melas and the wellcome reseption. Please also show the ID Card to committee before transportation to the conference venue.

Instruction for the Moderator

- Please ensure that the sessions and speaker presentations are kept stricly on time

Instruction for Speakers (Keynote Speaker and Oral Presenter)

- Speaker are requestes to submit their presentation to staff in the audio-visual room at the least 1 hours before each presentation, then upload and ensure that the proper presentation is in the computer provided
- 45 minutes have been allocated for each keynote speakers (please allow time within this period for answering the questions)
- Free oral presenter will last 10 minutes only (please allow time within this period for answering questions)
- Please be aware that the above times must be strictly adhered to

Instructions for Poster Presenter

- Poster presentations will be located in the front of the conference space along the second floor.
- Poster will be display throughout the conference, and presenters are responsible for putting them and removing them.

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DECOMPOSITON OF COFFEE PULP UNDER SOLID STATE FERMENTATION BY *Aspergillus* VT12

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Abstract

In coffee bean processing more than 40% coffee pulp hemicellulose wastes were produced with high C/N ratio so that difficult to decompose. An isolate *Aspergillus* VT12 can grew well and produced extracelluler enzyme during solid state fermentation on coffee pulp substrate based. The enzyme was observed actively breakdown or degrade coffee pulp substrate and released reducing sugars. The optimum activity of crude enzyme in pH 5 at 35^{o}C. At 18 hours incubation, the crude enzyme had hydrolysis eficiency 1.49 %.

Keyword: *Aspergillus* VT12, coffe pulp, solid state fermentation

1. Introduction

Coffee pulp is abundant agricultural waste in Indonesia. In every coffee processing will be produced less more 40 % coffee pulp [1]. In 2012, Indonesia produced about 336 tons coffee pulp from total capasity coffee production about 748 tons [2]. Coffee pulp has contain compounds of caffeine, tannins and polyphenol that show toxic effects on the environment [3]. In Addition, Coffee pulp also has a high C/N ratio namely less more 57.2 which cause this agricultural waste takes long time to decompose naturally [4]. So, for those reason, coffee pulp is very potentially to cause pollution. However, coffee pulp has some potensial material as well such as cellulose (18,65 %), hemicellulose 0,98 % nad lignin 12, 25 % [5].

The coffee pulp waste treatment can be helped using microorganism. Microorganism such as bacteria and fungi as a decomposer agent has some benefits, such as need little time to grow and has a cheap production cost relatively. Genus *Aspergillus* is a genus of filamenteous fungi which can decompose organic material from natural resource. *Arpesgillus* VT12 can produce selulase extarselluler enzyme to break polysaccharides chain in the CMC and TKKS substrats [6]. For this reason, need to further study about activity of *Arpesgillus* VT12 extraseluler crude enzyme to decompose polysaccharides contens in the coffee pulp.

2. Materials and Methods

Alkaline extract substrat of coffee pulp

A total of 100 grams of coffee pulp powder 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. Then added 97 % ethanol with ethanol filtrate ratio is 6 :4. The resulting mixture is centrifuged and taken it pellet and dried at 50 °C.

Optimization of the production of enzymes

1 ml suspension of *Aspergillus* VT12 containing 4,2 x 10^7 spora/ml into 10 grams of coffee pulp substrate and then incubated at 30°C for 7 days and continued harvesting enzymes begin day 1 to day 7. Enzyme harvesting is done by adding 20 ml of distilled water containing 1 % NaCl and 0.1 % Sodium Azide and shaker 10 rpm for 12 hours then filtered using filter paper. The filtrate centrifuged at a speed of 800 rpm for 10 minutes [14, 15]. Supernatant was taken and tested the it activity using *Somogyi-Nelson* method.

Large -scale production of crude enzyme.

A total of 20 ml suspension of *Aspergillus* VT12 containing 4,2 x 10^7 spora/ml added into 200 grams of coffee pulp substrate, 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. Supernatant was taken and tested using *Somogyi – Nelson* method.

