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Original Article

Decontamination of Water Polluted with Phenol Using *Raphanus sativus* Root

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Abstract

Plant materials have been found useful in decontamination of water polluted with phenolic compounds. The detoxification effect is due to peroxidases contained in plant tissue. Enzyme mediated oxidative coupling of phenol is followed by precipitation of the formed polymer and its removal from the aqueous phase. A synthetic wastewater buffered at pH = 7.4 containing 0.9 mM phenol was treated in this research with cut *Raphanus sativus* root and its juice. Cut Raphanus or Raphanus juice were added separately as enzyme source to phenol solution in buffer and in tap water in two series of experiments. The reaction was initiated by the addition of hydrogen peroxide. After three hours stirring the phenol content of the mixtures was determined. More than 90% of phenol was removed in both cases.

Keywords: *Raphanus sativus*; peroxidase; phenol decontamination.

Introduction

Phenols and anilines are toxic contaminants in the wastewater of different industries such as plastics, resins, steels, dyes and organic chemicals. Therefore, removal of these contaminants from industrial wastewater should be taken into consideration. Conventional processes for removing these compounds include extraction, adsorption on activated carbon, steam distillation, bacterial and chemical techniques, irradiation, etc. All of these methods suffer from high cost, incompleteness of purification, formation of dangerous by-products, or low efficiency (1-5). Using different sources of peroxidase to oxidize and polymerize the chemicals were previously reported (6-8). Peroxidases in their catalytic cycle oxidize phenol with hydrogen peroxide generating phenoxy radicals, which then react to form polymer. Klibanov et al (9) reported that horseradish peroxidase can eliminate aromatic contaminations of water. They used pure horseradish peroxidase and the results showed a good eliminating potential of the enzyme for decontamination of phenols, anilines and other aromatic compounds from aqueous solutions. Horseradish peroxidase is the most studied enzyme for using in decontamination processes but its high cost is one of the limitations. Using microbial sources of enzyme and plant material directly without purification for removal of phenols from wastewater could be considered as two solutions (8). The results from Nicell et al (5) showed similar ability for peroxidase obtained from *Coprinus macrorhizus* as a microbial source. Dec et al (8) proposed an additional method to facilitate enzymatic treatment. They proved horseradish plant material could be reused up to 30 times for decontamination. These studies were performed in a specific

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condition according to pH and presence of alum.

In this study, phenol eliminating potential of *Raphanus sativus* root was evaluated in two different ways (juice and cut pieces). It was attempted to adjust an appropriate condition to obtain an optimum elimination yield.

Experimental

Material

Phenol, sodium bicarbonate, potassium dihydrogen phosphate, sodium hydroxide, alum [Al_2 $(SO_4)_3$. 16 H_2O], 4-aminoantipyrine (4-AAP), ferricyanide and hydrogen peroxide were purchased from Merck Company.

Enzyme source preparation

Raphanus sativus was purchased from vegetable market and used as peroxidase enzyme source. It was stored in -10^{o}C and before performing the reaction, washed with water. It was used after peeling in two different ways in separate experiments. In fact *Raphanus sativus* root was cut into pieces (cut root) or minced and then its juice was extracted.

Removal reaction

Measurements were performed on an UV Spectrophotometer Pharmacia LKB- NiaspecII RS232C. Phenol polymerization and removal from the synthetic wastewater were carried out in a container agitated with a magnetic stirrer at room temperature. The initial reaction solution contained 20 ml phosphate buffer (pH = 7.4) or 20 ml water without buffer and 0.9 mM phenol for all of the experiments while juice and pieces of *Raphanus sativus* root had a range from 0.1 ml to 7 ml and 0.6 g to 8 g respectively in separate reactions. The reaction was initiated by adding the necessary quantity of a hydrogen peroxide stock solution and stopped after 3 h stirring. On the other hand, the mixture of the reaction was provided in presence or absence of alum. After centrifuging the reaction mixture within a period of 30 min (5000 rpm/min) the supernatant was separated and assayed with a phenol/ 4-AAP chromogen system as described previously (6). The absorbance of supernatants was determined at 510 nm by UV spectrophotometric method. Several experiments were carried out to find appropriate conditions for the reaction: amount of horseradish juice or pieces, effect of pH and effect of buffer.

Results and discussion

Phenol removal activity of *Raphanus sativus* root

In this study *Raphanus sativus* root was used in order to eliminate phenol contamination from water. Root juice and pieces without any purification and separation were used as enzymatic materials. In both experiments, comparison of results showed a good phenol

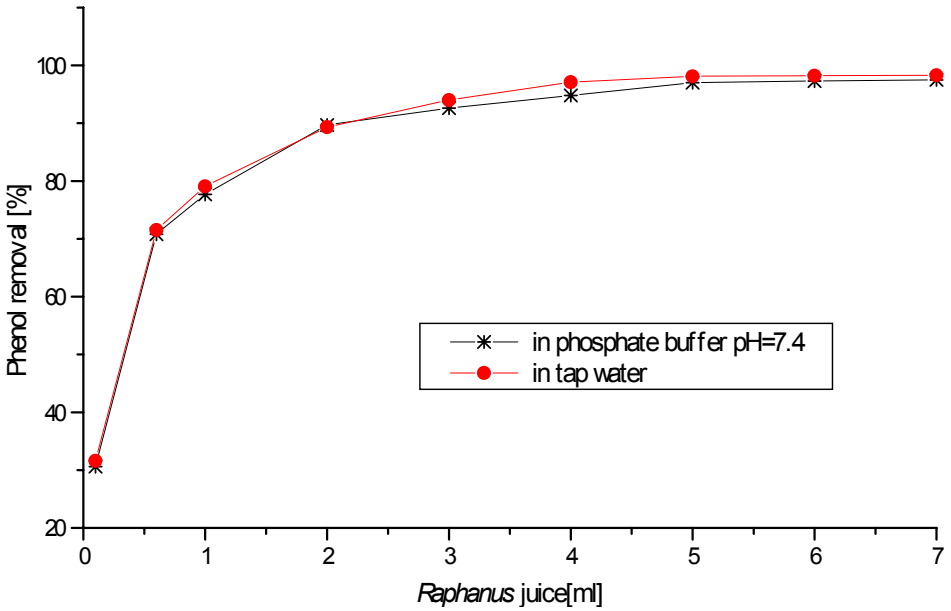

Figure 1. Comparison of phenol removal using raphanus juice in buffered medium and tap water in presence of 1 mM H_2O_2.

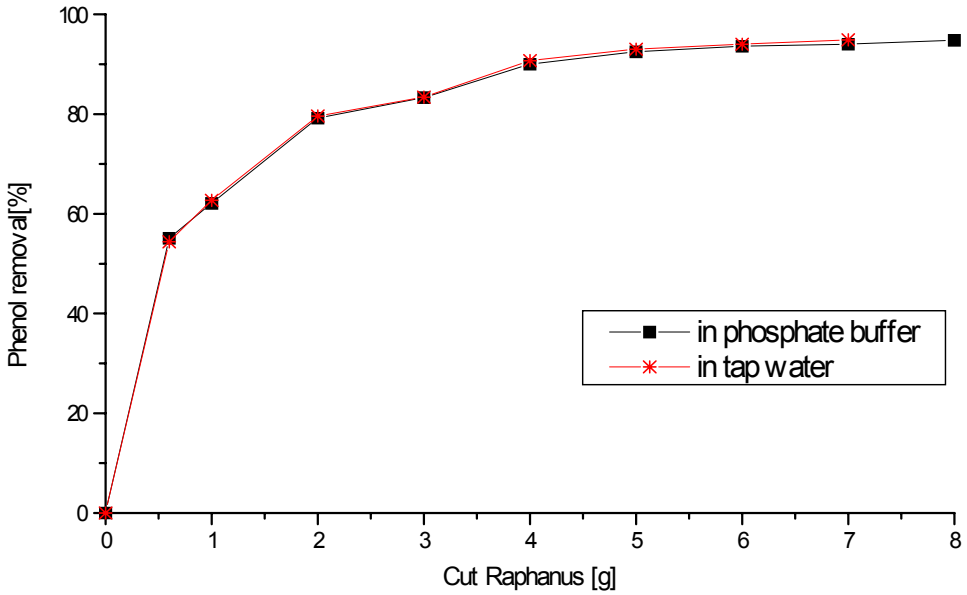

Figure 2. Comparison of phenol removal using cut Raphanus in tap water and buffered medium in presence of 1 mM H_2O_2.

removal from aqueous solutions. In fact *Raphanus sativus* tissues in both forms had the capability to accomplish the phenolic removal reaction. The results have been shown in figures 1 and 2. Increasing the extent of Raphanus sativus root juice from 0.1 ml to 7 ml caused a removal equivalent of 33% to 98%. Using pieces of *Raphanus sativus* root at amount of 0.6 g to 8 g led to 58% to 95% of removal.

It seems that using plant root juice instead of cut root in order to eliminate the aromatic contamination leads to higher removal yields. That can be due to higher contact area between reaction components and/or higher enzyme concentration. As it can be seen in figure 1 only 2 ml of root juice is capable to remove 90% of phenol and increasing juice amount from 2 ml to 7 ml causes up to 98% removal. Using 4 g of root pieces (Figure 2) caused 90% phenol elimination and increasing the amount of root up to 8 g caused only small increase in elimination.

Effect of pH, buffer, hydrogen peroxide/ phenol ratio and alum

Considering the point that *Raphanus sativus* was used to remove phenol from water for the first time, it is necessary to optimize the method considering the effect of pH and stoichiometry in reaction.

The effect of hydrogen peroxide concentration was studied, too. In general the consumption ratio of hydrogen peroxide per phenol seems to be 1:1 or 1:1.1 (Figure 3).

In previous publications, purified enzyme were used at an optimum pH and good removal activity was obtained (6-10).

In our research, in both cases of using root juice and pieces, results showed a significant difference at various pH values if the reaction was carried out in buffer ($p<0.05$). As it can be seen in figure 4, the optimum removal is 7.4.

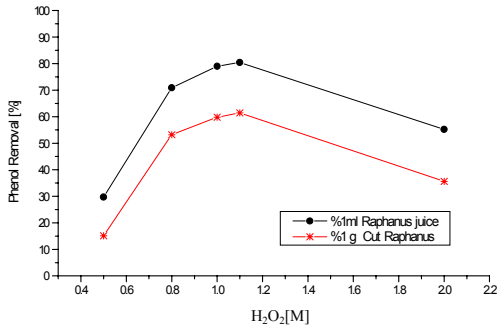

Figure 3. The effect of stoichiometry on removal of 1 mM phenol from phosphate buffer pH=7.4.

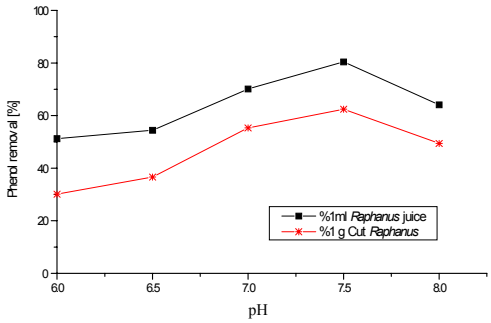

Figure 4. The effect of pH on removal of 1 mM phenol in the presence of 1 mM H_2O_2.

On the other hand a group of reactions were performed in tap water with similar results (Figures 1 and 2). It could be concluded that removal reaction can be carried out in unbuffered water. This finding has a great value in view of reaction development and lowering the high cost of enzymatic treatment and helping to perform the reaction in a more economic way.

The important points of this study can be summarized as follows:

-Using plant material directly without separation and purification of enzyme makes enzymatic treatment economically feasible.

-High yield of phenol removal makes the process useful for this purpose.

-There is no need for buffering the reaction mixture.

-Finally, *Raphanus sativus* root juice and pieces have a potential activity for removal of phenol (> 90%) from a synthetic wastewater.

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