Immobilization of Castor Plant Peroxidase by polyethylene Glycol

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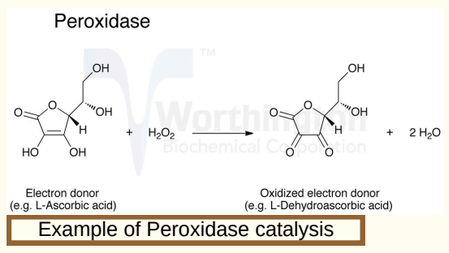
Abstract

Peroxidase is an enzyme in the oxido-reductase group that catalyzes the oxidation reaction of various substrates, both organic and inorganic, with hydrogen peroxide as the electron acceptor. Immobilization of this enzyme helps save the cost by being able to reuse it for catalysis in bioremediation and biotechnological applications. The objectives of this study were to Immobilize peroxidase from castor bean leaves using polyethylene glycol (PEG) and sodium sulfate at different percentages of weight per weight via aqueous two-phase systems, and to investigate the effect of pH on phase partitioning. Peroxidase activity was determined using guaiacol and hydrogen peroxide as substrates at pH 6. Our aim was to focus on the upper PEG layer which was our phase of interest under five conditions resulting in the corresponding activity values: (1) 14% PEG 1500 with 12% sodium sulfate (1.02 units), (2) 18% PEG 1500 with 8% sodium sulfate (1.21 units), (3) 18% PEG 1500 with 10% sodium sulfate, (3.22 units), (4) 18% PEG 1500 with 12% sodium sulfate (2.03 units). and (5) 18% PEG 6000 with 12% sodium sulfate (0.96 units). Among these, 18% PEG 1500 with 10% sodium sulfate demonstrated the highest enzymatic activity in the upper phase with specific activity of 38.08 units/mg of protein. Further studies were conducted at pH 8 and pH 10 to determine the effect of higher pHs on this condition which resulted in specific activity of 0.26 units/mg of protein and 0.15 units/mg of protein, respectively. In the next step, additional investigation will be carried out on the effect of lower pHs on this condition.

Introduction

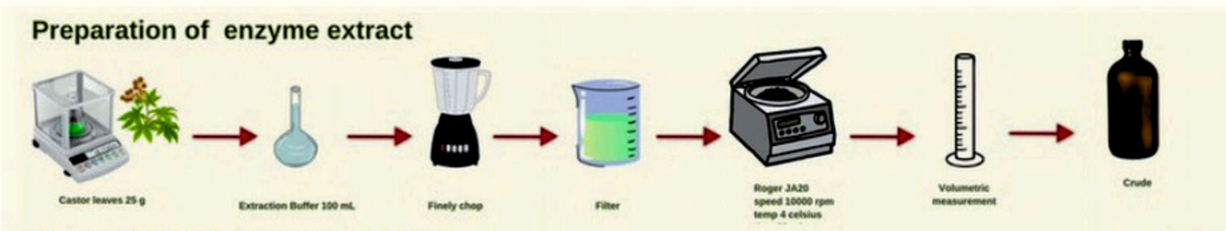
Peroxidases are widely utilized enzymes in biotechnological applications due to their ability to catalyze oxidation-reduction reactions. The castor plant (*Ricinus communis*) is a valuable source of peroxidase, an enzyme known for its stability and catalytic efficiency. However, the free enzyme often suffers from reduced activity and stability in industrial applications. Immobilization techniques, such as the use of polyethylene glycol (PEG), offer a promising solution by enhancing enzyme stability, reusability, and overall efficiency. This study focuses on the immobilization of castor plant peroxidase using polyethylene glycol, aiming to improve its performance in biocatalytic processes.

