Modification of Cu$^{2+}$ in polyphenol oxidase extract from purple eggplant for phenol degradation in coal wastewater treatment

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Abstract. Cracking coal-forming organic compounds during the gasification process produces liquid waste-containing phenolic compounds that require special handling based on their toxicity. As one of the components, there is liquid waste resulting from the coal gasification process. The purpose of this research was to study the activity of polyphenol oxidase (PPO) on phenol after the addition of Cu$^{2+}$ to purple eggplant (Solanum melongena L.) extract and its potential to work more effectively in phenol biodegradation for coal wastewater containing phenol. Enzyme activity and phenol determination were carried out spectrophotometrically. The results showed PPO activity of 25.90-38.10 U/mL; 4.0 mM phenol and the activity of PPO-Cu$^{2+}$ was 21.58-46.32 U/mL; 2.0-4.0 mM CuSO$_4$; 2.0-4.0 mM phenol. Based on Michaelis Menten's graph, the initial rate of PPO-Cu$^{2+}$ was 0.015 mM/min and the initial rate of PPO was 0.15 mM/min using 2 mM phenol as a substrate. Lineweaver-Burk’s graph shows the $K_M$ of PPO-Cu$^{2+}$ = 6.92 mM, which is lower than $K_M$ of PPO = 13.05 mM. Its means that the phenol response has a higher affinity for PPO-Cu$^{2+}$ than PPO. The application of PPO-Cu$^{2+}$ in purple eggplant extract works effectively as much as 46.7% for artificial coal liquid waste containing phenol.

1. Introduction
Coal chemical wastewater is difficult to decompose because it contains organic pollutants, including phenols, polycyclic aromatics, hydrocarbons and heterocyclic compounds containing nitrogen and sulfur [1]. The gasification process in practice not only produces gas but also produces solid and liquid waste. Generally, the handling and utilization of solid waste can be done well. Phenol and tar are a
problem in that coal liquid waste. Coal liquid waste contains the total phenol 6,700 mg/kg (from Palimanan) and 42,600 mg/kg (from Medan) [2]. Phenolic compounds and their derivatives are water pollutants that include a wide variety of organic chemicals. Coal tar is an important product produced from the pyrolysis of coal and phenol as an anoxicogenic compound, one of the components in coal tar content [3]. Phenolic compound poisoning can occur through skin absorption, inhalation, ingestion, and various other methods which can cause health effects. High phenol exposure can be fatal to humans [4].

Polyphenol oxidase (PPO) is one of the critical factors contributing to the browning reaction during the post-harvest process [5]. The PPO has been reported as one of the important factors that contribute to browning during postharvest processing. Enzymatic browning is caused by the oxidation of natural phenolic compounds in fruit tissue in the presence of oxygen. Oxidation of phenolic substances to quinones, which are catalyzed by PPO [6]. Eggplant is a very rich source of PPO, which reduces fruit quality in cutting and postharvest processing due to enzymatic browning. An active PPO from eggplant is precipitated in 50 – 70% saturated ammonium sulfate, 259-fold purified and characterized to a 56 kDa homodimer. Specificity of the substrate shows its nature as catechol oxidase (EC 1.10.3.2), as a maximum activity with 4-methyl catechol [5]. Physiological disturbances in harvested eggplant resulted in decreased quality, short postharvest life and significant economic losses. Methyl jasmonate treatment maintains higher quality, phenolic content, and delayed browning in eggplant during ten days of storage at 20 °C [7].

PPO is one type of multicopper protein widely distributed throughout bacteria to mammals. Copper plays an important role as a cofactor in many enzyme systems, including cytochrome oxidase, super oxidase dismutase, PPO, etc., due to the multiple variables Cu⁺ and Cu²⁺. Multiple oxidases such as PPO, ascorbate oxidase, and laccase catalyze the reduction of dioxygen to water [8]. The main components of polar groups in coal tar consist of phenol, methyl phenol, dimethylphenol, naphthol, and derivatives [3]. The adsorption process with an active ceramic alternative biofiltration media has been used to remove phenol as an organic compound and also some inorganic metals such as zinc (Zn⁺²), copper (Cu⁺²), and lead (Pb⁺²). The active ceramic was modified by ozonation with a mixture of crushed coal, Kaolin, and Bentonite, which was then treated in a vacuum furnace at temperatures ranging between 300 and 600°C [9].

