

Detoxification of pesticides aqueous solution using horseradish peroxidase

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ABSTRACT

There are pesticide residues in agriculture wastewaters and that compounds must be removed before discharge of wastewaters 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 to 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 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 to 221 mg L⁻¹ (at pH 8). Tests based upon horseradish peroxidase, at optimized parameters, show the reduction of toxicity to non-toxic levels. © 2013 Trade Science Inc. - INDIA

KEYWORDS

Pesticide residues;
COD removal;
Agriculture wastewaters;
Horseradish peroxidase.

INTRODUCTION

An important quantity of pesticide is released from pesticide production plants are toxic and carcinogenic in nature even at low concentration^[7].

Organophosphate pesticides like methyl parathion and triazophos can be harmful to aquatic life even at very low concentrations^[4]. Atrazine is a xenobiotic compound that was recognized by EPA as Group C – “possible” human carcinogen^[11].

Along with municipal point sources and industrial discharges, urban stormwater runoff has been identi-

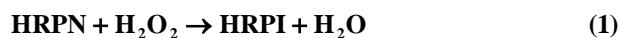
fied as a primary source of pollution of surface waters with pesticides^[2,12].

Removal of methyl parathion by in aqueous solutions by electrochemical oxidation was reported^[2]. To remove p-hydroxybenzoic acid and atrazine from agriculture waste water^[9], used Fenton’s like system Fe (III) with H₂O₂. Atrazine was removed from contaminated water using activated carbon^[5].

Peroxidases like horse radish peroxidase (HRP) (E.C.1.1.1.7), 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

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for their functioning. Lignin peroxidase^[8] and manganese peroxidase are obtained from fungi. There are various plant sources of peroxidases – like horse radish^[10], soyabean^[6].



The aim of the present study is 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 H_2O_2 (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) 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 3mM 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) and H_2O_2 (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^[1]. 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 chromatograph model.

RESULTS

Influence of enzyme and H_2O_2 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 units/ml to 2.5 units/ml 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 Units/ml 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^[13]. Also, Figure 1 shows no methyl parathion, atrazine or triazophos removal was observed when either the peroxidase or the H_2O_2 were omitted from the reaction mixture. Thus, the disappearance of the pesticides was due to a combined action of HRP and H_2O_2 , thereby indicating that these compounds were oxidized under the catalytic action of HRP. Further experiments were performed to determine how much H_2O_2 would be required to accomplish the reduction of methyl parathion, atrazine and triazophos at a constant enzyme dose of 2.0 U/mL. 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 H_2O_2 concentration, a set of experiments was carried out for the investigated synthetic wastewater for which the concentration of H_2O_2 was progressively increased while maintaining the concentration of HRP enzyme constant at an arbitrary value of 2.0 Uml⁻¹. All experiments were carried out for 6.0 h of

reaction time and at an initial pesticide concentration 2.0mM at pH 8. The hydrogen peroxide concentrations studied were in the range of 5-20 mM. The optimum H_2O_2 concentration was determined as 10 mM as shown in figure 2a. A COD removal efficiencies ob-

tained after HRP enzyme treatment of wastewater at varying initial H_2O_2 concentrations are presented in Figure 2b. Therefore, H_2O_2 and HRP enzyme dose should be added at the optimal concentration to achieve the best degradation.

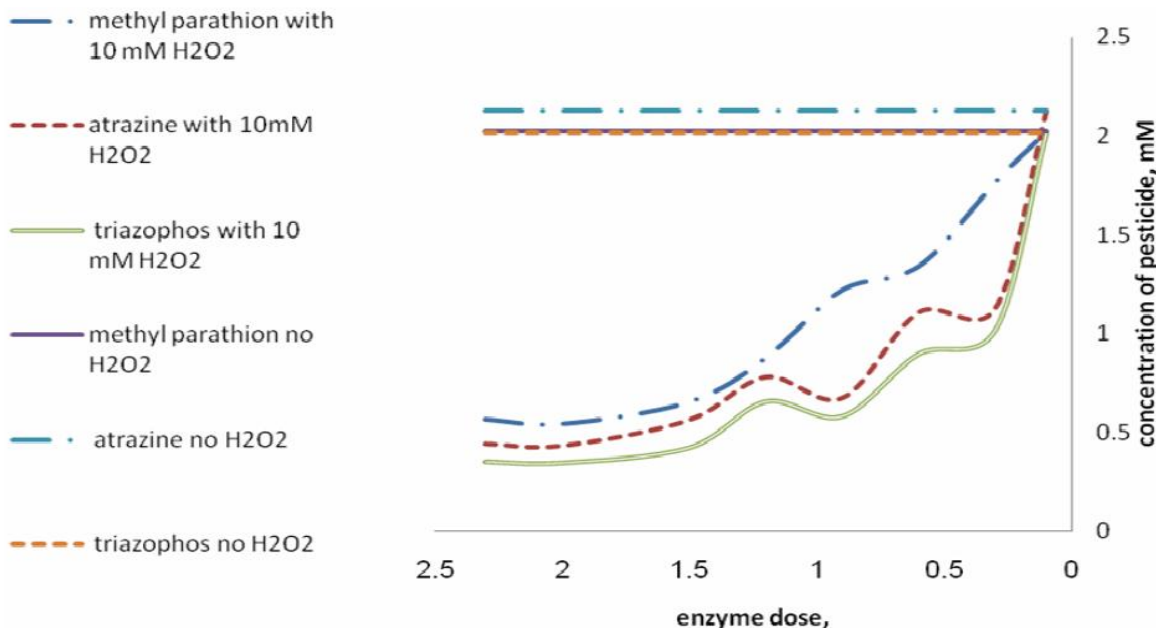


Fig. 1. Residual methyl parathion, atrazine and triazophos remaining after treatment with HRP/ H_2O_2 with a retention time of 6 h . Residual concentrations in control samples (no HRP; no H_2O_2) were the same as initial concentrations.

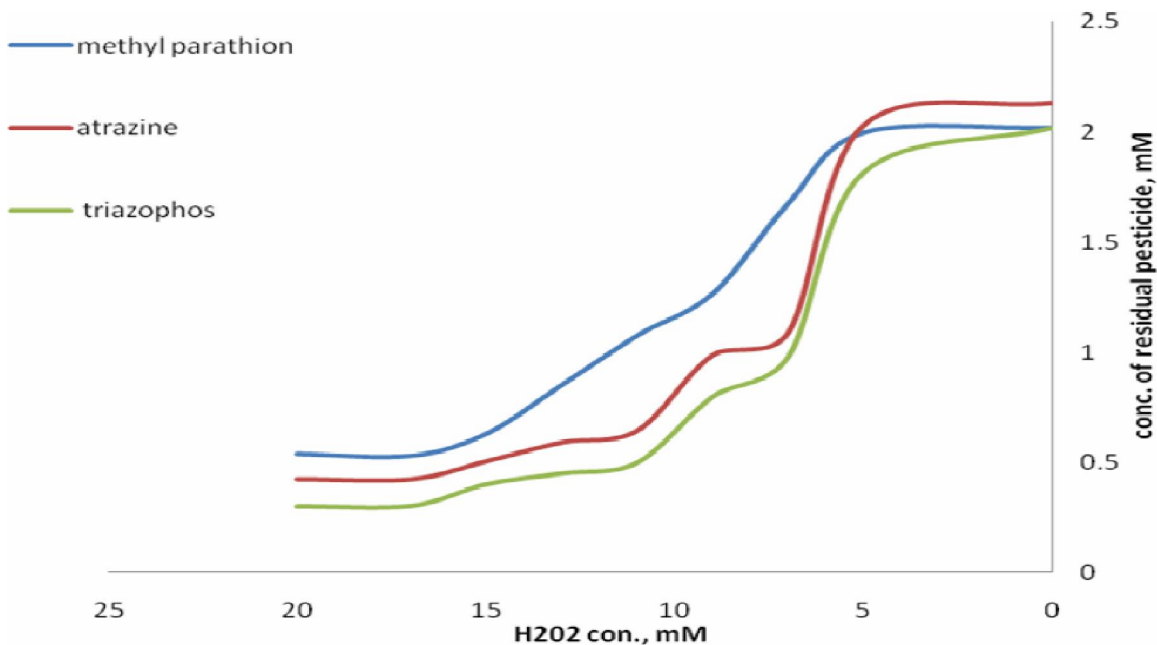


Fig. 2a pesticides degradation rates for differing doses of H_2O_2 . Enzyme dose = 2.0 U mL^{-1} ; $t = 6 \text{ h}$

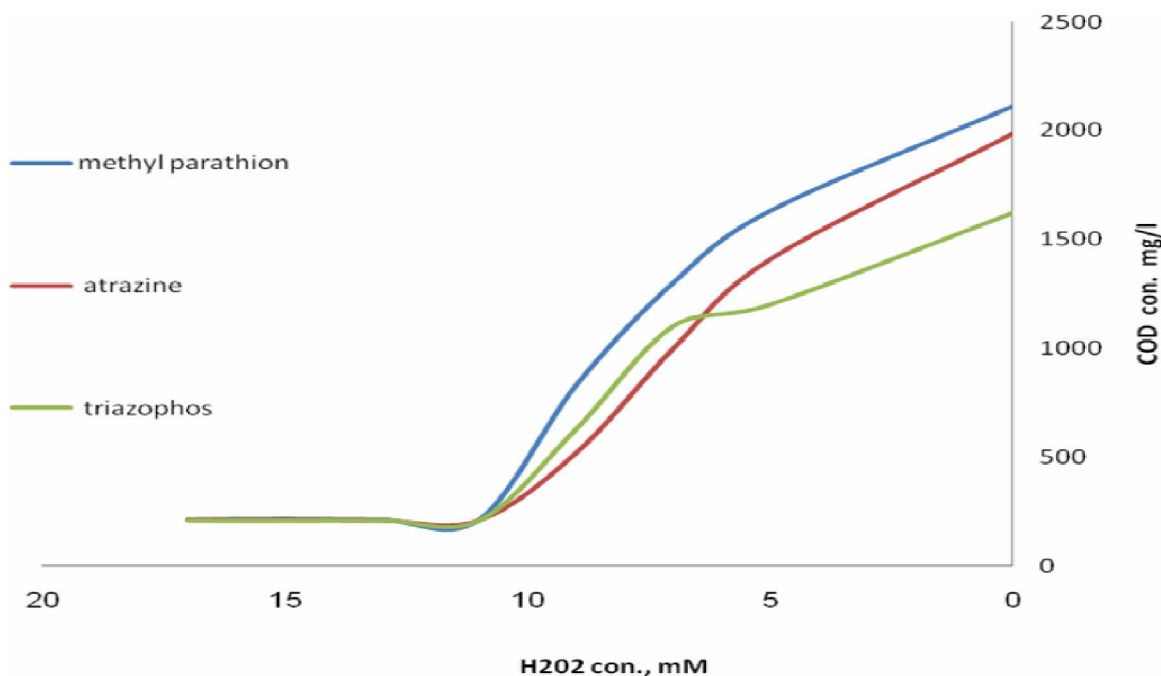


Fig. 2b Chemical oxygen demand (COD) removal rates for differing doses of H₂O₂. Enzyme dose = 2.0 U mL⁻¹ ; t = 6h

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 Figures 3a and 3b. The experiments were carried out at pH range from 2 to 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 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.

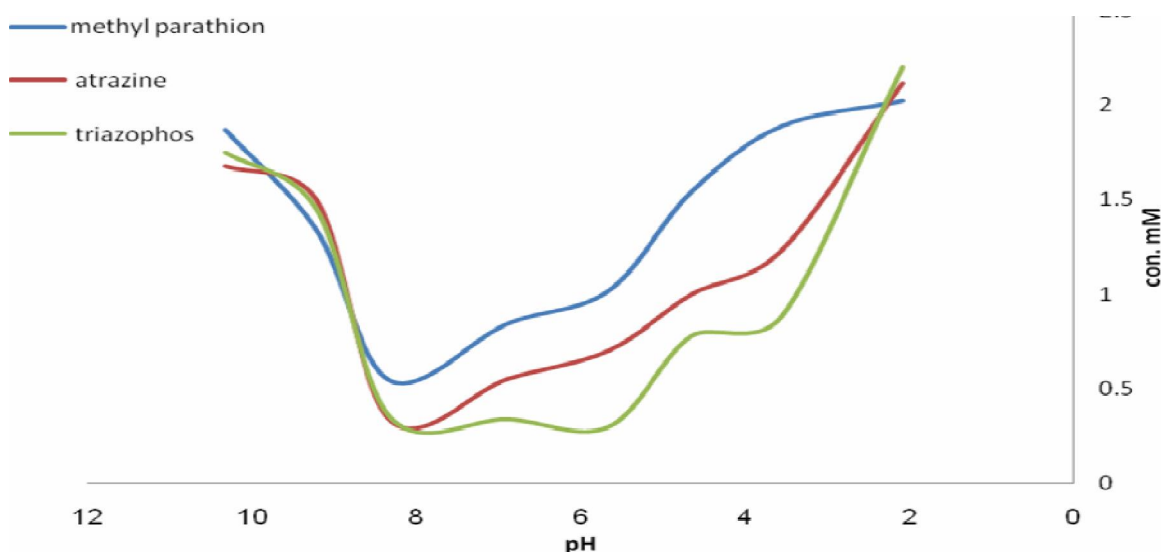


Fig. 3a. pesticide removal from aqueous solution as a function of pH. Enzyme dose = 2.0 U mL⁻¹; t = 6h; [H₂O₂] = 10 mM

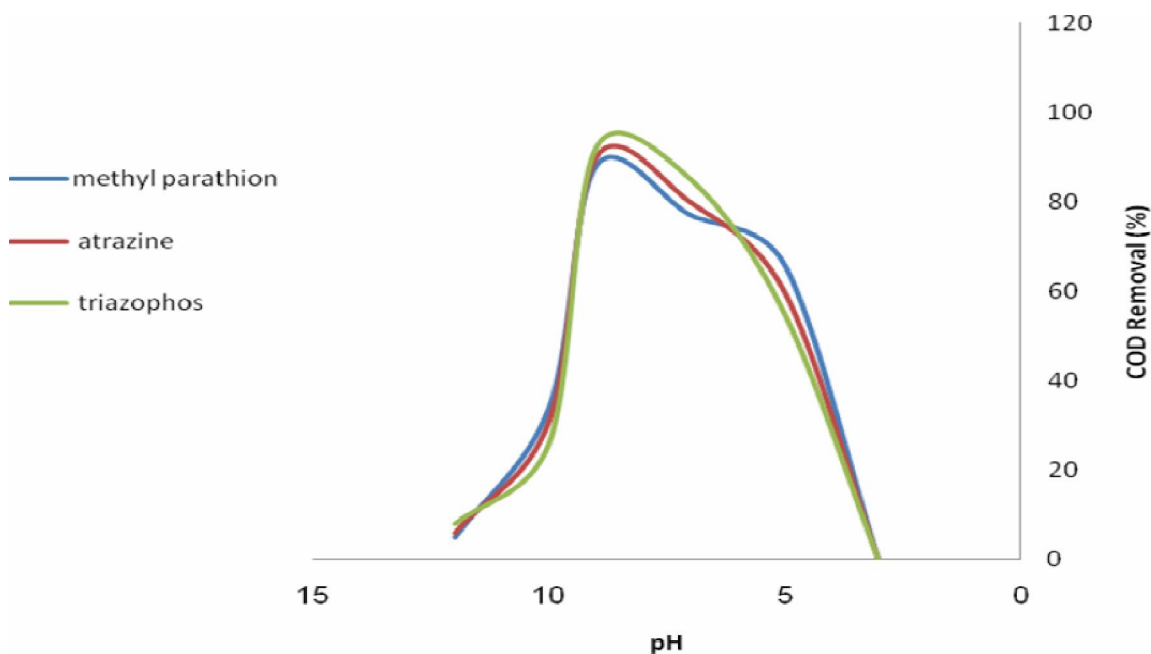


fig 3b. Effect of pH on the COD reduction , enzyme dosage = 2.0 U/mL-1 ; t = 6h

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) were added and reaction

media (25 °C, pH=8.0) was agitated for a period of 6 hours. Every 20 minutes, 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 (Figure 4a). Furthermore Percent COD removal efficiencies obtained after HRP treatment of wastewater at varying contact time are presented in Figure 4b.

