

ENZYMATIC TREATMENT REDUCES THE TOTAL HARDNESS OF PRODUCED WATER

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ABSTRACT

Produced water from oil reservoirs were produced with huge amounts, it contains several pollutants. 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 contains the following 4.5 mg/l phenol, 250 mg/l total hardness, Cl^- 62 mg/l, pH 6.0. The effect of addition of calcium ions on the activity of horseradish peroxidase was determined. It was found that calcium ions activate the horseradish peroxidase at concentrations up to 1.2 moles of calcium chloride CaCl_2 , and further addition of calcium ions reducing little the activity of horseradish peroxidase. Studying the effect of treating the produced water with enzyme peroxidase, extracted from horseradish plant, and peroxide (H_2O_2) and poly ethylene glycol has been occurred. The enzymatically treated/untreated produced water samples were treated with different reagents quick lime, potassium hydroxide and sodium carbonate to reduce the water hardness. A new characteristic of the enzymatic treatment has been explored. The peroxidase-peroxide system decreases the total hardness of produced water beside the other characteristics which are the catalyzing effect of phenol. The horseradish peroxidase–hydrogen peroxide system reduces the amount required of caustics for reducing the total hardness. Quick lime (CaO) was the most active and economic reagent to reduce the total hardness.

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

1. INTRODUCTION

Produced water is the water pumped from aquifers associated with petroleum oil production, gas production and 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 thounds of years, and the type of hydrocarbon product being produced. Produced water properties and volume can vary throughout the lifetime of a reservoir. Major components of produced water are oil and grease, total dissolved salts, total hardness and poly aromatic hydrocarbons (phenol) [1].

Discharging produced water in marine environment without previous treatment has severe effect on the marine environment and produces aqua-toxicological effects, which is deleterious to aquatic life. Conventional processes have proven to be efficient in the treatment of many pollutants from the produced water. 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.

The use of microbial organisms to break down and remove oil and aromatics from produced water has some disadvantages. 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. Produced water re-injection into a disposal well, or preferably the same reservoir from whence 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 [2].

The following potential advantages of an enzyme-based treatment over conventional biological treatment were noted [3]: 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.

An enzymatic approach was developed using horseradish peroxidase and H_2O_2 for the removal of phenols from coal-conversion aqueous effluents. Treatment with horseradish peroxidase and hydrogen peroxide precipitates 97-99 percent of the phenol in a wide range of pH and phenol concentrations [4].

This produced water contains several difficulties, a high amount of total hardness 250 mg/l that causes this produced water very hard, high amount of oil and grease and high chemical oxygen demand, the effect of total hardness on the activity of HRP and enzymatic treatment is not clear in the previous literatures. In this research we used the same technique (horseradish peroxidase HRP and H_2O_2) for assessing the treatment of produced water (with the aim to decrease the phenol content in produced water) before its discharge to the marine environment. But we find a new characteristic of the enzymatic treatment is that the peroxidase-peroxide system decreases the total hardness of produced water beside the other characteristics which are the catalyzing effect of phenol to less toxic form.

The aims of this study were exploring the new characteristic of the enzymatic treatment. Studying the effect of total hardness on the enzyme activity and studying

the effect of enzymatic treatment process on the total hardness removal of produced water.

2. MATERIALS AND EQUIPMENTS

Horseradish peroxidase 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 FEKTON (Russian). Hydrogen peroxide (30% w/v), 4-aminoantipyrine and solid phenol were used for analysis of activity of horseradish peroxidase. Quick lime (CaO), sodium carbonate hexahydrate ($\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$) and potassium hydroxide (KOH) were used for hardness tests.

Photoelectrocolorimeter (VEK-2) (wavelength 300 to 700 nm) was used for absorbance measurements. Glass cuvettes with an optical path length of 1.0 cm and a volume of 5ml were used. Measurements of pH were made using a pH probe. Magnetic stirrer with coated magnetic bar was used for mixing reactants with the produced water.

3. ANALYTICAL METHODS AND EXPERIMENTAL PROCEDURE

To determine the horseradish peroxidase enzyme concentration/activity, colorimetric assay was used. 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 $7311 \text{ M}^{-1}\text{cm}^{-1}$. The rate of reaction is proportional to the rate of color formation [5].

Titrametric analysis was occurred for the determination of total hardness, alkalinity, acidity and chlorine. Grease and oil, suspended solids and chemical oxygen demand (COD) were determined according to Standard methods [6].