This research focuses on studying phenol’s response as a substrate with a higher affinity for PPO-Cu²⁺. Thus, adding Cu²⁺ ions as an activator of PPO as a biocatalyst is expected to work more effectively in the degradation of phenol to a quinone. The PPO and PPO-Cu²⁺ activities of purple eggplant were studied for artificial coal wastewater containing phenol. Several studies related to phenol liquid waste produced by coal gasification are used to reference PPO and PPO-Cu²⁺ simulations from purple eggplant extract. The amount of PPO and PPO-Cu²⁺ activity correlated with the amount of PPO in purple eggplant and depended on crude PPO-Cu²⁺ in purple eggplant.

2. Materials and methods

2.1. Chemicals, laboratory apparatus and instrumentations
Artificial coal chemical wastewater containing phenol. Purple eggplant is obtained from the traditional market of Cimahi (West-Java Indonesia) and frozen at 20°C. Some of the primary reagents were used in previous studies [10], copper sulfate (CuSO₄·5H₂O), sodium hydroxide (NaOH), and hydrochloric acid (HCl). All solutions were prepared with doubly distilled water.

2.2. Preparation of crude PPO-Cu²⁺ extract
PPO from purple eggplant extract obtained 50.00 g purple eggplant and added 50 mL of citrate buffer solution pH = 7, and then other reagents were added as done by previous researchers [10][11][12]. Purple eggplant PPO extract was made into two samples, namely without Cu²⁺ and with Cu²⁺. PPO and PPO-
Cu\(^{2+}\) from purple eggplant extract were determined by PPO activity, protein content and specific activity, with phenol and catechol substrates.

2.3. PPO activity assay, protein content and specific activity determination

PPO and PPO-Cu\(^{2+}\) from purple eggplant extract were determined by their activity measured by the UV-VIS spectrophotometry method at the maximum wavelength (\(\lambda_{\text{max}}\)) modified based on the Sigma procedure with a phenol calibration curve as the substrate. Protein content from purple extract eggplant was determined based on the Biuret method based on the Bovine Serum Albumin (BSA) calibration curve (0.2; 0.4; 0.6; 0.8 to 1 mg/mL) measured on \(\lambda_{\text{max}}\) BSA= 400 – 600 nm. Based on PPO activity data and protein content from purple eggplant extract, then calculated PPO-specific activity.

2.4. Enzyme kinetic determination

Michaelis constant (\(K_{\text{M}}\)) and the maximum rate (\(V_{\text{max}}\)): The enzymatic activity was measured using phenol as substrates and the concentration range of 1.0 to 5.0 mM. A better method for determining the values of \(V_{\text{max}}\) and \(K_{\text{M}}\) was formulated by Hans Lineweaver and Dean Burk [12].

2.5. Removal of phenolic compounds from artificial coal wastewater containing phenol

The amino antipyrine method was used to determine phenol levels in a sample of artificial coal chemical wastewater containing phenol by direct photometric method [12], using a calibration curve (phenol 2.0; 4.0; 6.0; 8.0; up to 10.0 mg/mL) at phenol \(\lambda_{\text{max}}\). A total of 100 ml of the sample was inserted into a 250 mL beaker. The primary reagent was added as has been done in previous studies [10][12][13] and added (0.5, 1.0, and 2.0 mL) the PPO and PPO-Cu\(^{2+}\) of the purple eggplant extract, respectively. It was allowed to stand for 24 hours at pH 6.80 to 7.20.

2.6. Characterization of crude PPO eggplant extract with fourier transform infrared

The PPO from purple eggplant extract was analyzed by FTIR, which aims to observe the wavenumber of functional groups of proteins based on FTIR spectra.