Stability and Optimum pH

PH stability test is divided into 2 test, test X and Y. A total of 500 mL 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. Then, Each mixture is incubated 4 hours at 37 ° C and reducing sugar testing methods Somogy nelson using coffee pulp substrate 0.5 % and 500 ul enzyme . 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, mixed with vortex engine. Each of these is taken into a 1 ml eppendorf and centrifuged at a speed of 800 rpm for 10 minutes. The supernatant is taken and the reduction of sugar measured with a spectrophotometer wavelength of 500 nm as much as 2 repetitions. Reduction sugar test results are 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 andcontrol. 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.

Coffee pulp hydrolysis optimization by *Aspergillus* sp. VT12 extraseluler enzyme

The aim of this method to determine the highest activity of *Aspergillus* sp. VT12 extracellular enzymes in decomposing coffee pulp waste. A total of 2.5 grams of coffee pulp substrates added into 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 micture between substrate and enzyme at the incubation time 0,6, 12, 18, 24, 30, 36, 48, 60 and 72 hours . Each sample is tested the reducing sugar by Somogyi - Nelson method. Efficiency hydrolysis was calculated using the formula :

$$hydolysis\ efficiency = \frac{\sum reduction\ sugars\ was\ produced}{\sum polysaccharides\ substrats} x\ 100\ \%$$

3. Results

Optimization of *Aspergillus* sp. VT12 extraseluler enzyme production

Enzyme prodution time was known at 4-5 day with reduction sugar less more 1, 122 ug/ml and 1, 127 um/ml (figure 1).

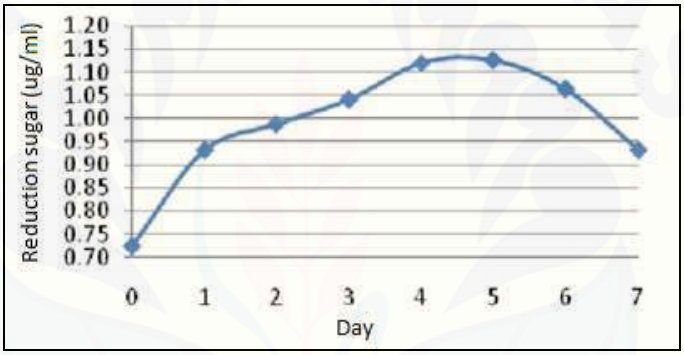

Figure 1. Curve of enzyme production time

[7] said that high relative activity of *Asperigillus sp.* selulase enzyme was obtained after stationary phase at 4 day fermentation . The relative activity decrease at 6nd day to 7nd day, this is happen because *Aspergillus* sp. VT 12 use a simple carbon source from the break of polysaccharides chain in the coffe pulp. [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 of pH

Crude extaselluler enzyme of *Aspergillus* sp. VT 12 has a stable activity at range pH 3,5 -7 with % activity less more 85-95 % and the optimum ph was obtained at 5 with 1,2 ug/ml reduction sugars (figure 2).

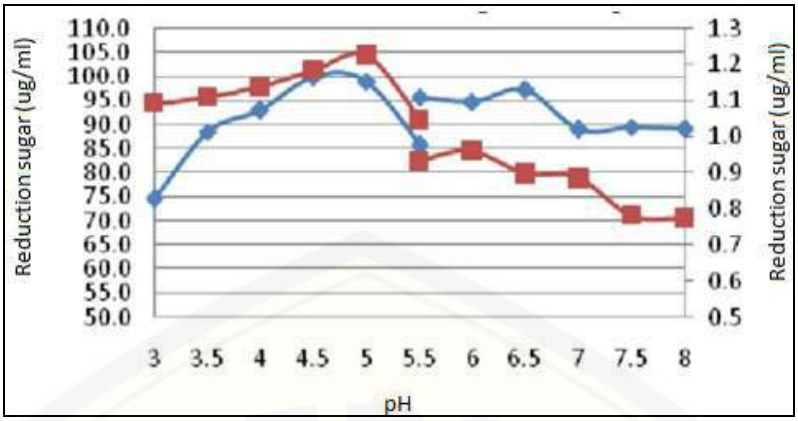

Figure 2. Curve of stability and optimum pH

The enzyme has a structure wich very sensitive to changes in pH. Too acidic pH 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 pH optimum, then fell back when the pH the more alkaline environment [9].

