

Enzyme activities in the spent mushroom substrate from *Pleurotus ostreatus*

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Abstract

Spent mushroom substrate (SMS) from *Pleurotus ostreatus* is residue of mushroom cultivation that is known to have lignocellulolytic enzyme activity in it. Enzyme activities provides information on evidence of fungi converting complex substrates to simple ones as nutrients for them. In this study, the enzyme was found cellulase, lignin peroxidase, and protease. The samples used consisted of three samples of SMS were collected from Padang Panjang (sample 1), Padang (sample 2), Payakumbuh (sample 3). Research was conducted to determine and compare the activity of cellulase, protease, and lignin peroxidase enzymes were evaluated as potential source of enzyme and also to determine the levels of reducing sugar, ph, and C/N ratio. Maximum activities in the SMS were 0.179 U/g cellulase, 3584.229 U/g LiP, and 1400 U/g. The value range 16,459 mg/ml to 19,845 mg/ml of reducing sugar, 5.78 to 8.08 of pH and 24.053% to 81.345% of C/N ratio. The result reveal that SMS from *P. ostreatus* was a good and could be a potential source of enzymes for alternative in the circular economy concept.

Keywords: Enzyme; Lignocellulolytic; *Pleurotus ostreatus*; Potential; Spent Mushroom Substrate

1. Introduction

Mushroom of *Pleurotus ostreatus* is one of the cultivated mushrooms from the basidiomycetes group. This fungus can grow in cold and hot climates ranging from 18-30 °C [1] and harvest process for 4 months [2]. Harvesting of *P. ostreatus* leaves the mushroom growing medium in the substrate. The substrate media includes 95% sawdust, 3% rice bran, lime or CaCO₃ 1%, and gypsum or CaSO₄ 1% [3].

The increasing production of *P. ostreatus* mushrooms is directly proportional to the waste generated called spent mushroom substrate (SMS). SMS has high organic matter, mineral nutrients and enzymes [4]. SMS from *P. ostreatus* is the part of white-rot fungi that produce several enzymes (lacasse, peroxidases, cellulases, and xylanases) that degrade lignocellulose [5]. Anisa et.al [6] reported that SMS from *P. pulmonarius* were found enzymes such as xylanase 2.3 U/g, laccase 4.1 U/g, endoglucanase 14.6 U/g, lignin peroxidase 214.1 U/g and -glucosidase 915.4 U/g. In addition, Karthika et al [7] reported that the protease and amylase enzymes contained liquid waste from the florida strain of white oyster (*Pleurotus florida*), 0.042 U/ml and 3.42 U/ml.

The C/N ratio had an effect on mushroom mycelium, mushroom weight, and protein content in mushroom fruiting bodies. Several previous studies have found that the protein content in mushroom fruiting bodies depends on the chemical composition and C/N ratio of the substrate. Agro-industrial waste will be obtained with low nitrogen content, one of which is in spent mushroom substrate of white oyster mushrooms [8]

Furthermore, the SMS from *P. ostreatus* exhibits great potential to be reused and needs to do exploration of several enzymes activity of SMS from *P.ostreatus*. It would be more information that has high organic matter. The aim of this

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study was to explore enzymes and recent advances in enzyme production for various applications such as bioremediation, animal feeding, and potential usage in alternative energy.

2. Material and methods

2.1. Collection of sample

The samples used in this study was the remaining planting medium for mushroom cultivation which could no longer be productive in producing fruit bodies of aged up to 4 months after harvest. Samples were obtained from 3 white oyster mushroom cultivation sites located in Padang Panjang (sample 1), Padang (sample 2), and Payakumbuh (sample 3.) The process was performed at the Research Laboratory of Microbiology Andalas University, Padang.

