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Detoxification of Pesticides Aqueous Solution Using Horseradish Peroxidase

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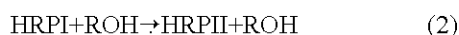
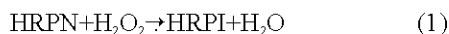
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Abstract: There are pesticide residues in agriculture wastewater and that compounds must be removed before discharge of wastewater in native waters. Thus the aim of this study was to remove toxic pesticide in waste water by the addition of horseradish peroxidase enzyme. The process of pesticide (methyl-parathion (O,O-Diethyl- O-4-nitro-phenylthiophosphate), atrazine (1-chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine) and triazophos (O,O-diethyl O-1-phenyl-1H-1,2,4- triazol-3-yl phosphorothioate) removal from synthetic wastewater using horseradish peroxidase and hydrogen peroxide has been analyzed. The technical feasibility of the process was studied using 0.001-3.0 mM synthetic pesticides solutions. Experiments were carried out at different time, HRP and H₂O₂ dose and pH to determine the optimum removing conditions. The removal of the three pesticides increases with an increase in HRP and hydrogen peroxide dose. The optimum HRP dose is 2.0 U L⁻¹ and 10 mM for H₂O₂. The contact needed to reach equilibrium was found to be 360 min. Maximum removal was achieved up to 74% at pH 8. Also, Chemical Oxygen Demand (COD) of the effluent reduced at the end of 6 h from 2111-221 mg L⁻¹ (at pH 8). Tests based upon horseradish peroxidase, at optimized parameters, show the reduction of toxicity to non-toxic levels.

Key words: Pesticide residues, COD removal, agriculture wastewater, horseradish peroxidase

INTRODUCTION

An important quantity of pesticide is released from pesticide production plants. These compounds are toxic and carcinogenic in nature even at low concentration (Nicell *et al.*, 1993). Along with municipal point sources and industrial discharges, urban stormwater runoff has been identified as a primary source of pollution of surface waters with pesticides (USEPA, 2000; Auriol *et al.*, 2006). Peroxidases like Horse Radish Peroxidase (HRP) (E.C. 1.11.1.7), manganese peroxidase (E.C.1.11.1.13) and lignin peroxidase (E.C. 1.11.1.14) are ferric ion containing heme proteins and require peroxides like H₂O₂ for their functioning. Lignin peroxidase (Kersten *et al.*, 1990) and manganese peroxidase are obtained from fungi. There are various plant sources of peroxidases-like horse radish (De Souza *et al.*, 2007), soyabean (Johnson and Pokora, 1994):



The aim of the present study was to determine the efficiency of HRP in the process of pesticide removal from

wastewater. Various organochlorine as atrazine and organophosphate, methyl-parathion and triazophos pesticides were the subject of experiments.

MATERIALS AND METHODS

Reagents and equipment: Horseradish peroxidase (Type EC 1.11.1.7 and MW = approximately 44 kDa) and H₂O₂ (35% w/w; 8.82 mol L⁻¹) were purchased from Sigma Chemicals (St. Louis, MO). Methyl-parathion, atrazine and triazophos were purchased from Tung Fong Ltd., Co., Taiwan. HRP stock solutions (10 U mL⁻¹) were prepared by dissolving the solid enzyme in distilled deionized water (18 MΩcm) and were stored at 4°C. Acetate (pH 4, 5 and 6), phosphate (pH 7 and 8) and bicarbonate (pH 9 and 10) buffers were used for pH control. An individual stock solution of the synthetic wastewater of methyl-parathion, atrazine and triazophos of 3 mM was prepared by dissolving the weighted methyl-parathion, atrazine and triazophos in bi distilled water.

Degradation studies: Pesticides-HRP reactions were carried out in 100 mL borosilicate glass vials. Reactions were initiated by the addition of aliquots of enzyme (U mL⁻¹) and H₂O₂ (5-10 mM) stock solutions. The reaction mixtures were briefly mixed and allowed to incubate for 6 h at room temperature

(20°C). During the reaction the glass vials and beakers remained sealed with screw caps or parafilm, respectively.

