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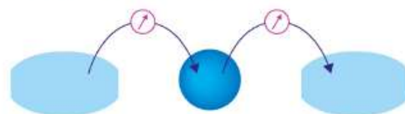

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Utilization of Coffee Pulp as a Substrate for Pectinase Production by *Aspergillus* sp. VTM5 Through Solid State Fermentation

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Abstract. Coffee pulp is an agro-industrial waste that has an abundant presence in Indonesia, up to 612.000 tons in 2018. Up to 6,5% of coffee pulp's dry weight is pectin. So, the coffee pulp has potential as a substrate for pectinase production. This study aims to acknowledge the utilization of coffee pulp for pectinase production using a solid-state fermentation method by *Aspergillus* sp. VTM5. Spore suspensions of *Aspergillus* sp. VTM5 with 10^8 spores/ml density are inoculated to coffee pulp substrate and incubated for 0 to 168 hours at 30°C. Characterization of pH testing with 250 µl of crude pectinase plus 250 µl of acetate buffer (pH 3-5) and phosphate buffer (pH 5.5-8). Enzyme activity assay using the Somogyi-Nelson Method. Optimum incubation time Aspergillus sp. VTM5 in producing pectinase is at 72 hours and the reducing sugar concentrations are 85,849 µg/ml. The optimum pH for pectinase activity is 4,5 and stable at pH 3-5. These results indicate that coffee pulp can be utilized as a substrate for pectinase production using a solid-state fermentation method by *Aspergillus* sp. VTM5. Purification of pectin content in the coffee pulp, purification, and characterization of the enzyme is needed to improve enzyme activity.

INTRODUCTION

Coffee is one of the leading export commodities in Indonesia, the amount of which according to International coffee organization export data, in 2018 [1] is up to 612,000 tons. Coffee production produces the main byproduct of coffee pulp by 40% of its wet weight. The abundant amount of coffee pulp with less utilization is a threat and a source of pollutants for the environment [2], [3]. The pectin content (6.5%) in the coffee pulp has the potential to use it as a substrate for pectinase production [4].

Pectinase is a group of enzymes that break down pectin through enzymatic reactions into simpler molecules. Pectinase catalyzes the degradation of pectin through depolymerization (hydrolase and lyase) and de-esterification (esterase) reactions [5]. Pectin esterase is an enzyme that catalyzes the de-esterification of the methyl ester bond in pectin. Hydrolase is an enzyme that catalyzes the hydrolysis of the α-1,4 glycosidic pectic acid and pectin bonds. Pectin lyase catalyzes the process of breaking the α-1,4 glycosidic bond of pectin acid through a trans-elimination reaction and forms unsaturated galacturonic acid and methyl galacturonate [6]. The ability to break down pectin is often needed by various industries such as the textile industry, food, the bioethanol industry, pre-treatment for liquid waste before environmental discharges, and paper industry [7]. Based on the total enzyme production in the market as much as 10% is the production of pectinase [5] and 25% of pectinase production is used in the food industry [7].

The main source of industrial enzyme production is microorganisms, including 50% derived from fungi and yeast while 35% derived from bacteria [8]. Pectinase production is widely used from fungi because it has a high yield value and can withstand the conditions of mild environmental changes, such as temperature, pH, and pressure [9]. One of the fungi that are widely used in enzyme production is *the Aspergillus* genus [10]. *Aspergillus* sp.VTM5 is a fungus that isolated from the waste of the Palm Oil Empty Fruit Bunch (POEFB) [11].

Pectinase production in this study uses the Solid-state fermentation method. Solid-state fermentation (SSF) is a fermentation process that occurs in solid material and with almost no free water (free liquid). However, the substrate

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must have enough moisture to support the growth and metabolic activity of microorganisms. The advantages of this method are higher productivity, high product concentration, simple methods, and the possibility of low contamination [12]. Besides, the advantages of SSF are low production costs, low energy requirements, as well as less liquid waste, generated [13]. This study aims to acknowledge the utilization of coffee pulp as a substrate for low-cost pectinase production from *Aspergillus* sp.VTM5 using a solid-state fermentation method.

