Effect of Enzyme Concentration On The Rate Of An Enzymatic Reaction .

Method:

1- Prepare 8 tubes in the following manner table ( 1 ) :

|  |  |  |
| --- | --- | --- |
| Tube | Acetate buffer (ml) |  0.18M Sucrose (ml) |
| Blank | 1.0 | 2.0  |
| A | 1.0 | 2.0  |
| B | 1.0 | 2.0  |
| C | 1.0 | 2.0  |
| D | 1.0 | 2.0  |
| E | 1.0 | 2.0  |
| F | 1.0 | 2.0  |

2- Mix each tube then add 0.05ml of diluted enzyme according to the following table (2) , EXCEPT FOR THE BLANK ADD 0.05ml OF DISTILLED WATER INSTEAD , mix and start the stop clock immediately , incubate each tube for 10 min , then stop the reaction by adding 2.0ml of the DNS reagent to each tube.

Note : Mix each tube frequently during the incubation time .

Table (2).

|  |  |  |
| --- | --- | --- |
| Tube | Enzyme Solution | Enzyme concentration x 10-3 |
| Blank | --- | 0 |
| A | E1 | 4.0X |
| B | E2 |  6.0X |
| C | E3 |  8.0X |
| D | E4 | 10X |
| E | E5 | 15X |
| F | E6 | 20X |

Table(3).

|  |  |  |
| --- | --- | --- |
| Tube | Start (min) | Stop (min) |
| Blank | 0.0 | 0.0 |
| A | 1.0 | 11.0 |
| B | 2.0 | 12.0 |
| C | 3.0 | 13.0 |
| D | 4.0 | 14.0 |
| F | 5.0 | 15.0 |
| G | 6.0 | 16.0 |

3- Mix properly , cover each tube by aluminium foil and place in a boiling water bath for 5min to allow the colour to develop .

4- Then remove from water bath cool under tap water , add 20ml of distilled water to each tube , mix properly then measure the absorbance at 540nm .

5- Record the absorbance of each test tube in the following table ( 4),

6- Convert the Absorbance reading obtained to micromoles of sucrose hydrolyzed making use of the standard reducing sugars calibration curve , then divide by 10 to obtain the number of micromoles of sucrose hydrolyzed /min (vi ).

7 – Draw a graph between the (vi ) the micromoles of sucrose hydrolyzed /min and enzyme concentration .

Table (4)

|  |  |  |  |
| --- | --- | --- | --- |
| Tube | Absorbance 540nm | µmoles of sucrose hydrolyzed | µmoles /min( vi )  |
| A |   |  |  |
| B |   |  |  |
| C |   |  |  |
| D |   |  |  |
| E |   |  |  |
| F |   |  |  |
|   |  |  |  |