The Utilization of Laccase-Functionalized Graphene Oxide as an Effective Biodegradation of Pharmaceutical Industry Waste: Diclofenac and Ibuprofen

Yusnidar Yusuf
Faculty of Pharmacy, Universitas Muhammadiyah Prof. Dr. Hamka, Jakarta, Indonesia
yusnidar_yusuf@yahoo.co.id

Abstract: Laccases are multi-copper oxidases that can act as a catalyst the one-electron oxidation of aromatic and non-aromatic compounds which include substituted phenols, several inorganic ions, and various non-phenolic compounds with the concomitant reduction of O2 to H2O. This research aims to utilize laccase-functionalized graphene oxide (GO) as effective biodegradation of pharmaceutical industry waste: diclofenac and ibuprofen. In this study, Laccase enzyme was isolated from white oyster mushroom and immobilized with GO that was synthesized from graphite powder. Afterward, GO-laccase would be applied in degradation of diclofenac and ibuprofen. The experimental results indicated the GO-laccase had a good biodegradability to degrade pharmaceutical industry waste. Some of the compounds derived from diclofenac are N-(2,6-Dichlorophenyl) indolin-2-one, 2-(2,6-Dichloro-phenylamino)-benzaldehyde, and 2,6-Dichloroaniline, and ibuprofen is 4-Isobutylacetophenone.

Keywords: Biodegradation, Laccase enzyme, graphene oxide, diclofenac, ibuprofen

1. INTRODUCTION

Waste is the residue of a product that is usually not used or desired anymore by humans. This waste comes from human activities, both individually and in groups such as commercial activities, industry, agriculture, and mining. This waste will become a problem when discharged into the environment without proper treatment first. Nowadays, the need for consumption and growth of the pharmaceutical industry is rapidly increasing and becomes one of the sources of world pollution. This is caused by several factors such as the high prevalence of chronic diseases, advances in science and technology, and increasing world population. In addition to the usefulness of advances in the pharmaceutical field drugs sewage disposal of liquid and solid waste into the environment (soil, rivers, and lakes) is an issue of growing concern. Propranolol, diclofenac, gemfibrozil, ibuprofen, and fluoxetine are examples of drugs that are known to have an influence on aquatic organisms at the level of laboratory experiments.

Wasted drug residues contain BOD, COD, volatile organic compounds (VOCs), antibiotics (PhACs), surfactants, toxic substances, and active compounds such as hormones. In large quantities, pharmaceutical waste poses a dangerous threat and requires special treatment. Diclofenac and ibuprofen are the most common substances found in the aquatic environment among non-steroidal inflammatory drugs. These drugs are usually used to fight pain, inflammation, and fever and can be used without a doctor’s prescription. In one report, only 15% of the diclofenac excreted was able to be processed by the body after being used in therapy. Because of the large amount of diclofenac and ibuprofen in the aquatic environment, it causes ecological problems in the water. The results showed the influence of these drugs on invertebrate and vertebrate reproduction, genetic and systemic damage in several species of fish, shellfish, and cytogenetic properties in freshwater bivalves, and the growth of fungal and bacterial species. Diclofenac and ibuprofen are considered as pollutants which must be removed immediately.
Conventional wastewater treatment, usually not designed to eliminate or reduce drugs. These drugs can be eliminated in biological, chemical or physical (absorption) treatment systems. One treatment that can be done is by using enzymes to degrade these compounds into derivatives which are safer for the environment. Enzymes are biological catalysts that have catalytic properties that can be used very well in various fields. The enzyme chosen in this study was the **laccase enzyme**. The laccase enzyme (**o-diphenol: oxygen oxidoreductase E.C.1.10.3.2**) is produced by various types of fungi and higher plants which are multi-copper oxidases. The most common laccase enzyme is extracted from white or red rot fungi, such as *Trametes versicolor* or *Trametes vilosa* that grows on dead/fallen tree trunks. This enzyme can catalyze one-electron oxidation of phenolic molecules to oxidize various aromatic and non-aromatic compounds which include substituted phenols, several inorganic ions, and various non-phenolic compounds by concurrent reduction of oxygen to water.