MATERIAL AND METHOD

Inoculum preparation

Aspergillus sp.VTM5 subcultured on Potato Dextrose Agar slant for 72 hours (3 days) as pre-culture. After 3 days *Aspergillus* sp.VTM5 was inoculated to M9+coffee pulp extract 0,5% slant and incubated for 0-168 hours at 30°C. The spore was harvested by adding 5 ml aquadest sterile. The spore suspension was counted using a haemacytometer to get 10^8 density [17].

Coffee pulp extraction

Coffee pulp extraction in this study was modified from [14]. Coffee pulp (150 g) were delignification using 30 g NaOH with 500 ml of distilled water and then homogenized using a magnetic stirrer for 24 hours. The mixture is filtered and adjusted the pH to 7 (neutral) with the addition of acetic acid (CH_3COOH). It was precipitated using 97% alcohol in a ratio of 4:6 (filtrate: alcohol) and then centrifuged for 10 minutes at 8000 rpm. The pellet is dried at 50°C. This coffee pulp extract is used as a media substance for *Aspergillus* sp. VTM5.

Optimization of pectinase production

Solid-state fermentation (SSF) substrate is made by adding distilled water to 10 grams of coffee pulp substrate [15]. Suspension of *Aspergillus* sp. VTM5 spores with a density of 10^8 spores/ml of 1 ml were inoculated in 10 g of SSF media and incubated for 0-168 hours at 30°C. A crude enzyme of pectinase was harvested every 24 hours by adding 20 ml of H_2O containing 1% NaCl and 0.01% Na-Azide and shaker for 12 hours. Every culture was centrifuged at 8000 rpm for 5 minutes. Filtrate enzyme activity was tested using the Somogyi-Nelson method [16].

Characterization of pectinase enzyme

Enzyme (250 µl) plus 250 µl acetate buffer 20 mM pH 3,5; 4; 4,5; 5 and phosphate buffer 20 mM pH 5,5; 6; 6,5; 7; 7,5; 8. Each pH variation for enzyme pH stability assay was incubated for 4 hours at 37°C. The assay for optimum pH was carried out without 4 hours incubation. Each sample was analyzed by the Somogyi-Nelson method without substrate incubation for 20 minutes [17].

Pectinase activity assay

Pectinase activity is calculated from the reducing sugars produced in hydrolyzing the pectin substrate. The method used is Somogyi-Nelson that modified [19]. The crude enzymes were used 50 µl, Somogyi reagent (500 µl), and Nelson reagent (500 µl).

RESULT AND DISCUSSION

Inoculum preparation

Spore calculation is performed to determine the incubation time required for *Aspergillus* sp. VTM5 in producing spores with a certain density as an inoculum. The known spore density is then used to source the inoculum in the pectinase production process. The density of spores *Aspergillus* sp. VTM5 in the M9 + medium of 0.1% coffee fruit soft skin extract can be seen in the following curve (Figure 1)

