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PHENOL REMOVAL FROM WASTEWATERS USING POLYPHENOLOXIDASE FROM POTATO

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ABSTRACT. An easy available enzyme preparation for bioremediation of phenol containing wastewaters was tested. The influence of most important parameters (pH, substrate-enzyme ratio) was studied.

Keywords: *Polyphenol oxidase, phenol removal, bioremediation*

INTRODUCTION

The rapid expansion and technological improvement in industrial fields increase the amount and complexity of toxic waste effluents. At the same time, regulatory authorities have been paying more attention to problems of contamination of the environment. There is a growing recognition that enzymes can be used in many industrial remediation processes to target specific pollutants for treatment [1]. The potential advantages of enzymatic treatment as compared with conventional treatments include: application to recalcitrant materials, operation at high and low contaminant concentrations over a wide pH, temperature and salinity range and the easy control of the process among others.

Among xenobiotic contaminants, aromatic compounds constitute one of the major classes of pollutants and are heavily regulated in many countries. This implies that the presence of phenols in industrial residues is an area of environmental concern, since toxic phenols enter the environment in wastewater streams, released by numerous industries, including pulp and paper mills, coal and steel works, wood preservation plants, and various chemical and petrochemical industries [2]. Moreover the presence of these compounds in drinking and irrigation water, or in cultivated land, also represents a significant

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health hazard and not only environmental: phenol concentrations greater than 50 ppb are toxic to some forms of aquatic life and ingestion of 1 g of phenol can be fatal in humans [3].

Conventional processes for removal of phenols and aromatic amines from industrial wastewaters include extraction, adsorption on activated carbon, steam distillation, bacterial and chemical oxidation, electrochemical techniques, irradiation, etc. All of these methods suffer from serious drawbacks as high costs, incompleteness of purification, formation of hazardous by-products, low efficiency and applicability to a limited concentration range [4]. Due to these drawbacks alternative methods may become important in a large scale in the near future. The treatment through enzymatic catalysis seems to have the potential to substitute conventional methods [5]. Bioremediation represents an important environmental remediation strategy because naturally occurring biological processes are used.

Polyphenole oxidase (PPO) from potato [6], a multi-copper-containing enzyme, belong to the classe of laccases (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) which catalyze the oxidation of a wide range of phenolic and aniline compounds. In its reaction, substrates are oxidized by one electron to generate the corresponding phenoxy radicals, which are further oxidized to a quinone. This enzyme was isolated from a large variety of plants and fungi [7]. Due to the high price, its use in industrial wastewater treatment is still limited. Recently the presence laccases (LACs) and polyphenol oxidases (PPOs) in marine cyanobacteria was revealed [8]. These strains were efficient in decolourizing of synthetic aromatic dyes.

In this work the crude polyphenole oxidase from potato was investigated as an alternative catalyst for the phenol removal from aqueous solutions.

RESULTS AND DISCUSSION

Isolation of PPO from potato

Enzyme extracts were prepared by homogenizing diced peeled potato samples with L-ascorbic acid as antioxidant in cold 0.2 M sodium phosphate buffer (pH 6) and centrifugation. The collected solution was lyophilized for further use or storage. PPO activity of crude enzyme preparation was measured as the initial rate of oxygen uptake at 25°C in 50 mM sodium phosphate, pH 6.0. The reaction was measured using the colorimetric method with 4-aminoantipyrine (4-Amino-1,2-Dihydro-1,5-Dimethyl-2-Phenyl-3H-Pyrazol-3-One, AAP) in presence of potassium ferricyanide [9]. The analytic range covers phenol concentrations from 0.03 to 0.12 mM.

Enzyme stability

The activity of crude enzyme during the storage at -80 ºC was studied (Figure 1). After approximately 4 days, it decrease with 50 % and after 15 days only 2% from the initial activity (16.9 UI/mg) was retained. In conclusion, even

at low temperature, the polyphenol oxidase is an unstable biocatalyst and in order to improve its stability the immobilization could be a convenient method.

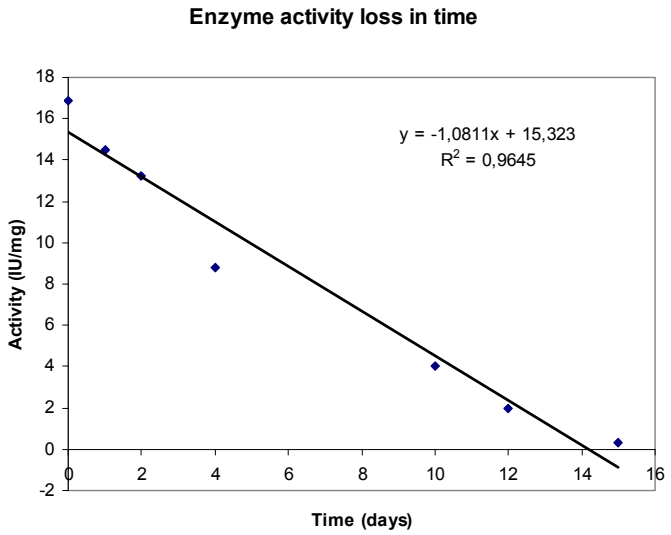


Figure 1. The stability of crude polyphenol oxidase at low temperature

The influence of pH on the bioremediation process

Several sets of samples are prepared, varying the pH of phenol solution, using acid solutions of sodium phosphate buffer 0.2 M. From the calibration curve determine the concentration of phenol in solution, before Adding to the enzyme preparation. Then add the same amount of enzyme, with same concentration (2 mg enzyme / ml sol.) and constant temperature (30°C) to phenolic solutions with different pH's. The solution will be put in to the shaker. The absorbance of each sample will be read, after certain intervals of time. For absorbance reading is used the same method as for determining the concentration of phenol from water.

