Effect of various factors on polyphenol oxidase activity

BCH303 [Practical]

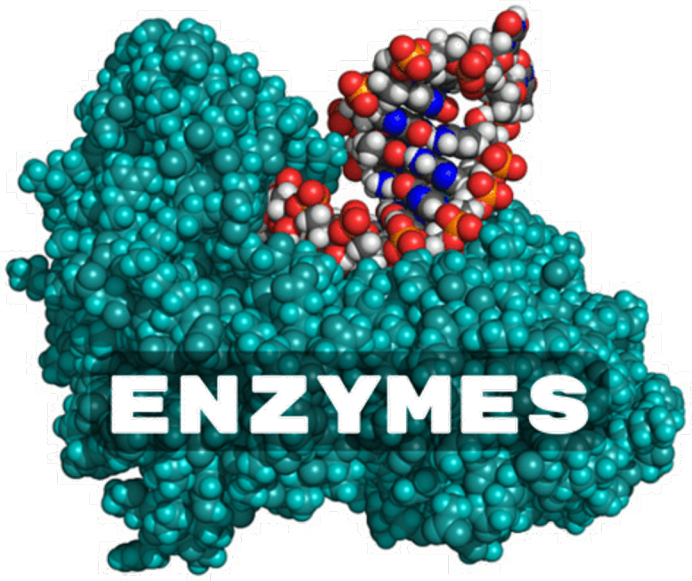
Catalyst:

- A substance that speeds up a chemical reaction.
- Not consumed in the reaction.
- The catalysts for biochemical reactions that occur in the living organisms are called **enzymes.**

Enzymes:

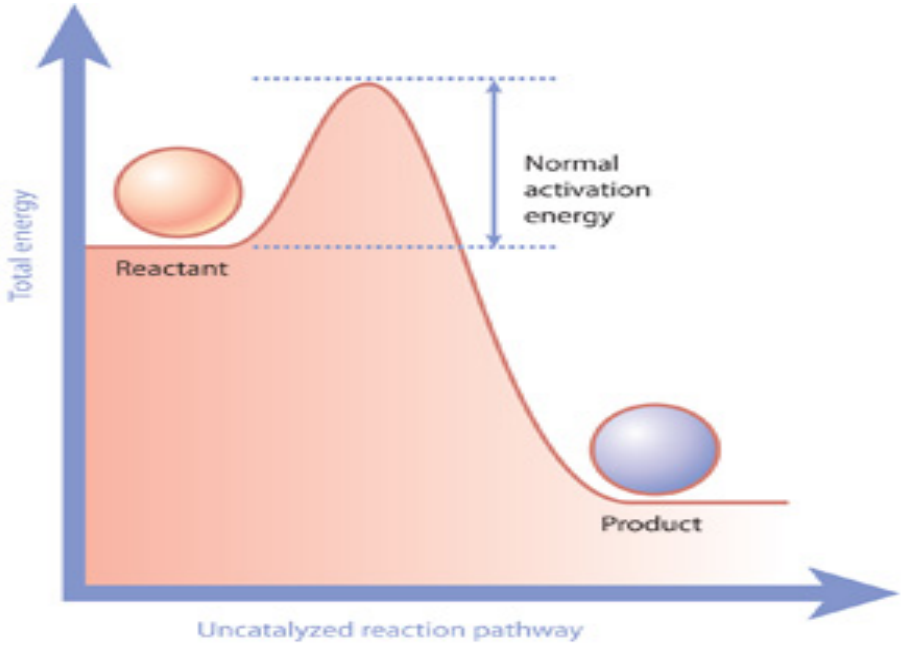
- Highly specialized **proteins.**
- Protein and RNA.
- -ase.
- Accelerate chemical reactions (INCREASE RATE OF REACTION).

