International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2015): 6.391

Effect of Physical Pretreatment on Microstructure of Cassava Stem Fibers and *Aspergillus niger* FNCC 6114 Growth through Solid State Fermentation

Pramesti Dewi[1], Ria Millati[2], Retno Indrati[3], Sardjono[4]

[1]Biology Department, Faculty of Mathematics and Natural Science, Universitas Negeri Semarang, Semarang, Central Java, Indonesia

[2, 3, 4]Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta, Indonesia

Abstract: ***Aims: To investigate the influence of physical pretreatment methods toward microstructure of cassava stem fiber and Aspergillus niger FNCC 6114 growth through solid state fermentation. Methodology and Results: The potential of cassava stems as raw materials for bioconversion into valuable products can be monitored through the feasibility test as a growth medium for microorganism used. The feasibility test as growth medium for Aspegillus niger FNCC 6114 was done by evaluating precise pretreatment method to cassava stem. The cassava stem were pretreated physically (size reduction). The pretreated cassava stem was used as solid substrate for growth of Aspegillus niger FNCC 6114. The effect of physical pretreatment method on microstructure of cassava stem fiber was evaluated through SEM micrograph. Furthermore, during the fermentation period, growth and metabolism activities of Aspegillus niger FNCC 6114 were monitored through SEM micrograph of cultured media at 6th days fermentation, changes in glucosamine and reducing sugar levels, as well as the number of spores. Results of this research was: Smaller size of cassava stem gave better growth of Aspergillus niger FNCC 6144 based on higher level of glucosamine and reducing sugar, and also amount of spores . Conclusion, Significance and Impact of study: Aspergillus niger FNCC 6114 was able to grow on cassava stems through solid state fermentation. For better growth of Aspergillus niger FNCC 6144 cassava stems should be physically pretreated. It can be suggested that cassava stem in small size could be used as raw material for bioconversion into sugar-based products using Aspergillus niger as its inoculum. This positive effect is also important in relation to the utilization of lignocellulosic waste from agroindustry.***

Keywords: cassava stem, physical pretreatment, *Aspergillus niger*, solid state fermentation

1. Introduction

Cassava stems are one of the most abundant agroindustrial lignocellulosic wastes in the world. This cassava stem contains cellulose and hemicelluloses, and small amount of lignin. These can be converted into simple sugars and further into valuable products by suitable microorganisms.

Utilization of cassava stem requires pretreatment to remove physical and chemical barriers caused by lignin. Pre-treatment is a key step in the bioconversion process of lignocellulose. The possitive effect of pretreatment in improving efficiency of bioconversion is due to easier access of microorganism involve in the cellulose, hemicelluloses and/or lignin release process (Jönsson & Martín, 2016).

Physical pretreatment by reducing the size of biomass can decrease cellulose crystalinity and improve digestibility. The size reduction method can be a combination of chipping, grinding, and/or milling (Kumar *et al*., 2009). Decreasing particle size will increase the accessibility of polymer components in depolymerization processes, both chemically and biochemically (Shi *et al*., 2009).

However, Wan & Li (2010) in Meehnian *et al*., (2016) stated that small size of biomass particle will inhibits oxygen penetration and hampers fungal growth due to reducing distance of particles. In contrast, large particle size gives an inhibitory effect on the ease of accessing nutrients. In other word, particle size will give impact on fungal growth and metabolism. There are less publication about the effect of particle size media on fungal growth, especially *Aspergillus niger.*

The ability of *Aspergillus niger* to grow in lignocellulosic biomass is based on its ability to produce enzymes such as cellulases, hemicellulases and ligninolytic enzymes (Reddy *et al*., 2015; Pensupa *et al*., 2013; Ibrahim *et al*., 2012; and Dhakar *et al*., 2015) . Appropriate pretreatment process will increase the ability of *Aspergillus niger* to grow (Salihu *et al*., 2015; Sridevi *et al*., 2015)

The purpose of this study is to investigate the influence of physical pretreatment method, i.e size reduction toward microstructure of cassava stem fibers and *Aspergillus niger* FNCC 6144 growth through solid state fermentation.

2. Materials and Methods

Microorganism and Starter Powder Preparation

Aspergillus niger FNCC 6114 was obtained from Laboratory of Biotechnology, Faculty of Agricultural Technology, Universitas Gadjah Mada as a pure culture on agar slant. Cassava stem obtained from Sleman, Yogyakarta. Firstly, the culture was grown on PDA for 5-days, and then the resulting spores were harvested using 0,05 % Tween 80 and used in starter powder preparation.