The development of competitive new approaches to immobilizing enzymes in multilayer systems through layer-by-layer deposition is underway. Enzyme immobilization is the process of combining an enzyme with a solid physical matrix (support) so that it can be used continuously and repeatedly. In this study, graphene oxide was used to be functional in order to immobilize the Laccase enzyme. Graphene oxide (GO) is glimpsed because it has a surface filled with active groups. Besides its easy manufacturing, active functional groups such as carboxyl, hydroxyl, and epoxy are the main assets that are often used in various chemical reaction purposes. In addition, GO has a large surface, high adsorption capacity, excellent stability, and good biocompatibility. The enzyme immobilization process is carried out with the stages of carrier binding, cross-linking, physical adsorption, and covalent bonds. In this study, laccase immobilized with graphene oxide was synthesized and applied in the degradation of chemical compounds of pharmaceutical waste, diclofenac and ibuprofen.

2. EXPERIMENTAL

2.1 Tools and Materials

All Chemicals and reagents in this research were obtained from Merck Chemical Company and used for an analytical grade. The materials used in this study are NaNO₃, H₂SO₄, KMnO₄, ammonium sulfate, ethanol, ethyl acetate, BSA, Zn, amino antipyrine, graphite, Lowry reagent, acetate buffer, sodium phosphate buffer 7.0, vanillin, sodium diclofenac, ibuprofen, TLC silica gel, n-hexane, methanol, H₂O₂, distilled water. The equipment used is laboratory glassware as well as several instruments such as UV-Vis spectrophotometers, FTIR, and GC-MS.

2.2 Laccase Extraction from White Oyster Mushroom (*Trametes sp.*)

White oyster mushrooms (250 grams) were extracted with 500 mL acetate buffer solution (pH 4.6) in a flask in a shaker for 1 hour. The solution and substrate are filtered and transferred into centrifugation tubes and cooled. Centrifugation was carried out for 15 minutes at 2000 rpm. The solids obtained are separated by decantation and stored in the refrigerator before use.

2.3 Partial Purification with Ammonium Sulfate

The crude extract of the laccase enzyme is added distilled water. Then precipitated using ammonium sulfate. The amount of (NH₄)₂SO₄ is added to the crude extract of laccase in the following order: 0-40%, and 40-70%. Salt is added slowly under cold conditions and while stirring. The solution is incubated for 12 hours at 4°C, then centrifuged for 30 minutes. The formed sludge is separated and dissolved into 0.5 M acetate buffer (pH 5.0) as much as 10% of the total volume obtained. Then the enzyme activity and protein content were determined.

2.4 Determination of Protein Content by Bradford Method
Determination of protein content of the crude laccase enzyme was carried out by mixing 25 μL of the enzyme with 250 μL of Bradford reagent into the microplate and incubated for 10 minutes. Furthermore, the absorbance of the enzyme solution was measured at λmax of 595 nm. The absorbance obtained is plotted in the linear regression equation of the protein standard curve. In determining the protein content of laccase, BSA is used as a standard.

2.5 Determination of Laccase Activity

The determination of the laccase activity test was carried out using 100 μL of 0.5 M acetate buffer (pH 5.0) and 40 μL of 1 mM ABTS reagent in a microplate. Then 50 μL of the crude extract is added to the mixture. The mixture was measured for absorbance at a wavelength of 420 nm with UV-Vis spectrophotometer. One unit of laccase activity is defined as the number of enzymes needed to oxidize 1 mmol ABTS per minute.