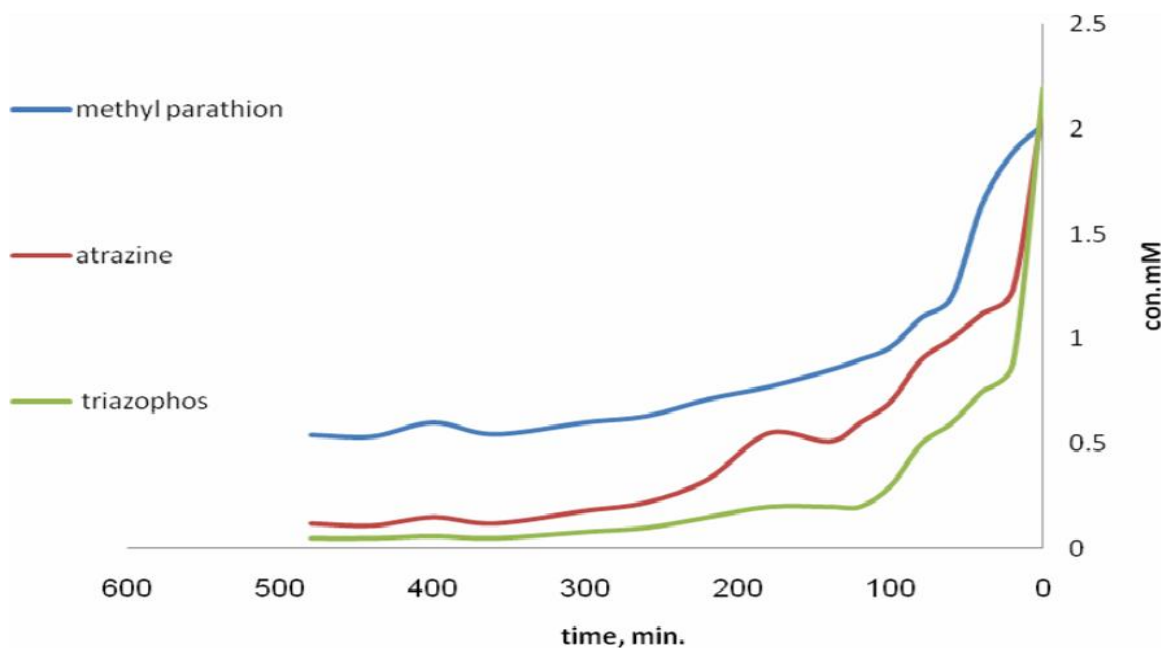


Fig. 4a. Rate of pesticide reduction by HRP from the initial concentration (C0) of 2.0 mM ; enzyme dosage 2.0 U/mL-1

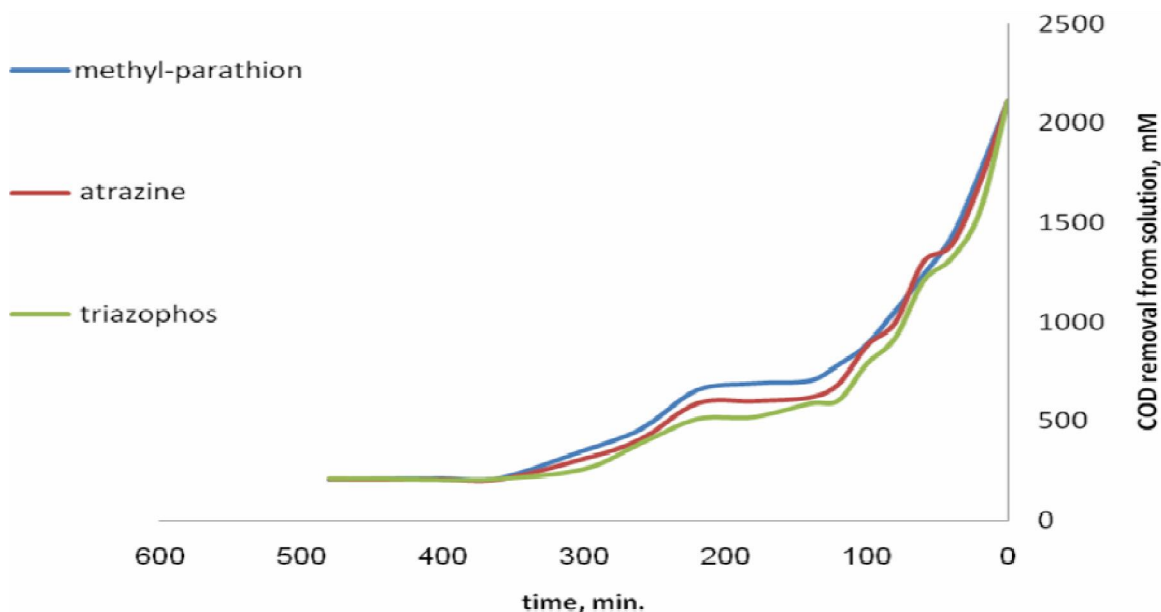


Fig. 4b. Chemical oxygen demand (COD) removal obtained at varying contact time

CONCLUSION

The experimental results obtained in the present work 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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