Starter culture medium contain rice bran, rice grain and cassava stem powder in a ratio of 1 : 1 : 2. Rice grain (50 g) was previously cooked in distilled water (50 ml) which was added with 0.25 ml lactic acid for avoiding bacterial growth. The cooked rice was mixed with 50 g rice bran and 100 g

cassava stem powder and sterilized at 121 ^{o}C for 30 min. This medium was then inoculated with spore suspension and incubated at 30 ^{o}C for 6 days. After that the culture media were dried, ground into rough powder and ready for use as inoculums powder.

Inoculums powder contained 3.5 x 10^9 spores/g and 0,003 g of inoculum was inoculated into 30 g sterilized cassava stem (0.01%, w/w) to reach 10^5 spores density. The remaining inoculums were packed with resealed plastic bag and stored in refrigerator for further use.

Substrate Preparation and Pretreatment
Cassava stem were chopped and dried in cabinet drier until 10 % water contain reached, followed by grinding by hammer mill into fine (0.149-0.297 mm) and coarse (4.76 – 9.51 mm) particle size. These pretreated cassava stems were ready to use for solid substrate.

Solid State Fermentation
Solid state fermentation was done on a 300 mL plastic boxes (6 x 8.5 x12 cm3). The bottoms of boxes were perforated in 1 mm diameter for each 5-10 cm distance. Every box filled with 30 g sterilized pretreated media with a thickness of 1.8 cm. The moisture content were maintained to be 53 % by addition of 30 mL distilled water before sterilization. These media were inoculated with 10 % (w/w) diluted inoculums powder. Every box covered with sterilized paper.

Fermentations were carried out for 7 days in covered plastic boxes incubator. Sampling was done every 24 h by taken 1 box of pretreated media. Humidity inside the incubators were maintained by putting a beaker glass of water.

Effect of pretreatment on microstructure of cassava stem fibers

These effect were observed by Scanning Electron Microscope (SEM) micrograph againts pretreated media before fermentation. A powdered sample of uncultured media were mounted on to brass stubs using double-sided adhesive carbon tape. A gold-paladium coating was done by using sputtering tools. After that the samples were examined with SEM (FEI, Type Inspect S50) at 15kV, high vaccuum and 9.6 mm distance according to the Standard Operating Procedure of SEM analysis, Central Laboratory, State University of Malang, Indonesia. The SEM result was used for explaining growth differences of *Aspergillus niger* on pretreated cassava stem media.

Effect of physical pretreatment on *Aspergillus niger* FNCC 6114 growth

Effect of physical pretreatment on *Aspergillus niger* FNCC 6114 growth also evaluated using SEM micrograph of cultured media at 6th days fermentation, glucosamine and reducing sugar analysis, and also spores quantity produced per gram media.

a) SEM micrograph at 6th days fermentation
Cultured media at 6th days fermentation were dried and prepared for SEM analysis to observe mycellium growth after inoculation of *Aspergillus niger* FNCC 6114. A powdered sample of cultured media were mounted on to brass stubs using double-sided adhesive carbon tape. A gold-palladium coating was done according to the method mentioned above and SEM analysis was done at 20 kV, high vaccuum and 10 mm distance.

b) Glucosamine
The biomass of *Aspergillus niger* FNCC 6114 in media during fermentation was estimated by determining glucosamine content. The glucosamine content was measured by the method of Jahromi *et al*. (2011) (Jahromi et al., 2011) based on colorimetry at λ =530 nm. The sample was dried previously in oven at 50 ^{o}C, milled with waring blender, and weighed to 0.2 g for each.

c) Reducing sugar
Metabolic activity of *Aspergillus niger* FNCC 6114 in utilizing cellulose and hemicelluloses was estimated by determining reducing sugar content. The reducing sugar content was measured spectrophotometrically by the method of Miller (1959) using DNS reagent. Sample (1 g) in sterile plastic bag was added to 50 mL distilled water, homogenized in stomacher for 60 sec and filtered. The filtrate was used as a sample in spectrophotometric assay.

d) Spores quantity
Cultured media (1 g) was added to 9 mL of 0.1 % Tween 80 solution, mixed thoroughly, and then calculated for spores content using Neubauer haemacytometer under light microscope (Olympus BX 41) at magnification of 520 x.