3. Results and discussion

3.1. Effect of Cu\(^{2+}\) ion PPO activity, protein content and specific activity

The initial rate curve profile of reduced substrate concentration based on the relationship curve Absorbance (Abs.) vs. time (second) (PPO \(\lambda_{\text{max}}\) = 270.4 nm) measured by spectrophotometry is shown in Figure 1a and 1b. This graph is the initial rate curve as the correlation between the decrease in phenol concentration with the change in time in the presence of PPO. These curves can be analyzed PPO activity data during catalysis by studying the standard curve of phenol. Meanwhile, PPO activity data can be observed based on Table 1. Previous researchers have studied the UV-Vis absorption spectra for PPO, which includes: Sc-Ms1 melanin, a melanin-like pigment synthesized by Sc-Ms1 tyrosinase, dopa, and cysteinyldopa, a melanin-like pigment synthesized by Tv laccase. Excess dopa is used as a substrate for enzyme synthesis. The peak at 274 nm depends on the unreacted medium and its presence of protein [14]. During the initial stage of the enzyme catalyst reaction, the conversion of the substrate to the product is small. It thus can be considered to remain constant and effectively equal to the concentration of the initial substrate. Enzymes can be regarded as remaining stable during the initial stages of a reaction. The progress curve is usually linear under 20% conversion of the substrate to product [15].

Based on this assumption, observations in Figures 2a and 2b show that PPO activity in purple eggplant extract was measured based on its affinity for phenol as a substrate. The addition of Cu\(^{2+}\) metal ions may affect the activity of PPO-Cu\(^{2+}\) as an enzyme cofactor at optimum conditions. Based on the data analysis, the PPO activity was obtained before the addition of the Cu\(^{2+}\)ion (25.90 – 38.10 U/mL; 2.0-4.0 mM phenol), and the optimum conditions for PPO activity (38.10 U/mL; 4 mM phenol). The
addition of Cu$^{2+}$ can increase the PPO activity achieved at (46.32 U/mL; 4 mM phenol; 10 mM Cu$^{2+}$). Other researchers found that supplementation of sprouts with metal ions (Zn$^{2+}$, Mn$^{2+}$, Fe$^{3+}$) and/or inhibitors (ascorbic acid, citric acid) may be used to decrease the activity of PPOs [16].

(a) without Cu$^{2+}$; 4 mM phenol (PPO-purple eggplant polyphenol oxidase extract); (b) with 10 mM Cu$^{2+}$; 4 mM phenol (PPO-Cu$^{2+}$ purple eggplant polyphenol oxidase extract)

Figure 1. The initial rates of spectrophotometry data of Absorbance (Abs.) vs time (second). This graph is the initial rate curve as the correlation between the decrease in phenol concentration with the change in time in the presence of PPO at pH 7.0; PPO $\lambda_{\text{max}} = 270.4$ nm.

The optimization of pH treatment was carried out on the stability of the PPO activity in purple eggplant extract. The PPO activity stability in purple eggplant extract was achieved with a citrate buffer of 50 mM pH 7.0. In this study, the temperature setting was also set at 20$^\circ$C which also affected the performance of PPO and PPO-Cu$^{2+}$. Other researchers have studied that PPO activity monitoring uses 0.5-50 mM L-DOPA at pH 7.0 in a 50 mM phosphate buffer [17]. The use of 5 mM methyl jasmonate at 20$^\circ$C is useful for maintaining fruit quality during storage, inhibits increased browning of petals and weight loss, and decreases sensory quality, firmness, and anthocyanin content. Other authors have studied PPO extract of apricots, apples, eggplants, and potatoes in a potassium phosphate buffer. The optimum pH of PPO activity for potatoes was 6.4 and the PPO activity for other fruits was 7. The optimum temperature of activity was 20$^\circ$C for the PPO activity of apricots and apples, while eggplant and potatoes were 22$^\circ$C was stable at neutral pH [18]. The temperature is also another important factor influencing the enzymatic activity of PPOs, depending on the type of plant. Previous authors stated that PPO activity of some eggplant cultivar have determined at 2$^\circ$C and 80$^\circ$C. Eggplant PPO remained active even at 80$^\circ$C, with relative activity above 30% [7].
Table 1. PPO activity and specific activity (protein = 6.17 mg/mL) in crude purple eggplant extract with variations in substrate concentration without the addition of Cu²⁺

| Substrate (mM) | PPO activity (U/mL) | Specific activity (U/mg) |
|---------------|---------------------|-------------------------|
| 2.00          | 36.96 ±4.86         | 1848.03±243.23          |
| 2.50          | 33.14 ±4.87         | 1656.99±243.58          |
| 3.00          | 25.81 ±5.14         | 1290.75±257.20          |
| 3.50          | 38.13 ±2.21         | 1906.43±257.20          |
| 4.00          | 36.96 ±4.86         | 1497.62±273.09          |

