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A study on the efficiency of Polysorbate 20 as additive for phenol removal from aqueous solutions catalyzed by horseradish peroxidase

Merlin Thomas

ABSTRACT

In the present study, the efficiency of Polysorbate 20 (Tween 20) as additive on the removal of phenol catalyzed by horseradish peroxidase was done as an alternative to the conventional phenol removal processes. Tween 20 was found to increase the activity of horseradish peroxidase. Various studies were done by varying Tween 20 concentration, initial phenol concentration, pH and time.

Key words: Surfactant, Tween 20, Phenol Removal, Horseradish Peroxidase, Enzymes

1. INTRODUCTION

Phenol and phenolic compounds are ubiquitous pollutants which come to the natural water resources from the effluents of a variety of chemical industrial such as refineries, phenol manufacturing, pharmaceuticals and industries of resin paint, dyeing, textile wood, petrochemical, pulp mill, etc. (Nahed & Amel, 2008). There has been a rapid increase in environment pollution by phenolic compounds due to urbanization and industrialization. Phenols must be removed from the contaminated water before being discharged. Even at low concentrations, phenolic compounds are toxic, carcinogenic, mutagenic and teratogenic. If released into the environment, they may accumulate in ground surface water or soil thus creating environmental problems. Phenol is a strong corrosive effect on skin and mucous membrane, skin contact causes severe burns of the local skin swelling of the skin, black necrosis, can also be absorbed through the skin into the body caused by heart, liver, kidney, nervous system toxic effects. Phenols as a class of organics are similar in structure to the more common herbicides and insecticides in that they are resistant to bio degradation. Some phenols are highly soluble in water and their presence in water is noticed due to taste and odour. The conventional processes like extraction, adsorption, electro chemical techniques, irradiation etc suffer from serious drawbacks like high cost, incomplete purification, and

formation of hazardous by-products, low efficiency and applicability to a limited concentration range (Miodrag & Lidija, 2004).

Biological processes are widely being used over physicochemical processes as they are more effective and due to non toxic end products (Pradeep et al. 2012). Enzymes are biological catalysts that increase the rate of chemical reactions taking place within living cells. Horseradish peroxidase is an enzyme peroxidase isolated from the roots of horseradish. Horseradish peroxidase is used as a substitute for conventional methods of phenol removal because of its ability to catalyze oxidation of a wide variety of aromatic compounds. It initiates the oxidation of a wide variety of aromatic compounds. This enzyme catalyzes the oxidation of aqueous phenols resulting in the formation and precipitation of polymeric products. The reaction products undergo a non-enzymatic polymerization to form water insoluble aggregates which are readily separated from the solution. Horseradish peroxidase is well known for its stability and retention of catalytic ability over a wide range of operating conditions (Kim & Kim, 2011; Ibrahim et al. 2011; Monica & James, 2002). Tween 20 is a polyoxyethylene derivative of sorbitan monolaurate, and is distinguished from the other members in the polysorbate range by the length of the polyoxyethylene chain and the fatty acid ester moiety (Ayorinde et al., 2000). Tween 20 is widely used as additive for enhancement of phenol removal using *Coprinus cinereus* peroxidase. It was found that the requirement of the enzyme for almost 100% removal of 100 mg dm⁻³ phenol decreased to one-fourth by the addition of 30 mg dm⁻³ Tween 20 (Sakurai et al., 2003). The objective of this present study is to investigate the effect of non-ionic surfactant Tween 20 on phenol removal catalyzed by horseradish peroxidase. Effect of various parameters like surfactant concentration, phenol concentration, pH value and time were studied.

2. MATERIALS AND METHODS

Horseradish peroxidase and 4-Aminoantipyrine (98%) were purchased from MERCK. All reagents used were of analytical grade. Stock solutions of horseradish peroxidase (10 mg/l) were stored at 4°C and allowed to equilibrate to 28°C before using.