Statistical Analysis
All data were collected through duplicate measurements and analyzed using the Excell Program (Microsoft). Average result were expressed as the mean ± standard deviation.

3. Result and Discussion

Effect of physical pretreatment on microstructure of cassava stems fibers
Figure 1 shows the differences of cassava stem microstructures after physical pretreatments observed with SEM analysis at magnification of 1000 x. Physical pretreatment by reducing cassava stem size (into fine and coarse size) have a real impact in the fibers microstructure (1A and 1B).

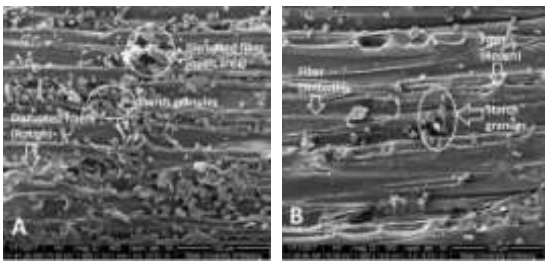

Figure 1: SEM analysis (at magnification of 1000 x) of cassava stem after physical pretreatment (A. Fine particle size; B. Coarse particle size)

The fine-sized cassava stem caused the formation of more rough stripes, this resulted in brokening and opening the structure (Figure 1A). This condition will bring up the starch

granules that previously buried on the structure. As a result, the growth of *Aspergillus niger* FNCC 6114 increased.

On the contrary, fibers microstructure of coarse-sized particle exhibited more smooth fibers than rough ones (Figure 1B). This indicated that the fibers just a little bit disrupted. Starch granules still buried and the growth of *Aspergillus niger* FNCC 6114 hampered.

Effect of pretreatment on the growth of *Aspergillus niger* FNCC 6114

a) SEM micrograph at 6th days fermentation

Figure 2 shows SEM micrograph of cultured media at 6th days fermentation that mycelium were abundant in coarse-sized media. This mean that the growth of *Aspergillus niger* FNCC 6114 was better in coarse-sized media than fine-sized at 6th days of incubation.

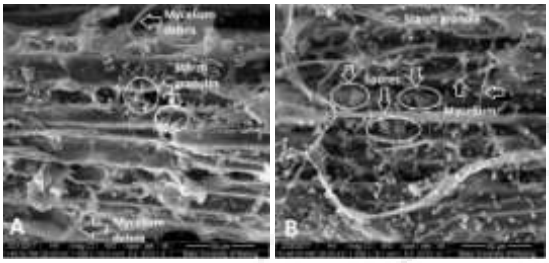

Figure 2: SEM analysis (at magnification of 1500 x) of cassava stem after 6 days fermentation (A. Fine particle size; B. Coarse particle size)

Figure 2 also indicated that after fermentation starch granules were reduced rapidly. These starch (amylum) used for *Aspergillus niger* metabolism. This result correspond to the research result obtained by Pooja & Padmaja (2015). Starch granules in fine-sized media were more abundant at the beginning and still observed after fermentation (Figure 2A). As an opposite, Figure 2B showed that starch granules just a few and spores appeared plentiful.

b) Glucosamine content

The result of glucosamine analysis was shown on Figure 3. Glucosamine obtained from fine size media was higher than that from coarse size media. This result was in line with the dense mycelia growth of *Aspergillus niger* according to visual observation (data not shown). Glucosamine was a component of mycelium or fungal cell wall. The higher the glucosamine is, the better the growth of fungi.

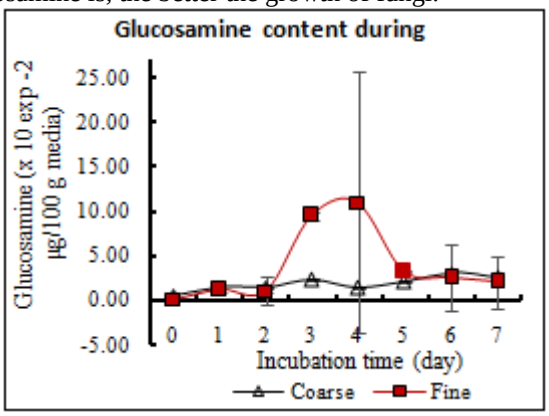

Figure 3: Glucosamine content during fermentation of cassava stem by *Aspergillus niger* FNCC 6114. Effect of physical pretreatment (fine & coarse)

The highest value of glucosamine was reached from fine size media at 4 days of incubation, which was 10.8785 x 10^{-2} µg/100 g media (dry basis). This value was higher than that of coarse size, which was 1.5285 x 10^{-2} µg/100 g media (dry basis). This may cause by the surface area of fine size media was more opened (Figure 2), this resulting in the better growth of *Aspergillus niger* caused by the availability of amylum granules. The growth of *Aspergillus niger* will induced the biochemical depolymerization of cassava stem components (Shi *et al*., 2009).

