PEROXIDASE CATALYZED THE REMOVAL OF PHENOL FROM SYNTHETIC WASTE WATER

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ABSTRACT

The implementation of increasingly stringent standards for the discharge of waste water and wastes into the environment has motivated the development of alternative processes (enzymatic treatment) for the treatment and the disposal of wastes. These processes are developed to minimize the need for effluent disposal and to reduce the quantity and maximize the quality of effluent waste streams that are created during production processes. Phenols in our environment come from various sources. For example, many are found in the waste waters of industries such as petroleum refineries, glue and resin manufacturers, coal processing, pulp and paper mills, and from the leaching of municipal landfills. Phenol is toxic to fish at a level of 0.05 mg/l, therefore the removing of phenols from waste water is therefore of great importance. The aim of this research were studying the effect of treating aqueous solutions contains high concentrations of phenol, 8mM phenol and low concentration of phenol, 1m mole phenol with the enzyme peroxidase extracted from horseradish, hydrogen peroxide (H_2O_2) and poly ethylene glycol (PEG). It was found that the most effective addition of horseradish peroxidase and hydrogen peroxide were 1 U/ml and 10.0 m. mole respectively at neutral pH, for removing 70% of the phenol from aqueous solutions contains 8mM phenol. It was found also that the most effective addition of horseradish peroxidase and hydrogen peroxide were 0.3 U/ml and 3.0 m. mole respectively at neutral pH, for removing 80% of the phenol from aqueous solutions contains 1 m. mole phenol. Precipitation the phenolic oxidation products that resulted from the enzymatic treatment, by coagulation and precipitation by different coagulants alumina and quick lime has been studied.

Keywords: horseradish peroxidase, enzymatic treatment, phenolic polymers, coagulation, quick lime.

1. INTRODUCTION

Phenols in our environment come from various sources. For example, many are found in the waste waters of industries such as petroleum refineries, glue and resin manufacturers, coal processing, pulp and paper mills, and from the leaching of

municipal landfills. Phenol is toxic to fish at a level of 0.05 mg/l, therefore the removing of phenols from waste water is therefore of great importance.

Most of the methods used for transformation of wastes and pollutants and treating wastewaters are physical, chemical or biological. Chemical transformations involve the application of reagents and reaction conditions to transform and treat target species.

Chemical methods involve the application of reagents and reaction conditions to transform and treat target species. Chemical processes often require the presence of excess quantities of reagents to accomplish the transformation to the desired extent. In addition harsh conditions (e.g., high temperature or extreme of pH) are sometimes required to facilitate the chemical transformation. Many chemical treatment processes are not highly selective in terms of the type of pollutants that are transformed during treatment. The high cost and disposal of contaminated media are the disadvantages of chemical treatment.

Biological processes make use of the natural metabolism of cells to accomplish the transformation or production of chemical species. The metabolic processes occur as a result of a sequence of reactions conducted inside the cell that are catalyzed by proteins called enzymes. An important advantage of biological systems is that they can be used to carry out processes for which no efficient chemical transformations have been devised. In addition, biological processes can often be conducted without the harsh conditions that are necessary during chemical transformations. Due to the large water hold-up volume and bacterial culture contact time, these systems are very large and heavy, and therefore only suitable offshore for low volume application. Also there are operational and bacterial inhibition problems. The method is best suited to onshore installations where space and volume are not limitations [1].

The following potential advantages of an enzymatic treatment over conventional biological treatment were noted [2]: action on, or in the presence of, many substances which are toxic to bacteria; operation at both high and low concentrations; no shock loading effects; no delays associated with acclimatization of biomass; reduction in sludge volume (no biomass generation).

The aims of this study were: Extracting the enzyme peroxidase from horseradish roots by using mixing with tap water. Determination of the optimum parameters of enzymatic treatment (Horseradish peroxidase dose, peroxide dose (H_2O_2)) for oxidation of phenol at aqueous solutions contains high and low phenol concentrations. To remove the phenolic oxidation products by coagulation and precipitation by different coagulants alum and quick lime (CaO).

2. MATERIALS AND METHODS

2.1 Materials and Equipments

HRP enzyme (EC 1.11.1.7) was extracted from horseradish in our lab. All the following chemicals were of analytical grade and were purchased from FECTON (Russian). Hydrogen peroxide (30% w/v), solid phenol, 4-aminoantipyrine and potassium ferricyanide [$K_3Fe(CN)_6$] for analysis of phenol and activity of horseradish peroxidase. Quick lime (CaO) and alum (aluminum sulphate) were used as coagulants.

