ENZYMATIC REMOVAL OF PHENOL FROM PRODUCED WATER AND THE EFFECT OF PETROLEUM OIL CONTENT

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ABSTRACT

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. Produced water is the water pumped from aquifers associated with petroleum oil production, gas production or coal bed methane production. Produced water from petroleum oil reservoirs was produced in huge amounts and it contained several pollutants, such as phenol and petroleum oil droplets. New methods were required to reduce the amounts of toxic pollutants to permissible limits before rejecting this produced water to the environment. The produced water contained the following 4.5 mg/L phenol, 300 mg/l petroleum oil, Cl^- 62 mg/L and pH 6.0. The effect of addition of petroleum oil on the activity of horseradish peroxidase was determined. It was found that petroleum oil has strong depressing effect on the activity of the horseradish peroxidase at concentrations up to 1 gm/L of petroleum oil. The effects of treating the produced water with enzyme peroxidase extracted from horseradish plant, peroxide (H_2O_2) and polyethylene glycol have been studied. The horseradish peroxidase–hydrogen peroxide system decreased the amount of phenol present in produced water by 80%. The most effective concentrations for treating produced water were peroxidase at 2U/mL and 0.5mM hydrogen peroxide (H_2O_2) at neutral pH.

Keywords: horseradish peroxidase, produced water, enzymatic treatment, phenol, total hardness.

1. INTRODUCTION

Produced water is the water pumped from aquifers associated with petroleum oil production, gas production or coal bed methane production. The physical and chemical properties of produced water vary considerably depending on the geographic location of the field, the geological formation with which the produced water has been in contact for years and the type of hydrocarbon product being produced. Produced water properties and volume can vary throughout the lifetime of a reservoir. The major

components of produced water are oil and grease, total dissolved salts and poly aromatic hydrocarbons (phenol) (Veil et al., 2004).

Discharging produced water in marine environment without previous treatment has severe effects on the marine environment and produces aqua-toxicological effects, which are deleterious to aquatic life. The main contributors to acute toxicity of produced water have been found to be the aromatic and phenol fractions of the dissolved hydrocarbons. Phenol is toxic to fish at levels of 0.0045-0.05 mg/L, therefore the detoxification of phenols from produced water is therefore of great importance (CCME, 2002).

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. Conventional processes have proven to be efficient in the detoxification of phenolic compounds. However, these processes have certain disadvantages and limitations. The high cost and disposal of contaminated media are the disadvantages of solvent extraction and activated carbon adsorption.

Biological processes make use of the natural metabolism of cells to break down and remove oil and aromatics from produced water. 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 (IAOGP, 2002).

The following potential advantages of an enzyme-based treatment over conventional biological treatment were noted by Nicell et al. (1993): application to a broad range of compounds; action on, or in the presence of, many substances which are toxic to microbes; operation at both high and low concentrations of contaminants; operation over wide temperature, pH and salinity ranges; no shock loading effects; no delays associated with acclimatization of biomass; reduction in sludge volume (no biomass generation) and better defined system with simpler process control.

Produced water re-injection into a disposal well, or preferably the same reservoir from where the water originated is widely applied across the industry in a number of regions, particularly onshore, but the method has some disadvantages. Re-injection can be very energy intensive due to high pump pressure requirements, and thereby cause increased greenhouse gas emissions (IAOGP, 2002).

Klibanov et al. (1980, 1983) developed an enzymatic approach using horseradish peroxidase (HRP) and H_2O_2 for the removal of phenols from coal-conversion aqueous effluents. Treatment with horseradish peroxidase and hydrogen peroxide precipitates 97-99% of the phenol in a wide range of pH and phenol concentrations.

Wagner et al. (2001) used the same enzymatic technique (horseradish peroxidase and H_2O_2) for the treatment of a petroleum refinery wastewater which contains high amount of phenols. As a result of the treatment with enzyme HRP, the phenol content of a refinery was reduced below the discharge limit. Phenols were transformed to less biodegradable compounds and approximately 95% of toxicity was removed.

