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Efficiency of Cellulase Production Using Coffee Pulp Waste under Solid State Fermentation by *Aspergillus* sp. VT12

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Abstract. Activities in the production of coffee beans cause abundant coffee pulp wastes in many areas in Indonesia which can be an environmental problem. In contrast, this a huge agricultural waste can potentially be used through microbial utilization because of its cellulose polysaccharides content up to 63%. The fungus, known as cellulase producer *Aspergillus* sp. VT12 under solid-state fermentation (SSF) using coffee pulp based has been isolated. Investigation showed that optimum cellulase production by this isolate when SSF of 10 g coffee pulp, inoculated with 10^8 spores/ml, incubated at 30°C for 120 hours was done. Cellulase activity reached 1,8 U/ml based on reducing sugar released assayed at 37°C for 96 hours against 0,5% carboxymethyl cellulose (CMC) substrate in acetate buffer 20 mM pH 5. This cellulase stable at pH range i.e. pH 3-8 and optimum at pH 7. In this preliminary study of microbial utilisation of coffee pulp is a cheap-way to produce cellulase and may efficiently because without any nutrition either mineral added in the medium during production.

INTRODUCTION

Indonesia is the 4th largest country in the world which produced coffee after Brazil, Vietnam, and Columbia, i.e. 612.000 tons [1]. Coffee is the second largest traded commodity in the world and it generates large amounts of by-products, residues, and waste during processing. The first by-product that produced by wet processing is coffee pulp [2]. Amount of coffee pulp waste is up to 50% from coffee weight that processed. The organic components present in coffee pulp is rich in carbohydrates, especially cellulose, proteins, minerals, and it also contains appreciable amounts of tannins, caffeine, and polyphenols. The presence of tannins and caffeine in coffee pulp, if it is not utilized immediately, environmental pollution can occur [3]. The efficient utilization and value-addition of agro-industrial residue such as coffee pulp has been an increasing trend in recent years. One of agro-industrial residues application in bioprocess is as an alternative substrate for enzyme production and the other side, it helps to solve pollution problem [4].

Coffee pulp has high potency as a substrate to produce cellulases, because of the highest presence component in coffee pulp is cellulose (63%) [2]. The cellulase system in fungi is a group of hydrolytic enzymes that consists of endo-(1,4)-β-D-glucanase, exo-(1,4)-β-D-glucanase, and β-glucosidase. The complex enzymes work synergistically and have an important role in hydrolyzing cellulose to glucose as source carbon to the life cycle and microorganism growth [5,6,7]. Cellulase is often used in many industries purpose like food and brewery production, animal feed processing, textile processing, paper pulp manufacture, detergent processing, biorefinery, among others [8,9].

One of the microorganism which has been known can produced cellulase was *Aspergillus* sp. VT12. This isolate was obtained from the vermicomposting process of oil palm empty fruit bunches. *Aspergillus* sp. VT12 has high cellulolytic activity at carboxymethyl cellulose (CMC) and oil palm empty fruit bunches substrates namely by producing the reducing sugar respectively 10,18 µg/ml and 4,4 µg/ml [10]. Therefore, *Aspergillus* sp. VT12 has high potential to produce cellulase with coffee pulp which has high cellulose content (63%). The suitable method in this study is solid-state fermentation because this method uses the culture of fungi so it has a higher capacity to produce enzyme [11].

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This study is expected to be an alternative way for cellulase production that obtainable from the utilization of coffee pulp, so that the cellulase production can be efficiently and can help to solve pollution problem.

MATERIALS AND METHOD

Inoculum Preparation

Aspergillus sp. VT12 was pre-cultured on Potato Dextrose Agar medium and it was incubated at 30°C for 72 hours. After that, the isolate was cultured on M9+coffee pulp extract medium and it was incubated at 30°C for 0-168 hours. Then the density of spores were counted using Haemocytometer every 24 hours. It was supposed to know the spores density of *Aspergillus* sp. VT12 which reached 10^8 spores/ml as ideal inoculum for cellulase production [12], the density of spores was counted using Haemocytometer every 24 hours for 0-168 hours.

Solid-State Fermentation (SSF)

Solid-state fermentation was performed in 250 ml erlenmeyer flasks which contained 10 g of coffee pulp as a substrate with 20 ml water content (1:2). The flasks were sterilized at 121°C for 25 minutes and then cooled to room temperature. Inoculum (*Aspergillus* sp. VT12 which have reached 10^8 spores/ml) was added 1 ml on SSF medium and it incubated at 30°C for 0-168 hours [13,14].