2.2. Enzymes activity assay

2.2.1. Cellulase activity

Cellulase enzyme activity test begins with the manufacture of enzyme filtrate. Then 1 ml of enzyme filtrate extract was added and incubated at 40°C for 30 minutes. The mixture was put into boiling water for 20 minutes and 1 ml of Nelson-somogyi solution was added. Then, it was vortexed and heated again in boiling water for 20 minutes. The solution was cooled immediately in ice water until it reached 26°C, then 1 ml of arsenomolybdate reagent was added. The solution was shaken until no gas was seen. Then the volume of the solution was made up to 10 ml with distilled water. The solution mixture was shaken again until no air bubbles were seen. Furthermore, absorbance measurements were carried out with a spectrophotometer at a wavelength of 500 nm. The control measurement is the same as the sample treatment. However, in the enzyme control, 1 ml of the enzyme filtrate was first deactivated by heating in boiling water for 20 minutes without adding 1% CMC substrate, while for the substrate control, 1 preincubated CMC solution was incubated again at room temperature for 30 minutes. Furthermore, the treatment was continued without enzyme filtrate [9]. Cellulase enzyme activity ($\mu\text{mol/ml}$) was calculated by the formula [9]:

$$AE = \frac{MG}{BM \times t}$$

AE is enzyme activity ($\mu\text{mol/ml}$), MG is weight of glucose, BM is Molecular weight of glucose (180) and t is incubation time.

2.2.2. Protease activity

Protease enzyme activity test begins with the manufacture of enzyme filtrate. The protease enzyme activity test was measured based on the Northrop method. 50 ml of casein substrate solution was pipetted into a 125 ml Enlemeyer flask, closed and heated on a water bath at 40°C. Then, 1 ml of enzyme solution was added and the mixed solution was incubated for 25 minutes at 40°C. The reaction was stopped by adding 25 ml of acetate buffer solution and filtered. Pipette 2 ml of the filtrate and add 3 ml of NaOH into the test tube and homogenize. After that, 1 ml of Folin Ciocalteus Phenol was added. Then, after 10 minutes, the absorbance value was measured with a wavelength of 660 nm. One unit of enzyme activity or NU (Eine Northrop-Unit) is equivalent to the amount of enzyme that can hydrolyze 40% of a 20% casein solution [9].

The activity of the protease enzyme can be calculated by the formula [9] :

$$Nu/g = \frac{E660 \cdot F}{Ew}$$

Where E660 is absorbance at 660 nm, EW is enzyme in g/ml solution, F is Ratio factor of color development of Folin Ciocalteus reagent phenol. Fungal protease factor 200

2.2.3. Lignin Peroxidase (LiP) activity

Lignin Peroxidase (LiP) enzyme activity test begins with the manufacture of enzyme filtrate. LiP activity test was carried out using the method according to [10]. The steps are, as much as 0.2 ml of enzyme filtrate; 0.05 ml H2O2 5 Mm; 0.1 ml veratrhy alcohol 8 Mm; 0.2 ml of 0.05 M acetate buffer pH 5; and 0.45 ml of distilled water was put into a cuvette and then shaken. The solution was read for absorbance at a wavelength of 310 nm at intervals of 0-30 minutes.

LiP activity test was by the formula [10]:

$$Unit(U/ml) = \frac{\Delta C \times V \text{ total } (ml) \times 10^9}{t \times V \text{ enzyme } (ml)}$$

$$\Delta C = \frac{(A_t - A_0)}{(k \times b)}$$

Where ΔC is amount of veratraldehyde formed during t minutes, A_t is absorbance value at t min of the reaction, k is constant (veratraldehyde; 9300/M/cm), A_0 is absorbance value at the beginning, V total is total solution volume, V enzyme is volume of enzyme filtrate, t is incubation time, and b is cuvette diameter

2.3. Measurement of/ pH value

Measurement of pH value using a digital pH meter. Weighed 10 grams of the sample from the rest of the growing media in the SMS twice, each put into a shake bottle. Then, added 50 ml of pH H₂O-free water in one bottle and 50 ml of 1 M KCl pH KCl into the other bottle. Shake with a whisk for 30 minutes. Then, the sample suspension was measured with a pH meter that had been calibrated with a buffer solution of pH 7 and pH 4. Then the pH value was written [11].