Analytical methods: The change in pH of treated wastewater was monitored using pH meter (ELICO-L1127, India). Chemical Oxygen Demand (COD) concentration was estimated as per the standard procedure (APHA, 1998). High Performance Liquid Chromatography methods uv detector at 205 nm, array detection for determination of methyl parathion atrazine and triazophos were optimized with the aid of a Hitachi liquid chromatography model.

RESULTS AND DISCUSSION

Influence of enzyme and H₂O₂ concentration: Since, the biocatalyst has a finite lifetime, normally removal of pesticides is dependent on the amount of catalyst added. To study the effect of enzyme concentration on methyl parathion, atrazine and triazophos pesticides removal, seven different enzyme concentrations (0.3, 0.6, 0.9, 1.2, 1.5, 0.2 and 2.5 U mL⁻¹ were used to compare the efficiency of HRP enzyme. Figure 1 depicts the effect of enzyme concentration on pesticide removal. It is found that for a 2.0 mM pesticide solution, increasing enzyme concentration from 0.3-2.5 U mL⁻¹ results in gradual increase in pesticide removal. Further increases in enzyme concentration have no significant effect on pesticide removal. The enzyme concentration of 2.0 U mL⁻¹ was found to be the optimal dose for the experiment condition. Increasing the HRP dose produced higher pesticides removals in a manner similar to phenol reductions reported with HRP (Wu *et al.*, 1997). Also, Fig. 1 shows no methyl parathion, atrazine or triazophos removal was observed when either the peroxidase or the H₂O₂ were omitted from the reaction mixture. Thus, the disappearance of the pesticides was due to a combined action of HRP and H₂O₂, thereby indicating that these compounds were oxidized under the catalytic action of HRP. Further experiments were performed to determine how much H₂O₂ would be required to accomplish the reduction of methyl parathion, atrazine and triazophos at a constant enzyme dose of 2.0 U mL⁻¹. Also the different factors were studied, contact time and pH to reach the optimum conditions at which Chemical Oxygen Demand (COD) and pesticides concentrations reduced. In order to determine the optimum initial H₂O₂ concentration, a set of experiments was carried out for the investigated

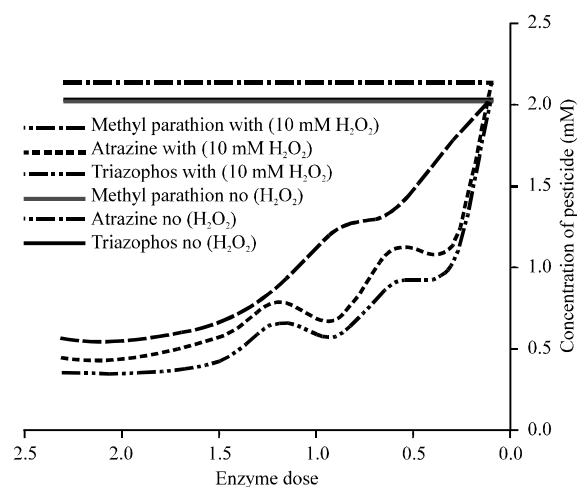


Fig. 1: Residual methyl parathion, atrazine and triazophos remaining after treatment with HRP/H₂O₂ with a retention time of 6 h. Residual concentrations in control samples (no HRP; no H₂O₂) were the same as initial concentrations

synthetic wastewater for which the concentration of H₂O₂ was progressively increased while maintaining the concentration of HRP enzyme constant at an arbitrary value of 2.0 U mL⁻¹. All experiments were carried out for 6.0 h of reaction time and at an initial pesticide concentration 2.0 mM at pH 8. The hydrogen peroxide concentrations studied were in the range of 5-20 mM. The optimum H₂O₂ concentration was determined as 10 mM as shown in Fig. 2a. A COD removal efficiencies obtained after HRP enzyme treatment of wastewater at varying initial H₂O₂ concentrations are presented in Fig. 2b. Therefore, H₂O₂ and HRP enzyme dose should be added at the optimal concentration to achieve the best degradation.