Photoelectrocolrimeter (VEK-II) (wavelength 300 to 700 nm) carried out for absorbance measurements. Glass cuvettes with an optical path length of 1.0 cm and a volume of 5 mL were used. Measurements of pH were made using a pH meter (Russian). Centrifuge with 6000 max revolution per minutes (rpm), and with a capacity 200 ml. Magnetic stirrer with coated magnetic bar was used for mixing reactants with the produced water.

2.2 Analytical Methods

The activity of the horseradish peroxidase enzyme was measured by colorimetric method. Fixed concentrations of phenol, hydrogen peroxide and 4-aminoantipyrine (AAP) were reacted with the enzyme solution under controlled pH and temperature conditions. The pH was maintained constant with the addition of a buffer solution of pH 7.4. The reaction resulted in the formation of a non-precipitating product which absorbed light at a peak wavelength of 510 nm with an extinction coefficient of 7311 $M^{-1}cm^{-1}$. The rate of reaction is proportional to the rate of color formation [3].

The concentration of total phenols was measured using a colorimetric method. Phenolic materials react with 4-aminoantipyrine in the presence of potassium ferricyanide at a pH of 10 to form a stable reddish-brown colored antipyrine dye. The amount of color produced is a function of the concentration of phenolic material [4].

2.3 Experimental Procedure

Batch experiments for enzymatic treatment were conducted at room temperature (approximately 25℃). The batch reactors were glass vials of capacity 100 mL, which contained 50 mL of synthetic waste water (phenol - distilled water) and predetermined doses of each of horseradish peroxidase enzyme (HRP), hydrogen peroxide (H_2O_2) and poly ethylene glycol (PEG) has been added. A magnetic stirrer with a magnetic bar was used for mixing agitation of the synthetic waste water with the reactants for a specific time and at medium speed. After treatment the resulting solution was centrifuged for 30 minutes at 6000 rpm. The supernatant was analyzed for phenol as described earlier.

For coagulation studies, jar tests were carried out. The objective of the jar test was to determine the optimum dose and the pH value at which a coagulant should be introduced to the waste water. Alum and quick lime were used as coagulants. After treatment of phenol with horseradish peroxidase HRP, H_2O_2 and PEG, the resulting solution contains colored products (phenolic polymers). Four samples of this solution were treated by a specific dose of alum in a jar test. Each sample of this solution has a 50 ml volume, alum was added with a specific dose, and stirring occurred for 15 minutes and then stopped. The change in the color of the solution sample (clarification percentage) was measured at a specific time by measuring the relative absorbance of the solution sample at 400 nm by a photoelectrocolorimeter.

3. RESULTS AND DISCUSSION

3.1 Extraction of Peroxidase from Horseradish Roots

Extraction of horseradish peroxidase enzyme from minced horseradish roots using soft tap water has been occurred in a mixer at high speed for a specific time (1-4 hours). Two different ratios of horseradish to tap water were used 50 gram minced horseradish roots to 500 milliliter of tap water, and 100 gram minced horseradish roots to 500 milliliter of tap water. The resulting solution from mixing horseradish with water, for predetermined time, was filtered and the supernatant was centrifuged at 4000 rev/min. The supernatant was stored at -4°C.

Every day the activity of HRP was analyzed before using the enzyme in the tests. The activity of the enzyme is defined in units; one unit of activity (U) is defined as the number of micromoles of hydrogen peroxide which are consumed in one minute at pH 7.4 and 25°C [3].

Figure 1 demonstrates the dependence of the activity of peroxidase on the extraction time. The parameters that were studied were horseradish roots weight to water ratio and time of extraction of enzyme. The activity of enzyme per milliliter extract was dependent on the ratio of horseradish roots weight to water, and the time of mixing the horseradish in tap water.

With increasing the time of mixing, the activity of the enzyme in the extract becomes higher, and reached 7U/ml for ratio of horseradish roots (HR) to water equal 0.1 (50 gram HR per 500 ml water), and 8 U/ml for ratio of HR to water equal 0.2 (100 gram HR per 500 ml water). The total units of enzyme extracted were dependent on the volume of the water used for extraction. This means that the optimum ratio of HR to water was 0.1 (50 gram per 500 milliliter water) and also the optimum activity of HRP was 7 U/ ml extract.