Based on the initial rate reaction curve shown in Figures 1a and 1b, further data on PPO activity with phenol as a substrate was obtained, as shown in Table 1. PPO activity and specific activity (protein = 6.17 mg/mL) in crude purple eggplant extract with variations in substrate concentration without the addition of Cu²⁺. The optimum condition of PPO activity before adding Cu²⁺ has been achieved at 1906.43±257.20 U; 3.50 mM of phenol and specific activity at 308.75 U; 3.5 mM of phenol. Furthermore, Figures 2a and 2b verified the effect of adding Cu²⁺ and without Cu²⁺ to purple eggplant extract on PPO activity. Metaloenzim Cu can increase or decrease the enzyme activity when interacting physically or chemically between PPO and the substrate.

The addition of Cu²⁺ to purple eggplant extract increased PPO activity by using phenols as substrate (Figure 2a and 2b). The addition of Cu²⁺ ions to purple eggplant extract can increase the PPO activity in catalyzing phenol to quinone. PPO activity of PPO-Cu²⁺ was obtained (22 – 46 U/mL; from 2.0 – 4.0 mM phenol; 2-10 mM CuSO⁴); (phenol λ_max = 280.80 nm; y = 1.5154x + 0.0102; R = 0.9998). In Figure 2a, PPO activity without Cu²⁺ can be shown (38.13 U/mL; 3.50 mM phenol;). In Figure 2b, PPO activity with Cu²⁺ can increase (42.46 U/mL; 4.00 mM phenol; 8 mM Cu²⁺). The results of this study are more in line with previous researchers that Cu²⁺ and Fe²⁺ can increase the PPO activity contained in dill (Anethum graveolens L). However, Hg²⁺, Sn²⁺ had the maximum inhibitory effect, and Zn²⁺ and Pb²⁺ had no significant effect on enzyme activity [18].

In other conditions, Cu²⁺ acts as an inhibitor by assuming that the Cu²⁺ is the central atom. It is possible there is competition between the Cu²⁺ and Cu²⁺ as a PPO's cofactor. These can be covalently bound in coordination with the phenolic compound’s oxygen-lone pair. The addition of Cu²⁺ did not give a significant effect on PPO activity in several conditions. By using phenol as the substrate, before the addition of Cu²⁺, so that the PPO activity (38.13 U/mL; 3.50 mM phenol) and the decreased of PPO-Cu²⁺ activity (33.75 U/mL; 3.50 mM phenol; 10.00 mM Cu²⁺). The protein content obtained from purple eggplant extract was 6.17 mg/mL, or 1.23% of 50 g extract of purple eggplants by Biuret method (BSA λ_max = 545 nm; y = 0.244 x + 0.003; R² = 0.999). The results of previous studies on PPO in lentil sprouts showed that Ba²⁺, Fe³⁺, and Mn²⁺ (10 mM) inhibited PPO activity. Studies on the effect of antibrowning compounds and cations on PPO activity can be used to protect lentil sprouts from enzymatic browning during storage and processing [16]. In theory, PPO catalyzed browning of fruit and vegetables can be prevented by heat inactivation of the enzyme, removing one or both substrates (O₂ and phenol), lowering the pH= 2 or more units below the optimum pH, by enzyme inactivation reactions or adding compounds that inhibit PPO or prevent melanin formation [19].
3.2. **PPO kinetics in purple eggplant extract with and without Cu$$^{2+}$$**

PPO in purple eggplant extract was also studied based on the plot of the reaction rate according to Michaelis Menten's enzymatic reaction kinetics with measuring parameters $V_{\text{max}}$ and $K_M$, which found the characteristic reaction of an enzyme. The plot shows that the rate increases with increasing substrate concentration until it reaches a maximum as $V_{\text{max}}$. After this, the addition of the substrate concentration does not increase the reaction rate significantly. The reaction rates of PPO and PPO-Cu$$^{2+}$$ from purple eggplant extract were determined for their activity under optimum conditions with 50 mM citrate buffer at pH = 7 and 20°C. The relationship between initial rates ($V_0$), ($V_{\text{max}}$), ($K_M$) of phenol was studied based...
on the Michaelis Menten curve, with PPO and PPO-Cu$^{2+}$ biocatalysts from purple eggplant extract (Figures 3a).