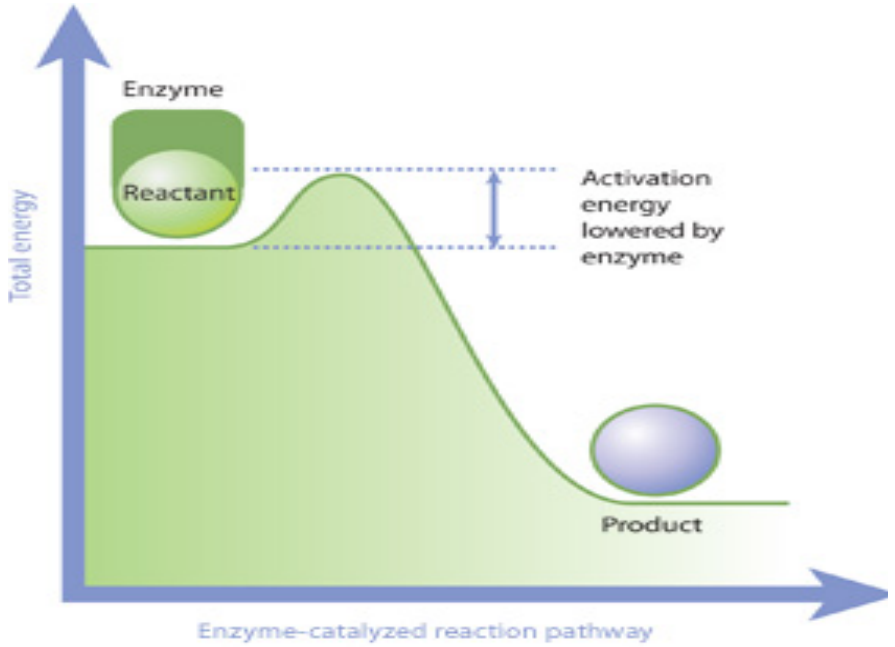
➔ **HOW ?**



How can enzyme increase the rate of a biochemical reactions ?

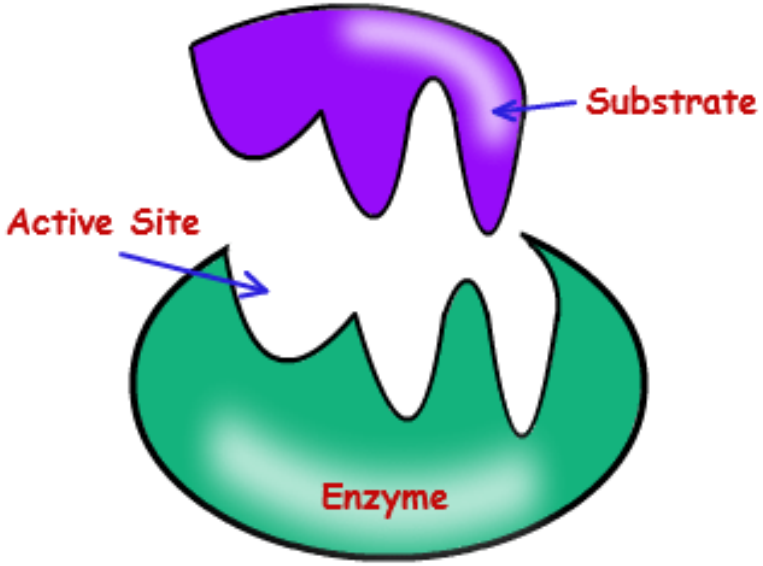
- Enzymes perform the critical task of lowering a reaction's activation energy.





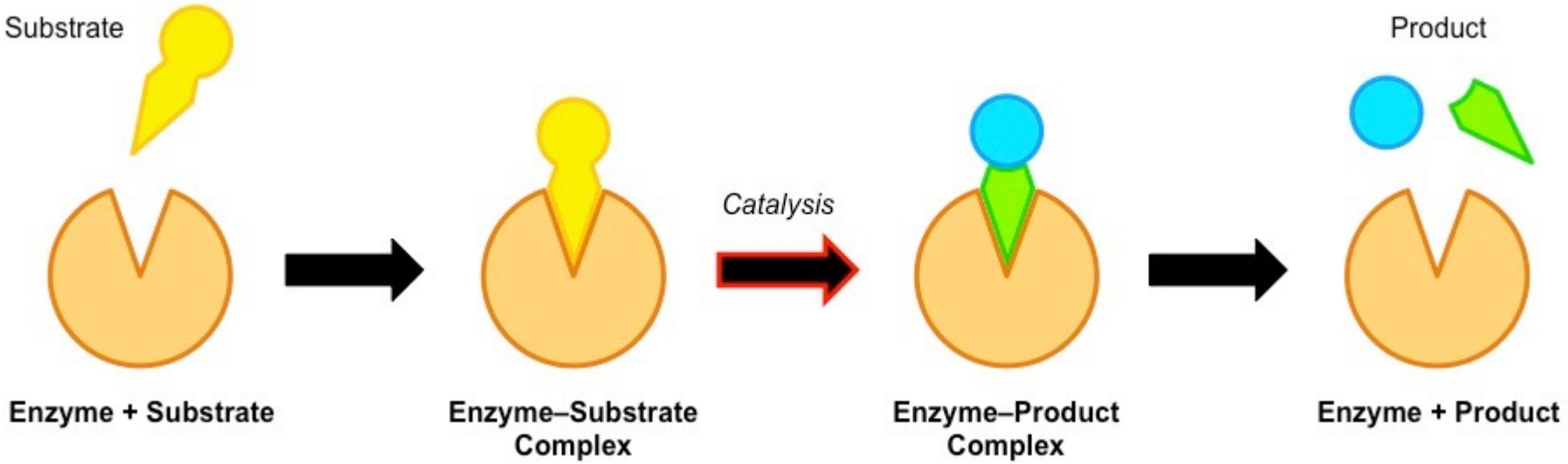
Enzyme active site and substrate:

