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Extraction of PPO Enzyme from Ridge Gourd and its Potential Use in Decolorizing Dyes used in Laboratories at Undergraduate Level to Reduce Organic Pollutants

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Abstract: ***In agricultural country like India, we generate abundant crop waste and food processing waste. Degradation and disposing of this waste is a major challenge as it contains phenolic and quinone compounds- the major contaminants. Phenolic contamination can be decreased by various techniques such as chemical methods or biological method using enzymes or microorganisms. In this study the enzyme Polyphenol oxidase was extracted from peels of the Ridge gourd using potassium phosphate buffer, was partially purified by precipitating with ammonium sulphate followed by dialysis. Protein and enzyme assays were performed for crude, salt precipitated and dialysis sample. Phenol content was estimated. Effort has been made to study the potential of PPO extracted from peel of ridge gourd to decolorize residual and disposable dyes used in the undergraduate laboratories. The extent of decolorization of dyes gives the measure of effectiveness of PPO in reducing the organic contaminants. Hazardous effect of dyes can be minimized by treating with PPO before draining into sewage system. Comparitive decolorisation of dyes with synthesized dye in chemistry lab was studied using extracted PPO from Ridge gourd. An attempt to treat disposal of hazardous chemical washings from laboratories is made.***

Keywords: PPO - Poly phenol Oxidase, ridge gourd, organic contaminants, dye decolorization

1. Introduction

Phenolic compounds are major concern for the ecosystem which needs immediate attention. Accumulation of phenolic pollutants over a long period have adverse effect on humans, animals and plants and their interaction with other chemical bodies produce more toxic effect. Polyphenol oxidase is a copper enzyme that catalyzes two distinct reactions involving molecular oxygen as a co-substrate a) the o-hydroxylation of monophenols to o-di phenols (cresolase activity) and b) the subsequent oxidation of o-diphenols to o- quinones (catecholase activity) which are subsequently polymerized into red,brown or black pigments[3] and non-toxic intermediates. These enzymes are formed in all living organisms and plays a role in plant resistance against diseases.Many plants species have been the major target to isolate PPO's. It is also shown to have an important application such as its use in synthesis of L- Dopa[8] and in waste water treatment containing polyphenol as contaminants.

On the other side, agricultural waste: like vegetable and fruit peel, is the major source of polyphenol oxidase which can be used to remove phenolic compounds from the dyes used in the labs at undergraduate level. Extent of decolorization gives the efficiency of PPO in minimizing the organic pollutants[9]. Hence, in the present study polyphenol oxidase is extracted from peels of ridge gourd, partially purified, its enzyme activity and total phenol content is estimated and the isolated enzyme was used in the treatment of residual dyes before disposing into the sewage system to reduce the toxic effect of phenol.

2. Materials and methods

Fresh ridge gourds from local market were chosen, washed well. Catechol was used as the substrate. Reagents used were 0.02M potassium phosphate buffer (KH_2PO_4 + K_2HPO_4) distilled water, Catechol, Follin's reagent, ammonium sulphate, the chemicals used were of analytical grade. Spectrophotometer was used to measure the optical density in each step.

Enzyme Extraction:

10gms each of ridge gourd peels were homogenized separately using 50ml of phosphate buffer solution (pH-7) at 4^0c. The homogenates were filtered through 8 layered gauze cloth and collected separately into 10ml centrifuge tubes which are centrifuged at 12000rpm for 15mins at 4^0c.The supernatants were collected and stored at 8^0c for further use.

Ammonium sulphate precipitation: The supernatants were pooled and concentrated by adding 4.5gms of ammonium sulphate to 10ml of crude sample to obtain 70% saturation. Samples were centrifuged at 10000rpm for 15mins at 4^0c and the pellets were dissolved in phosphate buffer.

Dialysis:
About 3ml of each sample is loaded into activated dialysis tubes and placed in 400ml of 0.01N phosphate buffer for 24hrs, ensuring stirring throughout the procedure and then were stored at 4^0c. Sample volumes were measured before and after dialysis.

Protein Estimation:
Protein content in the samples were determined using Lowry's method using Bovine serum albumin as standard. For ridge gourd – crude, salt saturated and dialysis samples were analyzed for protein content to obtain a comparative study at 660nm.