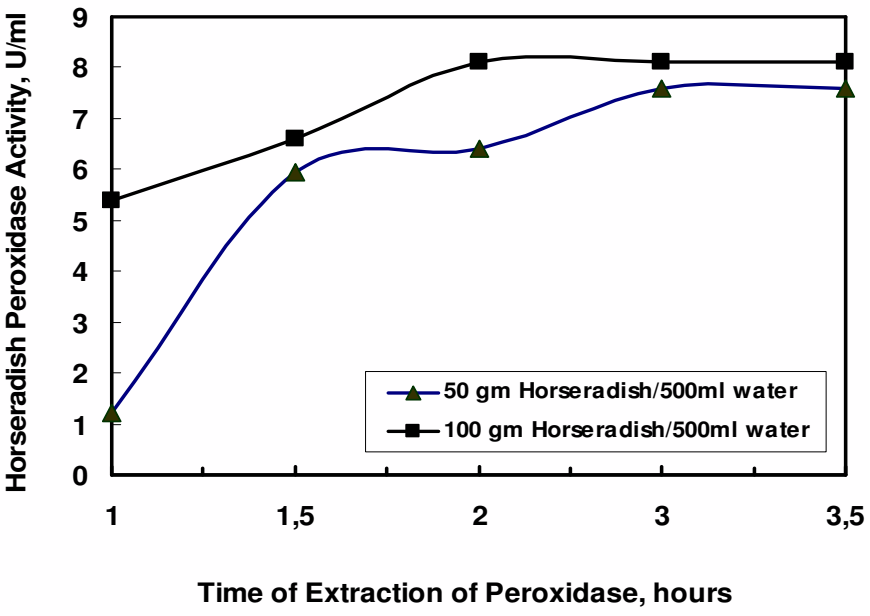

Figure 1. The dependence of the activity of peroxidase on the mixing time of horseradish roots in water

3.2 Mechanism of HRP- H_2O_2-Phenol Reaction

Horseradish peroxidase undergoes a cyclic reaction when reacting with phenolic substrates [5]. This sequence is summarized in the following reactions:

$$E + H_2O_2 \rightarrow Ei + H_2O \quad (1)$$

$$Ei + PhOH' \rightarrow Eii + PhO \quad (2)$$

$$Eii + PhOH'' \rightarrow E + PhO + H_2O \quad (3)$$

The enzyme starts in its native form (E) and is oxidized by hydrogen peroxide (H_2O_2) to form an active intermediate compound known as compound 1 (Ei). Compound 1 oxidizes one molecule of phenol (PhOH) to form a phenol free radical (PhO) and become compound II (Eii). Compound II oxidizes a second phenol molecule to produce another phenol free radical and complete the cycle by returning to its native form E. The free radicals polymerize and form insoluble compounds which precipitate from solution.

The polymerization reaction is illustrated in equation (4):

$$PhO + PhO \rightarrow \text{Polymer of aromatic products} \quad (4)$$

3.3 Effect of pH

The pH of each sample was adjusted to be between pH 2 and pH 10 using concentrated HCl or NaOH. The pH of the solution was adjusted before stirring and after addition of the phenol, PEG, H_2O_2, and HRP. Stirring of the aqueous solution in the presence of the chemical substances has been conducted in the sake of oxidation of phenol by using H_2O_2 in the presence of HRP enzyme. As a result of oxidation of phenol and formation of the oxidation products (phenolic polymers), which with less toxicity to the environment, the remaining phenol in the reaction solution was decreased and a measurement of the removal percentage of phenol has been achieved.

Figure 2 shows that the optimal removal of phenol from the aqueous solution occurred between pH 4 and 8, with optimum removal percentage 60% of phenol (Final phenol concentration 3.2 m. mole). The removal of phenol decreased at high acidic and alkaline conditions, this may be due to the effect of OH and H ions on the oxidation reaction of phenol by using H_2O_2 and HRP. This study demonstrated that HRP is slightly less susceptible to pH changes and is probably suitable for the treatment of phenol at slightly acidic and alkaline conditions.