PPO enzymatic kinetics profile with phenol substrate was observed by a plot of correlation between the reaction rate PPO from purple eggplant extract, V (mol/s) vs. phenol [mM], and PPO-Cu$^{2+}$ of the purple eggplant extract. The addition of Cu$^{2+}$ in purple eggplant extract was possible as an activator, which was observed based on the reaction rate with phenol as a substrate. The contributions of PPO and PPO-Cu$^{2+}$ in purple eggplant extract as a catalyst to the oxidation of phenols to quinones, the reaction rate observed in Figures 3a and 3b. An initial rate of the addition of Cu$^{2+}$ increases the reaction rate almost ten times faster than the performance of PPO without the addition of Cu$^{2+}$. These studies are based on comparing the y-axis of the profile in Figure 3a (0.015 to 0.15 mol/s), that curve to be increased linearly on condition concentrations below 2.00 mM phenol. The addition of Cu$^{2+}$ increased the reaction rate almost ten times faster than the PPO profile without the addition of Cu$^{2+}$ (0.15 µmol/s; 0.015 µmol/s) when the phenol concentration was twice as large as the concentration without the addition of Cu$^{2+}$ (2.00 mM phenol; 1.10 mM phenol).

Figure 3a shows the PPO reaction rate increases, and the curve increases (2.00 – 4.00 mM phenol; 0.020 – 0.035 µmol/s), so the $V_{\text{max}}$ achieved between 0.020-0.035 µmol/s. The reaction rate above 0.035 µmol/s, the addition of phenol with high concentrations shows that it is not sufficient for PPO performance in phenol catalysis so that the $K_M$ PPO reaction rate (0.010-0.015 µmol/l; 0.50 – 1.50 mM phenol). The PPO-Cu$^{2+}$ reaction rate is increased and the curve increased (2.00 – 4.00 mM phenol; 0.20 – 0.25 µmol/s), and $V_{\text{M}}$ achieved between 0.20 – 0.25 µmol/s. The reaction rate above 0.25 µmol/s, the addition of phenol is not effective for the performance of PPO-Cu$^{2+}$ in phenol catalysis so that $K_M$ the PPO-Cu$^{2+}$ reaction rate (0.015-0.050 µmol/l; 1.50 – 2.00 mM phenol).

On the lower area of the curve, the reaction approximates the first-order reaction kinetics, because the active molecules of the enzyme are not saturated. On a higher curve, the upper part of the reaction plot closer to the reaction kinetics is zero order, because the active site of the enzyme molecule is saturated. Thus, the reaction rate does not depend on increasing the substrate concentration. In the intermediate part of the curve, when the enzyme reaches substrate saturation, its kinetics is a mixture of first-order and zero-order reactions in the substrate concentration. At this concentration point, the reaction rate does not increase even though the concentration of the substrate continues to increase, this indicates that $V_{\text{M}}$ has been reached in the catalytic reaction. In this condition, the enzyme has reached a saturated condition with the substrate so that it cannot function properly.

**Figure 3a.** Michaelis Menten graph. The plot of initial velocity vs. phenol as a substrate. The reaction rate for PPO (---) and PPO-Cu$^{2+}$ (---)

**Figure 3b.** Lineweaver-Burk graph. The plot of 1/V vs. 1/[S] PPO (---) and PPO-Cu$^{2+}$ (---)
Figure 3b describes the graph of the Lineweaver-Burk curve equation. The Lineweaver-Burk curve is a plot of 1/[v] vs. 1/[S] which correlates with the Michaelis-Menten curve. This picture shows the PPO reaction rate without Cu$^{2+}$ and the PPO-Cu$^{2+}$ reaction rate with Cu$^{2+}$ during catalytic reaction in the oxidation of phenol to quinones. Based on the two figures above, the $V_{\text{max}}$ and $K_M$ values obtained from Michaelis-Menten can be compared with the Lineweaver-Burk plot. This statement will allow them to see the graph boundaries. In the highest substrate concentration in this experiment, $V_{\text{max}}$ is not achieved. Thus, it has been estimated that $V_{\text{max}}$ from the $v$ vs. [S] will be lower than the Lineweaver-Burk plot [20].