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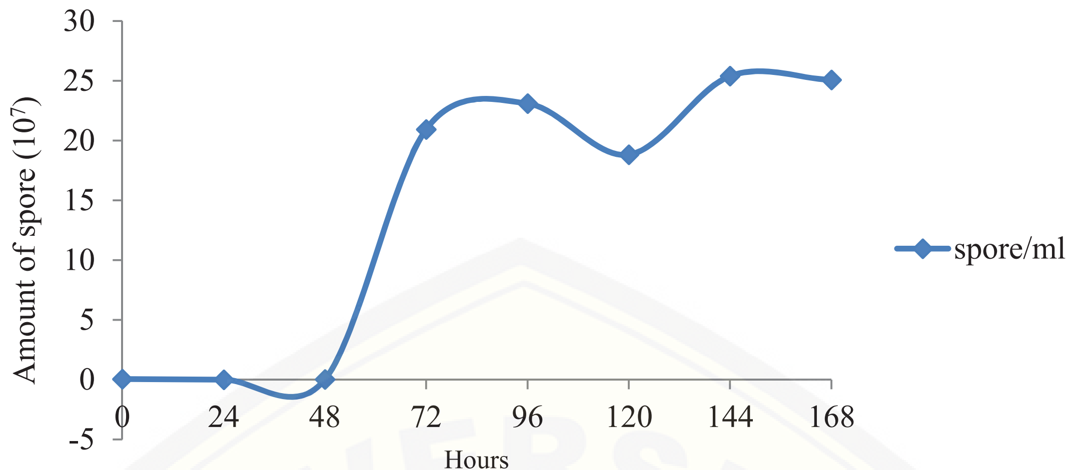

FIGURE 1. Effect of incubation time for spore production by *Aspergillus* sp. VTM5

The density of spores used as inoculum was 10^8 spores/ml in which *Aspergillus* sp.VTM5 reached the spore density at 72-hour incubation by 2.1 x 10^8 spores/ml. According to [19] spore density of 10^8 can produce enzymes with high yield values in solid media than lower density.

Optimization of enzyme production

Optimization of enzyme production was carried out to determine the optimum incubation time of *Aspergillus* sp. VTM5 in producing pectinase. To find the optimum time by using reducing sugar as a reference like the following curve (Figure 2):

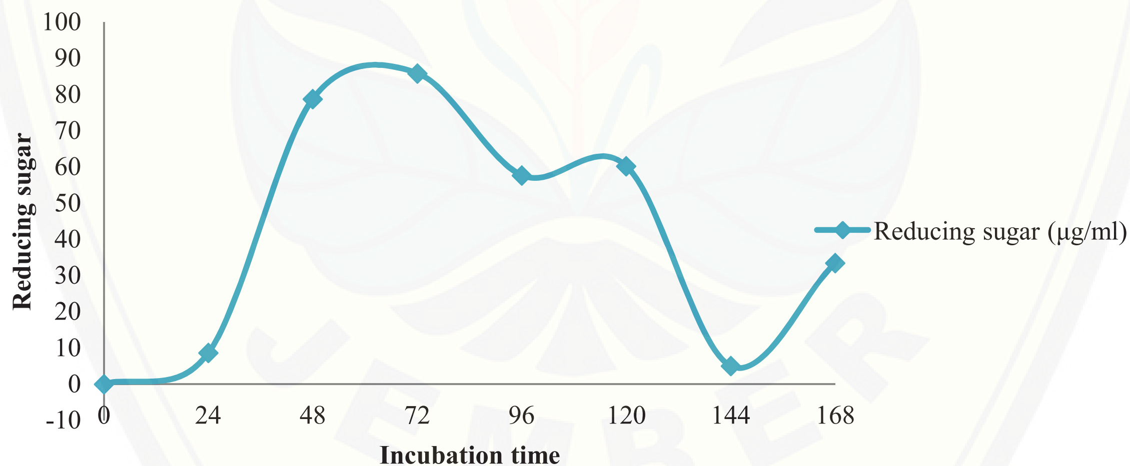

FIGURE 2. Effect of incubation time for reducing sugars production by *Aspergillus* sp. VTM5

Based on the results obtained by *Aspergillus* sp.VTM5 in producing the most optimum pectinase at 72 hours. The enzyme activity at that hour was 85.85 µg/ml. Based on [20] research, the highest activity of pectinase from *Aspergillus niger* is 96 hours. In other studies, the highest activity of *Aspergillus fumigatus* pectinase and polygalacturonase was 48 and 72 hours [21]. In this study, pectinase activity continued to increase along with the length of fermentation time and reached its optimum at 72 hours. Pectinase activity after 72 hours decreased. This decrease in activity is possible due to changes in pH during denaturation, fermentation, or decomposition of enzymes due to interactions with other components of medium and nutrient content in the medium which continues to decrease [22].