2.6 Synthesis of Graphene Oxide (GO) and Enzyme Immobilization

Synthesis of graphene oxide (GO) used Hummer’s method which has been modified. 5 g of graphite powder and 125 mL H₂SO₄ 98% in three-neck round bottom flask were stirred slowly. Then 10 g of NaNO₃ was added during the stirring process for 1 hour and 3 g KMnO₄ was added slowly, stirring continued for 2 hours and kept the temperature at 20°C (the mixture will turn greenish-black). The stirring was continued for 24 hours at 35°C. After that, the mixture was washed with 200 mL distilled water, continued with the addition of 20 mL H₂O₂ 30% (the solution turned yellowish-green). The results obtained were cooled and centrifuged. Then the precipitate was washed with HCl, distilled water, and acetone. Subsequently, the mixture was dried at 110°C for 12 hours to obtain graphite oxide sheets. Then, 80 mg of graphite oxides were added with 80 mL of distilled water and stirred for 1 hour for homogeneous solution, then ultrasonically performed at 50/60 Hz ultrasonic waves for 120 minutes. Graphite oxide is reduced by adding 15 grams of Zn metal and 25 mL of 35% HCl, then stirring for 1 hour. After 1 hour of stirring, another 25 mL HCl 35% solution is added and followed by the stirring process for 5-30 minutes, then washed using distilled water and 5% HCl. After washing until neutral the precipitate in the washing process is put into the furnace and heated at 160°C for 12 hours.

The immobilization of the enzyme with graphene oxide was carried out by mixing the partially purified laccase enzyme with 70% ammonium sulfate with GO and adding 0.1 M phosphate buffer. The mixture was incubated for 1 hour with the help of a shaker in cold conditions. After completion, the mixture is decanted. The solid phase is GO-laccase and washed with the same buffer for 3 times.

2.7 Biodegradation Reaction of Pharmaceutical Waste Solution Models

0.1 g of the GO mobilized laccase enzyme was added with 10 mL of the aqueous solution: ethanol (1:1) for 1000 ppm ibuprofen and sodium diclofenac respectively. Then 10 mL vanillin was added as the mediator reagent. The mixture is stirred with a magnetic stirrer at room temperature for 8 hours. After stirring, the mixture is transferred to a separating funnel and 10 mL ethyl acetate is added. The extraction is carried out, and the ethyl acetate phase is separated, and sodium sulfate is added then filtered. The filtrate of each sample was tested by UV spectrophotometer at 276 nm. Ibuprofen and sodium diclofenac solutions that have not been added to the mobilized laccase enzyme are determined the absorbance at the same wavelength. The degree of degradation is calculated using the equation:

\[
\frac{C_0 - C}{C} \times 100\%
\]

C₀ = initial concentration; C = final concentration of the reaction
3. RESULT AND DISCUSSION

3.1 Isolation of Laccase Enzyme

Laccase enzyme was isolated from white oyster mushroom (Trametes sp.) obtained from fallen dead trees (Fig. 1). Extraction of protein components (including enzymes) is carried out under acidic conditions by adding acetate buffer. Then, the enzyme is partially purified by the gradual addition of ammonium sulfate. The salt addition aims to make the instability of components of proteins that are stable in water. In this case, ammonium sulfate acts as salting-out in a homogeneous mixture. It will bind more closely to its water component, and material which is initially more soluble in water will be released. This condition will cause instability so that sediment will form. The precipitate formed from this step is thought to be a component of proteins.

![Image of mushroom and enzyme structure]

Fig. 1. White oyster mushroom (Trametes sp.) and laccase enzyme structure

From the results of this partial isolation, an identification test was carried out to identify the presence of enzymes (laccase) from the white oyster rot weathering fungus by analyzing its protein content. The results of testing protein contents by Bradford method will be obtained by the enzyme activities (Table 1).

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<th>Total activity (U)</th>
<th>Specific activity (U/mg)</th>
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From Table 1, the initial extract of white oyster mushroom protein was found to be 548.9 mg. This stage is considered as the ability of the level of separation carried out in this study calculated the amount of mass obtained by 100%. The second stage of purification is carried out by adding 70% ammonium sulfate. From this treatment, the non-enzyme protein component is reduced, which identifies that there is a protein that has been separated. The concentration of the protein-enzyme component has increased marked by the result of higher enzyme activity. The results of theoretical calculations can be concluded that there is an increase in enzyme activity 4 times, while the mass decreases to a third. This mass will be interpreted as laccase enzyme, which will be further investigated.