Horseradish peroxidase enzyme was extracted in our 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 was 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 rev/min. The supernatant was the crude enzyme and stored 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, 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, approximately 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 (H₂O₂) and poly ethylene glycol (PEG) has been added and mixing time of the produced water with the reactants was 4 hours. After treatment the resulting solution was centrifuged for 30 minutes at 6000 rev/min. The supernatant was analyzed for total hardness by titration.

4. RESULTS AND DISCUSSION

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

Table 1. Characteristics of produced water

Total hardness	250 mg-equ/l
Ca ⁺²	210 mg-equ/l
Mg ⁺²	40 mg-equ/l
pH	6.0
Cl ⁻¹	62 mg/l
Phenol	4.5 mg/l
Alkalinity	10.5 mg-equ/l
Acidity	12.5 mg-equ/l
Grease and oil	300 mg/l
Chemical oxygen demand	640 mg/l
Suspended solids	700 mg/l

4.1 Effect of CaCl₂ addition on HRP activity

Total hardness of produced water was 250 mg/l which specifies it as hard water. The effect of addition of Ca⁺² ions on the activity of HRP was tested as appears in Figure 1. A model of five vial glasses each contains 50ml distilled water were agitated with 0.0, 0.34, 0.81, 1.1, 1.6 mole CaCl₂ for 30 minutes. A dose of HRP equivalent to 1 U/ml was added to each of the five sets and agitation occurred further for four hours. Determination of the HRP activity has occurred as discussed earlier for each of the five vial glass.

Figure 1 presents the data obtained for HRP activity relative to 0.0 mole CaCl_2 in the first vial. At a dose of CaCl_2 0.33 mole, the HRP activity was increased up to 140%, a further increase in the CaCl_2 addition caused a little effect on the activity of HRP. At a dose of 1.6 mole CaCl_2 the HRP activity was decreased to 87%. As apparent from these results, low doses of CaCl_2 has an activating or little effect on HRP activity.

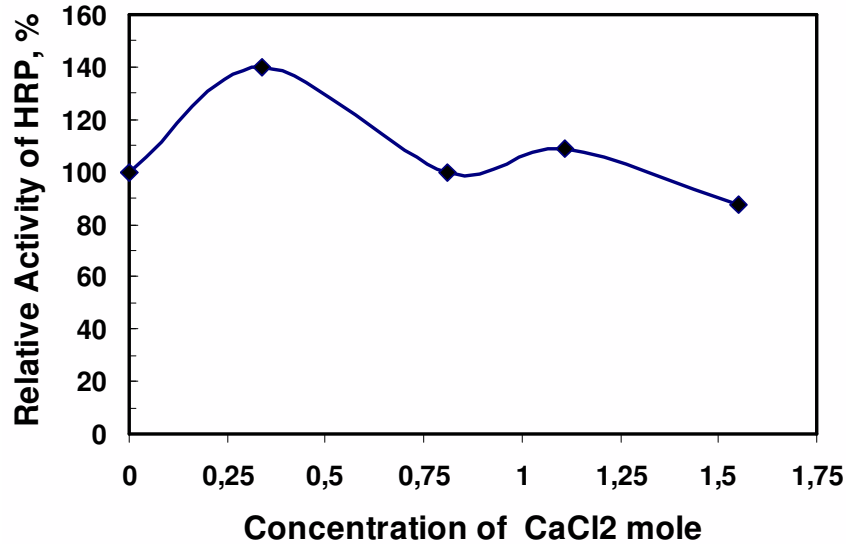
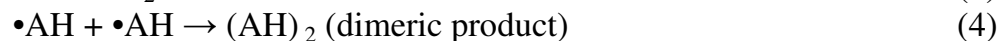
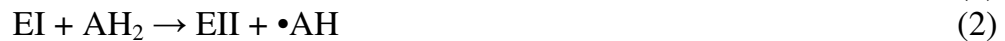


Figure 1. The effect of calcium ions addition on the relative activity of HRP

The amount of calcium ions in the produced water sample was 210 mg-eq/l which means that the presence of calcium ions in the produced water would causes an activating effect on the Horseradish peroxidase treatment.