In this research, the same technique (horseradish peroxidase HRP and H_2O_2) for assessing the treatment of produced water in order to decrease the phenol content in produced water before its discharge to the marine environment has been used. The produced water contains several pollutants, a high amount of total hardness of 250mg/L, a high amount of oil and grease, a high chemical oxygen demand (COD), and contains 4.5mg/l phenol. However, the effects of petroleum oil content on the activity of HRP and enzymatic treatment are not clear in the previous literature.

The aim of this study was to study the effect of petroleum oil content on the enzyme activity. At the same time, the optimum parameters of enzymatic treatment (HRP and H_2O_2) for removing the phenol from the produced water have been determined.

2. MATERIALS AND METHODS

2.1 Materials and Equipments

HRP enzyme (EC 1.11.1.7) was extracted from horseradish in our laboratory. All the following chemicals - Hydrogen peroxide (30% w/v), solid phenol, potassium ferricyanide ($K_3Fe(CN)_6$) and 4-aminoantipyrine were used for analysis of phenol and activity of horseradish peroxidase and are of analytical grade and were purchased from FEKTON (Russia).

Photoelectrocolorimeter (FEK-II) (wavelength 300 to 700 nm) was used for the 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 probe. Centrifuge with 6000 max revolution per minutes (rpm) and with a capacity 200 mL was used. Magnetic stirrer with coated magnetic bar was used for mixing reactants with the produced water.

2.2 Analytical methods

The horseradish peroxidase enzyme concentration and activity was determined by colorimetric assay. 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 (Wright, 1995).

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 (Yiseon, 1998). Grease and oil, suspended solids (by filtration method) and COD were all determined according to the procedures described in Standard Methods (1975).

2.3 Experimental Procedure

Horseradish peroxidase enzyme was extracted in the laboratory from horseradish roots using soft tap water as a solvent and without buffers. The ratio of horseradish to tap water was 50 gram minced horseradish root to 500 milliliter of tap water. The horseradish roots were purchased from market, washed and cut to very small pieces. The minced horseradish-water mixture was vigorously mixed for 3 hours using an electric mixer at high speed. The resulting solution was filtered and the supernatant was centrifuged at 4000 revs./min. The supernatant liquid was the crude enzyme and was preserved at -4°C. Every day the activity of HRP was analyzed before using the enzyme in the research. The activity of the enzyme in each gram of horseradish root contains 70 units. The activity of the enzyme is defined in units where 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.

Batch experiments were conducted at room temperature (25°C). The batch reactors were glass vials of capacity 100 mL, which contained 50 mL of produced water and predetermined doses of each of HRP enzyme, hydrogen peroxide (H_2O_2) and polyethylene glycol (PEG) have been added and the mixing time of the produced water with the reactants was kept at 4 hours. Polyethylene glycol was added to minimize enzyme inactivation through interaction with the oil and grease present in the produced water samples or through phenol oxidation reaction products during the enzymatic treatment of produced water sample. PEG has been reported to exert a strong protective effect and is a nontoxic compound. After treatment, the resulting solution was centrifuged for 30 minutes at 6000 revs./min. The supernatant was then analyzed for phenol level.

3. RESULTS AND DISCUSSIONS

3.1 Effect of Petroleum Oil Addition on HRP Activity

The effect of adding petroleum oil on the activity of HRP was tested and is represented by data in Figure 1. A model of four vial glasses each containing 20mL distilled water were vigorously agitated with 0.0, 0.25, 0.75, 1.5 g/L petroleum oil for one hour. A dose of HRP equivalent to 2 U/mL was added to each of the four sets and agitation occurred further for another one hour.

Figure 1 presents the data obtained for HRP activity relative to 0.0 g/L of petroleum oil in the first vial. At a dose of 0.25 g/L petroleum oil, the HRP activity was decreased to 40%, a further increase in the petroleum oil addition caused a further decrease on the activity of HRP. At a dose of 1.5 g/L petroleum oil the HRP activity was decreased down to 10%. From these results, it may be deduced that high doses (0.75g/L -1.5 g/L) of petroleum oil have a strong depressing effect on HRP activity, which is mainly attributed to the physical adsorption of petroleum oil on the active sites of the enzyme peroxidase. The amount of petroleum oil in the produced water sample was 300 mg/L which means that the presence of petroleum oil in the produced water would cause a depressing effect on the Horseradish peroxidase treatment, which can be tolerated by using high doses of the enzyme peroxidase.