5.2 Characterization of Graphene Oxide-Immobilized Laccase

Graphene oxide (GO) has been synthesized using the Hummer’s method. GO was obtained in the form of black powder (Fig. 2a). Proving the formation of GO synthesized from graphite powder was analyzed using FTIR. The FTIR spectra of graphite (Fig. 2b) significantly represents the typical characteristic absorption peaks of graphite that only
has three peaks. Namely peak at 3100, 2900, and 1600 cm\(^{-1}\) as absorption of the O-H, C-H, and C=C stretching functional groups, respectively. The presence of O-H group peaks in this spectrum is due to the absorption of air. The FTIR graphene oxide spectrum is different from the graphite spectrum, and there is some absorption shift. The absorption differences favor the formation of GO. The peak at the absorption of 3100-3400 cm\(^{-1}\) that widens indicates the presence of O-H stretching vibration absorption as a result of the reaction of GO formation. This absorption is much broader than the O-H absorption in graphite. Then, a new peak also appeared at 1700 cm\(^{-1}\) which is the C=O vibration of COOH on GO followed by the vibration of the C=C group at 1600 cm\(^{-1}\) which also appears on the graphite spectrum. The vibrations of C-O-C (epoxide) groups present at the absorption of 1100 cm\(^{-1}\) also support that GO has been successfully synthesized.

![Graphene oxide (a) and FTIR spectrum of graphite and graphene oxide (b)](image)

The GO structure that has a two-dimensional sp\(^2\)-bonded carbon sheet and the presence of active groups such as O-H, -COOH, C-O-C, and C=O makes GO can be used for various purposes, including in immobilizing the laccase enzyme. The characteristic and structure of GO can be used as supporting material for enzymes. The surface of the graphene oxide sheet, which is rich in functional groups will interact with the active functional groups in enzymes. Enzymes are polymeric amino acids that are rich in organic functional groups such as carboxylic, hydroxyl, amine, and sulfhydryl. The presence of this group causes semi-permanent and electrostatic interactions between the surface of the graphene oxide sheet and the enzyme, without the need for cross-linking agents. Illustration of the proposed laccase-immobilized graphene oxide can be seen in Fig. 3. Interactions that occur do not affect the enzyme activity, because the catalytic performance of enzymes occurs in stereochemical aspects such as tertiary structures and quaternary proteins that produce a kind of active-side hole which later matches the specific substrate.
3.3 Biodegradation Ability of Pharmaceutical Waste Material Models

Laccase has a function to reduce oxygen to H2O species, and at the same time after oxidation will be reduced again because of the presence of organic substrate that is ready to reduce it, so that laccase will be active as before. This phenomenon can be used as a mechanism for the biodegradation mechanism of organic compounds, which seems to act as an enzyme reducing agent or known as a substrate. For this reason, the active compounds of the drug diclofenac and ibuprofen were selected in this study. Description of the biodegradation mechanism as shown in Fig. 4.

![Diagram of Laccase Reaction](image)

Fig. 4. Diclofenac and ibuprofen biodegradation reactions by laccase enzyme

For the effectiveness of the reaction, from previous studies, it was reported that an intermediary compound (mediator) is needed to help the reaction rate. In this research, the vanillin compound was used as the mediator. In this study, the variation of reaction time functions was carried out in three stages, namely 30, 60 and continued to be 120 minutes, as well as variations in reaction conditions in acidic, neutral and basic conditions (pH 5.7, 7.0, and 11). From this design, the initial data was obtained by observing changes in pH values. Fig. 5 shows an analysis of the pH conditions of the ibuprofen and diclofenac reaction models.
Fig. 5. Analysis of the pH conditions of the ibuprofen and diclofenac reaction models

Observations showed that the biodegradation process of the ibuprofen and diclofenac test model samples prepared under three conditions of pH values (5.0, 7.0 and 11.0), showed a decrease in pH values in all conditions of the variations prepared. This decrease is due to the formation of degradation results in the form of species of acids, namely hydrochloric acid, and carboxylic acids. This change in pH value indicates that the sample structure has changed and is characterized by a change in solubility and an increase in precipitation.