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Optimum and stability of pH

Enzymes have the optimum pH or range of pH where the maximum activity is. Changes in pH affect the concentration of H^+ ions that cause changes in the structure of the enzyme. Significant changes in pH can cause the enzyme to denaturation so that the enzyme loses its ability to break down the substrate normally [24]. Results from pectinase assay were demonstrated at (Figure 3) with a variation of pH 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8 at 37°C for 2 hours.

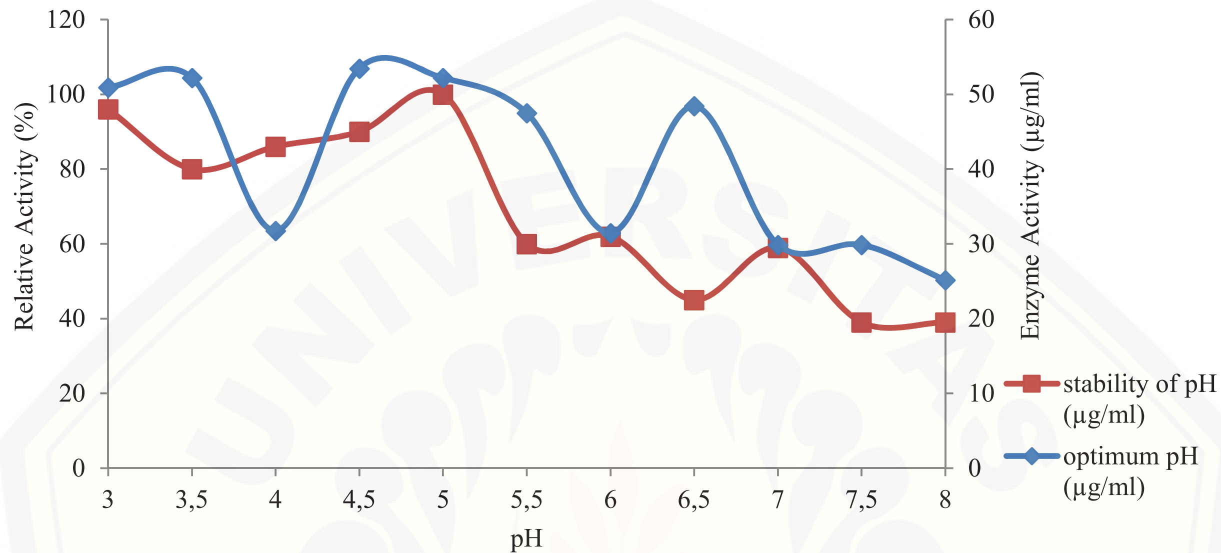

FIGURE 3. Effect variation of pH in reducing sugars production by *Aspergillus* sp. VTM5

The result shows that the optimum pH for pectinase activity is 4,5 with the concentration of reducing sugar .62,7 µg/ml. Based on reports from [21] pectinase from *Aspergillus fumigatus* it reaches the highest activity at pH 4 and polygalacturonase at pH 5. The other study mention that Endo-PGI from *Aspergillus kawachii* has optimum activity at pH 4,5 [23]. Stability of pH enzyme activity was a stable pH range at 3-5. This can be seen from relative enzyme activity and enzyme relative activity ≥ 75% find at pH 3-5.

SUMMARY

Aspergillus sp.VTM5 had higher reducing sugar after 72 hours of incubation. Pectinase activity in this study had a stable pH range at 3-5 and optimum at pH 4,5. It could be potentially cheap for enzyme production and management coffee pulp waste. Purification is needed to improve the enzyme activity

ACKNOWLEDGMENTS

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