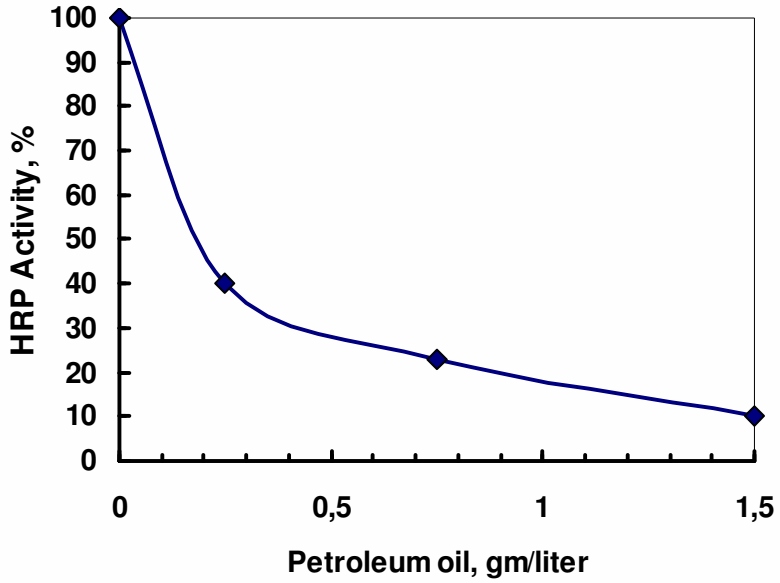

Figure 1 The effect of petroleum oil addition on the activity of horseradish peroxidase, at a HRP dose 2 U/ml

Figure 2 demonstrates the effect of increasing the dose of HRP on the activity of HRP at constant petroleum oil content equals 0.75gm/L. A model of four vial glasses each containing 20mL distilled water were vigorously agitated with 0.75 g/L petroleum oil for one hour. A doses of HRP equivalent to 2.0, 4.0, 6.0, 8.0 U/mL was added to the

corresponding of the four sets and the activity of HRP was determined after one hour and after two hours. As the time of agitating petroleum oil with the enzyme HRP in the distilled water increases the activity of HRP decreased due to increasing the chance for adsorption of petroleum oil droplets on the active sites of the enzymes. Also, by increasing the dose of HRP the activity of HRP increases.

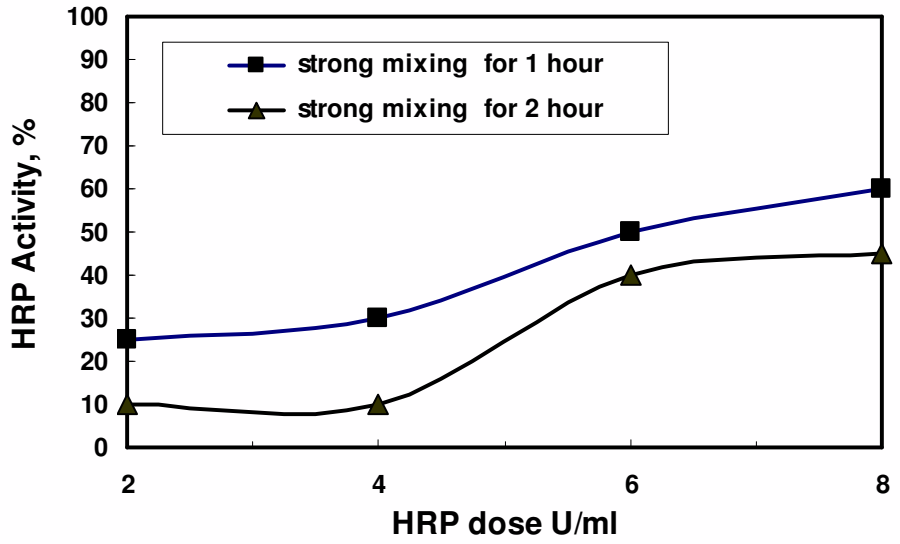

Figure 2 The effect of time of strong mixing the activity of horseradish peroxidase, at a petroleum oil addition 0.75 g/l