The results of ibuprofen and diclofenac biodegradation can be analyzed by thin layer chromatography (TLC). In this analysis, it will be seen whether a new compound is formed after treatment or not by the appearance of new spots on the TLC plate. Elution of ibuprofen and diclofenac compounds was carried out with hexane: ethyl acetate (3:1) eluent and viewed under UV light. From observations using TLC (Fig. 6), it appears that ibuprofen compounds are degraded into two derivative compounds (Rf=0.63 and Rf=0) and there are also intact ibuprofen compounds that are not degraded (Rf=0.87). This difference can be seen by comparing the results of ibuprofen TLC before (Fig. 6a) and after (Fig. 6b) treatment. The same treatment is conducted for diclofenac compounds. The results of observations with TLC showed that there were two compounds resulting from diclofenac degradation (Rf=0.69 and Rf=0.82). Here also can be seen diclofenac compounds that are not degraded at Rf = 0.45. From this TLC analysis, it can be concluded that the laccase enzyme can degrade ibuprofen and diclofenac compounds into their derivatives but not completely degraded so that there are still residual compounds that can be seen in the same Rf.

Fig. 6. TLC of ibuprofen and diclofenac before (I and D) and after (I' and D') degradation

Characterization of functional group changes that occur in ibuprofen and diclofenac before and after being degraded by the laccase enzyme can be analyzed using FTIR (Fig. 7). Information obtained from this instrument is
the vibrations of the ibuprofen and diclofenac functional groups that increase, shift, or disappear. These changes that identify the influence that occurs after treatment, in this case is degraded.

Fig. 7. TLC of ibuprofen and diclofenac before (I and D) and after (Ia and Da) degradation

The spectrum of ibuprofen compounds (Fig. 7a) did not show much change in the absorption of functional groups. Ibuprofen before degradation had absorption at 2952 cm\(^{-1}\) (C-H), 1720 cm\(^{-1}\) (C=O), the peak broad around 3000 cm\(^{-1}\) (O-H), and several peaks in the fingerprint area. After being degraded, the same peak between ibuprofen and its degradation compounds was still seen. The difference can be seen with the emergence of new peaks at the absorption of 1603 cm\(^{-1}\). This small difference does not identify that there was no change in ibuprofen before and after degradation. However, the change in the structure of ibuprofen into its derivatives is estimated still contain the same functional groups as the ibuprofen compound, so the absorption is measured at the FTIR with the same shift.

FTIR spectrum of diclofenac compound (Fig. 7b) shows several functional group vibrational peaks, including vibration secondary N-H stretching at 3389 cm\(^{-1}\), C-N stretching in secondary amines at 1200 cm\(^{-1}\), C=C stretching vibrations in the aromatic ring at 1578 cm\(^{-1}\), O-H vibration that broad from 3000-3600 cm\(^{-1}\), and several peaks in the fingerprint area. FTIR Spectrum of the diclofenac derivative compounds from the degradation results looks different from diclofenac. The vibration shift of the O-H stretching group is not as broad as the beginning. Then the secondary N-H group becomes sharper and shifts in the area of 3324 cm\(^{-1}\). The vibration of the C=O group is increasingly visible in the area of 1697 cm\(^{-1}\) and the vibration of the C=C group is shifted slightly from 1578 cm\(^{-1}\). Significant changes also occur in the fingerprint region, which supports that the compound is not just diclofenac compound.

Calculation of laccase biodegradation capacity can be calculated from the measurement of the decrease in \(\lambda_{max}\) value of each sample based on the amount of initial material (C) with the number of results of the material after reacting (Co). The C / Co ratio is the value of the ability of laccase to degrade model compounds. From the data (Fig. 8), it appears that ibuprofen after reacting for 30 minutes showed a decrease in sample quantity of about 0.3. As well as the reaction after 60 minutes, the decline occurred up to 0.5. In the reaction with a time of 120 minutes, there was a decrease again by 0.47, but not too significant from the reaction time of 60 minutes. Similar data can also be observed in diclofenac samples and have a similar decrease in character.
Fig. 8. Biodegradation of test model samples by laccase

