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RESEARCH ARTICLE

Purification and Characterization of Laccase Extracted from Cladophora sp. and Its Role in Decolonization of Dyes

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Abstract

The Laccase enzyme belongs to the oxidoreductases containing copper atoms, and it has an ability to oxidize a wide range of phenolic compounds. Laccase was extracted from local green algae *Cladophora* sp. and partially purified using 80% ammonium sulfate and ion exchange chromatography. The specific activity of Laccase in precipitation step was 11583.1 unit /mg protein, and was 33733.5 unit /mg protein after the washing step of ion exchange chromatography technique using DEAE- cellulose ion exchanger. The optimal pH of the partially purified enzyme was 5.5 and for the stability was 6.0 . The optimal temperature of the activity was 35 °C and the enzyme remained at full activity at temperatures ranging from 25-45 °C. The enzyme also had a large tendency towards the Orthotolidine compared to the other reaction materials (Catechol, Guaiacol and Para ansidine). It was observed that the activity significantly reduced when the partially purified enzyme was incubated with substrates of reaction (EDTA, L-Cysteine and 2- Merecaptoethanol) at concentrations of 5mM and 10mM separately. While, the enzyme was still active after the incubation with SDS solution. The enzyme activity increased in the case of incubation of the partially purified enzyme with solutions containing ions of Cu, Fe and Zn at concentrations of 5mM and 10mM individually. The enzyme has retained its activity with ions of Na and Ca, while the enzyme inhibited with ions of Ag, Hg and Mn. The partially purified enzyme has a suitable ability to decolorize four types of textile dyes compared with the crude enzyme, especially the black dye. The percentage of decolorization of the black dye was 91.6% after incubation of the partially purified enzyme for 28 hours at 35 °C.

Keywords: *Oxidoreductases, Algae, Orthotolidine, Temperature, PH.*

Introduction

The Laccase enzyme EC 1.10.3.2 is one of the copper-containing multi-phenolic oxidation enzymes [1]. The electron transport mechanism induced the oxidation of a wide range of substrates such as phenolic compounds and aromatic amines with reduction of O_2 into two water molecules [2].

Laccase has a wide distribution in the high plants, fungi, bacteria [3] insects [4], lichens [5] and algae [6]. Laccases have many applications in biotechnology due to their ability to oxidize phenolic and non-phenolic compounds, including treatment of liquid wastes of paper and textile industries, biological treatment of polychlorinated hydrocarbons and removing the contaminated dyes [7]. Purification of the enzyme is very important; it leads to increase in the specific activity of enzyme. The purification steps included two steps: Precipitation of ammonium sulfate and ion exchanger chromatography. Many of the Laccases have an optimal pH in the acidic medium (pH 3.0-6.0), and some of them in the alkaline medium and rare laccase has optimal pH at 7.0 and the optimal temperatures for the enzyme are between 40 and 70°C [8].

Also, some substrates and metals ions effect on the enzyme activity. The Algae have been

used as a source of laccase enzyme, Otto and Schlosser [9] purified and characterized the enzyme from the green algae Tetracystis aeria and identified the kinetic properties and the basic structure of the enzyme as well as the ability of the enzyme for decoralization of industrial dyes.

Caldophora sp. (Chlorophyta) has a novel source of natural antimicrobial, antioxidant and antifungal [10], furthermore ability to decolorize the pigment solution for wood, silk and cotton dyes as well as fungicides and insecticides [11]. The current study aimed to extract, partial purified and characterize of Laccase from green algae Cladophora sp. and study the ability of the crude and partial purified enzymes for decolorization of textile dyes.

Materials and Methods

Collection and Identification of Alga Cladophora sp

Cladophora sp. were collected from Baghdad (Abu Ghraib) provinces from September to December 2016, after collected algae were washed thoroughly in water to remove any contaminated material, small portion of fresh sample used for identification and the other were dried by the sun rays for 48 hours .

Laccase Assay

The activity of the Laccase enzyme was estimated using Orthotoldine as a substrate. The reaction solution of 2 ml of 0.1 M sodium acetate, pH 5.0 and 1 ml of 0.025 M Orthotoldine and 0.5 ml of 0.02 M hydrogen peroxide solution. Then absorbance of the substance solution was measured at a wavelength of 366 nm, then 1 ml of the enzyme extract added to the substrate solution in the cell and the absorbance was calculated after 1 min. The enzymatic activity was calculated according to following question: [12]

$$\text{Enzyme activity (unit / ml)} = \frac{V1 \, x \, A \, x \, 10^6}{V2 \, x \, \varepsilon \, x \, T}$$

A means absorption and measured by nanometers, V_1 means the total volume measured by ml, V_2 means the size of the enzyme measured by ml, T means time and measured by minute, Ɛ means the absorption constant of the base material and equals 27600 $mole^{-1}x\ cm^{-1}$ The enzyme activity unit is defined as the amount of enzyme required to oxidize 1mm of substrate per minute.

Protein Concentration Determination

The concentration of protein based on the Bradford method [13] was determined using the standard curve of bovine serum albumin.

Specific Activity of Enzyme (U/mg protein)

According to Bernard and Whitaker method [14], the specific activity of Laccase enzyme was estimated in algae extracts of the following relationship:

$$\text{Specific activity (unit / mg protein)} = \frac{\text{Enzymatic activity (unit / ml)}}{\text{Protein concentration (mg / ml)}}$$

Purification of Laccase

The extracted enzyme from *Cladophora* sp. was purified using two steps. The precipitation by ammonium sulfate and ion exchange chromatography technique.

Enzyme Precipitation by Ammonium Sulfate

Ammonium sulfate was added to a quantity of crude extract of enzyme with different saturation ratios (50%, 60%, 70%, and 80%) to determine the optimum saturation ratio of ammonium sulfate needed for precipitation of the enzyme. The enzymatic activity, protein concentration and the specific activity of enzyme were determined [15].

Ion Exchange Chromatography

The Ion exchanger column was prepared according to the Whitaker and Bernard method [14] to give an ion exchange with dimensions of 20 x 1.5 cm. The equilibrium process of the exchanger column was carried out by a buffer solution that ran at a flow rate of 20 ml / h. The enzymatic solution after precipitation step was added and washed by phosphate buffer with a concentration of 0.02 M, and pH 7.5, and passed through the column.

The separated fractions of the column were collected at a flow rate of 20 ml / h and by 3 ml for each separate fraction. The enzyme activity was assessed in the wash fragments. The elution step of the binding proteins with the ion exchanger used by phosphate buffer with a concentration of 0.02 M, and pH 7.5 washing step with linear salinity of 0.1-1.0 M sodium chloride the enzymatic activity and protein concentration were evaluated and then the specific activity was calculated.

Characterization of Laccase Enzyme

Determination of Optimal PH of Laccase Activity and Stability

The different buffers were used at 0.15 M concentration for Orthotolidine as a substrate preparation and included: sodium acetate buffer with pH 4.0, 4.5, 5.0, 5.5 and 6.0 and sodium phosphate buffer within pH 7.0 and 7.5 and Tris -HCl buffer within pH 8.0, 8.5 and 9.0, than 0.5 ml of the partially enzyme solution was added to the substrate solution and the enzymatic activity was measured. The effect of the pH on the enzyme stability was measured after incubation in the water bath at 30 °C for 20 min at different pH values. The remaining activity was measured after estimated enzyme activity.

Effect of Optimal Temperature on Laccase Activity and Stability

The optimum temperature for the enzyme was determined by the estimated the enzyme activity at different temperatures ranging from 25-75°C. The enzymatic activity of each temperature was calculated separately. The effect of temperature on the enzyme stability was determined by incubating the enzyme in a water bath for 20 min at temperatures ranging from 25-75°C and then the remaining activity was measured after estimated enzyme activity.

Laccase Specificity Towards Different Substrates

The different substrates included (orthotoldine, guaiacol, catechol and para-insidine) were prepared at a concentration of 0.025 M. The substance guaiacol was measured at a wavelength of 465 nm and the catechol and the para-insidine were measured at a wavelength of 450 nm, while the absorbance of orthotolidine was measured at a wavelength of 366 nm.

Effect of Some Chemical Compounds (Inhibitors and Activators) on Laccase Activity

The Ion chloride solutions (Na, Cu, Mn, Ca, Hg and Zn), Mg and Fe sulfate, as well as Ag nitrate with concentrations 5mM and 10mM were prepared with distilled water. The chemical compounds EDTA, 2-Mercaptoethanol, L-Cysteine and SDS were prepared at concentrations 5mM and 10MM. Then, the prepared solutions were mixed separately with the partially purified enzyme solution, and incubated at 30°C for 20 minutes. The remaining enzymatic activity was determined in pH 5.5.

The Role of Pure and Crude Enzyme in the Decolonization of Dyes

The dyes of the fabric (yellow, red, blue and black) were prepared (30 mg /l). The absorption of each pigment was measured for the reaction with crude and the partially purified enzyme when incubated for different times 1, 2 and 28 hours at 35 °C. The percentage of the dye decolorization was calculated for all times by applying the following relationship:

The percentage of the dye decolorization =

$$\frac{Initial\ Absorbance - Final\ Adsorbance}{Initial\ Absorbance} \times 100$$

Results and Discussion

Partial Purification of Laccase

Purification steps included precipitation using ammonium sulfate and ion exchange chromatography.

Ammonium Sulfate Precipitation

Ammonium sulfate salts were used with gradient rates ranging from 50% to 80% to achieve the best concentration ratio of the laccase extracted from the *Cladophora* sp. was significantly degraded and the results showed that the enzyme's specific activity increased gradually, the concentration of ammonium sulfate increased by 80% to 11803.6 unit / mg protein and by 1.42 purification fold with an enzyme content of 75.6%. Ammonium sulfate deposition depends on the size and shape of the enzyme as well as on the number and distribution of the ionic charge and loads on the surface of the enzyme molecule. The sedimentation mechanism occurs by the tendency of the enzyme molecules to combine with each other by salt, which pulls the adapted layer around the protein molecule, and then precipitated by the effect of the phenomenon of salting out [16].

Ion-Exchange Chromatography

DEAE-cellulose column (1.5 x 20 cm) is based on the principle of difference in charge, which is characterized by a number of properties including the easily of preparation and the possibility of reactivation for use several times and has high susceptibility to the separation of the biological and broad

absorption of proteins [17] the absorbance of the separated fractions in the wash step (unbounded proteins carrying the positive charge) was measured during the ion exchanger column at a wavelength of 280 nm. After absorption of the zero line, the elution of the proteins associated with the ion exchange (Proteins bearing negative charge) using a equilibrium solution (Sodium Phosphate concentration of 0.02 M and pH 7.5) and a linear saline gradient of 0.1-1.0 M sodium chloride at a wavelength of 280 nm, the results of the purification (Figure 3-1) showed the separation of one peak of the protein in the washing step at the separated fractions (5-25) with one peak-for the activity confined to the fractions 5-10. This summit represents non-binding proteins that carry a positive charge similar to the charge of the exchanger; in addition, two main peaks of protein were separated at the elution step, the first being between 29-58and the second between 59-73 with two low-activity peaks. The enzymatic activity was 268 and 241.9 (unit / ml) respectively, these two peaks return to the proteins that carry a negative charge opposite to the ion exchange. They have low activity and are isozymes of laccase. The low activity of these two peaks in the elution step has focused on the top separated by the wash step.

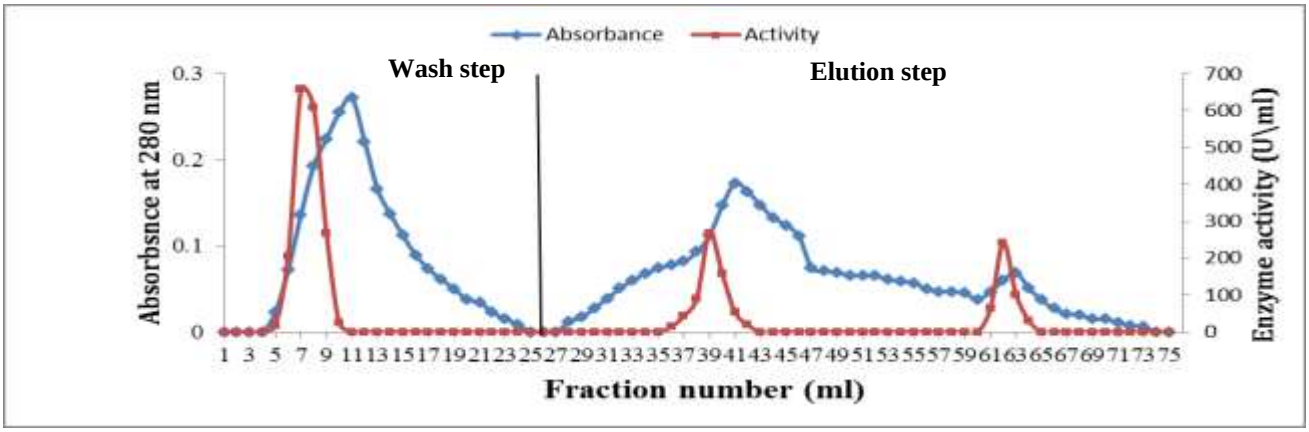


Figure 3-1: Ion exchange chromatography for laccase purification from *Cladophora* sp. using DEAE-cellulose column (1.5x20cm) equilibrated with sodium phosphate buffer (0.02M , pH 7.5), eluted with sodium phosphate buffer with sodium chloride (0.1-1.0M) in flow rate 30ml/ min., 3ml for each fraction

The enzymatic activity and protein concentration were obtained. A specific activity of 33733.5 unit/ ml protein was obtained, the number of purification times was 3.96 and the enzymatic yield was 26.4%. Table (3-1).Several studies have indicated the use of ion exchanger chromatography as a step in the purification of the enzyme. Khammuang and Sarnthima [18] used this technique (DEAE-cellulose) to purify the enzyme laccase, sodium phosphate of 0.02 M and pH 7.4 and linear salinity of 0-1 M NaCl and flow rate.0.5 ml / min to show three peaks, all of which gave activity of 2.54, 2.67, and 3.07 and final enzymatic yields of 0.76, 5.87 and 13.6% respectively. Studies have shown the purified enzyme of algae Tetracystis sp. using ion exchanger chromatography, the specific activity of 7 unit/mg protein, the number of purification fold 19 and the final enzymatic yield 33% [6].

Table 3-1 : The Purification steps of laccase from *Cladophora* sp.

Purification step	Volume (ml)	Enzyme activity (U/ml)	Protein concentration (mg/ml)	Specific activity (U/mg protein)	Total activity (U)	Purification fold	Yield %
Crude enzyme solution	10	3065.4	0.36	8515	30654	1	100
Precipitation with 80% saturation of ammonium sulfate	5	4633.2	0.40	11583.1	23166	1.36	75.6
Ion exchange chromatography (DEAE-cellulose) wash step	12	674.6	0.02	33733.5	8096	3.96	26.4

Characterization of Laccase

Substrate Specificity

The optimal substrate for the activity of laccase enzyme was identified by incubating it with different substrates, namely,

Orthotolidine, guaiacol, catechol and para ansidine at 35 °C and pH 5.5, the results indicated in Figure (3-2) showed that the activity of the enzyme increased when the use of orthotolidine as a substrate. The enzymatic activity was 320.3 unit / ml while the activity decreased when using the other substrates, with 112.5, 78.3 and 41.6 units for catechol, guaiacol and para ansidine respectively. This is evidence that the cleavage laccase enzyme from Cladophora sp. has a high potential for oxidation of the orthotolidine substrate than the other phenolic compounds used in the study. It has been observed that the refined laccase enzyme stimulates the oxidation of various substances; the enzyme gave the highest specific activity when using ABTS as a substrate 44 units / mg protein [5].

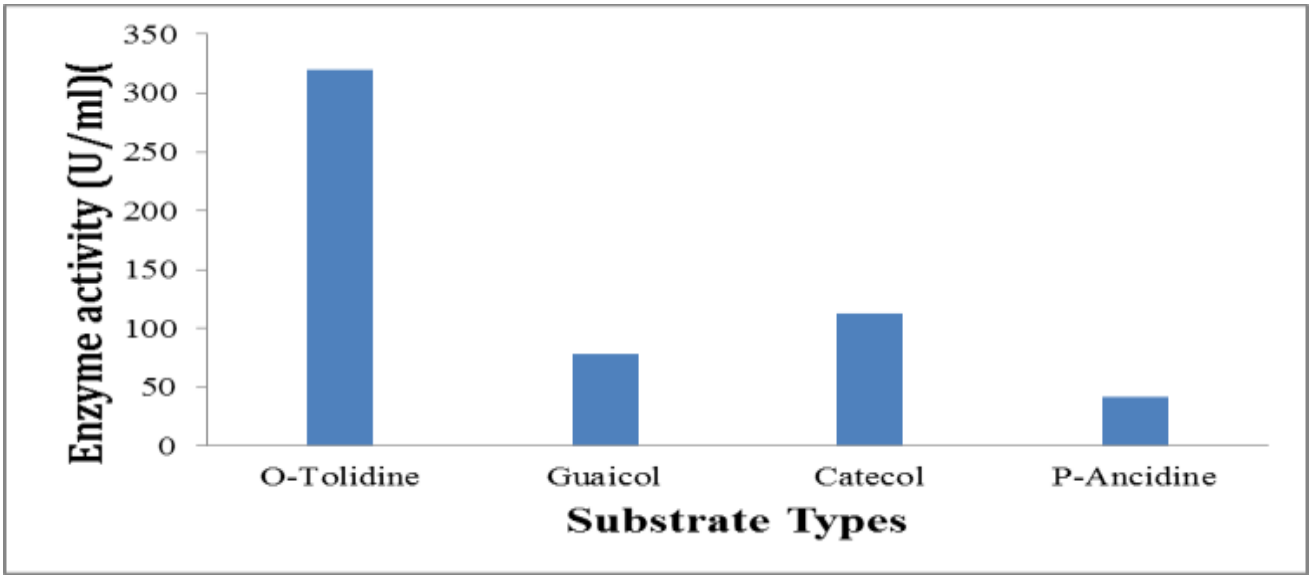


Figure 3-2: Enzyme activity extracted of Cladophora sp. towards various reaction substances at 35 °C pH 5.5

Determination of the optimal pH of the of the enzyme activity

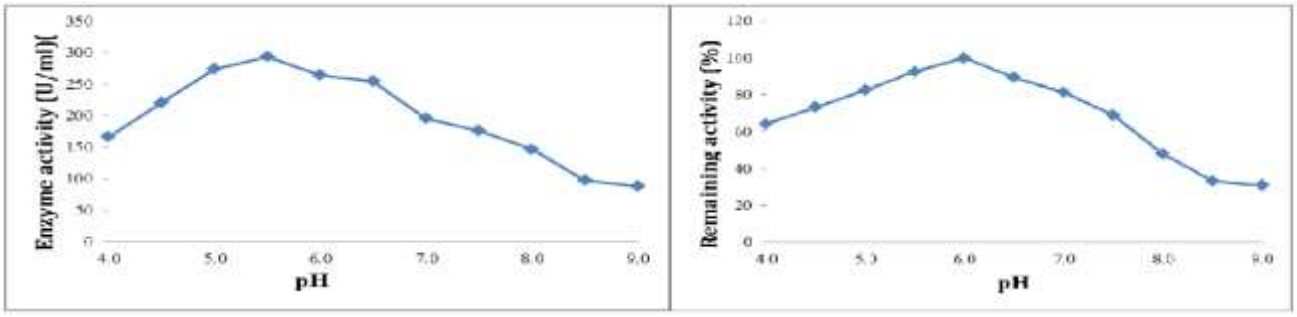
Optimal pH was determined for laccase activity from the Cladophora sp. after the reaction material was prepared with different values of pH 4.0–9.0 by interval0.5 °. The results showed that the optimal pH for enzymatic activity ranged from 5.0–6.5. It is active at pH 5.5 by 293.48 unit / ml (Figure 3-3 A), and it was noted that the activity has decreased in the extreme alkaline medium significantly more than in the extreme acid medium.

The activity of laccase enzymes is highly dependent on the nature of the substrate and its pH. It has been observed that the enzyme activity decreases at high pH values. Ion hydroxide is linked to the copper center T_2 /T_3 of the enzyme, which disrupts the electron transfer internally between T_1 and the copper center [19]. The results were approved for the study of Eichlerova et al. [20], they found that the optimal pH of the purified enzyme was 5.5 when using Dimethoxy Phenol and Dihydroxy-Phenyl Alanine as substrates. In a study of Vantamuri and Kaliwal [21], the highest activity of the purified enzyme was at pH 5.5 when Guaiacol was used as a substrate.

Determination of the Optimal PH of the Enzyme Stability

The effect of pH on the stability of laccase enzyme was studied by incubating the enzyme solution in a water bath at 30 ° C for 20 minutes with equal volumes of buffer solutions between 4.0-9.0 and by interval 0.5°. The enzymatic activity of all values, the results shown in (Figure 3-3 B) showed that the optimal pH for stability ranged between 5.5-6.5, with the enzyme remaining at 100% effective at pH 6.0 and with a high activity of 82.5%, 92.7% and 89.4% at pH 5.0, 5.5 and 6.5 respectively.

In the alkaline medium, the remaining activity was reduced to 30.9% at pH 9.0. The decrease in enzyme activity at the pH values is due to its effect on the synthesis of the enzyme that leads to an enzyme molecule or changes in the ionic state of the active site of the enzyme as well as its effect on the secondary and tertiary structure of the enzyme causing loss of enzymatic activity [22].



A B

Figure 3-3: Effect of different pH values (4.0-9.0) in *Cladophora* sp. laccase activity and stability using O-tolidine as a substrate

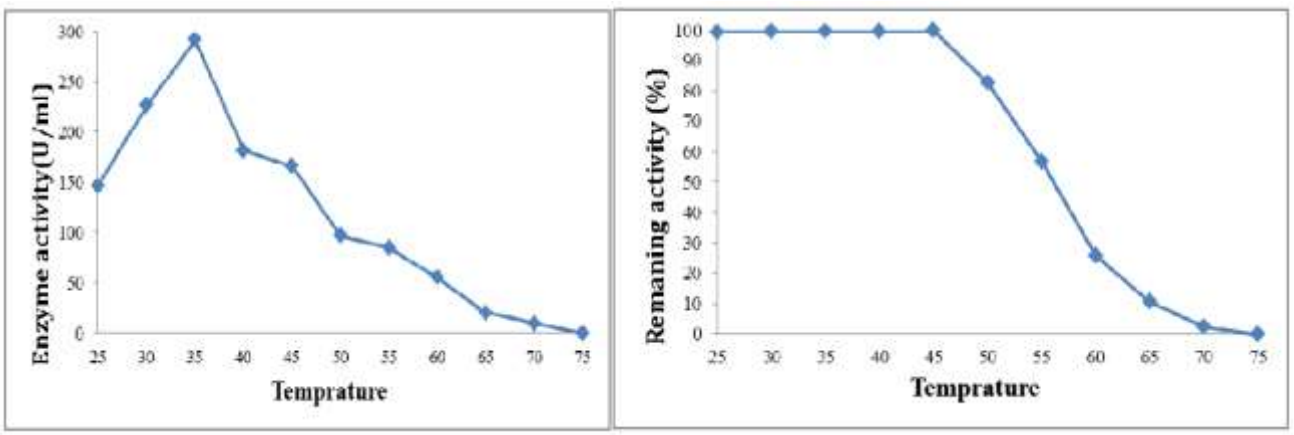
The results were consistent with previous studies, including a study of Abdullah [23] showing that the purified enzyme has enzymatic stability at pH 6.0. The results differed in other studies. Among these were the stability of the enzyme in a more acidic medium such as the study by Stiolova and others [24] on the purified laccase enzyme, they found that the optimal pH for enzyme stability was 4.5.

Determination of the Optimal Temperature for the Enzyme Activity

The enzymatic reaction was performed at temperatures ranging from (25-75) °C with difference of 5 degrees in a water bath. Enzyme activity was calculated after the addition of the enzymatic solution to the reaction solution. The results showed in (3-4A) that enzymatic activity increased with the temperature increased to 35 °C, with an enzymatic activity of 292.03 unit / ml, then decreased with continuous increase in temperature until it reached an enzymatic activity of 3.77 unit / ml at 70 °C. The reason for the increased rate of enzymatic interaction with an increase in temperature to 35 °C is due to increase the kinetic energy of molecules, increase the oxidation of enzyme molecules, substrate, and the temperature rise from certain limits leading to the enzyme monomer and loss of the tertiary structure[25], and the results were consistent with a study by Das *et al.* [26] where they found that the optimal temperature for the activity of the purified enzyme was 35° C when using ABTS as a substrate. Other studies showed that the optimum temperature of the enzyme was higher than 35 °C as in Al-Assadi [27] when using O-tolidine as a substrate.

Determination of the Optimal Temperature for the Enzyme Stability

The enzyme stabilization at different temperatures was estimated after incubation of the purified enzyme at 25-75 °C with difference of 5 degrees and the remaining activity was measured after 20 minutes of incubation. The results shown in Figure (3-4 B) showed that the optimum temperature of the enzyme stability ranged between 25-45 °C and after that the remaining activity started to decrease with the continued temperature rise up to 55 °C and the remaining enzymatic activity at 56.61%. There was a significant decrease in enzyme stability at high temperatures. The remaining activity was 10.7% and 2.5% at 65 and 70 °C respectively. Most enzymes are more stable at low temperatures, so they are stored at low temperatures.



A B

Figure 3-4: Effect of different ranges of temperature (25-70°C) on *Cladophora* sp. laccase activity and stability using O-tolidine as a substrate.

The cracking that happens to the protein due to temperature rise involves cracking of hydrogen bonds and weak covalent bonds, causing an enzyme monomer or loss of its three-dimensional structure [28]. Studies have shown that the thermal effect on enzyme stability is different in results, Wang and others [29] reported that the purified enzyme had an enzyme stability of between 25– 45 °C and the activity was maintained after incubation of the enzyme at temperatures greater than 60 °C.

The Effect of Reducing Factors and Activators on Enzyme Activity

The effect of reducing factors and activators on enzyme activity was studied after the incubation of the Cladophora sp. enzyme with some inhibitors prepared with concentrations of 10mM and 20mM containing the reducing agents and the SDS, L-Cysteine, 2-Merecaptoethanol, and EDTA). The results shown in Table (3-2) showed that the activity was reduced at the incubation of the purified enzyme with the EDTA reaction solution. The activity was reduced to 92.86% at 5mM and at a concentration of 10mM, it was observed that the activity was completely inhibited, where EDTA material, led to the withdrawal of the bionic ions found in the enzyme structure and that this decrease in activity confirms the association of the enzyme laccase to the group of Metalloenzymes that need some minerals in the active side of enzyme [30].

Table 3-2: Effect some of the reducing factors and activators on purified laccase from Cladophora sp

Reducing factors and activators	Remaining activity (%) 5mM	Remaining activity (%) 10mM
Control	100	100
EDTA	7.14	0
L-Cysteine	8.60	4.7
2-Mercaptoethanol	3.60	0
SDS	102.8	96.2

The results were agreed with studies that indicated that EDTA had a role in inhibiting enzyme activity; including study Dube et al. [31] found that the concentration of 5mM of EDTA inhibited activity. The results in Table (3-2) showed a significant reduction in enzymatic activity when incubating the enzymatic solution with the cysteine solution and the 2-merecaptoethanol solution. At concentration 5mM the remaining activity rate was (3.8 and 8.6%) respectively and in the concentration of 10mM was (4.7 and 0%), respectively. The reason for the high decline is the remaining activity at the incubation (cysteine and 2- merecaptoethanol) to reduce the two-sulfur bonds (S-S) in the protein molecule and change the protein's synthetic structure, thus dividing it into its components.

This negatively affects the activity of the enzyme [32] this means that the enzyme under study contains sulfur bridges at the active site or sites close to the active site. This is confirmed by Sondhi et al. [33]. The purified enzyme lost its activity when adding the cysteine and 2- merecaptoethanol to each reaction mixture separately, the remaining activity (1.8% and 0% respectively at the concentration of 5mM and 1.5% and 0% respectively at the concentration 10mM).

The results showed that the Sodium Dodecyl sulfate (SDS) don't effect on enzyme activity, the purified enzyme was kept active when incubated with SDS and the remaining activity rate was 102.8% at 5mM concentration and 96.2% at a concentration of 10mM, which can be interpreted according to Moore and Flurkey [34], since the association of these substances with the enzyme can cause a change in their enzymatic and physical properties. SDS increases enzymatic activity in low concentrations and reduces activity at high concentrations.

The Effect of Some Metal Ions on the Enzyme Activity

The effect of some ions on the enzymatic activity was studied after incubation of the purified enzymatic solution with some metal ions are represented by Na^{+}, Cu^{+2}, Mg^{+2} , Mn^{+2} Ca^{+2}, Zn^{+2}, Hg^{+2}, Fe^{+2} and Ag^{+} ions in the form of sulfate, chlorides and nitrates at a concentration of 5mM and 10mM. Table (3-3) the enzymatic activity of the enzyme in incubation with chloride Cu^{+2}, Zn^{+2} and sulfate Fe^{+2} solutions was increased at concentrations 5mM and 10mM.(151%, 107.7% and 129.2%, respectively, at 5mM and 178.3, 126.1 and 138.6%, respectively) at a concentration of 10mM.

Table 3-3: Effect some of the metal ions on purified laccase of *Cladophora* sp.

Metal Ions	Remaining Activity (%) 5mM	Remaining Activity (%) 10mM
Control	100	100
$CuCl_2$	151	178.3
$ZnCl_2$	107.7	126.1
$FeSO_4$	129.2	138.6
NaCl	104	95.6
$CaCl_2$	101	92.3
$AgNO_3$	54.2	31.8
$HgCl_2$	20.5	8.3
$MnCl_2$	36.1	11.5
$MgSO_4$	82.0	71.2

The results agreed with Sun et al. [35], noting that the remaining activity at the low concentrations of Cu^{+2} , Zn^{+2} and Fe^{+2} ions was high and found to be 101, 106 and 108% respectively, and Afreen et al. [36] In his bosom with ionic solutions, Cu^{+2} and Zn^{+2} they reported that the ratio was 136.6 and 120.3%, respectively, at a concentration of 1mM, the effect of metal ions on the enzyme's activity depends heavily on the origin of the enzyme as well as the type of metals used that strongly influence the catalysis of the enzyme [37] and Telke et al. [38] pointed to the role of ions Percipient to increase the rate of oxidation of the material orthotolidine, and that the activation of the positive ions Laccase copper can lead to fill the copper site T_2 linking sites with copper ions.

The results indicated in Table (3-3) that the enzyme retained a clear percentage of activity when incubating with chloride-containing Ca^{+2} and Na^{+} solutions. The remaining activity rate 106 and 101%, respectively, at the concentration of 5mM and reached 95.6 and 92.3%, respectively, at the concentration of 10mM. The results of some studies confirmed that there was no inhibitory effect of Ca^{+2} and Na^{+} chloride in the activity, which enabled it to be used in enzyme elution solutions during enzyme purification steps [39], such as ion exchange chromatography and gel filtration techniques.

The results of the inhibition of enzymatic activity shown in Table 3-3 were in solutions containing Mn^{+2} , Hg^{+2} , and Ag^{+} ions, and the remaining activity 54.2, 20.5 and 36.1% respectively at the concentration of 5mM and 31.8 and 8.3 and 11.5%, respectively, at a concentration of 10mM. These results show that there is a decrease in enzymatic activity with increasing concentration, so ionic solutions reduce the rate of oxidation of orthotolidine after incubation with the purified enzyme solution [40].

Inhibition of the enzyme in Hg^{+2} ions indicates the presence of the group (SH) at the active site of the enzyme leading to its oxidation. In addition, the presence of Hg^{+2} ions in the substrate solution can act as complexes with the enzyme that inhibits binding of the enzyme to the substrate and the formation of the product [41]. The results were consistent with results Al-Assadi [27] reported that Mn^{+2}, Ag^{+}, and Hg^{+2} ions have inhibited enzymatic activity to 50%, 56% and 24% respectively at 1mM concentration and 6.5, 20 and 4% respectively at 5mM concentration.

The Role of the Purified and Crude Laccase Enzyme in the Decolorizing of Contaminated Dyes

The viability of the pure and crude laccase enzyme was studied by removing four of the dyes at a concentration of 30 mg / l during 0, 1, 2 and 28 hour. The results indicated in Table 3-4 showed that the partially purified and crude enzyme had the ability to decolorize the dye to varying degrees, especially when incubating for prolonged periods in a 35 °C incubator, the partially purified enzyme has shown good activity in dye decolorization after 28 hours with 91.7, 82.1, 75 and 60% for black, blue, red and yellow dyes respectively.

The crude enzyme was also able to decolorize the dyes less than the partially purified enzyme. The percentage was 62.3, 47.6, 43.4 and 36.1% in the same order. The disparity in the enzyme's ability to decolorize dyes may be related to the nature of the chemical composition of the pigment and the purity of each dye, which may strongly affect the duration of decolorization [42]. The study mentioned that the blue dye was completely dissolved by the partially purified enzyme after a minute of incubation with the enzyme while the yellow dye was partially disrupted and this may be due to the complexity of the synthesis of this dye [43, 44].

In Al-Assadi [27] the decolorization using crude and partially purified enzyme gave close results after incubation of solutions for 3 hours. The percentage of yellow textile was 84.1% and 92% respectively for crude and puree respectively and the percentage of red textile was 91.1% and 94.6% respectively for crude and puree respectively, while the black textile dye had 88.1% and 92% for crude and puree respectively.

Table 3-4: The percentage of the dyes decolorizing using purified and crude laccase

The dye and the wavelength	Incubation time							
	0 h		1 h		2 h		28 h	
	Crude enzyme	Purified enzyme	Crude enzyme	Purified enzyme	Crude enzyme	Purified enzyme	Crude enzyme	Purified enzyme
yellow (450nm)	3.5	4.9	7.7	13.3	21.3	33.3	36.1	60
red (530nm)	3.8	5.5	8.4	9.4	30.5	34.4	43.4	75
black (530nm)	8.7	12.9	23.4	33.3	48.1	75	62.3	91.7
blue (610nm)	6.2	9.1	13.1	21.4	33.5	42.9	47.6	82.1

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