Figure 3b shows the Lineweaver-Burk curve for PPO $y = 2.8011 \times + 4.4417$ is obtained ($V_{\text{max}} = 1.07 \text{ mM/min}; K_M = 13.05 \text{ mM}$). The Lineweaver-Burk curve for PPO-Cu$^{2+}$ shows $y = 11.684 x + 0.592$ is obtained ($V_{\text{max}} = 0.59 \text{ mM/min}; K_M = 6.92 \text{ mM}$). The $K_M$ PPO is almost twice as high as that of PPO-Cu$^{2+}$. It means that PPO with a higher Km than $V_{\text{max}}$ is achieved with a high substrate concentration value. Meanwhile, PPO-Cu$^{2+}$ with low $K_M$ to achieve its optimal catalytic process only requires a low substrate concentration. The low $K_M$ of PPO-Cu$^{2+}$ indicates that the phenol response has a higher affinity for PPO-Cu$^{2+}$ than PPO. On this basis, it is possible that Cu contributed to PPO from purple eggplant. With the addition of Cu$^{2+}$ in the PPO extract from purple eggplant, it is hoped that PPO-Cu$^{2+}$ as a biocatalyst can work more effectively in the degradation of phenol to quinones. This research is also supported by the ratio of $V_{\text{max}}/K_M$ PPO-Cu$^{2+} = 0.085$ with a relatively greater value than $V_{\text{max}}/K_M$ PPO = 0.081.

According to Todaro et al. [21], the $V_{\text{max}}/K_M$ ratio is called "catalytic power" it is a better parameter to find the most effective substrate. Based on this research, the $V_{\text{max}}/K_M$ ratio for 3,4-dihydroxyhydroxysaminic acid and methyl catechol is the most suitable substrates for eggplant PPO activities [21]. In a previous paper, it was found that one PPO isoform from eggplant was purified 259-fold using standard chromatography procedures and found PPO a 112 kDa homodimer. This enzyme shows very low $K_M$ (0.34 mM) and high catalytic efficiency (3.3 x 10$^6$) with 4-methyl catechol as substrate [5]. In order to resolve the main problem of this kinetic model, the following conditions must be considered [15]; (i) the enzyme must be stable during the measurements used in determining reaction rates; (ii) reverse reactions (product to a substrate) should be ignored; (iii) the product should not inhibit enzyme activity.

### 3.3 Removal of phenolic compounds from artificial coal wastewater containing phenol using PPO-Cu$^{2+}$ extract in purple eggplant

The final target of the performance of PPO extract and PPO-Cu$^{2+}$ extract is to utilize them in their application to artificial coal wastewater containing phenol. The main goal is to work optimally and effectively in the degradation of phenol waste, especially on a large scale and environmentally friendly. Phenol in coal wastewater will react with oxygen in the presence of PPO so that the reaction takes place quickly to degrade the phenol into a brown melanin complex that is environmentally friendly. The phenol concentration was obtained based on Table 2 before the addition of Cu$^{2+}$ and after the addition of Cu$^{2+}$ by using the amino-antipyrine method and UV-Vis spectrophotometer (phenol $\lambda_{\text{max}}$= 500 nm; $Y = 0.125x - 0.0006$ $R = 0.9978$).

| Treatments                  | Phenol (mg/L) | Conversion of phenol to a quinone (%) |
|-----------------------------|--------------|-------------------------------------|
| Phenol initial              | 3.10         | -                                   |
| After PPO addition          | 2.04         | 34.30                               |
| After PPO Cu$^{2+}$ addition| 1.65         | 47.70                               |
In artificial coal wastewater containing phenols, the concentration was reduced by 1.45 mg/L or 46.7% when PPO-Cu\(^{2+}\) purple eggplant extract was added, compared with the addition of purple eggplant PPO extract without the addition of Cu\(^{2+}\) to the same liquid waste. Thus, the crude PPO-Cu\(^{2+}\) in artificial coal wastewater containing phenols successfully degraded the phenol content in a fairly effective percentage on a laboratory scale. The Cu\(^{2+}\) ion may be an activator that can improve its performance as a biocatalyst which is quite effective in degrading phenol artificial coal wastewater containing phenols, reaching 46.7%.