S. No	Crude	Salt Precipitation	Dialysis
	OD	OD	OD
1	0.52	0.28	0.25
2	0.5	0.275	0.24
3	0.52	0.27	0.24

Enzyme Assay:
PPO activity was assayed by measuring the increase in absorbance at 420nm using a spectrophotometer. Catechol was used as the substrate. In reaction mixture of 3ml -1.5ml of buffer, 1ml of 10mM catechol and 0.5ml of sample was taken and increase in absorbance was noted for every 1min. Blank consists of 2.5ml of buffer and 0.5ml of enzyme. One unit of PPO activity is defined as the amount of enzyme that causes an increase in absorbance of 0.001/min.

Phenol Content: Total phenol content was measured by Folin–Ciocalteu assay with catechol (1mg/ml),series of dilutions were made 0.2,0.4,0.6,0.8,1mland used as the standard.0.1ml test sample in test tube made up to 7ml with distilled water, 0.5ml folic reagent ,1ml of 35% of Na_2CO_3 is added, allowed to stand for one hour. Absorbance was measured at 760nm.

Sample	1	2	3	4	5	6	Phenol Content
Crude	0.33	0.375	0.42	0.45	0.48	0.5	0.36
Salt Precipitation	0.315	0.36	0.4	0.4	0.42	0.42	0.18
Dialysis	0.195	0.235	0.27	0.29	0.3	0.3	0.1

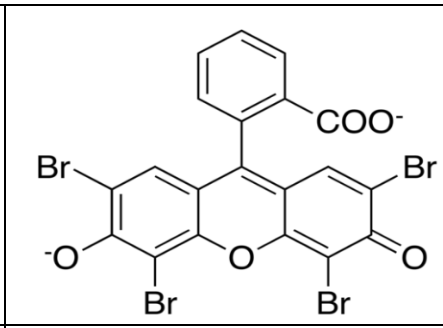
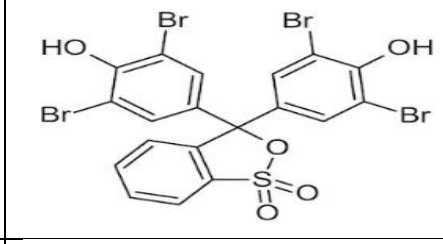
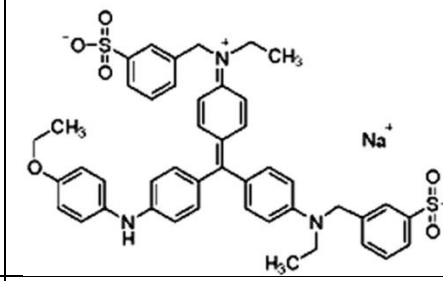
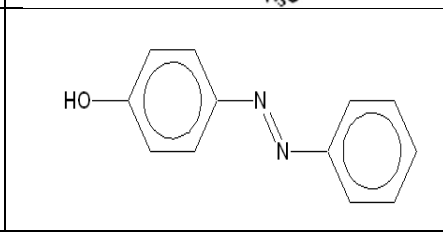
Concentration of protein and enzyme activity were calculated for crude, salt precipitated and dialysis samples of Ridge Gourd:

Sample	Protein content (µg/ml)	Enzyme activity
Crude	2550	18.7
Salt Precipitation	1100	11.072
Dialysis	1120	10.909

Polyphenol oxidase as decolorizing enzyme:
AcetoCarmine, Bromophenol blue, Coomassie brilliant blue, Azo dye dyes contain organic pollutants when their residue is disposed. Each sample of the dye was taken and a series of solutions of concentration range of 50mg-200mg/L were prepared in distilled water followed by treatment with equal volumes of PPO in potassium phosphate buffer and heated in hot water bath for 1hour at 37^0C.The intensity of the color decreased indicating PPO as an decolorizing enzyme. Untreated dye was used as control to get the percentage of decolorization and was monitored by decrease in absorbance at a particular wavelength of a dye[1].Decolorizing ability was determined spectro photometrically and the relative decrease in absorbance over time was noted. Decolourization efficiency was calculated using the following formula to obtain percentage of decolourization:

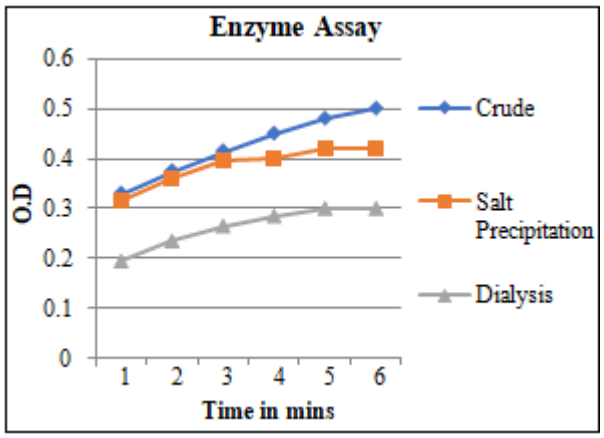
$$\text{Decolorization\%} = Ai - At/Ai \times 100$$

where A_i is the initial absorbance of dye, and A_t is the absorbance of dye after decolorization[6].