2.4. Measurement of total N content

Determination of total N there are several stages of testing according to the standard SNI 19-7030-2004 [11].

2.5. Measurement of C/N ratio

A comparison of C/N is obtained from the compared C and N values. Organic C test according to SNI 19-7030 2004. After the C-organic value has been obtained, then the existing C and N values are compared, so that the C/N ratio is obtained in the sample [11].

3. Results and discussion

Enzyme activities were measured in SMS from *P.ostreatus* that the presence of various enzymes such as cellulase, lignin peroxidase, and protease (Figure 1). The location of the average sample that has the highest enzyme is sample 3. Lignin peroxidase had the highest activity. Cause *P. ostreatus* is a type of white rot fungus. Supported by Abdel-Hamid *et al* [12] that white rot fungi attack lignin more than cellulose so that it plays a role in the production of extracellular ligninolytic enzymes and the degradation results leave white wood decay and a fibrous texture, namely cellulose.

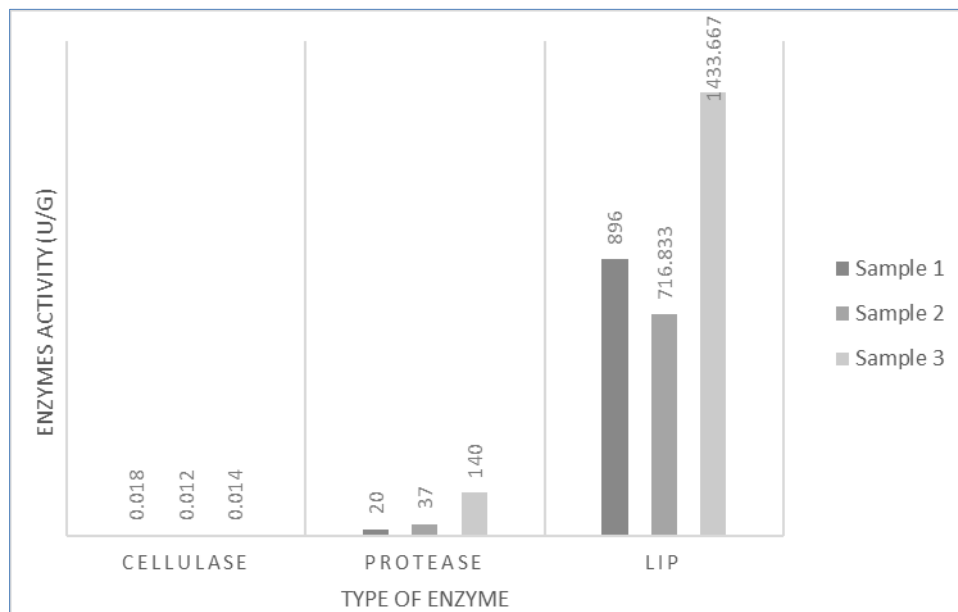


Figure 1 Enzymes activities in the spent mushroom substrate from *P. ostreatus*

The activity of the cellulase enzyme ranging from 0.114 mol/g to 0.179 mol/g. The remaining cellulase enzyme in the SMS from *P. ostreatus* was small. Wljayanti [13] reported that there is still a residual cellulase enzyme in the SMS from *P. ostreatus* which has a low activity of 0.009 U/ml. Enzymes as biocatalysts have natural properties that affect their work activities, namely conditions of temperature, pH, solvent, substrate concentration and other environmental factors [14]. The optimal temperature for the cellulase enzyme was between (20-50)°C and the optimal temperature choice to support the enzyme's work [15]

The remaining protease enzyme activity in the yield ranged from 200 NU/g to 1400 NU/g. There was influenced by the remaining mycelium in the substrate. Fungal mycelium secretes extracellular protease enzymes to hydrolyze protein substrates. Supported by Nakamura et al [16] that a protease enzyme was found in the test of six edible mushrooms, one of which was *P. ostreatus*. In addition, in this study the proteolytic activity of fungi was influenced by temperature, pH, and substrate.

