EKSTRAKCIJA ENZIMA IZ BILJNOG OTPADNOG MATERIJALA: PRIMENA U OBEZBOJAVANJU INDUSTRIJSKIH OTPADNIH VODA

ENZYME EXTRACTION FROM PLANT WASTE MATERIALS: IMPLEMENTATION IN DECOLORIZING OF INDUSTRIAL EFFLUENTS

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Otpadne vode iz tekstilne industrije sadrže različite zagađujuće materije. Antrahinonske boje, spadaju u jedne od glavnih polutanata i veoma su toksične za akvatični živi svet. Naime, u poslednjih nekoliko godina, enzimski tretman otpadnih voda tekstilne industrije, privlači sve više pažnje, u poređenju sa fizičkim i hemijskim tretmanima, usled ekološke i ekonomske opradavnosti. Dalje, enzimi se mogu izolovati iz različitih otpadnih materijala biljnog porekla, što je u skladu sa principima održivog razvoja i čistije proizvodnje. Peroksidaza je jedan od enzima, koji se može koristiti u tretmanu otpadnih voda, za degradaciju toksičnih antrahinonskih boja. U ovom radu ispitivana je ekstrakcija peroksidaze iz biljnog otpadnog materijala, kao što je: kora jabuke, banane, krompira i sojine ljuspice. Zatim je određivana aktivnost dobijenih ekstrakta i izvršena je selekcija najpogodnijeg enzima. Reakcija degradacije antrahinonske boje optimizovana je variranjem koncentracije rastvora boje i enzima.

Ključne reči: *peroksidaza; ekstrakcija; tretman otpadnih voda; antrahinonska boja; čistija proizvodnja*

Wastewaters from the textile industry contain various contaminants. Among them, anthraquinone dyes are considered to be highly toxic to the aquatic biota. Enzymatic treatment of wastewater from textile industry has drawn a great deal of interest since it is eco-friendly and a cost effective technology, comparing to chemical and physical decolorization methods. In addition to its environmental benefits, enzymes can be isolated from plant waste materials making them more alluring for utilization. One of plenty enzymes potentially used in purification of wastewater is peroxidase. It could be readily used for degradation of anthraquinone dyes. In this study, extraction of peroxidase from the following waste materials is performed: apple peel, banana peel, potato peel, and soybean hull. Next, enzyme activity is measured and from the results obtained, the most suitable waste material source is selected. Afterwards, the dye concentration is varied for the purpose of determining the optimal one for degradation.

Key words: *peroxidase, extraction; wastewater treatment; anthraquinone dye; cleaner production;*

1 Introduction

Effluents from textile industry are crucial environmental issue because of their volume and pollutants' content. Aside from processing the other contaminants from textile industry such as suspended solids and metals, treatment of dyes in textile waste water is perhaps the most challengeable. One of the most common dyes used in textile industry are anthraquinone dyes, whose acute and chronic toxic effect on living organisms is well known and studied. They cause inhibition of growth, development and reproduction of aquatic organisms [1,2]. Therefore, high level of degradation or degradation to compounds that are not as toxic as the dyes themselves is required.

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Conventional methods of waste water treatment such as biological treatment, adsorption and chemical oxidation, have some drawbacks when it comes to dye removal. These methods are time consuming, energy inefficient, non-specific and harmful for the environment with toxic byproducts. Main disadvantages of synthetic dyes removal by conventional methods is the chemical stability of these compounds and the generation of secondary pollutants. High concentration of dyes additionally makes the treatment more complex [3].

Enzymatic wastewater treatment is trending due to its cost - effectiveness and that is environmentally harmless [4]. Enzymes that catalyze removal of synthetic dyes are azoreductase, laccase, lignin-peroxidase, polyphenol-oxidase, horseradish and soybean peroxidase [5-7]. Common characteristic of these biocatalysts is their redox-active property with broad substrate specifity.

Despite the fact that laccase and azoreductase are most often used in enzymatic treatment, peroxidase has found its place among them because of its specifity, stability, abundance and low cost[8].

Peroxidase belongs to the oxidoreductase group of enzymes. It can be found in almost every plant but in different concentration in different part of the plant. The idea is to extract the peroxidase from chosen bio-waste material and use it in removal of anthraquinone dye Acid Violet 109 from textile industry effluent.