3.2 Mechanism of HRP- H_2O_2-Phenol Reaction

Horseradish peroxidase undergoes a cyclic reaction when reacting with phenolic substrates (Klibanov et al. 1980). 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 Chemical Analysis of Produced Water

Table 1 summarizes the produced water characteristics as determined in the laboratory. The produced water sample was produced from petroleum oil reservoirs at Baku.

Table 1 Characteristics of produced water

Total hardness Ca^{2+} Mg^{2+}	250 mg-equ/L 210 mg-equ/L 40 mg-equ/L
pH	6.0
Cl^-	62 mg/L
Phenol	4.5 mg/L
Alkalinity	10.5 mg-equ/L
Acidity	12.5 mg-equ/L
Petroleum oil and Grease	300 mg/L
COD	640 mg/L
Suspended solids	700 mg/L

3.4 Effect of pH on the Treatment of Produced Water

These tests were conducted to study the effect of pH on the removal percentage of the aromatic substance (phenol) from the produced water by using HRP enzyme and hydrogen peroxide (H_2O_2) system. For each test the produced water sample was 50 mL, the HRP dose was 1.0 U/mL of the solution, the H_2O_2 dose was 0.3 mM and the PEG dose was 400 mg/L. The time of mixing the produced water sample with the reactants was 4 hours. The pH of each sample was adjusted to be between pH 3.0 and pH 11 using concentrated HCl or NaOH. The pH of the sample was readjusted before stirring and after addition of PEG, H_2O_2 and HRP doses. Stirring of the produced water with the reactants (HRP, H_2O_2 and PEG) has been carried out at medium speed of approximately 200revs/min to allow for 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, which were less toxic to the environment, the remaining phenol in the treated sample was decreased and a measurement of the phenol by colorimetric method was thereafter performed.

Figure 3 shows that the optimal percentage of removal of phenol from the reaction solution occurring between pH 7.0 and 9.0, with an optimum removal percentage 65% of phenol. The removal percentage of phenol decreased at with increasing acidity and decreasing alkaline conditions. This could have possibly been due to the effect of oil and grease on the activity of HRP - a decrease in the suspended solids and oil and grease content with the increase in pH has been noticed (some clarification of

produced water is visible at high alkaline conditions). Also OH^- and H^+ ions may have some effect on the oxidation reaction of phenol by using H_2O_2 and HRP system. This study has therefore demonstrated that HRP is mostly active at neutral and medium alkaline conditions and is probably suitable for the treatment of phenol in the produced water at medium alkaline conditions (7.0 – 9.0).

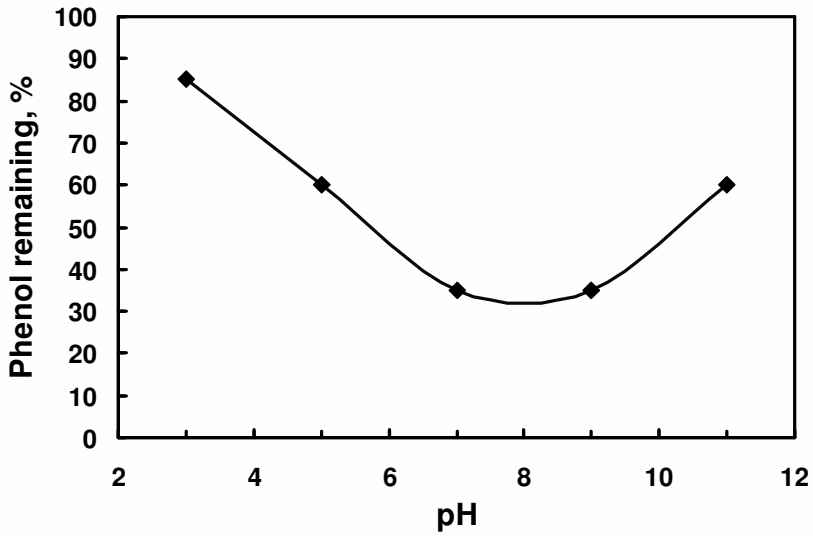

Figure 3 The effect of pH on the removal percentage of phenol from the treated sample

3.5 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 4 demonstrates the effect of stirring time of peroxidase on the remained phenol. Removing of 60% of phenol could be attained after sever mixing the peroxidase and peroxide with phenol for four hours.