Glucosamine content in 6th days of incubation shows that a little bit higher in coarse media than fine one. This result was in line with the better growth of *Aspergillus niger* (Figure 2). From this research it was assumed that on 4th days of incubation *Aspergillus niger* grown heavily (SEM data was not shown).

c. Reducing sugar content

Figure 4 shows that sugar content was higher in fine media. This result in line with research result obtained by Meehnian *et al*. (2016) using cotton stalk. Particle size reduction of cotton stalk increased surface area encouraging accessability of hollocellulose for enzymatic reaction by the fungi. The products of enzymatic reaction will be used for further fungal growth. Meanwhile, the maxium level from reached at 2 days of incubation, which was 3.341 % (w/w, dB).

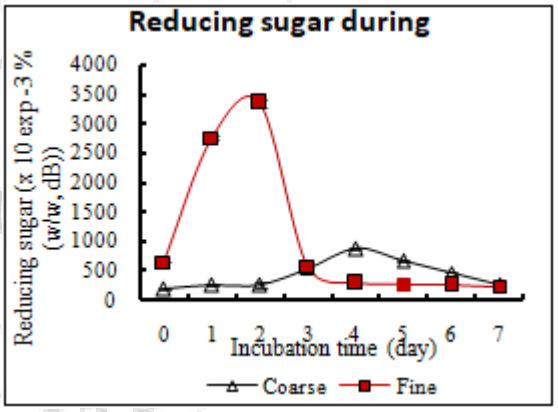

Figure 4: Reducing sugar content during fermentation of cassava stem by *Aspergillus niger* FNCC 6114. Effect of physical pretreatment (fine and coarse)

Furthermore observation on glucosamine content (Figure 3) compared to sugar content (Figure 4) indicate that at 2 days of incubation on fine size media, reducing sugar formation reached maximum level although glucosamine level was not at maximum level. This was in accordance with the Desai & Converse (1997) statement (in Sridevi *et al*., 2015) that polisaccharides breakdown into reducing sugar will be at maximum rate in early state of incubation since there still an amorf region of polisaccharides on the media.

c. Spores quantity

Number of spores from two kinds media during 7 days fermentation can be shown in Figure 5. Mycelium started to rise at 2 days of incubation (data not shown), and followed by sporulation. Fermentation will be more efficient if sporulation delayed (Nicolás-santiago *et al*., 2006). If the fermentation of cassava stem can be efficient then it will encourage efficient utilization of lignocellulosic biomass.

The level of sporulation increased with increasing day of incubation until reached maximum at 5th days. Pretreatment methods used were affect the spores quantity.

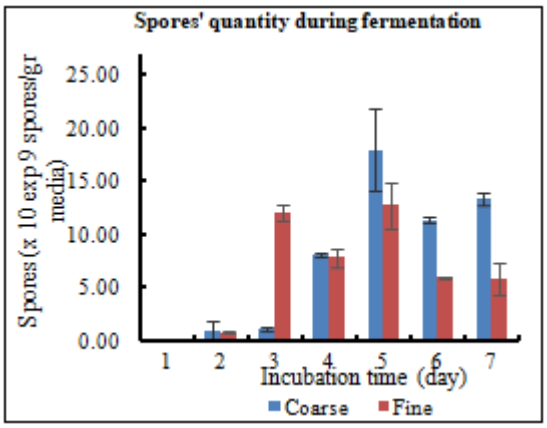

Figure 5: Spore's quantity during fermentation of cassava stem using *Aspergillus niger* FNCC 6114. Effect of physical pretreatment (fine and coarse)

Sporulation level in fine-sized media was lower than the coarse one. This data was in line with SEM micrograph at 6th days of incubation (Figure 2B). Currie (1917) stated that fermentation will conduct optimally when there are no spores formed, and mycelium will remain white. It was also indicated that fine-sized media was more suitable for fermentation to produce valuable product.