From the data, it is known that the pharmaceutical waste sample model that is ibuprofen and diclofenac with the addition of laccase treatment that has been immobilized with graphene oxide, shows the reaction. The ongoing reaction is thought to be an oxidation and reduction reaction because laccase is an oxidoreductase enzyme. Laccase will oxidize oxygen to H2O and vanillin (Laccase Reducer) will normalize and reactivate Laccase. The oxidized vanillin will be reduced again by the model compound, ibuprofen and diclofenac. From the graph data (figure. 8), it can be seen that ibuprofen or diclofenac tends to decrease the graph. This indicates that there is initial material that is changing. The formed product can be observed with the amount of starting material which has decreased. Then it can be discussed that laccase under these reaction conditions can chemically change the material of ibuprofen and diclofenac.

In order to find out the results of the biodegradation reaction with laccase, identification was made using Gas chromatography-mass spectrometry (GC-MS). In chromatogram of GC-MS ibuprofen compounds after degradation, there are four compounds that show at retention times 6.26; 8.127; 12.50; and 13.129 minutes (Fig. 9a). On the chromatogram, there is one compound with a high concentration at a retention time of 8.127 minutes which is a non-degraded ibuprofen compound. The molecular weight of ibuprofen is verified by the MS spectrum which is read at m/z = 206 (Fig. 9b). The high concentration of ibuprofen indicates that this compound is only degraded in small amounts. One of the compounds derived from ibuprofen degradation shows its peak at the retention time of 6.26 minutes. After further study with the MS spectrum (fig. 9c), this compound is the result of a reduction which is thought to be a 4-Isobutyloacetophene compound or 1- (4-Isobutylyphenyl) ethanone (m/z = 176). The change that occurs is the reduction of carboxylic acids into ketone compounds. From the peaks formed on the chromatogram, the formation of these compounds is only in small quantities.
Measurement of diclofenac compounds degraded by GC-MS showed more varied results compared with ibuprofen. On the chromatogram (Fig. 10 a), there are at least six peaks whose concentrations are not much different from each other. The peak is read at retention time 4.728; 9.879; 11.016; 11.450; 11.581; and 12.537 minutes. As with ibuprofen, the concentration of diclofenac compounds is the highest compared to its derivative compounds. The molecular weight of diclofenac compounds is 296 g/mol. Some diclofenac derivatives derived from degradation as measured by GC-MS have molecular weights m/z = 257, 162, and 277. The diclofenac degradation derivative compounds are N-(6-Dichlorophenyl) indolin-2-one (Fig. 10b), 2-(2,6-Dichloro-phenyl)ammo)–benzaldehyde (Fig. 10c), and 2,6-Dichloroaniline (Fig. 10d).

Fig. 9. Chromatogram ibuprofen after degradation (a), MS ibuprofen before (b), and after degradation (c)

Fig. 10. Chromatogram diclofenac after degradation (a), MS diclofenac after degradation (b,c,d)
4. Conclusions

The results showed that the local fungus of white oyster rot (Trametes sp.) contained laccase enzyme with partial purification results obtained specific activity 72,626 U / mg. Laccase is immobilized in graphene oxide synthesized from graphite powder has the ability to biodegrade samples of pharmaceutical waste models, namely ibuprofen and diclofenac. Identification of the results of degradation of ibuprofen and diclofenac compounds using GC-MS is known that laccase can convert ibuprofen into smaller mass products, one of the compounds that can be identified is 4-kobutylacetophenone, which is a product of ibuprofen reduction. Whereas for diclofenac samples biodegradable products were N- (2,6-Dichlorophenyl) indolin-2-one (Fig. 10b), 2-(2,6-Dichlorophenylamino)-benzaldehyde (Fig. 10c), and 2,6-Dichloroaniline.

Acknowledgment

References


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Ana Carla Medeiros Morato Aquino, Simone Carvalho Peixoto-Nogueira, João Atâlio Jorge, Hâctor Francisco Terenzi et al. "Characterisation of an acid trehalase produced by the thermotolerant fungus var. : Biochemical properties and immunochemical localisation ", FEMS Microbiology Letters, 2005

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