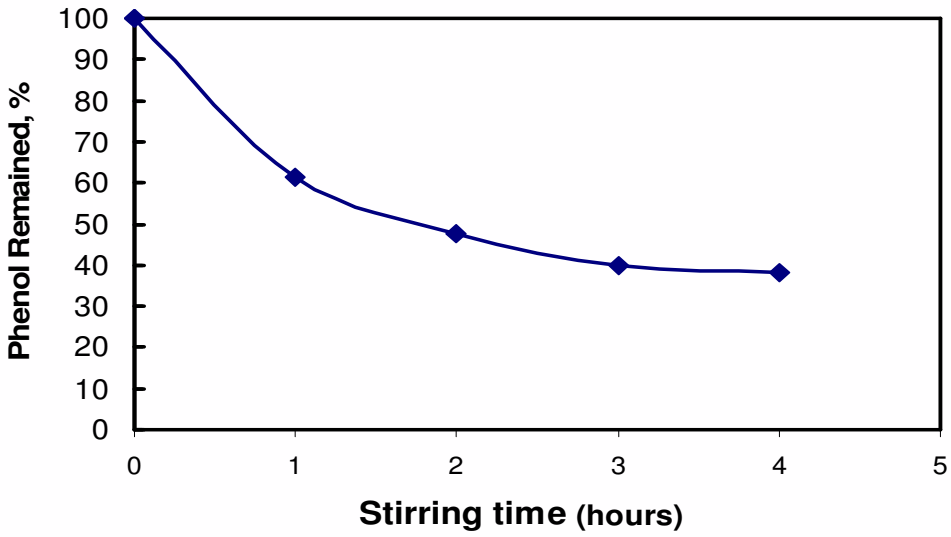

Figure 4 Effect of stirring time on the phenol remained in waste water

3.6 Effect of Peroxidase Dose on the Treatment of Produced Water

The optimum HRP dose was determined as 0.3 mM H_2O_2 and in the presence of PEG at 400 mg/L. These tests were conducted at the optimum pH value 7.0 determined in the previous set of experiments, and for time of mixing 4 hours. Horseradish Peroxidase was added in predetermined amounts in order to determine the effect of HRP dose on the phenol content in the produced water sample after enzymatic treatment.

Figure 5 shows that with an increase in the HRP dose, the removal of phenol increases and that the optimum HRP dose was 2.0 U/mL since the phenol concentration left in the solution started to be constant at this dose.

The initial phenol concentration was 4.5 mg/L and at a 0.3 mM H_2O_2 and the final phenol concentration of treated produced water was 1.1 mg/L. A high dose of HRP of 2.0 U/mL was required and this could have been necessary due to the inactivation effect of HRP by the high amount of suspended solids, and oil and grease in the produced water.