4. Conclusion

Based on the research result, it was known that *Aspergillus niger* FNCC 6114 able to grow on cassava stem through solid state fermentation and the better growth was on the media of fine particle size.

5. Acknowledgement

This publication is a part of the doctoral dissertation research of the first author. The authors are grateful for financial support of BPPS scholarship doctoral degree from the Indonesian Government through Directorate General of Higher Education (*DIKTI*). The authors are also grateful to Mr. Purwito and Mr. Anang Juni Yastanto for the kindness and technical support during the research.

References

[1] **Currie, J. (1917)**. The citric acid fermentation of *Aspergillus niger*. *The Journal of Biological Chemistry*, *xxx1*(1).

[2] **Dhakar, K; Kooliyotil, R; Joshi, A & Pandey, A. (2015).** Simultaneous production of ligninolytic enzymes by a temperature and pH tolerant strain of *Aspergillus niger* under different cultural conditions. *Indian Journal of Biotechnology*, *14*(January 2015), 81–86.

[3] **Ibrahim, D., Puspitaloka, H., Rahim, R. &, & Hong, L. (2012).** Characterization of Solid State Fermentation Culture Conditions for Growth and Mananase Production by *Aspergillus niger* USM F4 on Rice Husk in Tray System. *British Biotechnology Journal*, *2*(3), 133–145.

[4] **Jahromi, M. F., Liang, J. B., Rosfarizan, M., Goh, Y. M., Shokryazdan, P. &, & Ho, Y. W. (2011).** Efficiency of rice straw lignocelluloses degradability by Aspergillus terreus ATCC 74135 in solid state fermentation. *African Journal of Biotechnology*, *10*(21), 4428–4434. doi:10.5897/AJB10.2246

[5] **Jönsson, L. J., & Martín, C. (2016).** Pretreatment of lignocellulose : Formation of inhibitory by-products and strategies for minimizing their effects. *Bioresource Technology*, *199*, 103–112. doi:10.1016/j.biortech.2015.10.009

[6] **Kumar, P., Barrett, D. M., Delwiche, M. and, & Stroeve, P. (2009).** Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production. *Industrial & Engineering Chemistry Research*. doi:10.1021/ie801542g

[7] **Meehnian, H., Jana, A. K., & Jana, M. M. (2016)**. Effect of particle size , moisture content , and supplements on selective pretreatment of cotton stalks by Daedalea flavida and enzymatic saccharification. *3 Biotech*, *6*(235), 1–13. doi:10.1007/s13205-016-0548-x

[8] **Nicolás-santiago, S. D., Regalado-González, C., García-almendárez, B., & Téllez-jurado, A. (2006).** Physiological , morphological , and mannanase production studies on Aspergillus niger UAM-GS1 mutants, *9*(1). doi:10.2225/vol9-issue1-fulltext-2

[9] **Pensupa, N., Jin, M., Kokolski, M., Archer, D. B., & Du, C. (2013)**. A solid state fungal fermentation-based strategy for the hydrolysis of wheat straw. *Bioresource Technology*, *149*, 261–267. doi:10.1016/j.biortech.2013.09.061

[10] **Reddy, G. P. K., Narasimha, G., Kumar, K. D., Ramanjaneyulu, G., Ramya, A., Kumari, B., & Reddy, B. (2015)**. Original Research Article Cellulase production by Aspergillus niger on different natural lignocellulosic substrates, *4*(4), 835–845.

[11] **Salihu, A., Abbas, O., Balarabe, A., & Zahangir, S. (2015)**. Agricultural residues for cellulolytic enzyme production by Aspergillus niger : effects of pretreatment. *3 Biotech*, 1101–1106. doi:10.1007/s13205-015-0294-5

[12] **Shi, J., Hodge, D., Pryor, S., & Li, Y. (2009)**. Pretreatment of Lignocellulosic Biomass. *BEEMS Module B1*.

[13] **Sridevi, A. et al, Narasimha, G; Ramanjaneyulu, G., Dillepkumar, K., Reddy, B., & Devi, P. (2015).** Saccharification of pretreated sawdust by Aspergillus niger cellulase. *3 Biotech*, 883–892. doi:10.1007/s13205-015-0284-7