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

Editors

Kahar Muzakhar
Purwatiningsih
Eva Tyas Utami
Siswoyo
Fuad Bahrul Ulum
Rendy Setiawan
Syafiq Ubaidillah
Anis Barokah
Zakiyatul Khoiriyah
Arminatul jannah

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

HYDROLYSIS PROFILE of OIL PALM EMPTY FRUIT BUNCH by AN EXTRACELLULAR ENZYME from *Aspergillus niger*

Kahar Muzakhar[1], Ainul Latifah[1], Sutoyo[1], Siswoyo[2], and Rudju Winarsa[1]

[1]Biology Dept., Mathematic and Natural Sciences, The University of Jember
[2]Chemistry Dept., Mathematic and Natural Sciences, The University of Jember
kaharmzk@*unej.ac.id*

Abstract

Large quantities of oil palm empty fruit bunch (OPEFB) biomass with low economic value were released during oil palm production. This biomass components are cellulose (41-46%), hemicellulose (25-34%), lignin (27–32%), and C/N ratio 70-100 so that in nature OPEFB decomposition takes much time. To overcome this problem microbial utilization is needed. In this research, an extracellular enzyme from *Aspergillus niger* was used to hydrolyze OPEFB and gave the rate hydrolysis at 1.5% (756ug/ml) for 6 hours respectively. It was estimated by Gas Chromatograph (GC) analysis as alditol acetate proved that the main sugar in hydrolysate as monosaccharide was glucose with the concentration 74.2%. Suggested, much cellulase was released by *A. niger* during solid stated fermentation.

***Keywords**: Aspergillus niger*, extracellular enzymes, hydrolysis and OPEFB.

Introduction

Large quantities cellulose and lignocellulose which occurs as micro fibrils embedded in cell wall are most important component of plant biomass. However, during processing, generally all of these materials cannot be processed, and a significant amount remains as organic wastes (1,8). For example, the processing of OPEFB one of the important agriculture industry in Indonesia, which discard a huge amount of secondary organic products of material as OPEFB.

The degradation of lignocellulose to other forms of derivate polysaccharides can be efficiently done with appropriate hydrolyzing enzymes from fungi or bacteria (2,3), which is considered effective methods to manage the organic waste and other forms of pollution (1,9). By degradation followed by biosynthesis, the organic waste material can be transformed to other useful products which may decreased the process cost and solved some of in the environment problems (4,5). In nature, the genus of *Aspergillus* secreted wide range of enzymes and readily degraded a wide variety of polymers, such as cellulose (5), hemicellulose, pectin and lignin (4,6,7,8), and also storage compounds like starch (XX), sucrose and inulin (6,7,8). This capability is due to secrete on of a broad range of enzymes by different member of this genus that can degrade these complex polymers (7,9).

In this research, microbial utilization of OPEFB by introducing *Aspergillus niger* in solid state fermentation, which may degrade cellulose and lignocellulose was reported.

Methode And Materials

a. Crude enzyme production

Aspergillus niger was used for producing crude extracellular enzyme by inoculating to 100 g of sterilized OPEFB in a 5 liter Erlenmeyer flask and at 30^{o}C incubation. After 6 days, the culture was stopped by adding 200 ml water containing 1% NaCl and 0.1% toluene (v/v), followed by shaking at room temperature for 10 hours. The suspension were filtrated using filter paper and centrifuged to recover the supernatant as a crude extracellular enzyme. From this step, 180ml of filtrate as crude enzyme was collected. Then the crude enzyme were re-filtrated at 20 micron and concentrated to about one-tenth of the initial volume by dialysis using hollow fiber 10kD, eluted with acetate buffer 20mM at pH 5. The precipitate was dissolved and dialyzed against water for 3 days. This solution was stored at 4^{o}C till used for OPEFB hydrolysis.

b. Degree of hydrolysis and total sugar content analysis

The degree of hydrolysis was examined by incubating the reaction mixture of concentrated crude enzyme and OPEFB substrate at 37^{o}C. The OPEFB-hydrolyzate was obtained by centrifugation and the release of reducing sugars measured by the method of Nelson (10) as modified by Somogyi (11) using glucose as a standard sugar for calibration. The degree of hydrolysis of okara was calculated as follow:

where:

DH : Degree of hydrolysis (%)

TRSH : Total reducing sugar in hydrolyzate (w/v)

$$DH(\%) = \frac{TRSH}{TS} * 100\%$$

TS : Total substrate (w/v)

The total sugar of OPEFB-hydrolyzate was also measured by the phenol-sulphuric acid method (12).