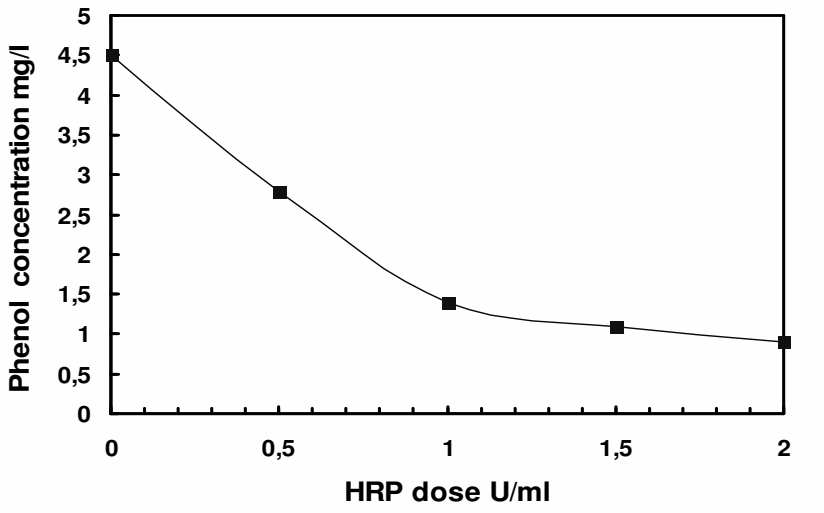

Figure 5 The effect of horseradish dose on the removal of phenol from the produced water sample

3.7 Effect of Hydrogen Peroxide Dose

These experiments were conducted to determine an optimum hydrogen peroxide dose at the previously determined optimum conditions (pH 7.0 and HRP dose 2U/mL).

Figure 6 shows that as the H_2O_2 dose was increased, the phenol content decreased down to an optimum value 0.9 mg/L. The high dose of H_2O_2 was due to the non-enzymatic oxidation of other dissolved contaminants present in the produced water and due to the enzymatic oxidation of phenol. From these results, it may be stated that the optimum doses and conditions for the enzymatic treatment of produced water were at pH 7.0, an HRP dose of 2 U/mL, an H_2O_2 dose 0.5 mM and a PEG concentration of 400 mg/L which a collectively achieved a final phenol concentration 0.9 mg/L corresponding to a phenol removal of phenol 80%. The final phenol concentration in the treated samples of 0.9 mg/L was still however still more than the required phenol concentration 0.0045-0.05 mg/L. This high value of the residual phenol may be set on account of the presence of high concentration of oil and grease in the produced water sample at a value of 300 mg/L, which necessitates a previous pre-treatment by air flotation to reduce the amount of oil and grease to the minimum concentration of less than 20 mg/L before the enzymatic treatment.

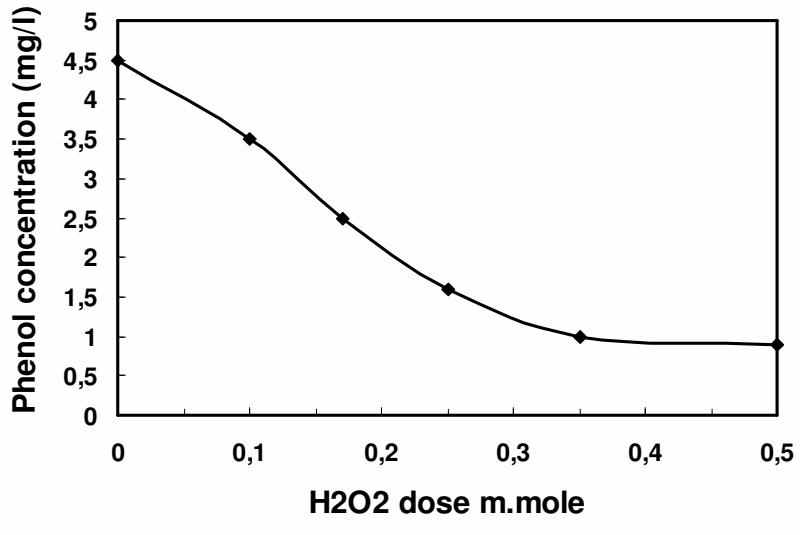

Figure 6 The effect of hydrogen peroxide dose on the phenol content in the produced water sample

4. CONCLUSIONS

This study has assessed the feasibility of treating produced water by the enzymatic system HRP- Hydrogen peroxide. Presence of petroleum oil in the produced water was found to have a strong depressing effect on the activity of horseradish peroxidase enzymatic activity, due to the physical adsorption of petroleum oil particles on the active sites of the enzyme. The most effective concentrations for treating produced water were a horseradish peroxidase dose of 2 U/mL, 0.5 mM hydrogen peroxide and a PEG concentration of 400 mg/L, at neutral pH. The enzymatic treatment of produced water gives a final phenol concentration 0.9 mg/L at a removal of 80%.

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