Harvesting and Extracting of Crude Enzyme

The crude enzyme was harvested every 24 hours. Harvesting the crude enzyme was started by adding 20 ml of aquades contained 0,01% Na Azide and 1% NaCl and incubating at rotary shaker (120 rpm) for 12 hours. The crude enzyme was extracted with filtration and centrifugation at 8000 rpm for 5 minutes. The supernatant was used as a source of the extracellular enzyme [13,15], then it was kept at 4°C [16].

Cellulase Activity Assay (Somogyi-Nelson)

The reducing sugar of crude enzyme was measured by Somogyi-Nelson method and verified with 0,5% carboxymethyl cellulose (CMC) substrate in acetate buffer 20 mM pH 5 to know the highest enzyme activity at optimum incubation time. 500 µl of CMC was incubated at 37°C for 20 minutes on waterbath. Then it was added with 50 µl of crude enzyme and incubated at 37°C for 2 hours. Somogyi reagent as much as 500 µl was added in the sample then it was boiled for 15 minutes. After the sample was cold, 500 µl of Nelson and 2.5 ml of H_2O were adding in it. The absorbance of the sample was measured using spectrophotometer with wavelength 500 nm. The absorbance value was converted to glucose standard curve formula to know the value of reduction sugar, then it was converted to enzyme activity formula to know the activity of cellulase [17].

Optimization and Stabilization of pH

To select the suitable pH for optimum and stability enzyme activity, used pH range 3-8 [18] (pH 3-5 of acetate buffer and pH 5.5-8 of phosphate buffer) by keeping all other parameters constant. The mix of enzyme and buffer were incubated at 37°C for 4 hours for stability treatment, but not for optimization treatment and then the next method as same as enzyme assay [15].

RESULT AND DISCUSSION

Spores Density of *Aspergillus* sp. VT12

Aspergillus sp. VT12 could reach the density 10^8 spores/ml of spores starting from 96 hours incubation time, exactly $2{,}06\times10^8$ spores/ml. The spores density of *Aspergillus* sp. VT12 increased significantly from 48 hours to 168 hours. Thus, the incubation time that used to cellulase production was 96 hours, because it has reached 10^8 spores/ml which as ideal inoculum under solid-state fermentation [12]. *Aspergillus* can grow well in an environment which is

relatively acid to alkaline [14], so the spores of *Aspergillus* sp. VT12 increase from day to day. The correlation between spores density and incubation time of spores calculation showed in Figure 1.

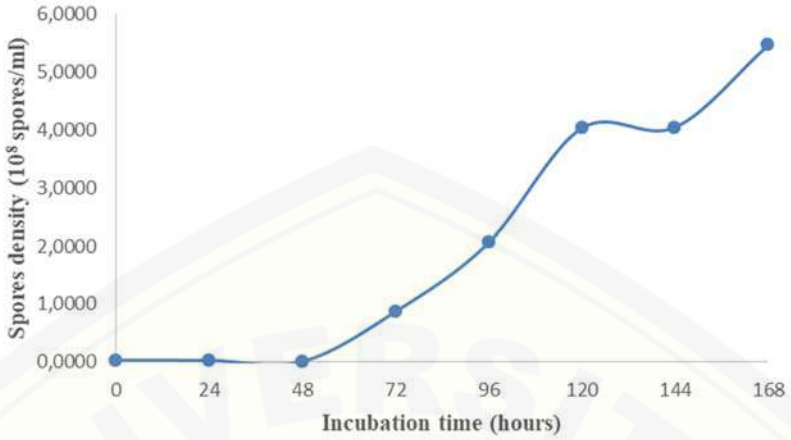

FIGURE 1. Spores density of *Aspergillus* sp. VT12 represented increasing from 48 hours to 168 hours

