ENZYMATIC DEGRADATION OF ACID BLUE 74

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Introduction. Synthetic dyes are one of the major classes of recalcitrant compounds and used in textile, food, cosmetic and pharmaceutical industries, and these dyes are released in the wastewater. A majority of such dyes are toxic to flora and fauna or mutagenic and carcinogenic. Phytoremediation is an interesting alternative for the treatment of wastewaters contaminated with colorants. Specifically, plant polyphenol oxidases (PPO) have great potential in the decolorization of textile and nontextile dyes[1,2].
The objective of this work was to determine the PPO activity of the green pea seed, and the discoloration of Acid Blue 74 under different reaction conditions using the green pea seed as PPO source.

Methods
Enzymatic extract. The green pea seeds where grinded in a blender with phosphates buffer solution (0.01 M, pH 7.6), the mixture was centrifuged and the supernatant was used as enzyme source.
Determination of PPO activity. The PPO activity of the green pea seed aqueous extract was tested using catechol, pyrogallic acid and caffeic acid as substrates, according to Khan[1] and Kabra[2].
Discoloration assays.
Effect of the colorant concentration. The colorant Acid Blue 74 (300, 1000, 1500 and 3000 ppm) was mixed with the green pea extract (pH 7.6), the mixture was stirred magnetically until complete discoloration was reached. The absorbance of the mixtures was determined at 610nm, to determine the discoloration extent. The degradation product was analyzed by HPLC, using a Nucleosil C18 column, and H_2O_2:MeOH as eluent.
Effect of NaCl and detergent concentration. Acid Blue 74 (300ppm) was mixed with the green pea extract (pH 7.6) in the presence of NaCl (0, 2, 4, 6, 8 and10%), the mixture was stirred until complete discoloration; then, under the same conditions but in the presence of a detergent (2%) instead of NaCl.

Results.
The results of the PPO activity of the green pea seed aqueous extract are shown in figure **1**. It can be observed that the PPOs in the extract are active towards the three substrates, but the activity is higher in the presence of catechol.
With regard to the concentration of the colorant, it take about 1h and a half to discolor 300 ppm, about 4h with 500 ppm, about 9 h for 1000 ppm, 17 h for 1500 ppm, and about 40 h for 3000 ppm. The results are very interesting,

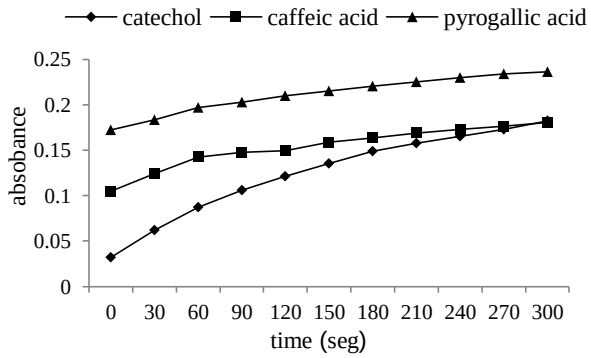

Figure 1. PPO activity of green pea seeds aqueous extract.

because most of the treatment methods to discolor Acid Blue 74 are not so efficient, because they need longer reaction times to discolor less concentrated solutions.

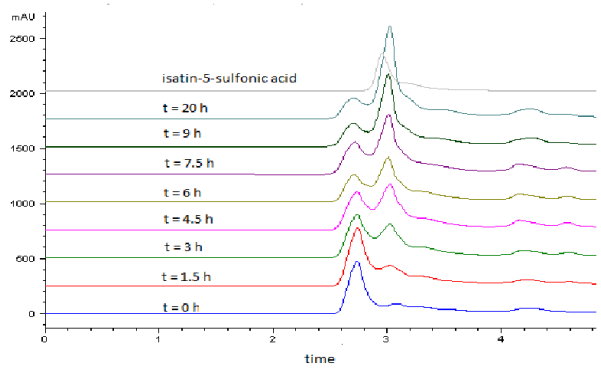

Figure 2. Degradation of Acid Blue 74 to isatin-5-sulfonic acid.

In Figure **2,** we can observe that PPO, from green pea seed aqueous extract, degrades Acid Blue 74 to isatin-5-sulfonic acid.
The degradation of Acid Blue 74 (300ppm) with the extract is slower in the presence of NaCl or detergent, but the PPO activity remains; with 10% of NaCl or 2% of detergent the discoloration was accomplished in about 4h.

Conclusions. The green pea seed is a PPO source, capable to degrade a high concentration of Acid Blue 74 to isatin-5- sulfonic acid, even in the presence of NaCl and detergent.

References.
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2. Kabra A, Khandare R, Waghmode T, Govindwar S Chemosphere. 87 (2012) 265-72.