2 Materials and Methods

2.1 Enzyme extraction

All plants were obtained from the local grocery store and the peel was separated from the fruit. Extraction was made with phosphate buffer: 0,1 M, pH 6.0 for soybean hull and potato peel; 0.05M pH 7.0 for banana peel and 0.1 M pH 7.8 for apple peel with 1:4 w:v ratio. The bio-waste material was homogenized for couple of minutes and left overnight at 4C. Next, the mixture was filtrated through cheesecloth, and the filtrate was heated 3 minutes at 65°C. Then, it was cooled with ice and centrifuged 10 min at 10^4 RPM. The supernatant was separated and subjected to activity measurement.

2.2 Assay on enzyme activity

Peroxidase activity is measured at 420 nm using pyrogallol as substrate. One unit of peroxidase activity is defined as the amount of enzyme that catalyzes the conversion of pyrogallol to 1 mg purpurogalline for 20s at 20°C and pH 7.0. For this assay, 13 mM solution of pyrogallol in phosphate buffer (0.1 M, pH 7.0) is prepared, 0.97 mM solution of H_2O_2. The procedure of peroxidase activity measurement is as following: the first cuvette is loaded with 1 mL of 13 mM pyrogallol in buffer solution and 10μL H_2O_2 as blank, and the second cuvette is loaded with 1 mL of 13 mM pyrogallol in buffer solution, 10 μL 0.97 mM H_2O_2 and 10 μL of crude enzyme extract. The absorbance is noted every 30 s for 3 minutes. The enzyme activity is calculated by the following equation:

$$\frac{IU}{mL} = \frac{\Delta A\, V_t d_f}{\Delta t \cdot 12 \cdot 3 \cdot V_u} \quad \text{where:} \tag{1}$$

$\frac{\Delta A}{\Delta t}$ – rate of the reaction

12 – molar extinction coefficient of pyrogallol

3 – coefficient of proportionality

d_f – dilution factor

V_t – total volume of the reaction mixture

V_u – volume of the analyte

2.3 Optimization of enzyme concentration

The enzyme concentration is varied in range 0.1-1 U: 0.1, 0.2, 0.4, 0.6, 0.8 and 1 U. Dye concentration of 30 mg/L and H_2O_2 concentration of 0.1 mM are kept constant. Change in absorbance is measured at 590 nm every 5 min.

2.4 Optimization of dye concentration

Dye solutions of different concentration are prepared: 10 mg/L, 20 mg/L, 40 mg/L, 60 mg/L, 80 mg/L, 100 mg/L and 200 mg/L. The other parameters, enzyme concentration of 0.6 U and H_2O_2 concentration of 0.1 mM are kept constant. Change in absorbance is measured at 590 nm every 5 min.

3 Results and discussion

In this study determination of the enzyme activity of several crude enzyme extracts from different bio-waste material is made, followed by selection of the most active extracts. The enzyme activity is given in Table 1.

Table 1. Enzyme activity

Origin of enzyme extract	*Apple peel*	*Banana peel*	*Potato peel*	*Soybean hull*
Enzyme activity (U/mL)	0.034	0.076	0.918	220

From the results shown in the Table above, it is clearly that enzyme extract from soybean hull exhibited the highest activity, followed by the enzyme extract from potato peel of 0.9 U/mL. Apple and banana peel extracts showed very low activity and therefore these crude enzyme extracts were not taken into consideration in the next set of experiments.

The effect of enzyme concentration is given in Figure 1. It was found that optimal enzyme concentration of potato peel peroxidase is 0.6 U. With further increase of enzyme concentration, the degree of decolorization decreases. One possible explanation could be that as the number of enzyme molecules increases it comes to competition for substrate molecules, whose concentration remains the same. So, higher degree of dye oxidation cannot be achieved. Enzyme concentration of 0.6 U is used for latter optimization.

As for soybean hull peroxidase, the optimal concentration obtained is 1 U, with 86% decolorization. In this case, the increase of enzyme concentration is directly proportional to the degree of decolorization.

