Journal of Chemical, Biological and Physical Sciences

An International Peer Review E-3 Journal of Sciences

*Available online at*www.jcbsc.org

Section B: Environmental Biotechnology

CODEN (USA): JCBPAT Research Article

Discoloration of Indigo Carmine Using Vegetal Extracts

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Abstract: Peroxidases are used to degrade different compounds such as textile dye. We tested the ability of different extracts, including chayote, cucumber peel and fruit, spring onion stalk and fruit, and leek fruit, to discolor indigo carmine (IC). The extracts at different pHs were analyzed for peroxidase activity using the maximum for discoloration of IC. Vegetables were found to have the highest peroxidase activity at pH between 5 and 7. All extracts had the ability to discolor IC in different percentages. IC at 50 ppm was easily and completely discolored by all extracts, whereas onion fruit, onion stalk, and leek fruit extracts could discolor the IC solution at 100 and 200 ppm. Onion fruit extract could decolorize IC completely at 500 and 1000 ppm. Results are interesting because represent an alternative to reuse residues and resolve a contamination problem like discoloration of textile effluents.

Key words: indigo carmine, vegetal extracts, peroxidase, discoloration

INTRODUCTION

The release of dyes from textile industries into wastewater results in severe contamination of rivers and ground water, due to their persistent and recalcitrant nature. In addition, coloration of the water may have an inhibitory effect on photosynthesis, affecting aquatic ecosystems. Some of the compounds and biodegradation products of the textile dyes are toxic, mutagenic, and carcinogenic[1]. The methods available for removal of dyes from waters and wastewater are mainly physical or chemical like photocatalytic oxidation, ozonation, coagulation, flocculation, and membrane separation. These methods have important disadvantages, including high cost, the formation of hazardous by-products or intensive energy requirements[2]. Adsorption is an effective process to remove colorants from wastewater. Activated carbon is an effective but expensive adsorbent. Adsorption using plants is by far the most versatile and widely used method to eliminate pollutants because of its low cost and ease of operation[3]; several agricultural waste and by-products of cellulosic origin have been studied for their capacity to remove dyes from aqueous solutions, such as peanut hulls[4] and lemon peels[5]. Additionally, orange peel was used for the removal of acid dyes[6]; pummelo peel pretreated with sodium hydroxide adsorbed methylene blue[7]; kohlrabi peel was used as a sorbent for the uptake of methylene blue, neutral red and acridine orange, whereas banana fibers were used for the removal of methyl red[8].

On the other hand, enzymes from plants have remained less explored for their application in the bioremediation processes. Peroxidases from plants have been used to oxidize a wide variety of aromatic pollutants including dyes. Horseradish (*Armoracia rusticana* L.) is the most frequently used source of peroxidases and has been employed for the oxidation of contaminants such as chlorophenols[9], naphthalene sulfonate dyes[10] and direct yellow[11]. Other useful peroxidase tested sources are: chayote to remove phenols[12], turnip roots to decolorize acid dyes[13], and onion to oxidize caffeic acid and ferulic acid[14].

The aim of this work was to test the ability of extracts from some vegetables containing peroxidases to discolor IC (Fig. 1), instead of using the vegetables to absorb the colorant. The advantages of these biological materials are that they are inexpensive and easily accessible. In addition, some residues of the selected vegetables are considered as waste.

METHODS

Dye and biological material: The indigo carmine (5, 5'-indigosulfonic acid, disodium salt), guaiacol, and H_2O_2 were purchased from J.T. Baker. The vegetables were purchased in local grocery stores. The vegetables selected were: fruits and peel of chayote (*Sechium edule* Sw.), fruit and peel of cucumber (*Cucumis sativus* L.), leek fruit (*Allium ampeloprasum* L.) and spring onion fruit and stalk (*Allium fistulosum* L.).

Crude enzymatic extracts: The biological material was washed with soap and rinsed with distilled water, and then 20 g was blended using a food processor with 20 mL of buffer solution at different pHs (3, 4, 5, 6, 7, and 8). The mixtures were centrifuged with an Eppendorf 5804R at 3500 rpm for 15 min, and the supernatant was filtered. The filtrate was used as the enzymatic extract without any other purification. The pH of each extract was adjusted to the selected pH.

Peroxidase activity assay: One milliliter of phosphate buffer pH 6, 200 L of guaiacol (1% in methanol), 200 L of H_2O_2 (0.5%), 1 mL of water, and 40 L of the crude enzymatic extract at every pH value were mixed. Absorbance was measured at 470 nm immediately just after all of the reactants were mixed (A1), and after 1 min of reaction (A2). Peroxidase activity was calculated with formula 1 using a molar extinction coefficient[15] of 26.6 mM^{-1} cm^{-1}. Each experiment was performed in triplicate.
:

Activity = (A2-A1)/molar extinction coefficient x dilution …….Formula 1

Discoloration of indigo carmine at different pHs

Ten milliliters of the enzymatic extracts and 10 mL of IC at 200 ppm were mixed, to obtain a final IC concentration of 100 ppm, then they were stirred at room temperature for 24 hours. A sample was centrifuged, and the absorbance was measured[16] at 610 nm, at the beginning of the experiment (A1) and after 24 h (A2). Each experiment was performed in triplicate. The discoloration was determined according to formula 2:

% discoloration = (A1 – A2)/ A1 x 100…….. Formula 2

Discoloration at different concentrations of indigo carmine: In this experiment, we used the pH associated with the maximum activity of each extract. Ten milliliters of each enzymatic extract at the selected pH was mixed with IC at the same pH as the extract to obtain final concentrations of 50, 100, 200, 500, and 1000 ppm of the dye. The mixtures were stirred at room temperature for 24 hours; the sample was centrifuged, and the absorbance was measured at 610 nm at the beginning of the experiment (A1) and after 24 h (A2). Each experiment was performed in triplicate. The discoloration was determined according to formula 2.

RESULTS

Plants have been used successfully as bio adsorbents to remove colorants from water. In this study, the selected plants were blended, centrifuged, and filtered to remove the solids, and the enzymes present in the aqueous phase were used. In this way, we demonstrated that the discoloration was due to the enzymatic degradation of IC and not by an adsorption process.

Peroxidase activity: Figure 1 shows the results of the guaiacol peroxidase activity (GPA) assay of the vegetable extracts at different pHs (3, 4, 5, 6, 7, and 8). All aqueous extracts from the selected vegetables showed GPA and the enzymatic activity level was dependent upon pH, the optimum pH was between 5 and 7. The pH of the highest biocatalytic activity of the vegetables tested was similar to that reported for other peroxidases, for example, the Sigma Aldrich technical information for horseradish peroxidase (EC 1.11.1.7) indicates that the optimum pH ranges is from 6.0 to 6.5. Polete *et al*. [17] reported that peroxidase activity from litchi pericarp depends on the pH, the highest enzymatic activity was at pH 6.5.The peroxidase activity of cucumber peel, chayote peel, and chayote fruit were the highest. The fruit of the cucumber was less active than its peel. In the case of the onion, the stalk was more active than the fruit. The leek fruit was the least active of all the vegetables tested.

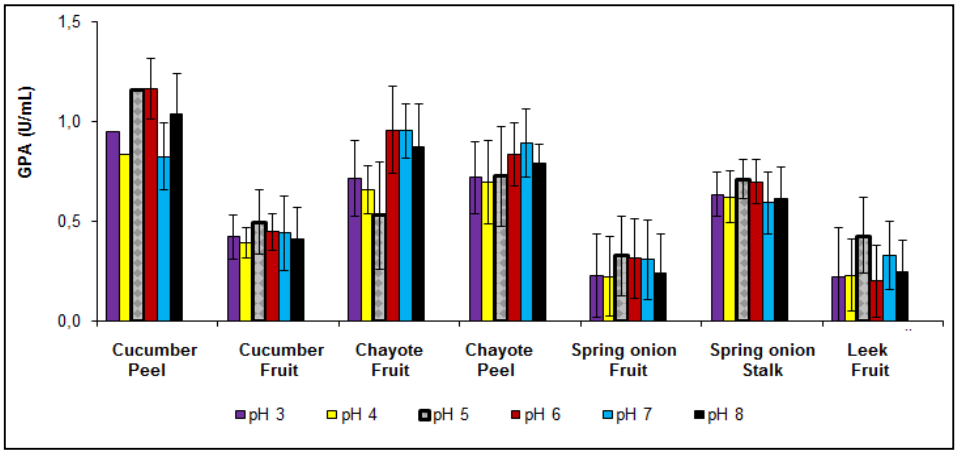

Fig. 1: Peroxidase activity for vegetal extracts at different pH values

Discoloration of indigo carmine at different pHs*:* To determine the correlation between the peroxidase activity and the extent of discoloration, we investigated the discoloration of an IC solution (100 ppm) with the vegetable extracts at the same pHs used to evaluate the peroxidase activity. The discoloration was measured at 610 nm after 24 h of reaction. All of the vegetable extracts tested had the ability to discolor the 100 ppm IC solution, but the magnitude of the discoloration was dependent on the pH and biocatalyst source. Complete discoloration was reached approximately at 24 h with spring onion fruit and stalk at pH 5 and 6 and leek fruit at pH 5, 6 and 7 (Fig. 2). Cucumber peel and chayote fruit and peel also discolored the IC solution but to a lesser extent.

