15. Investigation of the effect of substrate concentration on enzyme activities

Urease occurs in bacteria, moulds, higher plants (particularly in the Cucurbitaceae and Leguminosae) and in some lower animals. Urease is virtually substrate-specific. It acts on urea and converts it into ammonia and carbon dioxide by hydrolysis of the C-N bonds.

$H_2N - CO - NH_2 + H_2O \longrightarrow 2NH_3 + CO_2$

Urea

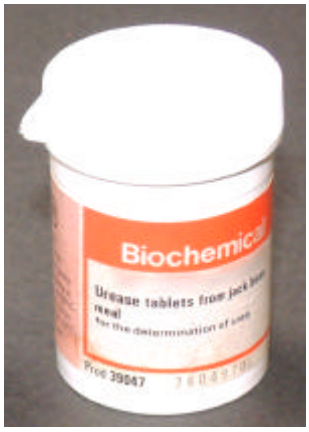

Fig.1 Urease

The ammonia liberated can be detected by using the indicator bromothymol blue which turns yellow/green in acid solution, but is blue in alkaline solution. As urease can retain its optimal activity over a wide pH range (between pH 5 and 10), the experiment can start with an acidic pH so that increase in pH due to the liberation of ammonia in the course of reaction has little effect on the rate of enzyme reaction.

In this investigation, the effect of different concentrations of urea on the rate of hydrolysis of urea by urease to carbon dioxide and ammonia is studied.

Procedure

A. Extraction of urease from soya beans

1. Weigh 1 g of soya beans.
2. Soak the beans in 5 cm^3 of distilled water in a test tube for one day.
3. Pour the softened beans into a mortar and grind them into a milky paste.

4. Add 25 cm^3 of distilled water to dilute the paste.
5. Filter the suspension through 4 layers of muslin cloth to remove cell debris and centrifuge for 5 minutes. Use the supernatant liquid as a source of urease.

B. Effect of urea concentrations on urease activity

1. Label 6 test tubes from 1 to 6.
2. Use graduated pipettes to add the following volumes of bromothymol blue solution, 2% urea solution and distilled water in tubes 1 to 6 as follow:

Tube	1	2	3	4	5	6
Bromothymol blue (cm^3)	1	1	1	1	1	1
2% urea solution (cm^3)	2	1.5	1	0.8	0.5	0.2
Distilled water (cm^3)	0	0.5	1	1.2	1.5	1.8

3. Mix the contents of each of the 6 test tubes well. Place them in a water bath at 35°C for 5 minutes.
4. Add 0.5 cm^3 of the enzyme extract into each of the tubes. Start the stopwatch immediately. Shake thoroughly to mix the contents of the tubes and quickly place them back in the water bath.
5. Record the time taken for the bromothymol blue to change from yellow/green to blue.