c. Analysis of sugar composition of OPEFB

Gas chromatograph (G-3000, Hitachi, Tokyo, Japan) was used to analysis sugar composition of OPEFB-hydrolyzate as alditol acetates (13,14) with a few modifications. A sample of 20 mg of OPEFB was fully hydrolyzed with 2 ml of 2 N HCl for 6 hours at 100ºC. The hydrolyzate was filtered, evaporated to dryness. One mg of 2-deoxy-D-glucose was added as an internal standard. The mixture was then reduced with 2 ml of 0.2 M $NaBH_4$ at room temperature, overnight. Five to six drops slurry of dowex resin H type 100-200 mesh (Bio-Rad Laboratories, CA) were then added to the mixture and incubated at room temperature for 1 h, followed by filtration. The filtrate was evaporated to dryness and remaining boric acid residue was removed by repeated evaporation using methanol. The sugar alcohols obtained were acetylated in 2 ml of acetic anhydride:pyridine (1:1) at 100ºC for 10 min. The mixture was then diluted with chloroform:water (1:4), shaken and the upper layer removed by centrifugation at 2000 rpm for 10 minutes. Remaining pyridine was removed from the chloroform extract by washing with water, followed by centrifugation. The resulting alditol acetates were dried and dissolved using chloroform. Gas chromatograph (GC) analysis was performed on a stainless steel column, 2 mm I.D. x 1.83 m, packed with 3% (w/w) ECNSS-M on Gas Chrom Q 100-120 Mesh (GL Sciences, Japan). Nitrogen gas flowing at 30 ml min^{-1} was used as carrier gas with the initial column temperature of 190ºC for 5 minutes and rapidly increased to 210ºC at a rate of 1ºC/min.

Results and Discussion

The hydrolyzing experiment using crude enzyme with 10% substrate of OPEFB unbuffered condition resulted in 1.5% degree of hydrolysis after 6 hours incubation at 37°C. The degree of hydrolysis was increased significantly, and nearly 45% degree of hydrolysis was after 36 hours if the mixture condition was added with 50 mM acetate buffer pH 5 and incubated at the same temperature.

It was reported in previous research (15) that the time delay in degree of hydrolysis was due to the enzyme tend to function at low pH condition with the optimum pH around 4, while the stability in a pH range of 2.5–7.5 (6,8). Thereafter 72 hours, the hydrolysis is still in progress and could presumably attained the same hydrolysis at very much later time. Whereas in control the hydrolysis is almost complete by 72 hours with the same substrate. Furthermore, in previous result (17), we found that the □-L-AFS and E-□GAL purified enzymes also tend to function at low pH condition and exhibited maximum activity at pH 4.5 and 3.6 and retained nearly 100% activity in a pH range of 2-7 and 3-6 respectively.

Analysis of reducing-sugars and total-sugar showed that OPEFB-hydrolyzate was rich in monosaccharides but poor in oligosaccharides, which constituted less than 0.5% of the total sugar content. By the TLC analysis, it was found that the major hydrolysis products of dried-OPEFB at the spots, are monosaccharides, while oligosaccharides were not detected (Fig.1). However, except for glucose spot, the presence of other monosaccharides could not be clearly identified.

A B C

Figure 1. Result of thin layer chromatography (TLC) analysis of OPEFB-hydrolyzates. Ten percent dried-OPEFB with no buffer condition was digested by crude-enzyme 42 hours at 37ºC. OPEFB-hydrolyzate with no enzyme as control (A), product (B) and glucose standards (C) was analyzed by TLC.

To quantification of sugars component of OPEFB-hydrolyzate, GC analysis was used. GC analysis revealed that OPEFB-hydrolyzate consists of four sugars component (Fig. 2) with the sugar constituents are glucose (74.2%), xylose (21.1%), arabinose (3.6%) and mannose (1.1%).

From the hydrolysis process results show that during solid state fermentation process, *A. niger* produced more than one enzyme. Furthermore, hydrolysis showed that the cellulase enzyme is very active as indicated by the high concentration of glucose produced as monosaccharides. Some researchs reported that *A. niger* can produced wide range of enzymes including cellulase (4,7).

Enzyme production by utilizing of OPEFB through solid-state fermentation was also investigated and found to be promising in bioconversion of biomass. Also reported that highest production of cellulase was noted at acid condition (pH 4-6) and 35 - 37 degrees C, under submerged conditions (1). Growth and enzyme production was affected by variations in temperature and pH (7,8)