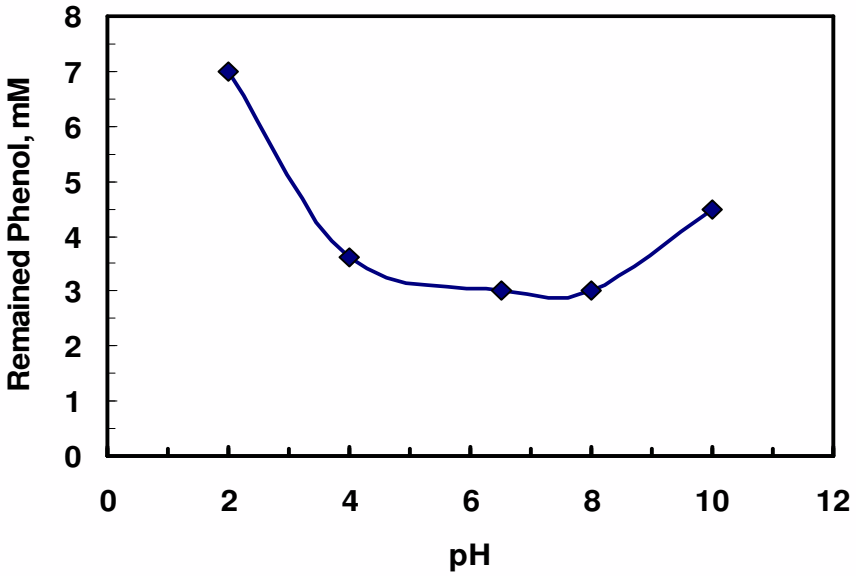

Figure 2. Effect of pH on the removal of phenol using crude HRP. Experiment conditions: 50ml aqueous solution (distilled water contains 8mM (752mg/l phenol), 8mM H_2O_2, 0.8U/ml HRP, 250 mg/l PEG, and mixing time 3 hours

3.4 Effect of Stirring Time

Mixing of the enzyme peroxidase with hydrogen peroxide and the phenol (at predetermined concentration) for a specific time causes oxidation of phenol to less toxic form.

Figure 3 demonstrates the effect of stirring time of peroxidase on the remained phenol. Removing of 60% of phenol after mixing the peroxidase with phenol in aqueous solutions has been occurred when the initial phenol concentration was 1 m. mole.

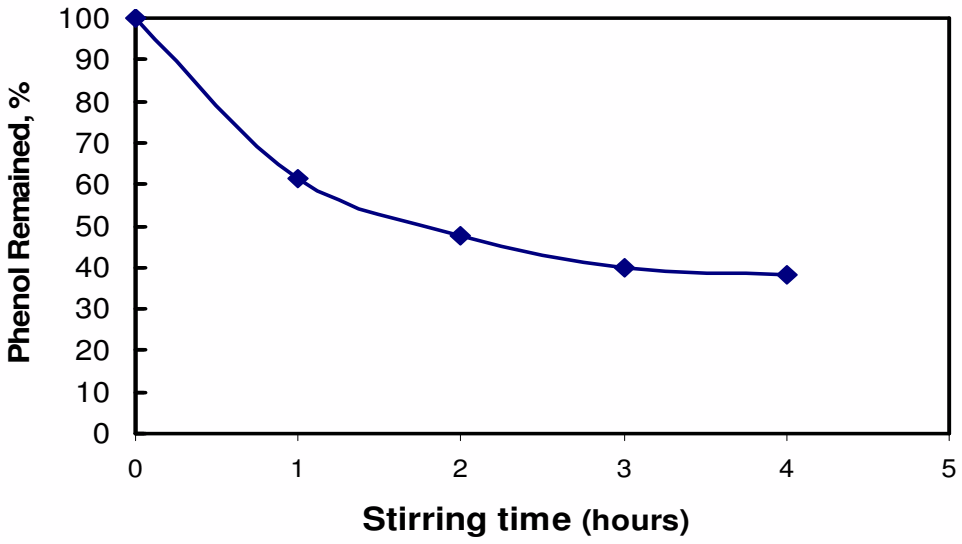

Figure 3 Effect of stirring time on the phenol remained in aqueous solution, when the initial phenol concentration was 1.0 m. mole

3.5 Hydrogen Peroxide (H_2O_2) Dose

Two different phenol concentrations 1mM (94mg/l) and 8mM were used in these tests. Hydrogen peroxide (H_2O_2) was added in predetermined amounts in order to determine the effect of hydrogen peroxide on extent the removal of phenol at initial phenol concentration 1 mM or 8 mM.

Figure 4 shows that, at initial phenol concentration 1m. mole (94 mg/l), as H_2O_2 dose increases the removal efficiency of phenol increased until the optimum value was attained. Maximal removal of phenol was 70% in the presence of 3 mM H_2O_2. The optimum peroxide concentration H_2O_2 is a function of the treated phenol concentration and the reaction conditions (HRP dose, pH). The increase of H_2O_2 dose leads to increase of the removal of phenol.

Figure 5 shows that, at initial phenol concentration 8.0m mole (752mg/l), as H_2O_2 dose increases the removal efficiency of phenol increased until the optimum value was attained. The optimum hydrogen peroxide dose was 10mM. Maximal removal of phenol was 65% in the presence of 10 mM H_2O_2. A final phenol concentration 2.4 mM, when the initial phenol concentration was 8 mM and optimum H_2O_2 concentration was 10mM.