The thing that affects the results of the protease enzyme activity above is the different substrate conditions in leaving a lot of mycelium. Mushroom mycelium contains protein [17]. The protein content of the mycelium is influenced by the composition of the growth medium. The presence of these protein content triggers protease enzymes to produce amino acids for fungal growth. Based on the research results obtained, different enzyme activities were associated with the protein content of the remaining mycelium in SMS.

Table 1 Reducing sugar, pH, N total, and C/N ratio in the SMS from *P.ostreatus*

No	Sample Types	Reducing Sugar(mg/ml)	pH	N total (%)	C/N ratio (%)
1	Sample 1	19.845	5.78	0.584	24.053
2	Sample 2	16.459	8.06	0.59	71.814
3	Sample 3	19.230	6.73	0.55	81.345

The average yield of reducing sugar (Table 1.) in the sample ranged from 16,459 mg/ml to 19,845 mg/ml. This is influenced by the content of SMS such as sawdust, bran, corn flour, sugar, and lime which contain carbohydrates. Corn flour affects the reducing sugar content of an ingredient [18]. Sucrose and starch (polysaccharides) are reducing sugars [19]. Sucrose or better known in the market as granulated sugar contains 94% sucrose [20]. The presence or absence of a reducing property of a sugar molecule is determined by the presence or absence of a free reactive hydroxyl (OH) group [21].

The pH value (Table 1.) above showed that the pH value of SMS was different for each sample location. The pH values in various samples ranged from 5.78 to 8.06. The pH o affects the growth of the fungal mycelium. The SMS will experience changes in pH during the substrate weathering process. Changes in pH in the growing media occur due to the reshuffle process of lignocellulose and other organic compounds that produce organic acids [22]. The initial treatment with pH 8 changed to pH 7 which caused brown oyster mushrooms to grow well [23]. The average fungal mycelium grows optimally at a neutral pH between 6.5-7.0 [24].

The value of total N (Table 1.) levels in the SMS from *P. ostreatus* levels ranged from 0.55% to 0.59%. The results obtained between sample locations did not differ much because the SMS had been completely absorbed for growth for the fungus. The total N value obtained in the sample is close to the opinion of Sulaeman [25] that the total N value in SMS from *P.ostreatus* is 0.6%. Nitrogen is the most important constituent of protein, nucleic acid synthesis, purines, pyrimidines, and the constituents of fungal cell walls consisting of (1-4)-N-acetylglucosamine [26].

The C/N ratio (Table 1.) of SMS from *P. ostreatus* that has been measured obtained a ratio ranging from 24.053% to 81.345%. This value is influenced by the remaining growing media so that the nutrients available are high organic carbon elements due to sources of lignocellulosic. The C/N ratio obtained based on the data above have a value above 24.053%. Based on Avnimelech et al [27] that a high C/N ratio of 24 from aquaculture waste can be utilized in the digestibility of catfish feed compared to a C/N ratio below 24 as in the treatment in this study. The presence of heterotrophic bacteria is expected for bacteria in the digestive system to obtain food from carbon and nitrogen sources in a certain ratio

4. Conclusion

The SMS from *P.ostreatus* had cellulase enzyme activity obtained from 3 locations ranging from 0.114 mol/g to 0.179 mol/g. The activity of Lignin Peroxidase (LiP) enzymes ranged from 1792,115 U/g to 3584,229 U/g. The activity of protease enzymes ranged from 200 U/g to 1400 U/g. The value of reducing sugar is at locations ranging from 16,459 mg/ml to 19,845 mg/ml, the pH value range from 5.78 to 8.08 the N total ranged from 0.55% to 0.59%. The value of the C/N ratio ranges from 24.053% to 81.345%.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest between the authors of this research work. The authors agreed and assigned in hand to all matters arising to this piece of research work.

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