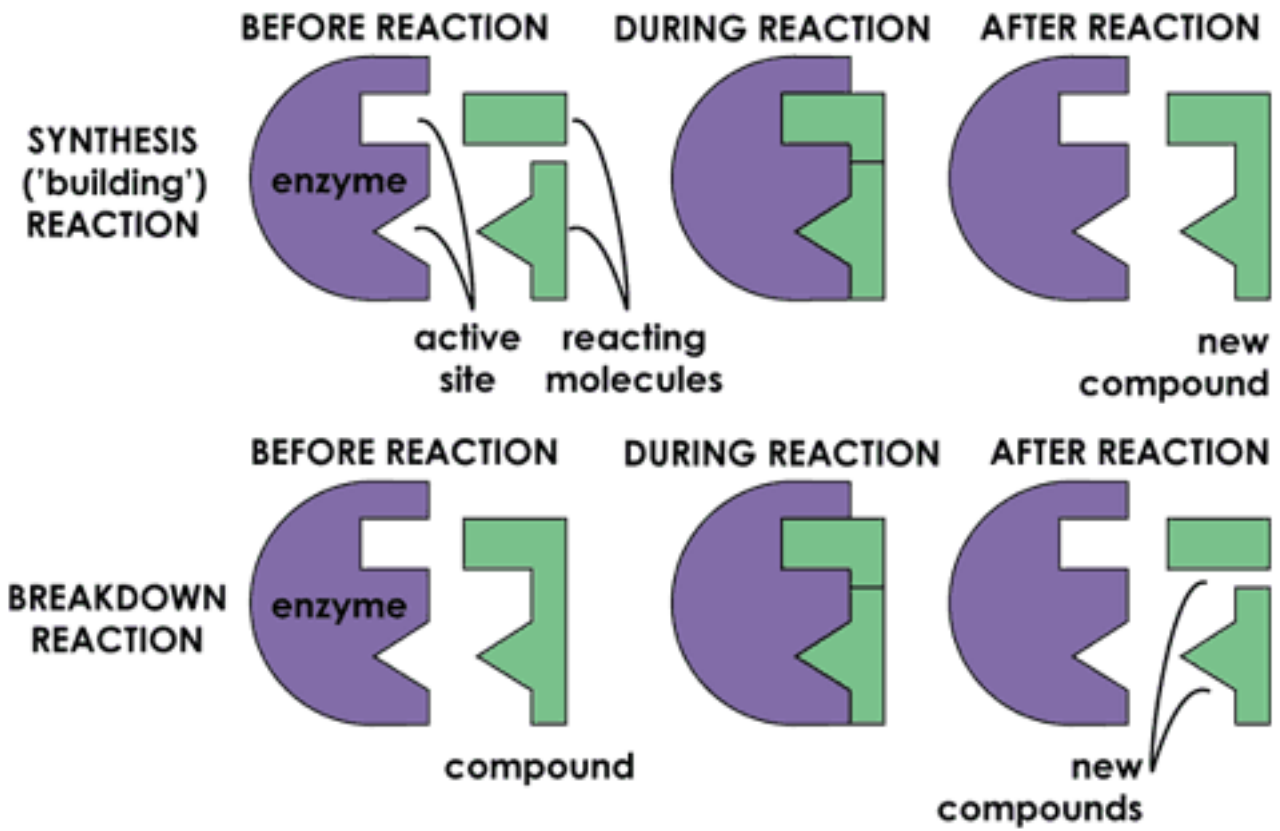
- The distinguishing feature of an enzyme-catalyzed reaction is that it takes place within the confines of a pocket on the enzyme called the **active site.**
- To catalyse a reaction, an enzyme will bind to one or more reactant molecules. These molecules are the enzyme's **substrates**



Enzymatic reaction :