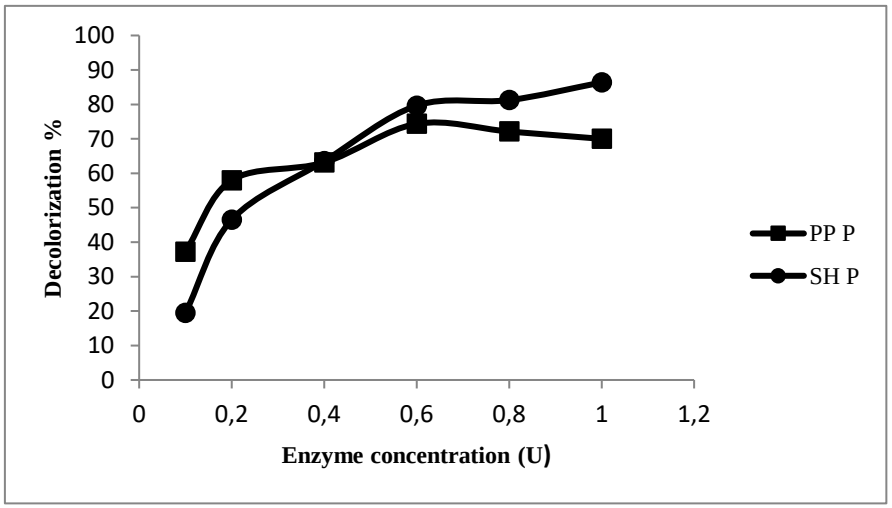

Fig. 1. Optimization of enzyme concentration (PP P-potato peel peroxidase, SH P-soybean hull peroxidase)

From Figure 2 it can be clearly seen that soybean hull peroxidase is most active at lower dye concentration. The degree of decolorization is highest (81%) at dye concentration of 10 mg/L. With increase of dye concentration, the degree of dye decolorization decreases. Therefore, this may be case where it comes to product inhibition.

Potato peel peroxidase behaves differently from soybean hull peroxidase. Linearity is not present, but it it is stable at wider range of dye concentration. Optimal dye concentration for potato peel peroxidase is 40 mg/L, where 68% decolorization is achieved.

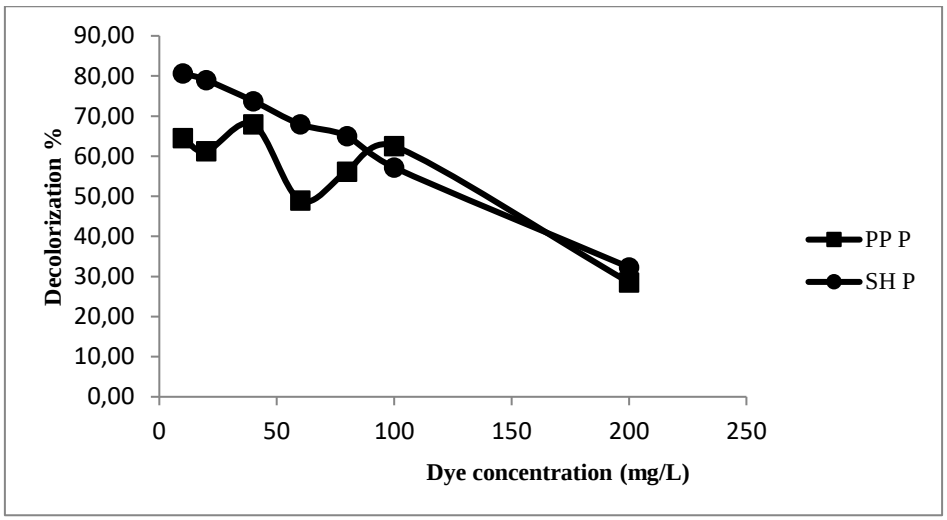

Fig. 2. Optimization of dye concentration (PP P-potato peel peroxidase, SH P-soybean hull peroxidase)

4 Conclusion

Aim of this study is to find the best source of bio-waste material where peroxidase is present at satisfactory level and to optimize the conditions of dye decolorization. In conclusion, highest concentration of peroxidase was found in soybean hull. It can achieve 81% decolorization, but at lower dye concentration. The peroxidase from potato peel is stable at higher concentration of dye, and more active at lower enzyme concentration. Both enzymes can be used for effluent treatment, depending on the waste water content. With more detailed optimization, higher degree of optimization can be achieved.

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