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Abstract

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Review Article

PRELIMINARY STUDY ON ENZYMATIC DEGRADATION OF MODEL OF PHARMACEUTICAL WASTE BY LACCASE FROM TRAMETES SP

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Abstract: Chemical synthesis processes of the pharmaceuticals industry produce wastewaters which are variable in character and highly strong. Disposal of waste materials such as unused drugs, pharmaceutical production facilities, hospital wastes are the origin of the pharmaceutical pollution. Laccase is a copper-containing phenol oxidase, which can oxidize electron-rich substrates of phenolic and non-phenolic origin with a concomitant reduction of oxygen to water through a radical-catalyzed reaction mechanism. Laccases are present in higher plants, fungi, bacteria and insects,and the most studied group of enzymes to date is from fungal origin, also the functions of laccases are diverse. The chemical model chosen for the study were diclofenac and ibuprofen, as it is one of the drugs that appears with the most frequency in the analyses of waste waters due to its high consumption as an anti-inflammatory and analgesic. A model sample for representative as common active materials was experimentally evaluated to assess the simple biological degradation, using crude laccase from *Trametes* species. Optimal results were achieved as the crude enzyme was precipitated with 60% acetone method ; the specific activity was found to be 0.67 (unit/mg), and showed can be exploited for handling the reduce of hazardous pharmaceutical waste materials.

Keywords: Enzymatic Degradation, Pharmaceutical Waste, Laccase, Trametes Sp.

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Introduction

Laccase enzyme (E.C. 1.0.10.3.2) is produced by various fungi and higher plants [1]. White decay fungus is known capable of degrading lignin by producing laccase enzyme [2]. This enzyme is first found in the sap of lacquer tree growing in Japan, i.e. *Rhus vernicira*, in 1883, and is then known distributed in plants and fungi. This enzyme has the structure of benzendiol, oxygen reductase and partly extracellular glycoprotein containing copper atom and its enzyme cluster is called blue copper oxidase. Laccase is a monomeric, dimeric, tetrameric enzyme with molecule weight of 60 – 80 kDa [3].

This enzyme is easily found on fungi in our surrounding and take no time to grow on dead/fallen tree trunk and are known as decay fungi of *trametes* species. In addition, laccase enzyme is also found on is consumable and non-toxic white oyster mushroom. This mushroom belongs to *pleurotus* species [4] [5].

Research Methods

This research is intended to be a preliminary study of the potential of white oyster mushroom locally growing in Jakarta to be used as an environmentally friendly catalyst in the reduction of waste content of drug materials containing ibuprofen and diclofenac with laccase enzyme simple extraction and analysis on the biodegradation of drug materials in waste by using UV-Visible spectrophotometer.

White oyster mushroom, ammonium sulfate, ethanol, ethyl acetate, BSA, Lowry reagent, acetate buffer. Diclofenac sodium, ibuprofen. Aquades. Tool: UV-Vis. FT-IR Spectrophotometer and GCMS.

Visible spectrophotometer. Any change in the dimer absorbance of guaiacol product is observed every minute for 10 minutes. One activity unit is defined as the amount of enzyme which may accommodate 1 µmol guaiacol every minute at 28°C.

Analysis on Protein Concentration

The protein is tested using Lowry solution. 100 µL supernatant (centrifuged liquid sample) is mixed with 2 mL Bradford solution in cuvette. This mixture is then homogenized using *vortexmixer* for 10 seconds and left for 10 minutes before measurement using spectrophotometry at wavelength of 595 nm.

Biodegradation reaction of pharmaceutical waste solution model

Water-ethanol based solution is prepared (1:1) respectively 100 mL, containing 10,000 ppm ibuprofen or diclofenac sodium compound. Isolate laccase enzyme 10% solution is then prepared in 20 mL water, mixed respectively with 50 mL ibuprofen or diclofenac sodium compound for 30 minutes, and added with 2500 ppm beta-naphthol as mediator. The mixture is inserted into separating funnel, added with 15 mL ethyl acetate, shaken, added with sodium sulfate dryer, and then filtered. The filtrate of each sample is evaporated, and then analyzed using KLT with hexane raising solvent: ethylacetate (2:1).

Results and Discussion

Laccase Rough Enzyme Extraction

Laccase Extraction.

Daily analysis on enzyme activity starts with enzyme extraction from 250 grams of white oyster mushroom, using 500 mL acetate buffer solution at pH 4.6 on *shaker* at speed of 100 rpm for 1 hour. The solution and substrate are decanted from the flask into centrifuge tube and chilled and centrifuged for 15 minutes at 2000 rpm.

Analysis on Laccase Activity.

Enzyme activity is measured by inserting 50 µL supernatant model into 150 µL, 0.5 mM guaiacol solution. The dimer absorbance is observed at wavelength of 500 nm using UV-

Laccase Rough Enzyme Extraction

Laccase enzyme extraction from trametes fungus starts with cutting of relatively hard mushroom body to small pieces, smashed using blender, added with aquades, and then filtered using rough cloth to withhold big sized flakes [6] [7].

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Figure 1.*Trametes sp.* sample

The filtrate produced is added with cool acetone and then left for deposition and the deposition result is separated from the filtrate. The filtrate is then added with ammonium sulfate 70% to obtain second phase deposition and measured for protein

substrate and enzyme. This oxidation result is known with biomodification [12].

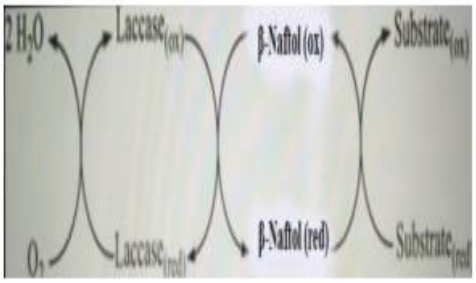

Figure 5. Utilization of intermediate compound beta-naphthol in biomodification reaction