Stability and Optimum of Temperature

Based on the analysis of the *Aspergillus* VT 12 extraselluler enzyme, known that stabilityof temperature at range between 30^{o}C-50 ^{o}C and the optimal of temperature at 35 ^{o}C with 0,9 ug/ml reduction sugars (figure 3).

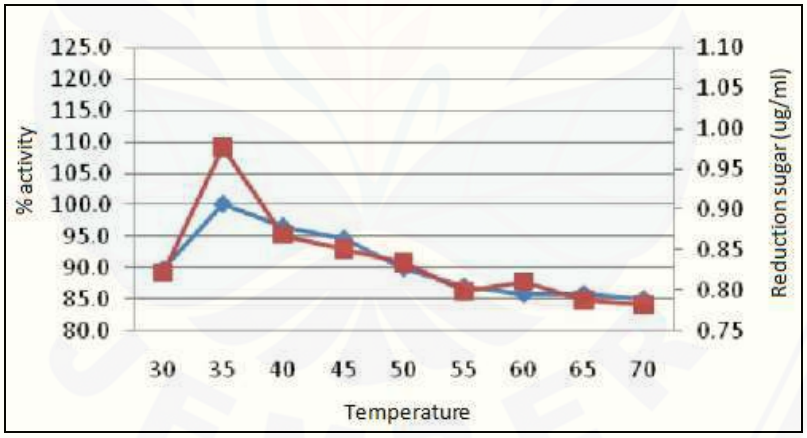

Figure 3. Curve of stability and optimum temperature

The enzyme basically can be a pure protein, or is the result of a combination of proteins with other chemical cluster [10]. 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 [10]. In the structure of enzymes, there are part thermosensitive easily 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]. [11] said that the genus Aspergillus such as *Aspergillus niger* has a temperature range of approximately between 6 °C- 47 °C and automatically influence the temperature range in which the enzymes produced work. Based on the above crude enzyme known that *Aspergillus* sp. VT12 is belong to mesophilic species [12].

Coffee pulp hydrolysis by *Aspergillus* sp. VT12. The highest hydrolysis activity was obtained at 18 hours incubations with hydrolysis efficiency 1,49 % and less more 750 ug/ml of reduction sugars (figure 4).

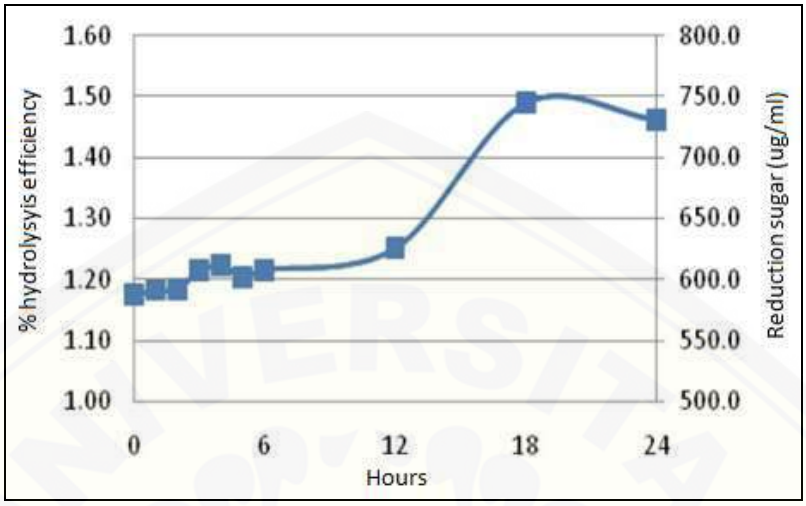

Figure 4. hydrolysis curve of coffe pulp using *Aspergillus* VT12 extracelluler enzyme

For curve above show that the relative activity increase at 3 hours and begin to decrease at 5 hours. This is happen may cause the anzyme break a simple substrat first, and then more complex substrats. This mechanism can produce a inhibitor molecul which stop its own syntesis, in this case cellulase. According to [13] 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.

4. Conclusion

Based on the our research was obtained that the optimum activity was obtained at pH 5 with temperature 35^{o}C and incubation time at-18. The eficiency value of hidrolysis is 1.49 %. The *Aspergillus* sp. VT12 extracelluler enzyme has stable pH between 3.5 -7 and stable temperature at range 30 ^{o}C to 50 ^{o}C.

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