Dependence of pH: Role of pH in mix reaction must be determined. The pesticide and COD degradation of model substances by HRP/H₂O₂ treatment as a function of pH are shown in Fig. 3a and b. The experiments were carried out at pH range from 2-10. The results clearly indicate that the extent of degradation increases with the increase in pH value for pH 2-10. At pH 2, free radical formation is very low; therefore, radical reactions are expected to be negligible. At pH 6, oxidation and °OH radical reactions are expected to be equally important. At pH 8, the formation of °OH radicals is fast, therefore, radical reactions are considerable and the

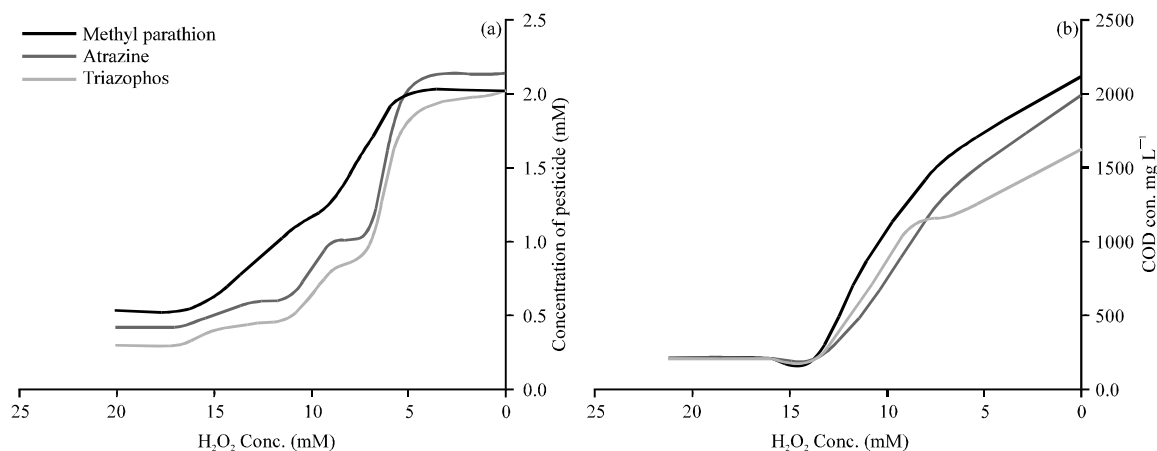


Fig. 2(a-b): (a) Pesticides degradation rates for differing doses of H₂O₂. Enzyme dose = 2.0 U mL⁻¹; t = 6 h and (b) Chemical oxygen demand (COD) removal rates for differing doses of H₂O₂. Enzyme dose = 2.0 U mL⁻¹; t = 6 h

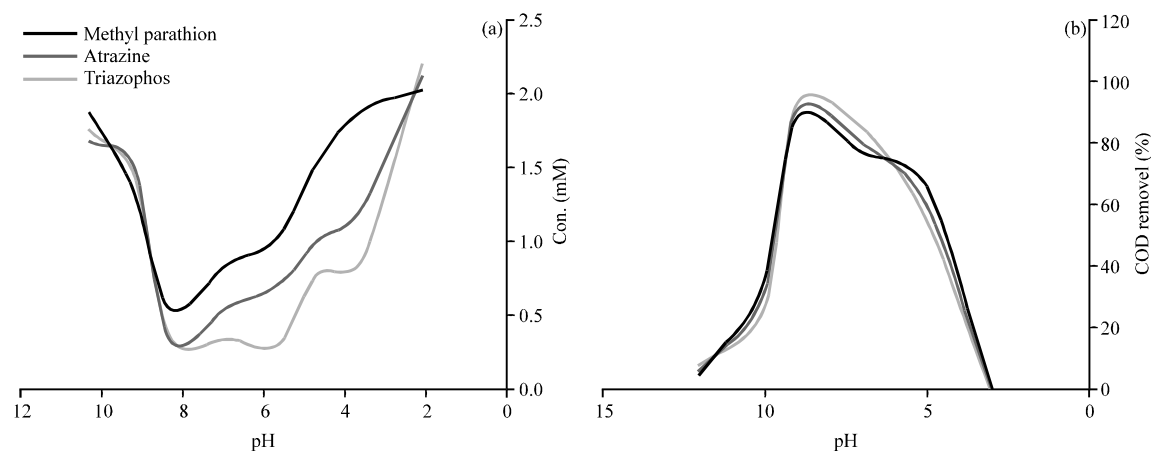


Fig. 3(a-b): (a) Pesticides removal from aqueous solution as a function of pH. Enzyme dose = 2.0 U mL⁻¹; t = 6 h, H₂O₂ = 10 mL and (b) Effect of pH on the COD reduction, enzyme dosage = 2.0 U mL⁻¹; t = 6 h