The bioremediation experiments were realized for phenolic solutions having the same phenol concentration (13 mM) and different pH (using various phosphate buffer), with the same amount of enzyme. Best results were obtained pH 6.15, when after 5 days the removal of phenol was almost quantitative (Figure 2).

At the optimum pH the rate of biodegradation was much higher: after 1 day the yield was similar with those of bioremediation in alkaline conditions after 5 days.

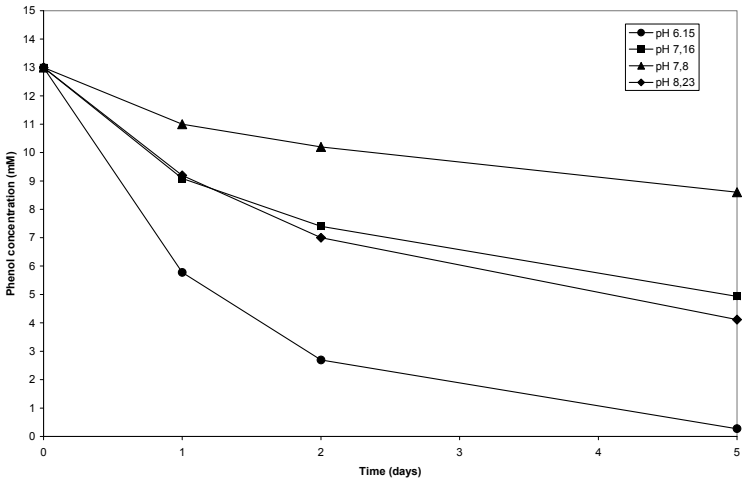


Figure 2. The inffluence of pH on phenol removal with soluble crude PPO

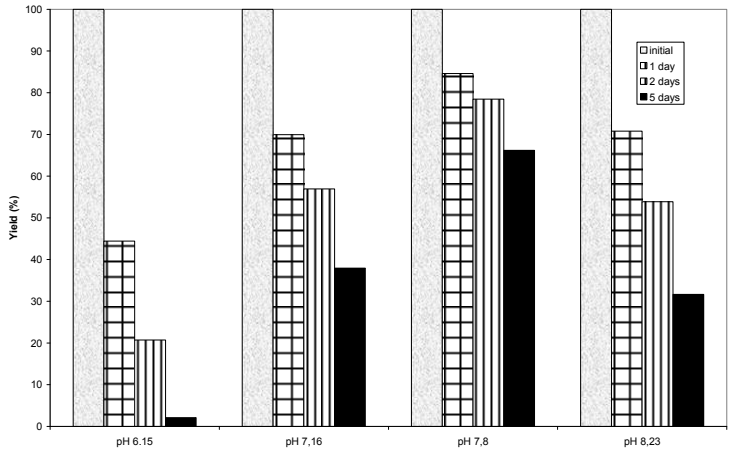


Figure 3. The efficiency of phenol removal with soluble crude PPO at different pH

The influence of enzyme-substrate ratio

Varying the amount of crude enzyme added to the solution having the optimum pH (6.15) and the same initial concentration (13 mM), using higher amounts of crude enzyme (2 mg enzyme/ml phenolic solution), the phenol was removed quantitatively in 6 days (Figure 4).