Many factors affect the performance of PPO for phenol degradation by 46.7%. The acidic condition of the waste is one of the important factors, where the performance of PPO is good in citrate buffer conditions pH 7. Another thing, there are impurities in the liquid waste, so it is necessary to do distillation to minimize disturbing ions that can interfere with the measurement that inhibits the biocatalyst performance of PPO in phenol degradation. Another factor is that the mixture of waste and crude-PPO is allowed to react in a closed state so that oxygen, which plays a role in oxidizing phenol, is limited and the PPO contribution may be less than optimal. It is also necessary to stabilize the PPO-Cu\(^{2+}\) position in a matrix. PPO enzymes from low purity sources, i.e., partially purified potato preparations, show good potential for phenol removal via polymerization. The experimental results show that the conversion of phenol in synthetic wastewater is possible using the carriers Eupergit C250L, Celite, and cellulose-M [22]. A conductive polymer is synthesized by pyrrole electropolymerization with platinum electrode [23] and a steel gauze support material [24].

3.4 Fourier transform infrared analysis of PPO in purple eggplant extract

The PPO FTIR spectrum of the purple eggplant extract is described in Figure 4. The FTIR spectrum to observe the wavenumber of functional groups of proteins. The PPO has carboxyl (-COOH) and amine (-NH\(_2\)) functional groups and other major groups characterized by absorption peaks. The FTIR spectrum can be studied and compared on the basis of the spectrum of pure PPO [23], PPO spectra can also be compared with previous researchers [10].

![Figure 4. Fourier Transform Infrared (FTIR) spectrum of PPO in purple eggplant extract](image-url)
The PPO spectra of this study were compared with those of previous researchers based on wavenumbers: -OH stretch vibration (3388.93 cm\(^{-1}\)), CH stretch vibration (2931.80 cm\(^{-1}\)), C=O stretch vibration (1658.78 cm\(^{-1}\)), C = C stretch vibration (1462.04 cm\(^{-1}\)), and C-N stretch vibration (1078.21 cm\(^{-1}\)). If the IR PPO spectra of the results of this study are compared with the results of the two previous studies, it is possible to shift the wavenumber [10][23]. Spectra shift wavenumber at PPOs can happen because there may be other influences matrix components contained in eggplant purple, pure PPO and other food.

The data from this study at least explain that an amino acid functional group characterizes the PPO contained in crude as a constituent of protein, so more detailed research is needed. Previous researchers [26] have successfully studied the conformational changes of fungal PPO, secondary structural changes of the enzymes during thermosonication treatment at different power combinations i.e. 60, 80, and 100%, temperature (20–60 °C), and time (0–30 minutes). The FTIR study demonstrated that the inactivation of PPO thermosonication was not due to insignificant changes in the active site but rather due to changes in the global conformation of the enzyme [25].

4. Conclusions
The effectiveness of PPO-Cu\(^{2+}\) purple eggplant extract performance increased by 26.55% compared to the PPO performance of purple eggplant extract in the degradation of artificial coal wastewater containing phenol. The addition of Cu\(^{2+}\) to the crude extract of purple eggplant’s PPO as an activator. In certain conditions the addition of Cu\(^{2+}\) did not give a significant meaning to PPO activity (38.13 U/mL; 3.50 mM phenol) after the addition of Cu\(^{2+}\), the PPO activity decreased (33.75 U/mL; 3.50 mM phenol; 10.00 mM Cu\(^{2+}\)). Michaelis Menten's graph showed that the PPO-Cu\(^{2+}\) reaction rate of purple eggplant extract was almost ten times faster than the PPO performance of purple eggplant extract when phenol was 2.00 mM (0.015 µmol/s to 0.15 µmol/s). Based on the Lineweaver-Burk graph, the low K\(_M\) value of PPO-Cu\(^{2+}\) shows the response of phenol as a substrate has a higher affinity for PPO-Cu\(^{2+}\) than PPO, which is expected as a biocatalyst can work more effectively in the degradation of phenol to quinones.

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