2.1. Experiments

All batch experiments were done in borosilicate glass vials. The vials were incubated in a water bath set. The concentration of horseradish peroxidase was kept constant in all the tests as 10 mg/l. The initial phenol concentration varied from 50 to 350 mg/l, pH from 4 to 9 and time from 1 to 24 hours. Blank experiments (control) without Tween 20 were also carried out.

2.2. Phenol concentration measurement

The stock solution of phenol was prepared by dissolving 1 gm of phenol in 100 ml of 0.1 N sodium hydroxide solution and diluting upto 1000 ml with distilled water. Phenol concentration was measured using a colorimetric assay using UV-Visible spectrophotometer (electronics India model 1372). 4-Aminoantipyrine was used as a complexing agent with phenol. Absorbance of the samples was measured at 500 nm.

3. RESULT AND DISCUSSION

3.1. Effect of Tween 20

The concentration of Tween 20 was varied from 0 to 300 µM while the horseradish peroxidase concentration was kept constant at 10 mg/l. An initial phenol concentration of 300 mg/l was used with a pH of 6.5 and time duration of 18 hours. The results (Figure 1) show that increase in the concentration of Tween 20 had a substantive positive effect on the phenol removal. The control sample (without Tween 20) could remove upto 63 % phenol while the maximum phenol removal of 87 % was observed with 250 µM Tween 20.

3.2. Effect of time

The time was varied from 1 to 24 hours while the horseradish peroxidase concentration and surfactant concentration were kept constant at 10 mg/l and 250 µM respectively. An initial phenol concentration of 300 mg/l was used with a pH of 6.5. The results (Figure 2) show that increase in the reaction time had a positive effect on the phenol removal. The maximum effect was found at 18 hours after which there was no substantial effect.

3.3. Effect of pH

The pH was varied from 5 to 10 while the horseradish peroxidase concentration and Tween 20 concentration were kept constant at 10 mg/l and 250 µM respectively. An initial phenol concentration of 300 mg/l was used with a reaction time of 18 hours. The results

(Figure 3) show that the maximum phenol removal was obtained at a pH of 6-7. The percentage removal of phenol decreases with further increase in pH value. This can be attributed to the dependence of phenol ionization on pH value.

3.4. Effect of initial phenol concentration

The initial phenol concentration was varied from 50 to 350 mg/l while the horseradish peroxidase concentration and Tween 20 concentration were kept constant at 10 mg/l and 250 μ M respectively. A pH of 6.5 and reaction time of 18 hours were used. Above the initial phenol concentration of 160 mg/l, no substantiate change in phenol removal was observed (Figure 4).