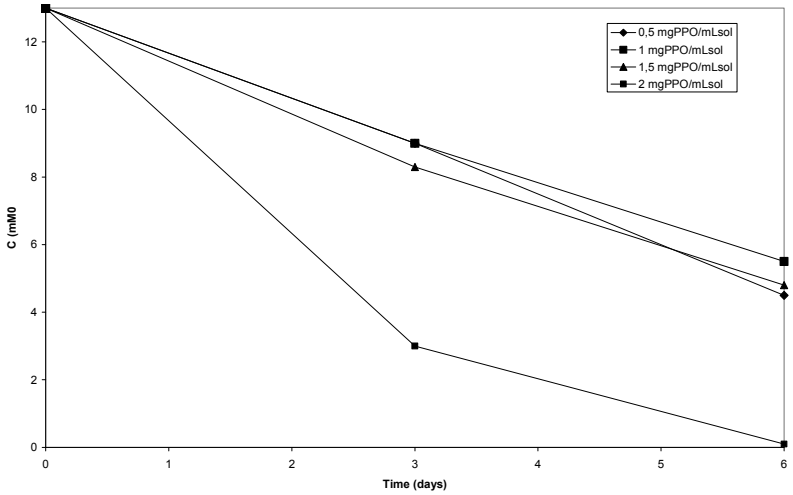


Figure 4. Influence of enzyme-subtrate ratio on phenol removal

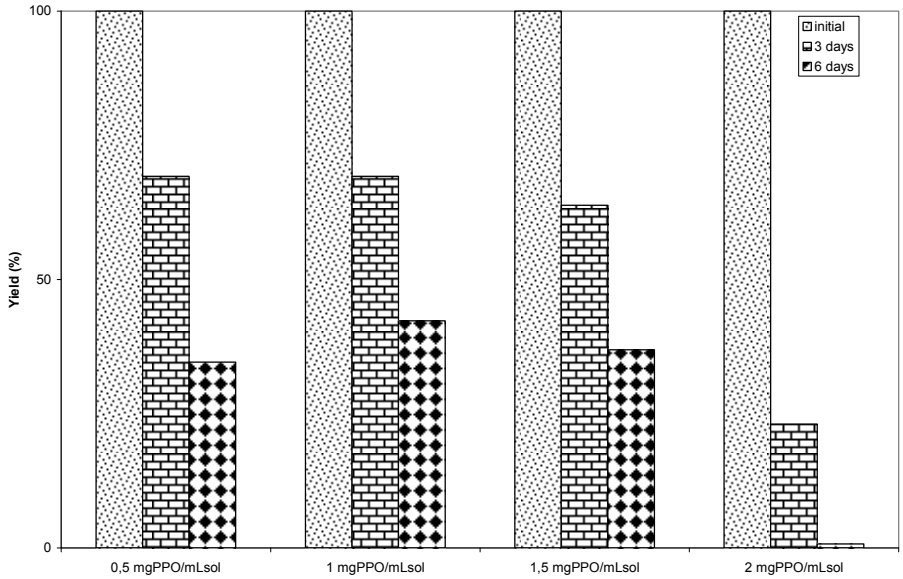


Figure 5. The efficiency of phenol removal with soluble crude PPO at different enzyme-substrate ratio

As expected, at lower enzyme concentrations the degree of bioremediation was in the field of 37-43 % after 6 days. The bioremediation process is also faster at higher enzyme concentration (Figure 5).

EXPERIMENTAL SECTION

Materials and methods

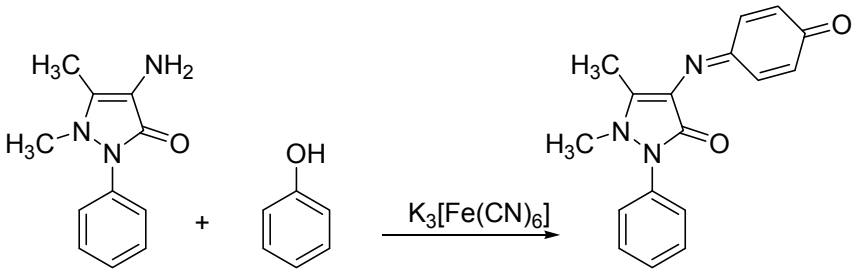
Potato *(Solanum tuberosum*) tubers were obtained from commercial fields in Romania at 3- to 4-week intervals. All inorganic and organic reagents were products of Aldrich or Merck. The UV-VIS spectra were recorded on a Agilent 8453 spectrophotometer at room temperature.

Preparation of potato extract (crude polyphenoloxidase)

Approximately 100 g of diced peeled potato sample were homogenized with L-ascorbic acid (0.3 g) and cold sodium phosphate buffer 0.2 M, pH 6 (100 ml). The mixture was centrifugated for 20 minutes at 10000 rpm. The collected supernatant (liquid phase) which contain the crude enzyme was lyophilized and stocked at –80°C.

Determination of phenol in water

Phenol concentration was measured using the colorimetric method with AAP in presence of potassium ferricyanide (Scheme 1). The analytic range covers phenol concentrations from 0.03 to 0.12 mM.



Scheme 1

Determination of Specific Activity

Enzyme activity in the stock enzyme solution was measured before use. 2 mL sample prepared from 1.9 mL phenol solution 0.2 M and 0.1 mL freshly preapared enzyme solution (100 mg crude enzyme in 25 mL phosphate buffer 0.2 M, pH 6) was mixed with 2 mL of 0.25 M sodium bicarbonate and 0.9 mL of 20.8 mM AAP. After vigorous mixing 0.9 mL of 83.4 mM potassium ferricyanide was added and mixed again. Samples absorbance was measured at 510 nm, 9 minutes after the ferricyanide addition and converted to concentration using the calibration curve. One international unit of activity represent the amount of enzyme which modify the absorbance with 0.001/min.

General experimental bioremediation procedure

Experiments were carried out in 200 mL beakers at 30º. Reaction medium was prepared by adding individually certain amounts of phenol and crude PPO into the corresponding phosphate buffer (0.2 M). The process was performed under continuously stirring at 200 rpm. Samples were taken at different intervals and the phenol concentration was determined colorimetric as described in section 2 of experimental part.

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