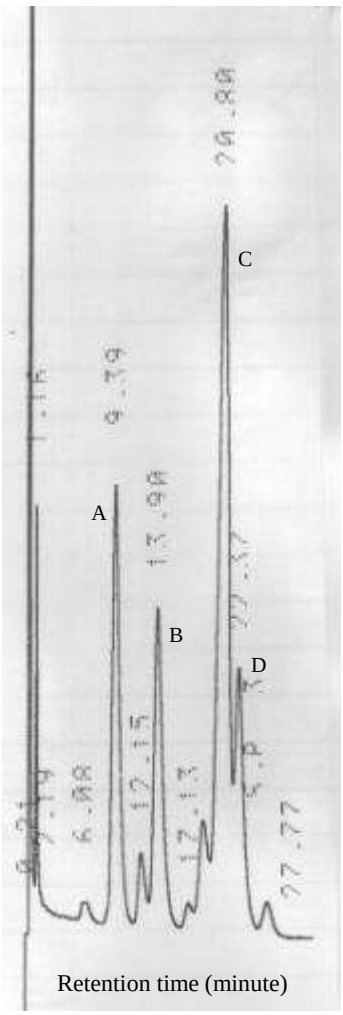

Figure 2. Chromatogram of sugar components in of OPEFB hydrolyzates which detected by Gas Chromatograph (GC). Four sugars were detected as xylose (A), arabinose (B), glucose (C) and mannose (D).

Conclusion

An extracellular enzyme from *A. niger* was used to hydrolyze OPEFB and gave the rate hydrolysis at 1.5% (756ug/ml) for 6 hours respectively. The main sugar in hydrolysate as monosaccharide was glucose with the concentration 74.2%, respectively. Suggested, much cellulase was released by *A. niger* during solid stated fermentation.

Aknowledgement

This research work was supported by Ministry of Research, Technology and Higher Education of the Republic of Indonesia under STRANAS scheme 2015.

References

[1] Sohail M., Siddiqi R., Ahmad A., Khan S. A., 2009, Cellulase production from *Aspergillus niger* MS82: effect of temperature and pH, N. Biotechnol. 25(6):437-41.

[2] Hurst P. L., Nielsen J., Sullivan P. A., and Shepherd M. G., 1977, Purification and properties of a cellulase from *Aspergillus niger*, 165(1): 33–41.

[3] Xie Y., Gao Y., Chen Z., 2004, Purification and characterization of an extracellular b-glucosidase with high transglucosylation activity and stability from *Aspergillus niger*, Appl. Biochem Biotechnol., 119(3):229-40.

[4] Andersen M. R., Giese M., de Vries R. P., Nielsen J., 2012, Mapping the polysaccharide degradation potential of Aspergillus niger, BMC Genomics., 16;13:313

[5] Martens-Uzunova E. S. and Schaap P. J., 2009, Assessment of the pectin degrading enzyme network of *Aspergillus niger* by functional genomics. Fungal Genet Biol., 46 Suppl 1:S170-S179.

[6] Guo Y., Zheng P., and Sun J., 2010, *Aspergillus niger* as a potential cellular factory: prior knowledge and key technology, Chinese journal of biotechnology, 26(10):1410-8.

[7] Acharya P. B., Acharya D. K., and Modi H. A., 2008, Optimization for cellulase production by *Aspergillus niger* using saw dust as substrate, African Journal of Biotechnology, 7:22, pp. 4147-4152.

[8] Reddy G. P. K., Narasimha G., Kumar K. D., Ramanjaneyulu G., Ramya A., Kumari B. S. S., and Reddy B. R., 2015, Cellulase production by *Aspergillus niger* on different natural lignocellulosic substrates, Int. J. Curr. Microbiol. App. Sci., 4(4): 835-845.

[9] Maria Papagianni, 2007, Advances in citric acid fermentation by *Aspergillus niger*: Biochemical aspects, membrane transport and modeling, Biotechnology Advances, 25:244–263.

[10] Nelson, N., 1944, A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. **153**, 375-380.

[11] Somogyi, M., 1952, Notes on sugar determination. J. Biol. Chem. **195**, 19-23.

[12] Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F., 1956, Colorimetric method for determination of sugars and related substances. Anal. Chem. **28**, 350-356.

[13] Arai, M. and Murao. S., 1978, Characterization of oligosaccharides from an enzymatic hydrolyzate of red yeast cell walls by lytic enzyme. Agric. Biol. Chem. **42**, 1651-1659.

[14] Spiro, G., 1972, Study of carbohydrates of glycoproteins, Vol.XXVIII. Part B. p. 1-11. Ginsburg I.V., (ed.), Methods in Enzymol. Academic Press, New York and London.

[15] Muzakhar, K., Hayashi H., Kawaguchi, T., Sumitani, J. and Arai, M., 1998, Purification and properties of

α-L-arabinofuranosidase and endo-β-D-1,4-galactanase from *Aspergillus niger* van Tieghem KF-267 which Liquefied the Okara. p. 134-143. In K. Ohmiya *et al*. (ed.), Genetics, Biochemistry and Ecology of Cellulose Degradation. Proceed. MIE BIOFORUM 98, Suzuka Japan. Uni Publisher Co., LTD. Tokyo Japan.