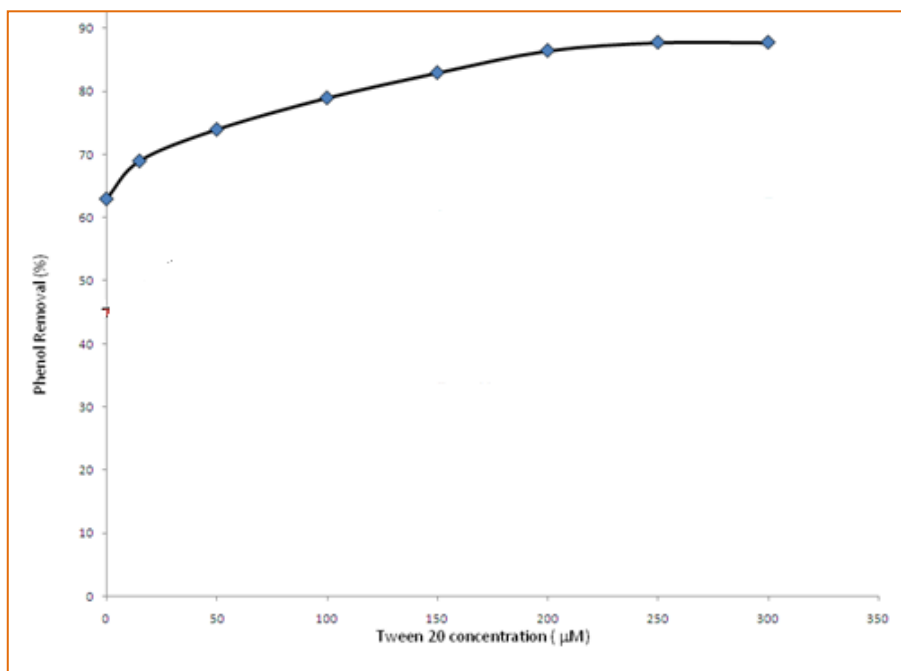


Figure 1

Effect of Tween 20 concentration on Phenol removal catalyzed by horseradish peroxidase (time: 18 hours, horseradish peroxidase: 12 mg/l, pH :6.5, initial phenol concentration: 300 mg/l)

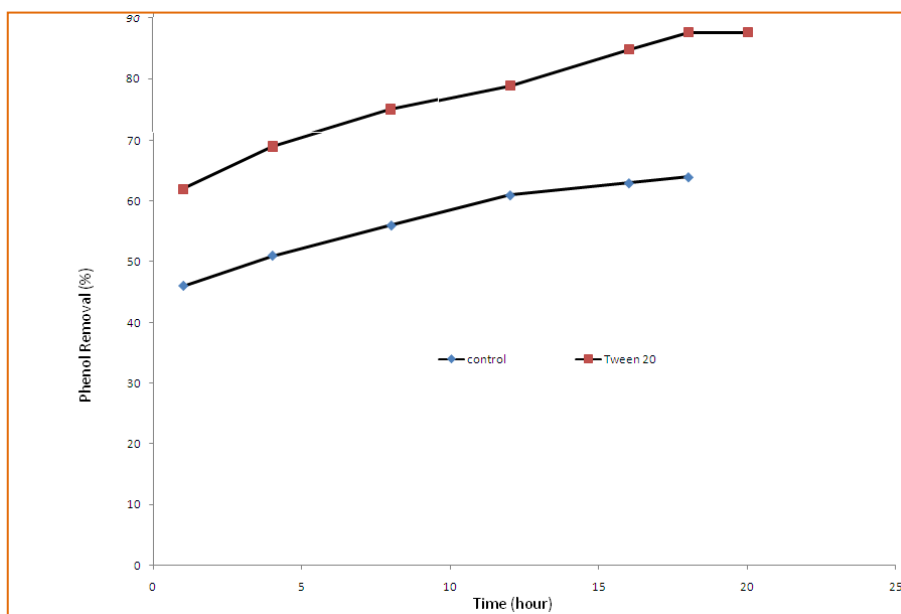


Figure 2

Effect of time on phenol removal catalyzed by horseradish peroxidase & Tween 20 (Tween 20: 250 μ M, horseradish peroxidase: 12 mg/l, initial phenol concentration: 300 mg/l, pH: 6.5)