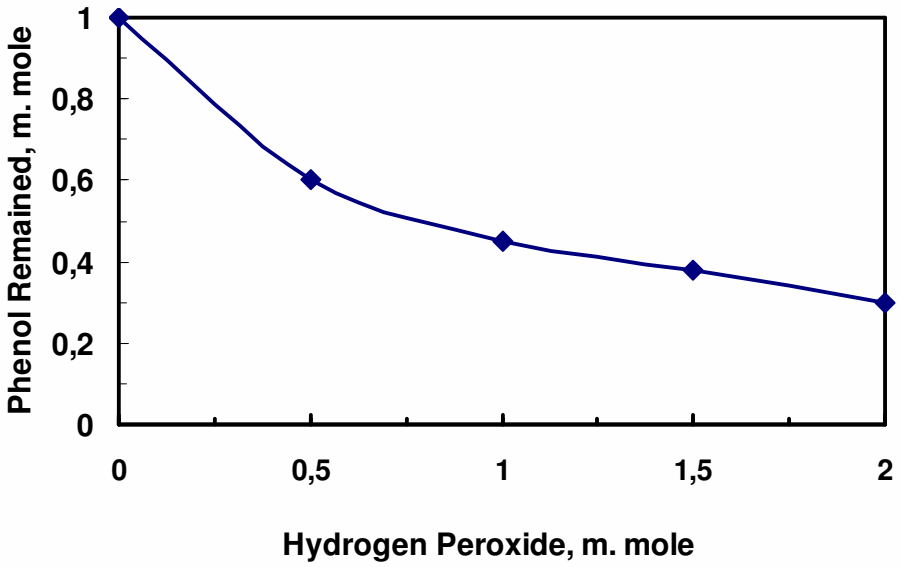

Figure 4. Effect of H_2O_2 additions on remaining phenol in synthetic waste water. Experiment conditions: 50ml aqueous solution (distilled water contains 1.0mM (94mg/l) phenol), 0.2U/ml HRP, 250 mg/l PEG, and mixing time 3 hours.

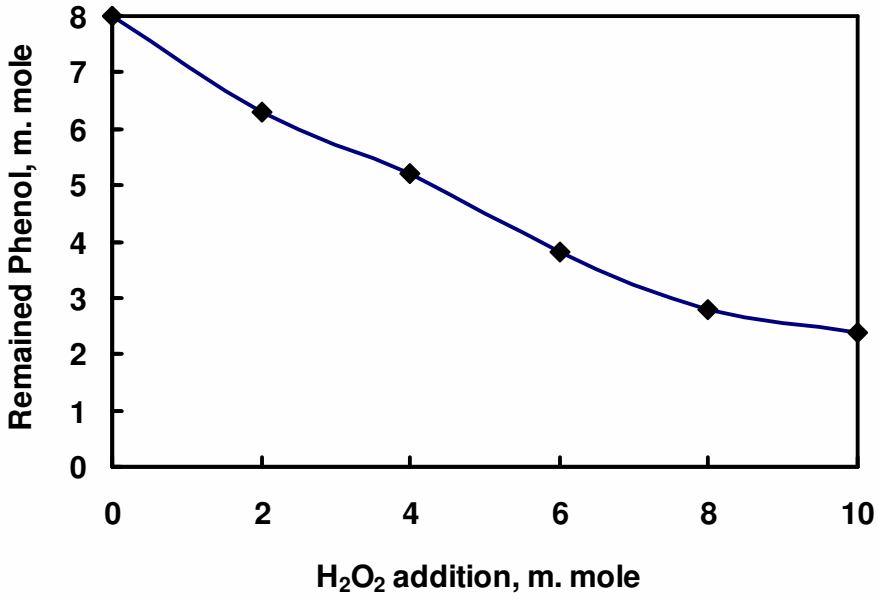

Figure 5. Effect of H_2O_2 additions on remaining phenol in synthetic waste water. Experiment conditions: 50ml aqueous solution (distilled water contains 8mM (752mg/l) phenol), 1U/ml HRP, 250 mg/l PEG, and mixing time 3 hours, without addition of PEG

3.6 Peroxidase Dose

Two different phenol concentrations 1 mM and 8 mM were used in these tests. Peroxidase was added in predetermined amounts in order to determine the effect of

HRP dose on extent the removal of phenol at initial phenol concentration 1 mM or 8 mM.

The optimum HRP dose was determined at 1mM phenol and 3 mM H_2O_2 concentration. As appeared at Figure 6, when the initial phenol concentration was 1mM, the effective HRP dose was 0.3 U/ml of the solution at H_2O_2 dose 3 mM, and the PEG dose 250 mg/L. Figure 6 shows that with the increase in HRP dose, the removal of phenol increases and that maximal removal of phenol were 80%, at optimum HRP dose 0.3 U/ml and a final phenol concentration 0.2 mM.