pesticide removal was (75 %) and 90% of COD reduction. This demonstrates that the most effective pH value for degradation of the selected model substrates by HRP treatment is 8. These results were contradicted with (Cooper and Nicell, 1996), they suggested that the optimum removal conditions for phenols from a foundry wastewater using horseradish peroxi-dase can be transformed to a high extent over a pH range 2-4. Specially, up to 95% removal of phenols using horseradish peroxi-dase was achieved at pH 2 and 5.

Optimum contact time: Initial experiments were performed in order to assess the optimum contact time

required for methyl-parathion, atrazine and triazophos removal. To a series of beakers each one containing 50 mL of 2.0 mM pesticides, 10 mM hydrogen peroxide along with enzyme concentration (2.0 units mL⁻¹) were added and reaction media (25°C, pH = 8.0) was agitated for a period of 6 h. Every 20 min, a 1 mL sample was taken from solution and was analyzed for the residual pesticides concentration. It was shown that 360 min is required to reach acceptable removal efficiency (Fig. 4a). Furthermore Percent COD removal efficiencies obtained after HRP treatment of wastewater at varying contact time are presented in Fig. 4b, these results were supported by Nicell (1994).

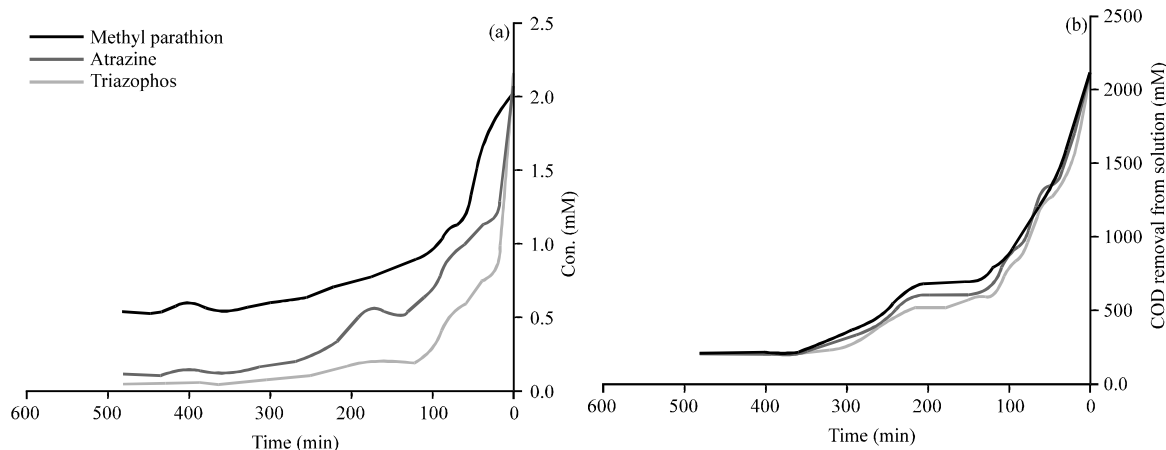


Fig. 4(a-b): (a) Rate of pesticides reduction by HRP from the initial concentration (C_0) of 2.0 mM; enzyme dosage 2.0U mL⁻¹ and (b) Chemical oxygen demand (COD) removal obtained at varying contact time

CONCLUSION

The experimental results obtained in the present study revealed the effectiveness of the horseradish peroxidase in pesticide and COD removal. The performance of pesticide removal was found to be highly dependent on aqueous pH, contact time and enzyme dose. The enzyme activity shows higher relative activity in basic solutions which are the most common conditions appeared in waste stream. Despite the advantages of enzymatic wastewater treatment, the major limitation in the use of enzymes is their prohibitive cost. Currently, effluent treatment using enzymes on a large scale is not economically viable. However, if maximum reusability of enzymes is achieved through the use of local enzyme manufacture, the running cost can be lowered considerably. The future research in this field should emphasize on the optimization of the activity of crude enzyme preparations and on the improvement of enzyme reusability to counteract the high start-up and running costs.

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