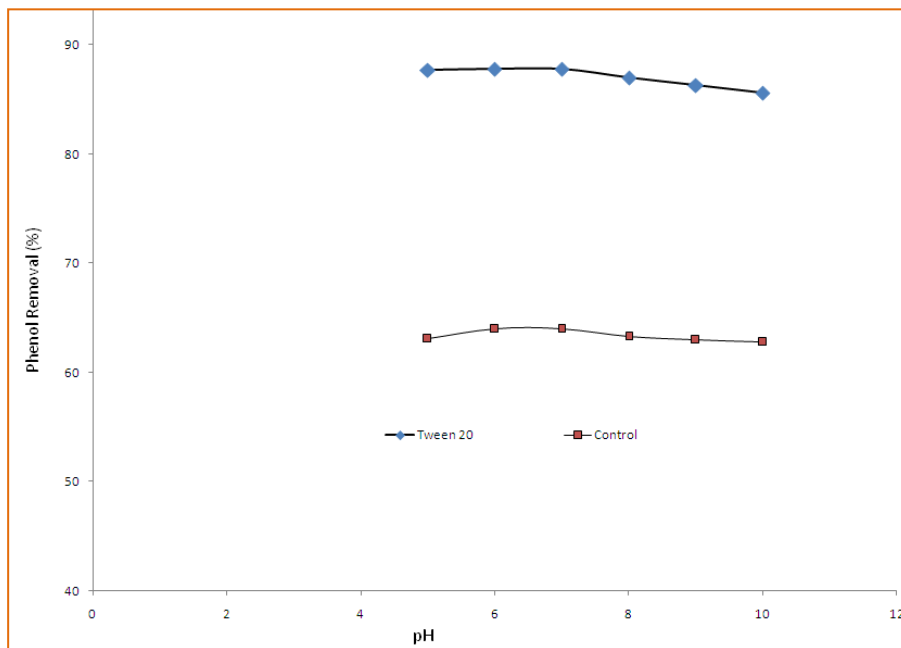


Figure 3
Effect of pH on phenol removal catalyzed by horseradish peroxidase & Tween 20(Tween 20: 250 μ M, horseradish peroxidase: 10 mg/l, initial phenol concentration: 300 mg/l, time: 18 hours)

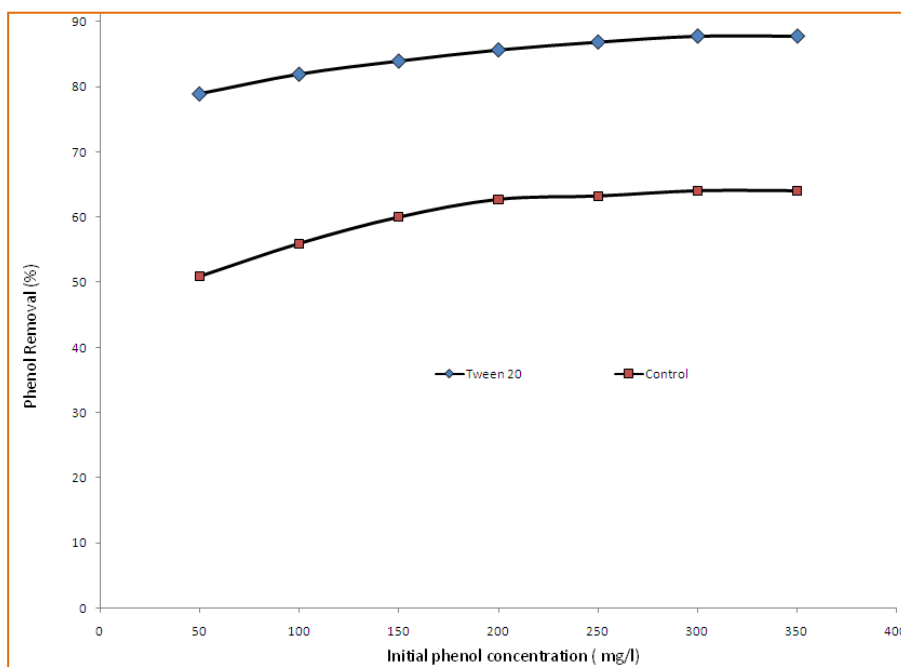


Figure 4
Effect of initial phenol concentration on phenol removal catalyzed by horseradish peroxidase & Tween 20

4. CONCLUSION

The enzymatic processes are now being widely used for phenol removal from its aqueous solution. The use of surfactant along with the enzymes significantly increases the activity of the enzymes. Tween 20 surfactant is found to be an excellent catalyst for increasing the activity of horseradish peroxidase for removal of phenol from aqueous solutions.

Ethical approval

Not applicable.

Informed consent

Not applicable.

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Conflict of Interest

The author declares that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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