4.2 Mechanism of HRP- H_2O_2 -phenol reaction

The generally accepted reaction mechanism of phenolic compounds catalyzed by peroxidases was described as follows [7]:



where E is the enzyme; EI and EII are intermediate forms of enzyme, called compound I and compound II, and AH_2 is the phenolic compound. If only the above catalytic cycle is taken into account the stoichiometry, for the overall reaction, would be:



and one mol of H_2O_2 should be able to convert two moles of AH_2 into the dimeric form $(\text{AH})_2$.

4.3 Removing the total hardness without enzymatic treatment

Three sets of produced water samples (each set composed of four vials and each vial contains 50 ml Produced water) were coagulated each with 3.5, 7.5, 12, 16 gram/liter quick lime (CaO), sodium carbonate hexahydrate ($\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$), potassium hydroxide (KOH). The produced water samples with the softeners were agitated for 15 minutes. Then the samples were allowed to set undisturbed for two hours, the pH and total hardness were determined as discussed earlier.

Figure 2 presents the effect of softener doses on the total hardness of produced water without previous treatment with the enzyme HRP- hydrogen peroxide H_2O_2 system. Quick lime which is inexpensive relatively, causes softening of produced water at doses less than the other softeners $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$ and KOH. Quick lime with a dose of 7 g/l causes the produced water soft (total hardness less than 100 mg/l, while very high doses of $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$ were required to achieve the same softening. This mainly due to the active constituent in $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$ is Na_2CO_3 forming 40% of the total weight of the reagent. KOH is very pure (purity is 99.9%) and expensive relative to quick lime (the activity of quick lime reagent, as labeled by the producing company, is 96%). Therefore KOH was used for the following comparison between enzymatically and non-enzymatically treated produced water samples (to minimize the effect of 1% impurities in quick lime).

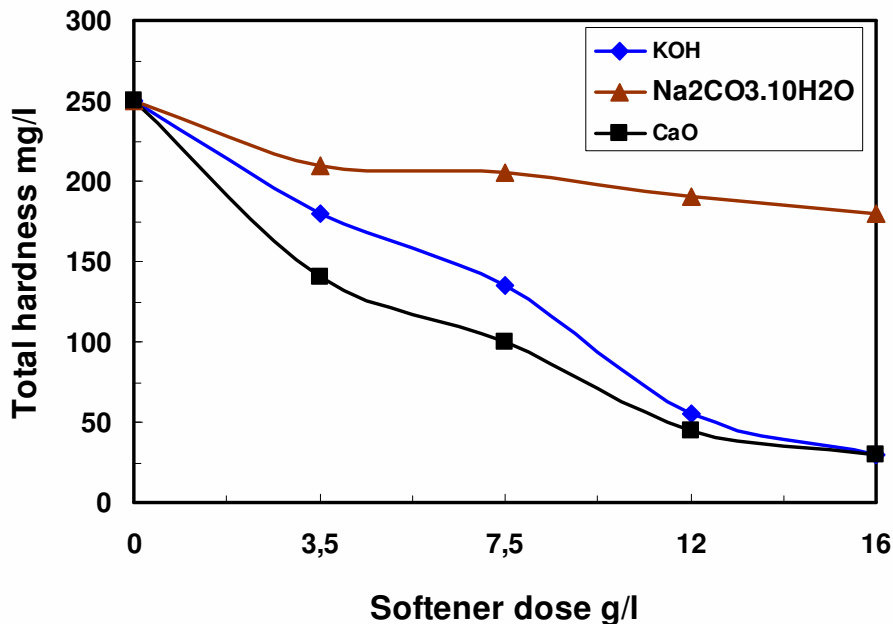


Figure 2. The effect of softener dose on the total hardness of the untreated produced water sample

Figure 3 presents the effect of softener doses on the pH of produced water. With the addition of softener to produced water sample, an increase in the pH has been occurred to a maximum value 10.5. With a further increase of the addition of the softener, a stabilization of pH values still occurred.