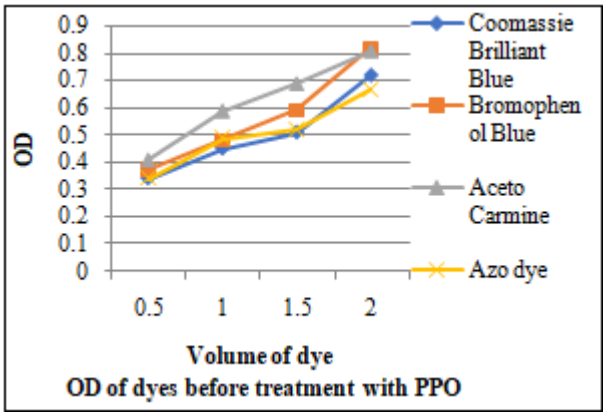
Aceto Carmine: Used to stain chromosomes leaving cytoplasm colourless. It is DNA specific stain which can be used in mitotic studies.it is mildly toxic but cost effective.	
Bromophenol Blue: Used in electrophoresis as a colour marker. Can stain used proteins and nucleic acids so acts as acid base indicator with pH range 3.4-6	
Coomassie Brilliant Blue: Used in colorimetric protein determination – Bradford assay.	
Azo dye – An organic compound prepared in the lab.Used to color pharmaceutical agents, cosmetic, textile, paper plastic etc .	

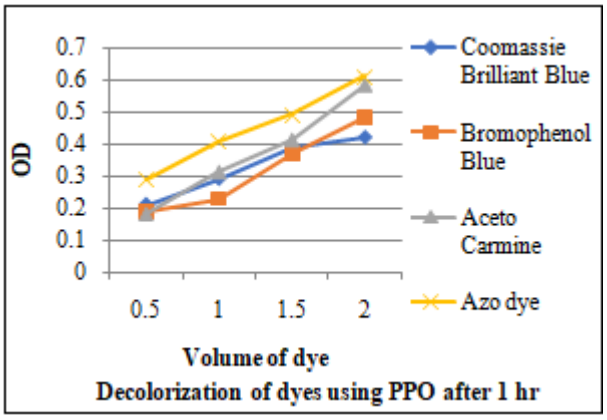
3. Results and Discussion

Extracted the biocatalyst polyphenol oxidase from the peels of ridge gourd which was further partially purified by ammonium sulphate precipitation and the enzyme was isolated by dialysis. Amount of protein was estimated for crude, ammonium sulphate precipitated and dialyzed samples and enzyme assay was performed to know the activity of the enzyme at each level using catechol as the substrate.Total phenol content was measured.



Isolated enzyme was added to various residual dyes collected from undergraduate laboratories. Decolorization efficiency was calculated and there was a relative decrease in the color which was measured using a spectrophotometer. The enzymatic degradation of phenolic compounds can be considered as the efficient method as these reactions occur at a faster rate when compared to other reactions and are cost effective as we are recycling the agricultural waste to treat the harmful and toxic organic remnants, there by safe guarding the water bodies.





4. Conclusion

PPO extracted from Ridge gourd was analysed for protein content and enzyme assay. Organic disposals from labs at undergraduate level, when enters the main sewage system turns out to be hazardeous. In this study attempt has been made to treat these surplus dyes with plant based enzymes such as PPO to minimize contamination. PPO is found to be effectively acting as decolourising agent for commonly used dyes in labs for various analysis. Coomassie Brilliant Blue, Bromophenol Blue Aceto Carmine and Azo dye were treated with PPO and it is found that above dyes shown considerable decolorisation with the enzyme except Azodye wherein the extent of decolorisation is not satisfactory. Combination of dilutions was tried to attain maximum decolorisation which needs further study. Usage of PPO to treat phenolic pollutants can be extended further to industrial effluents and house hold sewage systems. Treating lab disposals with enzymes or any sedimentation process to avoid contamination into water bodies has to be investigated further to inculcate social responsibilities among students, engaging them to be a part of problem solving issues of environment.

5. Acknowledgement

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