As appeared at Figure 7, the optimum HRP dose was determined when the initial phenol concentration was 8 mM, the effective HRP dose was 0.8 U/ml of the solution at H_2O_2 dose was 8 mM and PEG dose 250 mg/L. Figure 7 shows that with the increase in HRP dose, the removal of phenol increases and that maximal removal of phenol were 70%, at optimum HRP dose 1.0 U/ml and a final phenol concentration 2.4 m. mole.

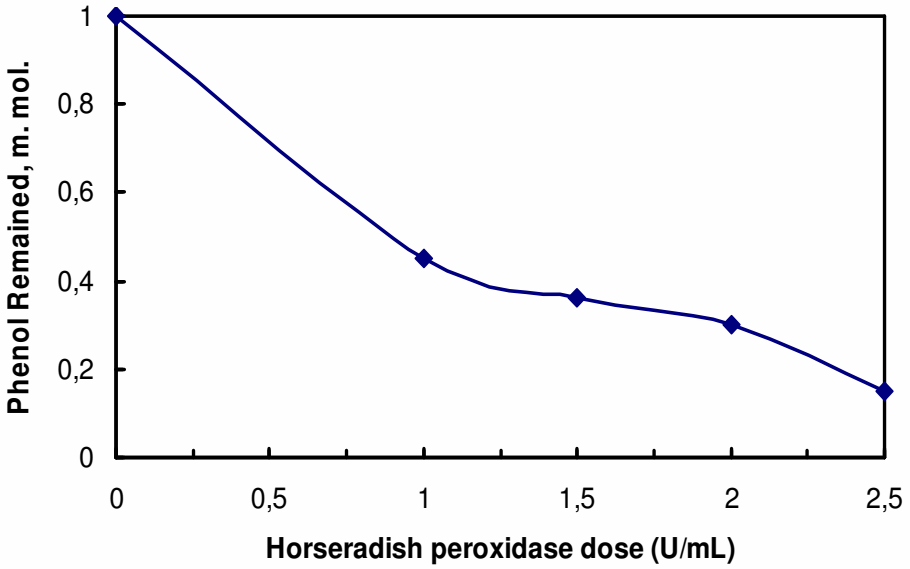

Figure 6 Effect of addition of HRP on the phenol remaining in synthetic waste water, at initial phenol concentration, 1m. mole.
Experiment conditions: 50ml aqueous solution (distilled water contains 1.0mM (94mg/l) phenol), 3.0mM H_2O_2, 250 mg/l PEG, and mixing time 3 hours

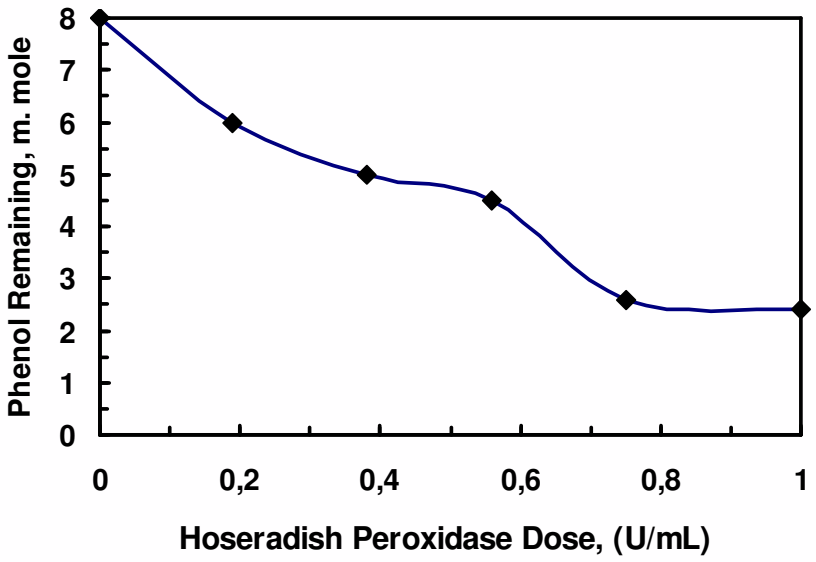

Figure 7. Effect of addition of HRP on the phenol remaining in synthetic waste water, at initial phenol concentration, 8.0 m. mole.
Experiment conditions: 50ml aqueous solution (distilled water contains 8mM (752mg/l) phenol), 8mM H_2O_2, 250 mg/l PEG, and mixing time 3 hours

3.7 Effect of Poly Ethylene Glycol Dose

Poly ethylene glycol was prepared in a concentration of 5 g/l, and stored in the refrigerator. A predetermined dose was added to each test to determine the effect of PEG on the enzymatic treatment efficiency. Figure 8 demonstrates the effect of poly ethylene glycol on the concentration of remained phenol in aqueous solution, when the initial phenol concentration was 8mM. The effective PEG dose was 275 mg/L, when HRP dose was 1.0 U/ml of the solution and H_2O_2 dose was 10 mM. Figure 8 shows that with the increase in PEG dose, the removal of phenol increases and that maximal removal of phenol were 75%, at optimum HRP dose 1.0 U/ml and a final phenol concentration 2 m. mole.