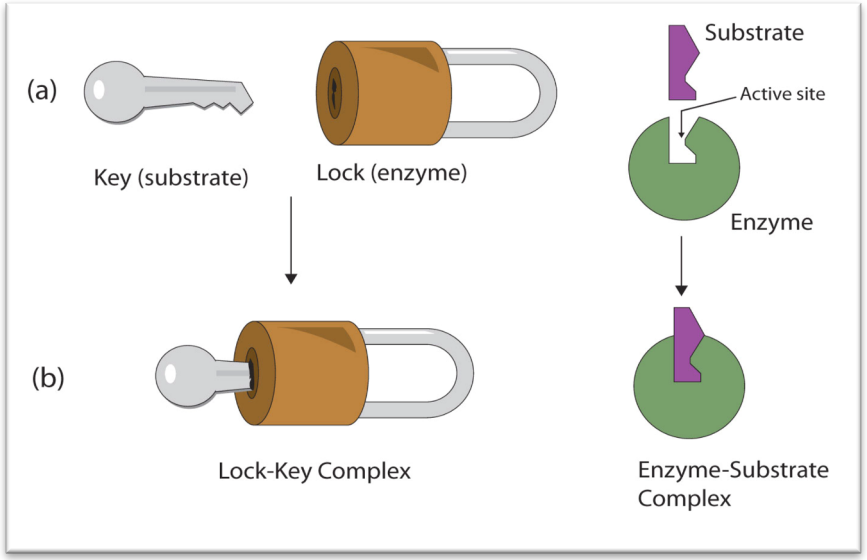
$$E + S \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P$$




BEFORE REACTION
DURING REACTION
AFTER REACTION
SYNTHESIS ('building') REACTION
enzyme
active site
reacting molecules
new compound
BEFORE REACTION
DURING REACTION
AFTER REACTION
BREAKDOWN REACTION
enzyme
compound
new compounds

How can substrate bind to the Enzyme?

(1)



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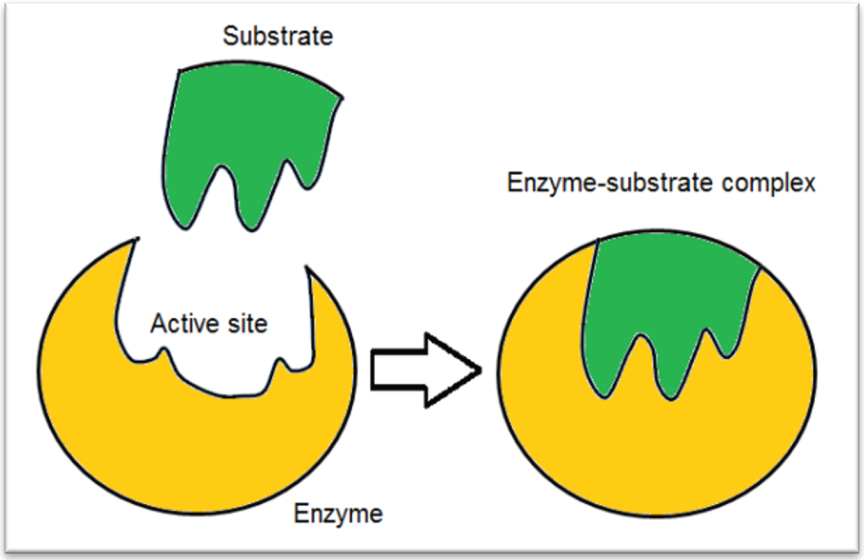


Importance of studying enzymes:

- Diseases.
- Food.
- Agriculture.
- Chemical industry.
- ..etc

Enzyme browning :

- This occurs when the phenolic compounds present in them react with **polyphenol oxidase.**

Polyphenol oxidase (PPO):

- Is a **copper-containing enzyme** that catalyse the oxidation of dihydroxy-and trihydroxy phenol to corresponding quinone which has a brown color.