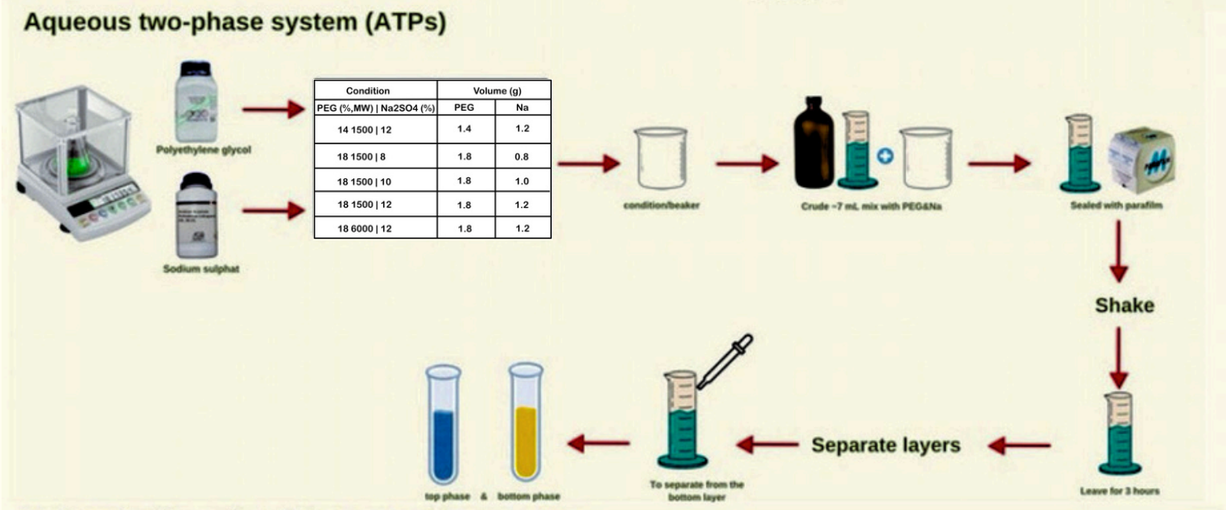


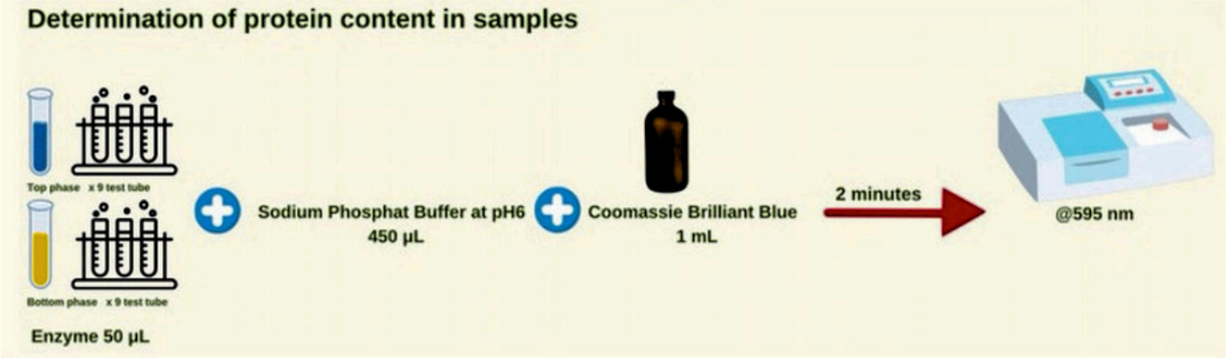
Example of Peroxidase catalysis

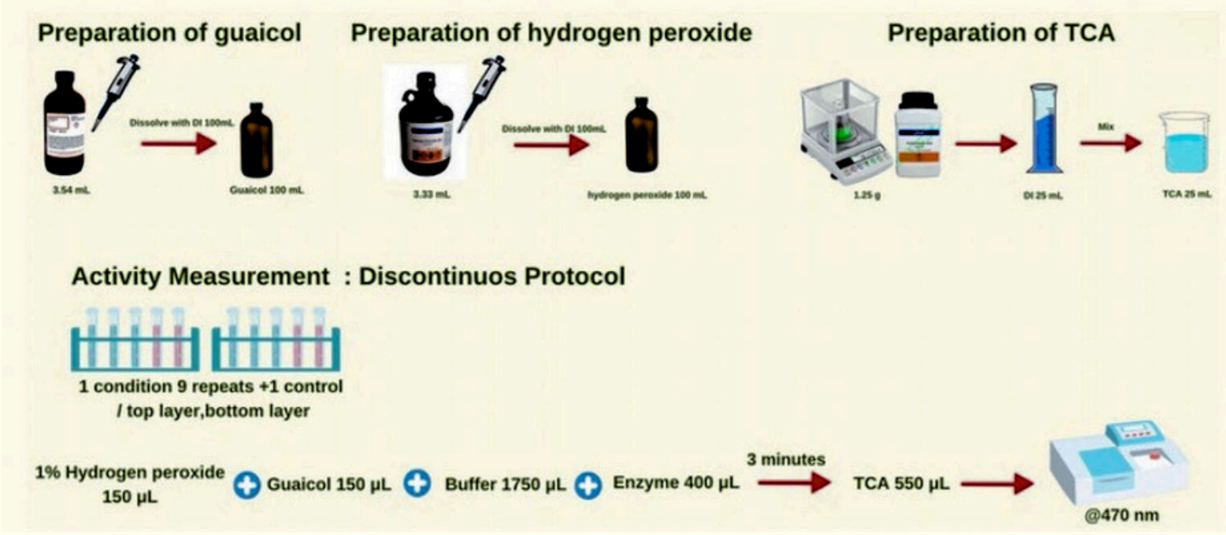
Castor leaves

Methodology









Results and discussion

Condition	Total Activity (unit)	
PEG (%, MW) | Na_2SO_4 (%)	Upper	Lower
14,1500 | 12	2.025	55.880
18,1500 | 8	1.206	67.852
18,1500 | 10	3.222	71.658
18,1500 | 12	1.017	80.429
18,6000 | 12	0.958	51.364

- Since the focus is on the top phase, which is the PEG phase , the experimental results indicate that at a condition of 18% PEG1500 to 10% sodium sulfate offers the highest activity

Condition			Total Activity (unit)		Total Protein (mg)		Specific Activity (unit/mg)	
PEG (%,MW)	Na_2SO_4 (%)	pH	Phase		Phase		Phase	
			Upper	Lower	Upper	Lower	Upper	Lower
18, 1500	10	6	3.222	71.658	11.02	9.37	0.292	7.647
18, 1500	10	8	0.372	0.636	16.78	12.45	0.022	0.051
18, 1500	10	10	0.564	0.294	12.87	10.12	0.044	0.029

- When 18% PEG1500 and 10% sodium sulfate at various pHs, the highest specific activity was found at pH6 at 0.292 units/mg protein.

Conclusions

Since the focus is on the top phase, which is the PEG phase , the experimental results show that when in the presence of 18% PEG1500 and 10% sodium sulfate at pH 6 the highest specific activity was found at were used 0.292 units/mg protein.

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