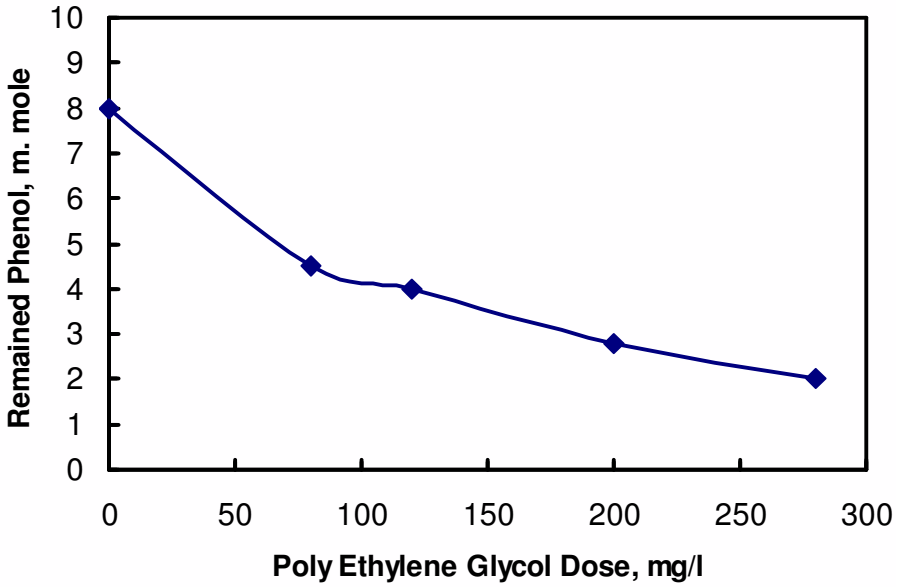

Figure 8. Effect of poly ethylene glycol dose on the remained phenol concentration in aqueous solution after enzymatic treatment, when the initial phenol concentration was 8 m. mole
Experiment conditions: 50ml aqueous solution (distilled water contains 8mM (752mg/l) phenol), 1U/ml HRP, 10mM H_2O_2 and mixing time 3 hours.

3.8 Coagulation of Phenolic Polymers

3.8.1 Precipitation of phenolic polymers using alum

Alum (aluminum sulphate) was used at two pH values, at slightly acidic conditions pH 5.6 and at slightly alkaline conditions, pH 8.0.

Figure 9 shows that at slightly alkaline condition, pH 8.0, the coagulation of the colored products was little by alum. Maximum color removal of the colored products (clarification percentage) was only 40%, at an alum dose 5.5 gm/l, after 18 hours (clarification time) from the moment of stopping the stirring in the jar test.

Also Figure 9 illustrates that when using alum at slightly acidic conditions, pH 5.6, the coagulation of the colored products were increased by increasing the alum dose and clarification time (time after the stopping the stirring). Maximum color removal of the colored products was only 50%, at an alum dose 3.0 gm/l, and after 3 hours, and increased to become 84% at the same dose and after time 18 hours from the moment of stopping the stirring in the jar test.