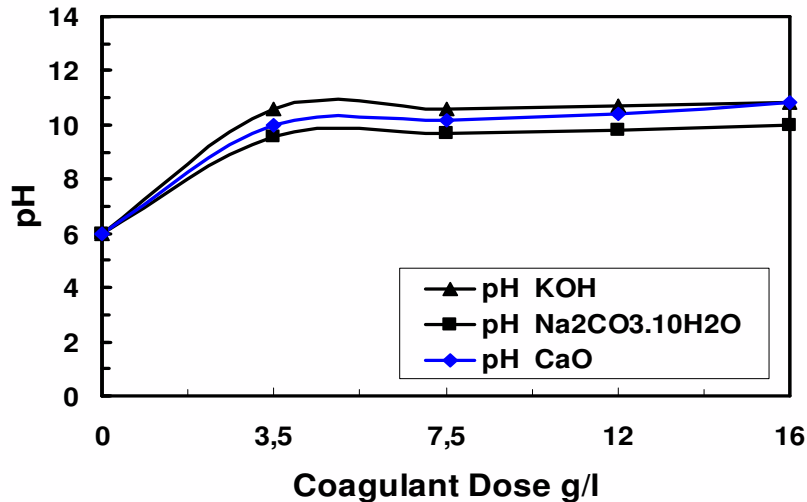


Figure 3. The effect of softener dose on the pH of untreated produced water sample

4.4 Effect of enzymatic treatment on the total hardness

A set of produced water samples was treated with the enzymatic-peroxide system at optimum doses of HRP, H₂O₂ and PEG without changing the pH of the produced water (pH adjusted to 6.0 after addition of the reagents). After enzymatic treatment, addition of various doses of caustic KOH for each tested sample and further agitation for 15 minutes. Then the samples were allowed to set undisturbed for two hours and total hardness were determined.

Figure 4 demonstrates a comparison between the total hardness of enzymatically treated and untreated produced water sample. As appeared from Figure 4, the enzymatically treated water sample caused a further decrease in the total hardness for the same caustic dose. A dose of KOH equal to 3.0 g/l is sufficient for softening of produced water (caused total hardness 100 mg/l). This may be attributed to a binding effect or co-precipitation of calcium ions with the removed phenol during treatment with enzyme and caustic. Since caustics has coagulation effect on polymerized phenols.

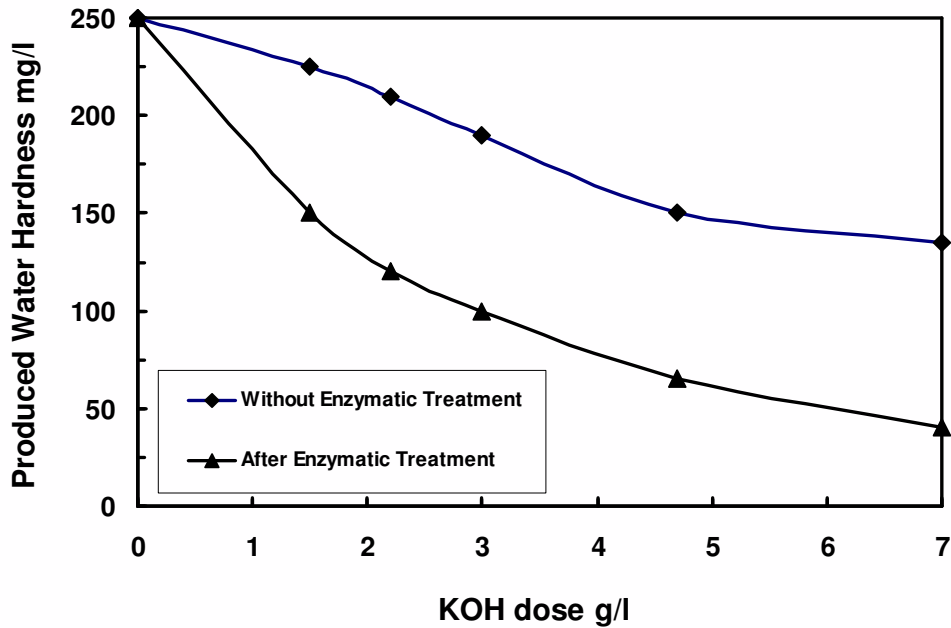


Figure 4. The effect of caustic dose on the total hardness of produced water before and after treatment with the enzyme

5. CONCLUSIONS

A new characteristic of the enzymatic treatment has been explored. The peroxidase-peroxide system decreases the total hardness of produced water beside the other characteristics which are the catalyzing effect of phenol to less toxic form. The effect of addition of calcium ions on the activity of horseradish peroxidase was cleared. It was found that calcium ions in the produced water have an activating effect on the horseradish peroxidase. The enzymatically treated produced water samples catalyzed the softening of produced water, and decreasing the amount required of caustics for softening.

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