- In this lab, activity of polyphenol oxidase extracted from **potato will be examined qualitatively.**

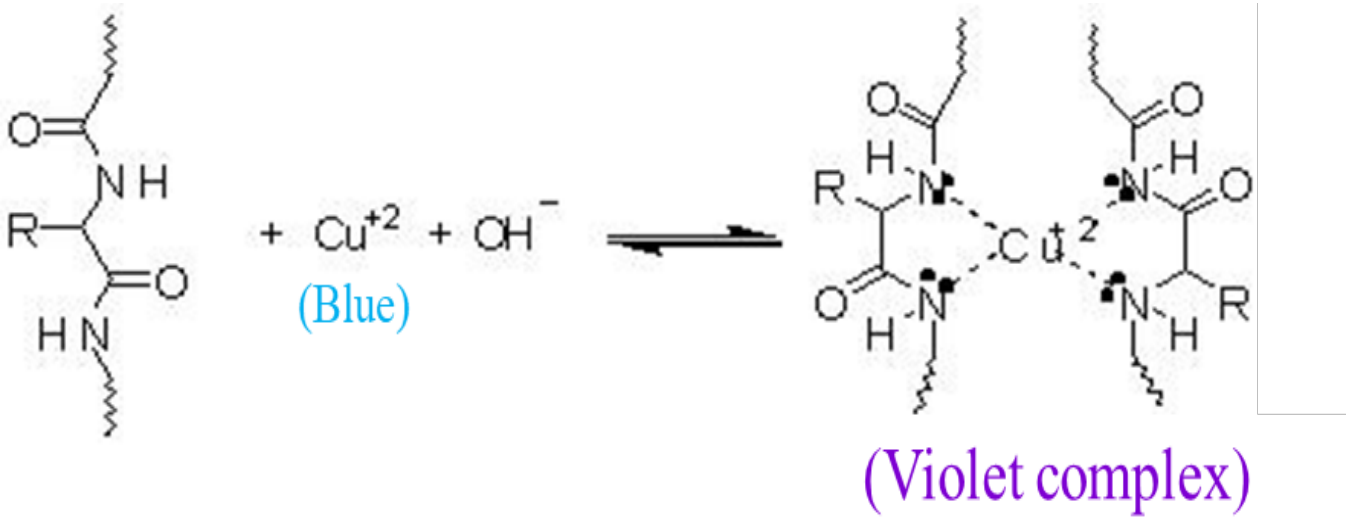
Practical part

Experiment (1): Examine the protein nature of polyphenol oxidase

Aim:

- Examine the protein nature of polyphenol oxidase by biuret test.

Principle:

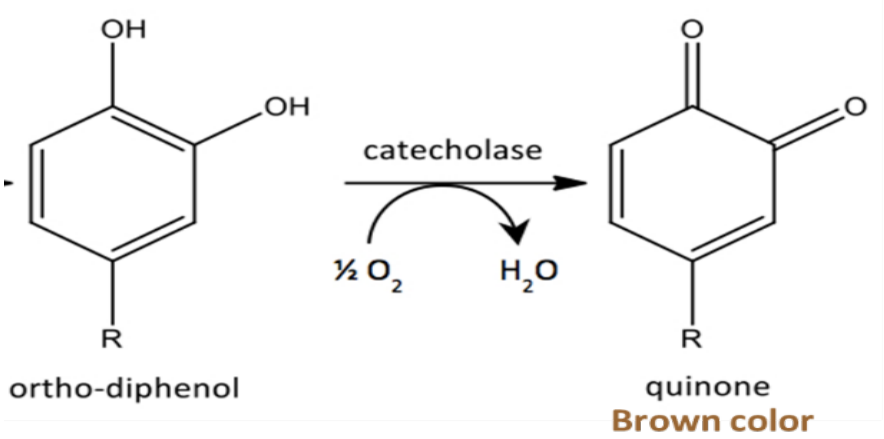


Experiment (2): Test the activity of polyphenol oxidase

Aim:

- To demonstrate activity of the enzyme.
- To investigate the effect of incubation time on enzyme activity.

Principle:



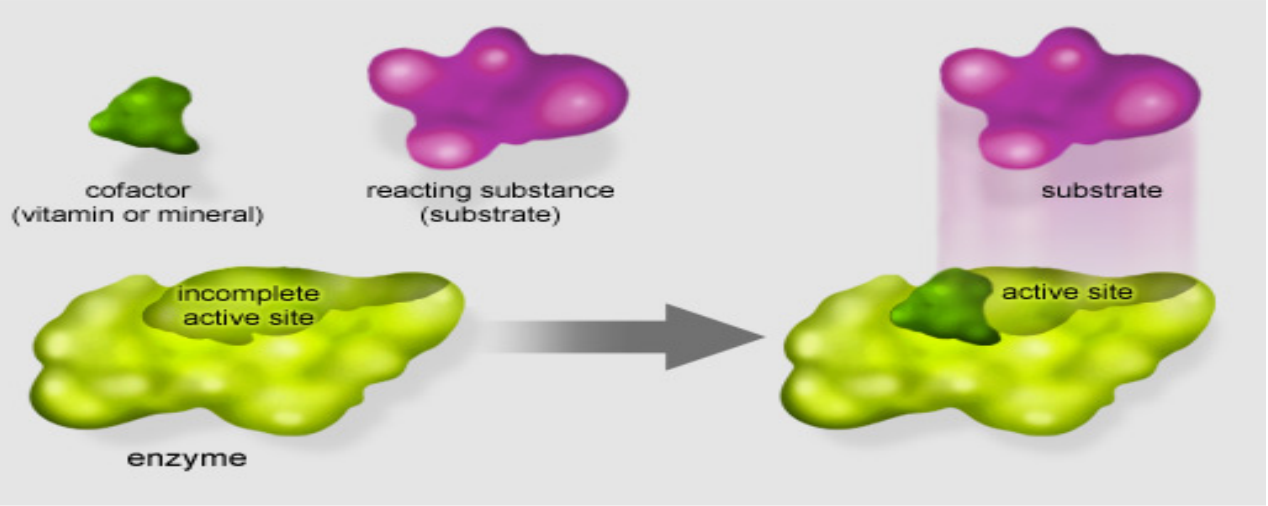
Experiment (3): Demonstrate the chemical nature of polyphenol oxidase

Aim:

- To examine the chemical nature of polyphenol oxidase.

Principle:

- pH.
- Cofactor.

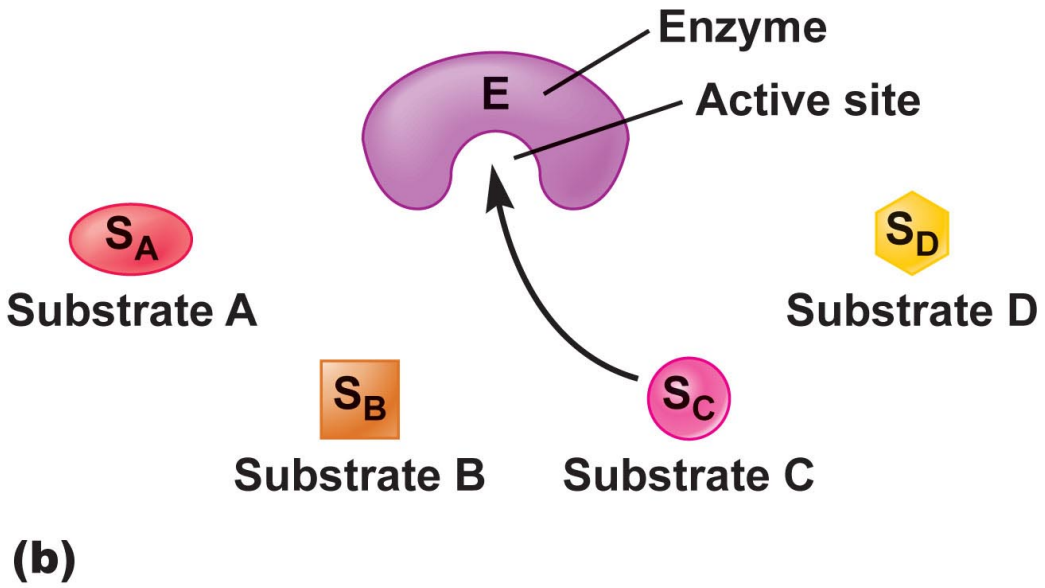


Experiment (4): Investigating the substrate specificity of polyphenol oxidase

Aim:

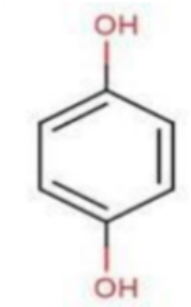
- To investigate the substrate specificity of the enzyme using structurally related chemicals.

Principle:

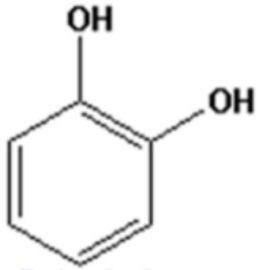


(b)

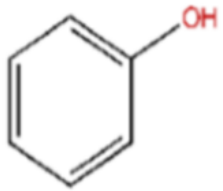
- The three compounds (mono-and di-hydroxyl phenol) will be used to find out which one of them is a substrate for PPO.



Hydroquinone



Catechol



Phenol

Experiment (5): Investigating the effect of temperature on polyphenol oxidase activity

Aim:

- To investigate the effects of temperature on the enzyme activity.

Principle: