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Removal of p-chlorophenols from aqueous solutions using tyrosinase extracted from mushroom *Lentinula edodes*

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ABSTRACT

Keeping clean environment is vital matter for human health and other organism. In last decade, the usage of living systems to treat pollution get a real attention. Bioremediation ia a way to treat wastewater using enzymes. This study was aimed to introduce a simple and inexpensive method for efficient removal of chlorine substituted phenolic compounds result from some industries that present in aqueous solution by use the enzyme tyrosinase extracted from mushroom Lentinula edodes from local area. The optimum conditions of enzyme activity were determined. Result of resent study indicate that tyrosinase effectively remove chlorine substituted phenolic compounds. Present study was concluded that mushrooms Lentinula edodes could be use as a source of enzyme tyrosinase which can effectively remove one of harmful hazardous by-products of industry a chlorine substituted phenols from aqueous solution in low-cost way.

KEY WORDS: p-Chlorophenols, Aqueous Solutions, Tyrosinase, Mushroom *Lentinula edodes*.

1. INTRODUCTION

One of the most important elements of life is the water as it enters into all walks of living system, in economy, in environment, in food security, in industry and in politics (Karakilcik, 2014).

Nowadays, the supply lack of water is considered a global problem and occupies critical role in political conflicts. Individuals and communities can survive for considerable periods with a deficiency of several fundamental goods, but they can not survive for more than few day without safe and clean drinking water (Dolatyar, 2016). Human living in destitution, especially those living in developing countries, facing a big trouble due to the lack of sufficient and safe water supplies, women suffer from hardship. This issue is seriously becoming threatening matter for life and, of course a concept of human right (Vivoda, 2016).

Industry is the backbone of life in developed societies, but its impact on the environment in general and the aquatic environment in particular, is very harmful, especially when the non-sutable procedures to be followed to maintain a safe living environment. Paint industries, plastics industries and oil refineries discharge harmful waste into rivers, which in turn convert to drinking water. For this reason, its very necessary to keep an efficient treatment facilities for waste water in this sites of industry before draining their wastes in rivers (Russo and Smith, 2013).

Water pollution and its negative impact on human health remain a major source of morbidity and mortality in indistrial and developing countries. The governments of these countries are exerting huge efforts to govern a healthy water environment, in addition to their potential in water treatment (Wang and Yang, 2016).

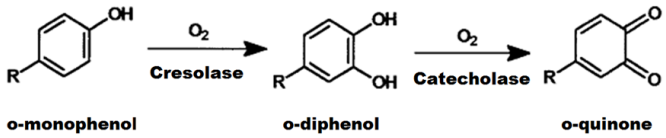
In last decade, the usage of living systems to treat restive pollutants for instance the use of plants and microorganisms are acquire importance as an applicable alternative to current physicochemical removal methods (Franciscon, 2009; Kagalkar, 2009). The development of simple and effective biological methods is necessary to overcome many challenges such as the stringent in government policies concerning allowed concentrations of pollutants in addition to expensive budget of chemical that use to remove pollutants as well as the problem of production of solid waste from physicochemical water treatment (Chung, 2003).

Many industries like pulp and paper manufacturing, coal conversion, metal casting and wood preservation produce many compounds including phenolic compounds as waste product and effluent from such operations. Phenolic compounds and effluent from these industries consider harmful and hazardous to human health, and therefore the evolution of an efficient method for their elimination is utterly important (Alta'ee, 2013).

The classical method of wastewater treatment, such as adsorption on activated carbon, solvent extraction and oxidation reduction reaction have a substantial drawbacks including expensive operating and production of harmful hazardous by-products (Chung, 2003; Alta'ee, 2013). The use of plants and microorganisms in pollution treatment is called bioremediation of wastewater (Mohapatra, 2006).

One of bioremediation of wastewater treatment is the use of enzymes. Enzymes are very important biomolecules to maintain the health of human and environmental aspects (Hadwan, 2014; Hadwan, 2014; Hadwan, 2014; Abdulsamie, 2015). Tyrosinases are metalloproteinase enzymes, containing copper in their active sites and are belonging to oxidoreductases class of enzymes, which mediate the catalysis of oxidation reactions in wide range of phenolic compounds using molecular oxygen as a substrate (Saeidian, 2016). Tyrosinase or monophenol, dihidroxyphenylalanine oxygen oxidoreductase (E.C.1.14.18.1) is an enzyme which catalyzes two types of

subsequent and separate reactions by use molecular oxygen as a substrate. The first reaction which so called (cresolase activity) is the hydroxylation in the ortho position of monophenols to ortho-diphenols, and the other one which so called (catecholase activity) is the subsequent oxidation of ortho-diphenols to produce ortho-quinones (Pellei, 2009), as shown in Scheme.1.



Scheme.1. Reaction Catalyzed by Tyrosinase

This study was aimed to introduce a simple and inexpensive method for efficient removal of chlorine substituted phenolic compounds present in aqueous solution by use tyrosinase extracted from mushroom *Lentinula edodes*.

2. MATERIALS AND METHODS

Materials: Fresh mushrooms *Lentinula edodes* were collected from Alraranjia farms, south of Hilla City, Iraq.

Preparation of Tyrosinase: The enzyme tyrosinase that use in this study were prepared from fresh mushrooms *Lentinula edodes* by precipitation with ammonium sulfate solution and converted to powder using freeze drier (lyophilizer).

Characterization of Tyrosinase Activity: L-tyrosine was used as a substrate to determined tyrosinase activity in sodium phosphate buffer (50 mM pH 6.5) at 25°C (Decker, 1977). The use of L-tyrosine as a substrate limits the measurement of tyrosinase activity to monophenolase action that maintains the monophenolase cycle. One unit of tyrosinase activity calculated from increase in absorption at 280 nm of 1 X 10^{-3} absorption unit per min. in a three milliliter reaction mixture that contain 0.181 mg of the amino acid L-tyrosine in one cm light path with quartz cuvette.

Dephenolization by Tyrosinase Activity: One liter of aqueous solution containing p-chlorophenol, was treated with a solution of tyrosinase prepared by dissolving 50 mg of tyrosinase powder in 50 ml of phosphate buffer (50 mM, pH 6.5). The mixture was incubated on a rotator at 25 °C and agitated at 250 rpm. Every five min, a one ml of mixture was taken for tyrosinase activity test. Tyrosinase activity assay was repeated three times for each sample. The p-chlorophenol removal efficiency from solution was determined using the conversion percentage as shown below:

$$\text{Conversion}(\%) = \frac{[\text{phenol}]_o - [\text{phenol}]_t}{[\text{phenol}]_o} \times 100\%$$

Where $[\text{phenol}]_o$ and $[\text{phenol}]_t$ are the primary and remaining levels of p-clorophenol in the reaction mixture, respectively (Decker, 1977).

Analysis of p-Chlorophenol: Determination of p-chlorophenol in the samples was performed using a spectrophotometric method, in which, the phenolic compounds present in sample react with 4-aminoantipyrine (4-AAP) under alkaline conditions and in the presence of potassium ferricyanide (Gomez, 2006). Reaction mixture contains one ml of sample, one ml of 2.0 mM 4-AAP and one ml of 6 mM potassium ferricyanide reagent. Potassium ferricyanide and 4-AAP were prepared by dissolving in a solution of 0.1 M, pH 10 of phosphate buffer. The absorption of the reaction mixture was read at 510 nm after 15 min of incubation at room temperature, using a spectrophotometer (Gomez , 2006).

3. RESULTS AND DISCUSSION

Dephenolization by Tyrosinase: Amount of 250, 500, 1000 internal units (IU) were used as amount of enzyme concentration, and shown increase in substrate removal with increase concentration of enzyme, Result of present study shows increase in p-chlorophenol removal with time by increase of tyrosinase amount in reaction mixture. as shown in figure.1.

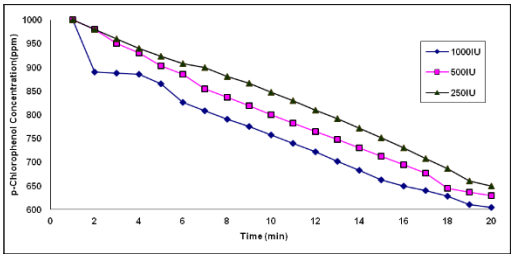


Figure.1. Effect of Tyrosinase Amount on p-Chlorophenol Removal.

The effect of pH on tyrosinase activity and as concequance on p-chlorophenol removal from reaction mixture was tested in a wide range of pH between (3-11) to determine the optimum pH of assay. Result of present study

shows that the optimum pH of tyrosinase activity is 7 to remove p-chlorophenol from aqueous solution, as shown in figure.2.

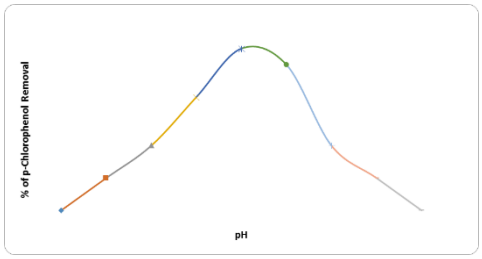


Figure.2. Effect of pH on Tysosinase Activity

The effect of incubation temperature on tyrosinase activity by use the removal of p-chlorophenol from aqueous solution was studied by carry out the enzymatic reaction in deferent temperature, range from (20-100) °C. Result of current study shows that 50 °C is the optimam temperature of incubation, as shown in figure 3.

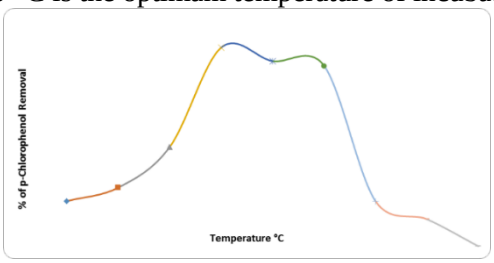
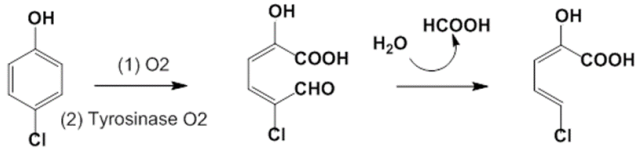


Figure.3. Effect of Temperature on the Tyrosinase Activity

Result of present study is agreed with Atlow (1984), study which reported that the efficiency of removal of p-chlorophenol was about one handred percent with 60 units/ml of tyrosinase activity. Present study was found that one units/ml of tyrosinase activity remove forty percent of p-chlorophenol from aqueous solution.

Generaly when p-chlorophenol undergoes biodegradation, this will accomplish in two phases; aerobic and anaerobic pathways. Firstly, p-chlorophenol undergoes oxidative dechlorination and p-chlorocatechol formation followed by reductive dechlorination and benzene ring cleavage in the next step (Atlow, 1984), as shown in scheme.2.



Scheme.2. Degradation Pathway of p-chlorophenol

White rot fungi such as *Phanerochaete chrysosporium* which contains the enzyme tyrosinase makes chlorine substituted aromatics to be converted to quinines and then to simpler products like CO_2, H_2O and inorganic chlorides (Mashkour, 2011). The gram negative bacteria *Flavobacterium scophthalmum* or *Ralstonia chlorophenolicusis* that contain tyrosinase can degraded polychlorophenols by initiation the p-hydroxylation to produce p-hydroquinone. Under anaerobic conditions such as in case of anoxic soil conditions, in marine, in lower layers of aquatic sediments, and in freshwater ecosystems, the reductive dechlorination of p-chlorophenol will result in replacement of chlorine by hydrogen atoms, where p-chlorophenol behave as an electron acceptors in anaerobic respiration reaction occur in microbes, threrefore poly chlorinated phenols could be degraded anaerobicly and resistant the aerobic breakdown (Filley, 2011).

Chlorophenolics, tannins and wood resins exhibt harmful impact on anaerobes like methanogenic bacteria and hence inhibit the biodegradable substrate metabolic breakdown. Therefore, anaerobic treatment followed by aerobic treatment will permit the residual toxic compounds to be degraded (Karam, 2015). Bleach sequence of wastewater include peroxide stage of the anaerobic treatment which pointed out that these wastewater underwent inhibitory impact on methanogenic bacteria but overcome this obstacle of degradation by acidogenic cultures (Raheem, 2016).

4. CONCLUSIONS

Mushrooms such as *Lentinula edodes* that grown in Alraranjia farms, south of Hilla City, Iraq could be use as a source of enzyme tyrosinase which can effectively remove one of harmful hazardous by-products of industry a chlorine substituted phenols from aqueous solution in low-cost way.

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