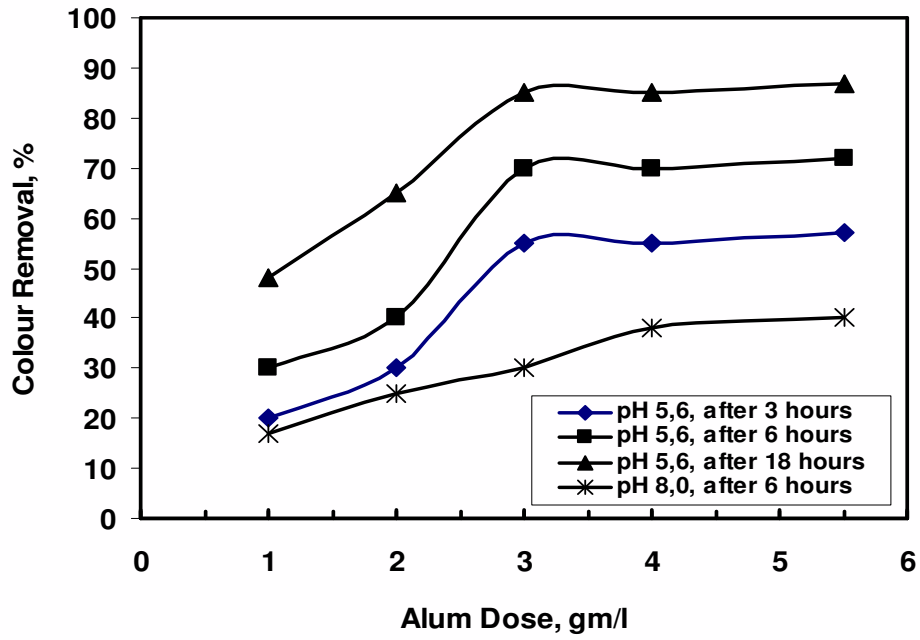

Figure 9 Effect of alum addition on the color removal (coagulation) of phenol polymers, at different pH values

3.8.2 Precipitation of phenolic polymers using lime

Lime (CaO) is used for color removal (precipitation of color products by lime) from pulping and bleaching effluents [6]. Molecular weight of color products is one of the major factors influencing the precipitation of color products. Pulping and bleaching effluents was treated by horseradish peroxidase and H_2O_2 and found that the colored products from this treatment have high molecular weights greater than 5000. They found that lime can almost completely remove color products with molecular weights greater than 5000. This previous work was encouraging us to demonstrate the capability of lime to precipitate the color products resulting from oxidation of phenols by HRP and H_2O_2 in the presence of poly ethylene glycol.

Figure 10 shows that as the addition of lime increases the pH of solution becomes above pH 10.5, and causes fast precipitation of the colored products. Maximum color removal (clarification) of the colored products was 60%, at a lime dose 3 gm/l, after 30 minutes from the moment of stopping the stirring in the jar test (clarification time). The percentage of color removal increases with the increase of the clarification time (after moment of stopping the stirring) in the jar test. Maximum clarification of the colored products was 85%, at a lime dose 3 gm/l, after two hours. Figure 7 shows fast and high efficiency of color removal using lime, as the lime doses increases and as the time after moment of stopping the stirring increases also.

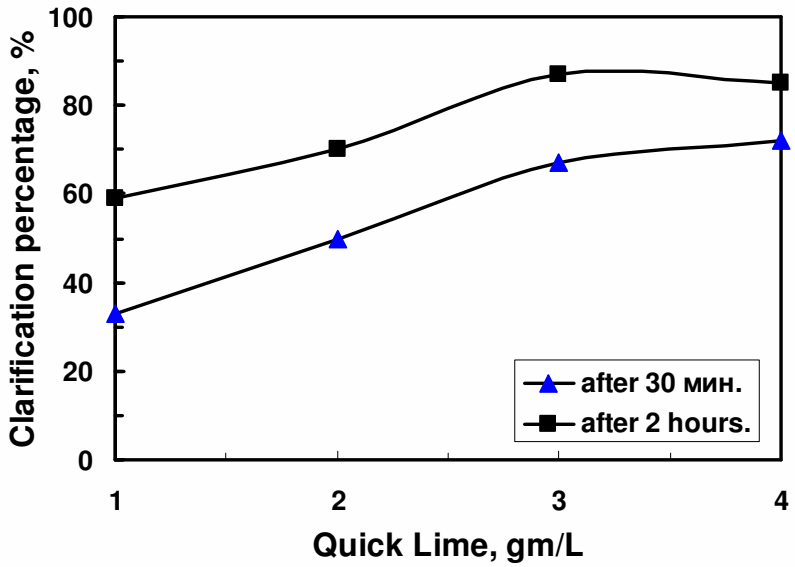

Figure 10 Effect of coagulation with quick lime for the phenolic polymers resulting from enzymatic treatment of phenol

4. CONCLUSIONS

This study has determined the possibility of treating synthetic waste water (distilled water contains both high amounts of phenol, 8 mM, and low amount of phenol 1.0 mM) by the enzymatic system (HRP-Peroxide). The optimum parameters of enzymatic treatment of synthetic waste water contains 1mM initial phenol concentration were at pH 7.0, 0.3 U/ml HRP dose, 3.0 mM H_2O_2 dose and PEG 275 mg/l achieved a final phenol concentration 0.2 mM with a removal percentage of phenol 80%.

The optimum parameters of enzymatic treatment of synthetic waste water contain 8 m. mole phenol were at pH 7.0, 1 U/ml HRP dose, 10.0 mM H_2O_2 dose and PEG 275 mg/l achieved a final phenol concentration 2.0 mM with a removal percentage of phenol 75%. The optimum coagulant was quick lime used for precipitation of colored oxidation products of phenol and 85% clarification could be attained after 2 hours.

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