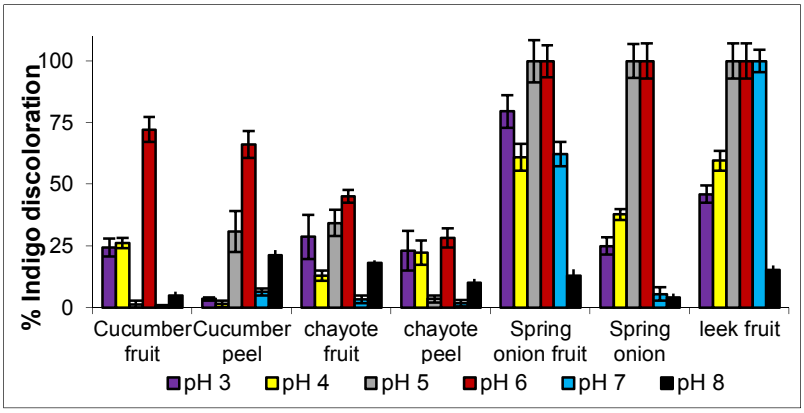

Fig. 2: Discoloration of IC at 100 ppm with vegetal extracts at different pH values

From the results, we observed that there was not always a connection between the GPA and the extent of discoloration. Spring onion fruit and leek fruit reached a complete discoloration after 24 h, although their peroxidase activity was lower than that from cucumber peel and chayote fruit and peel. In this work, we only quantified guaiacol peroxidase; however, other authors have reported the existence of different kinds of peroxidase isoenzymes in plants[18]. Discoloration observed could be due to a mixture of isoenzymes.

Discoloration of Indigo Carmine at different concentrations: From the data in Figure 2, we observed that chayote fruit and peel had the lowest biocatalytic activity towards the discoloration of IC, and thus, were not considered in the following experiment. All other extracts were tested at different concentrations of the dye at the pH showing the maximum discoloration of IC (Fig. 3).

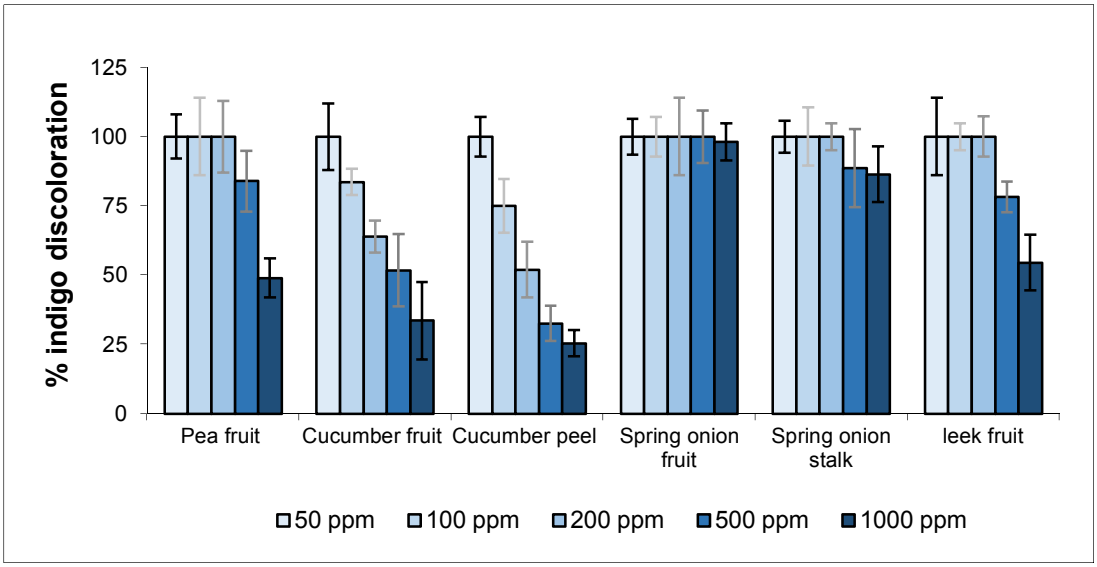

Fig. 3: Discoloration of IC with selected vegetal extracts

Both onion extracts showed lower peroxidase activity, but they discolored 1000 ppm IC by more than 90% in 24 h. Within 24 h, leek fruit discolored a little more than 50% of a 1000 ppm of IC solution, while both cucumber extracts discolored between 30 and 40% of this solution. Enzymatic oxidation of IC was due to its redox potential of 0.6 V, whereas the corresponding potential[19] for peroxidases was less than 1 V.

Results are interesting because the method using enzymatic extracts from cheap materials like vegetables, achieve similar or better results than those reported using more sophisticated and expensive processes to discolor indigo carmine. For example, Neelamegam[20] achieved a 90% discoloration of IC (100 ppm) in 6 days using *Pleurotus ostreatus,* Ramya *et al.* [21] used a liquid culture of *Paenibacillus larvae* with 100 ppm indigo carmine solutions, achieving a 100% color removal after 8 h. Podgornik *et al.* [22] reported the biodegradation of 30 ppm IC in 2 hours, using isozymes of LiP and MnP obtained from *P. chrysosporium*.

CONCLUSIONS

Vegetal extracts can be used to discolor dyes, like indigo carmine, using an enzymatic process rather than the adsorption of the dye. In this way, vegetal extracts have the potential to be used in the treatment of wastewater for textile industries. These materials are inexpensive, easily accessible, and considered waste (i.e., the peel of the vegetables). Consequently, this process represents an alternative option to those contaminating residues currently being used in disposal sites.

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