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ARTICLE

Comparison of the potential of *Ficus sycomorus* latex and horseradish peroxidases in the decolorization of synthetic and natural dyes

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Abstract The aim of this study was to compare the potential of *Ficus sycomorus* latex peroxidase (POL) and horseradish peroxidase (HRP) in the decolorization of a wide spectrum of eight synthetic dyes and two natural dyes, hibiscus flower color and pomegranate juice. We study for the first time the decolorization of natural dyes enzymatically. The highest decolorization percent was reported at 20 mg/l for all dyes treated with POL and HRP. Both the enzymes had lower decolorization % for azo-carmin (30–33%). During decolorization treatment, both natural dyes and titan yellow formed precipitates which settled down and were removed by centrifugation. The enhancement of the decolorization % of the most tested dyes by treatment with POL and HRP was reported in the presence of some redox mediators. The rate of decolorization was enhanced by increasing the time and the most significant changes were observed during the first 6 h of incubation. One hundred percent enhancement in decolorization was reported for azo-carmine in the presence of histidine and α-naphthol as redox mediators. A few of redox mediators caused no significant effect or decreases the decolorization % for a little number of tested dyes. The decolorization of dyes by POL and HRP in the presence of redox mediators appeared without the formation of precipitate. A similar decolorization % for all the tested dyes by POL and HRP was detected. The data suggested that the

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peroxidase/mediator system was an effective biocatalyst for the decolorization of synthetic and natural dyes, and POL could be used as a potential option for the application of dye decolorization.

1. Introduction

Synthetic dyes are extensively used in many industries. The problems associated with the discharge of colored effluents from various industries such as textile, paper, food, plastics and cosmetics have concerned both industrial and academic scientists [35]. Approximately, 100,000 different dyes and pigments are used industrially and over 0.7–0.8 million tons of synthetic dyes are produced annually worldwide [40,41,37,34]. Due to their chemical structure, dyes are resistant to fading on exposure to light, water and many chemicals [61,43,4]. During processing, up to 15% of the used dyestuffs discharged in wastewater cause extensive pollution. In addition to dye visual effect, some synthetic dyes cause allergy, dermatitis, skin irritation and they are toxic, mutagenic and carcinogenic in humans [34,55,14,39].

Anthocyanins, as natural dyes, are water-soluble phenolic compounds and the most diverse group of plant pigments derived from the phenylpropanoid pathway, ranging in color from red to violet and blue [53]. These pigments accumulate in the vacuoles and their stability and hue depend on conditions such as pH, enzymatic activity and formation of complexes with metal ions [29,49]. Very little information has been reported on the biodegradation of natural dyes of the fruit juice or pellet extract *in vitro*.

Conventional chemical and physical methods of dye decolorization are outdated because costs are high and they consume high amounts of chemicals and energy, other disadvantages are sludge formation and biomass accumulation [6,57]. Biological degradation of dyes included properties such as water solubility, large molecular weight and fused aromatic ring structures, which inhibit permeation through biological cell membranes. Other limitations of using microbes for treating pollutants were high costs of production of microbial culture, slow process of decolorization of dyes and metabolic inhibition [20,38].

Enzymatic systems fall between the two traditional categories of chemical and biological processes, since they involve chemical reactions based on the action of biological catalysts [5]. This was mainly because, unlike the chemical catalysts, the enzymatic catalysis showed its merits to convert complex chemical structures under mild environmental conditions with high efficiency [17,19,30]. Enzymes can act on specific recalcitrant pollutants to remove them by precipitation or transformation to other products [1,45]. Isolated enzymes were often preferred over intact organisms containing the enzymes because the isolated enzymes offered several advantages such as greater specificity, better standardization, easy handle, store and no dependence on bacterial growth rates [19,21].

Peroxidases (EC 1.11.1.7) are oxidoreductases which efficiently catalyze the oxidation of phenolic compounds and have great potential in treating a wide spectrum colored compounds [17,19,56,2,7]. These enzymes convert a broad range of substrates into less toxic insoluble compounds, which can be easily removed out of waste by a mechanism involving the formation of free radical followed by insoluble product [17,51]. Peroxidase based dye treatment still provides a reasonable basis for the development of biotechnological processes for continuous color and aromatic compound removal from various industrial effluents at a large scale [18]. Sometimes these enzymes cannot act on organic pollutants and dye effluents due to the recalcitrant nature of such compounds. These recalcitrant substrates get converted into less toxic forms in the presence of certain low molecular weight compounds that are known as redox mediators which enhanced the rate of enzyme-catalyzed reaction and increased the range of selection of their substrates [17,3,25].

The present study has been made to investigate the potential of *Ficus sycomorus* latex peroxidase (POL) as compared to horseradish peroxidase (HRP), which represents the commercial peroxidase, in the decolorization of a wide spectrum synthetic dyes and natural dyes (*Hibiscus sabdariffa* flower color and pomegranate *Punica granatum* juice). Evaluation of the role of various low molecular weight redox mediators in the decolorization of the selected dyes mediated by the action of peroxidases is the second goal.

2. Materials and methods

2.1. Materials

Bromophenol blue, catechol, vanilline, α-naphthol, L-histidine, methyl green, methylene blue, methyl orange were obtained from Sigma Chemicals Co. Azo-carmine, naphthylamine–azo-benzene, titan yellow were products of Fluka Co. Calyces of roselle (*Hibiscus sabdariffa* L.) and pomegranate (*Punica granatum* L.) were obtained from the local market. All other chemicals and reagents employed were of analytical grade and were used without any further purification.

2.2. Peroxidases

We previously purified and characterized the peroxidases from *F. sycomorus* latex (POL) [33] and from Japanese horseradish roots (HRP) [32].

2.3. Extraction of hibiscus pigments

Roselle extract was routinely prepared by boiling 3 g of dried roselle petals for 3 min with 100 ml of distilled water. The extract was rapidly filtered through a four layers of cheez cloth and kept at 4 °C [52].

2.4. Extraction of Punica granatum pigments

The method consisted of manually peeling the fruits, separating the seeds and extracting the juice by electric juice mixer, extract was rapidly filtered through a four layers of cheez cloth and kept at 4 °C [31].