Optimization of Cellulase Production

Optimization of cellulase production used 10^8 spores/ml of *Aspergillus* sp. VT12 at 96 hours incubation time. The optimum incubation time that produces the highest reducing sugar 231,057 µg/ml was at 96 hours. The cellulase activity of *Aspergillus* sp. VT12 on this incubation time was 1,8 U/ml and it was the optimum enzyme activity (Figure 2). *Aspergillus* sp. VT12 used cellulose of coffee pulp as a carbon source with the help of cellulase to hydrolyze cellulose to glucose. Most of fungi used glucose and sucrose as the most suitable carbon sources, so *Aspergillus* sp. VT12 can grow well on coffee pulp substrate because of the highest component of coffee pulp is cellulose (63%) [2,19]. The enzyme activity at 120-168 hours incubation times were up and down, that might because the crude enzyme become unstable if it left too long in the fermentation medium. The increase that followed by the decrease of enzyme activity was caused by changes on the enzyme conformation in such substrate. One of caused change of the enzyme conformation is pH [20]. The enzyme activity was decrease at 120 and 168 incubation times, it was suspected due to decreasing of coffee pulp concentration in the fermentation medium, so cellulase that secreted by *Aspergillus* sp. VT12 also decreased [21].

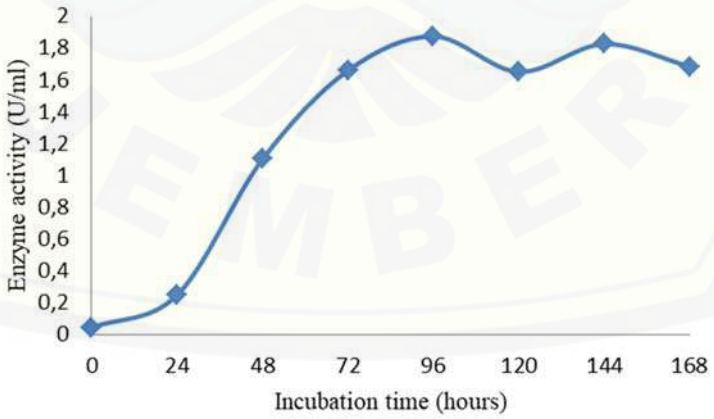

FIGURE 2. Cellulase activity of *Aspergillus* sp. VT12

Optimization and Stabilization of pH

Production of cellulase is affected by some factors, such as pH. Some microorganism can survive at various pH, because the pH may change from the initial scale during the fermentation process. Figure 3 showed that the optimum enzyme activity was at pH 7 and it was stable at pH 3 to 8 (above 75% of relative enzyme activity). pH has a strong effect on enzyme activity, the higher pH the higher enzyme activity [20]. The cellulase activity was not much different on each pH at the same temperature (37°C). Thus, it can be concluded that the cellulase production by *Aspergillus* sp. VT12 during fermentation on coffee pulp has wide pH range. The cellulase enzyme can be active at the pH range 3 to 9 [22].

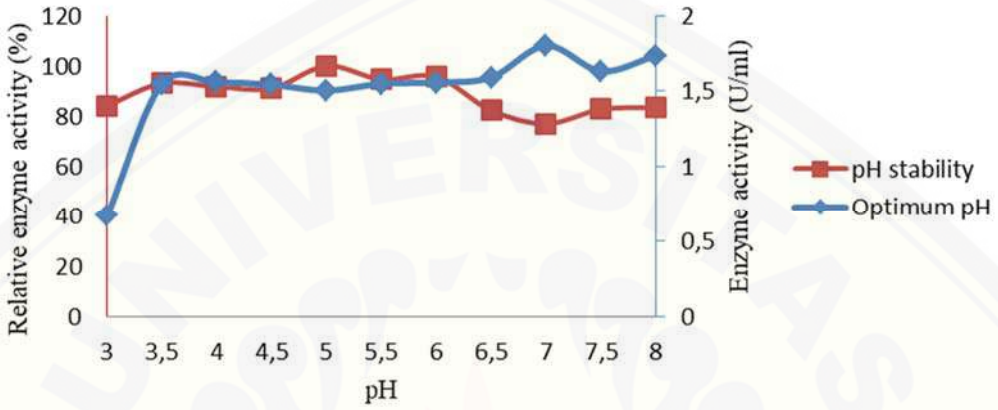

FIGURE 3. Effect of pH on cellulase production

CONCLUSION

The result showed that the best incubation period for cellulase production of *Aspergillus* sp. VT12 during fermentation on coffee pulp was 96 hours (1.8 U/ml) and it was stable at wide pH range 3 to 8 with the optimum activity at pH 7. So that cellulase production can be efficient because without any nutrion either mineral added in the medium during production, it can obtain high activity enzyme and help to solve pollution problem from the utilization of coffee pulp waste.

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