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A Comparative Study of Enzyme Kinetics of Amylase and Protease Produced from Isolated Microbial Strain of Soils of Tuljabhavani Temple of Tuljapur

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ABSTRACT - Michaelis-menten equation and Lineweaver Burk-double reciprocal plot can be studied for understanding the enzyme kinetics which has industrial importance such as amylase and protease from soil around Tulja-bhavani temple of Tuljapur. For analyzing activity on comparative mode with respect to substrate concentration, temperature and pH, the enzyme amylase are active at pH 6.0 with optimum temperature is 40°C with activity reached to 523.01 units/ml^{-1}, whereas protease are active at pH 8.0 and optimum temperature is 37°C with activity 549.23 units/ml^{-1}. This finding shows that extracellular amylase and protease have very little difference with their activity, and also follow the little difference in response to their enzyme catalyzed reaction.

Key –words- Enzyme catalyzed reaction, Enzyme Kinetics, Optimum temp & Ph

I. INTRODUCTION

Enzymes are remarkable and highly specialized proteins. Enzymes are catalysts of the biological system and are often much more efficient as composed to synthetic catalysts. Each enzyme has its own tertiary structure and particular confirmation which is necessary for its catalytic activity. The enzyme catalyzed reaction occurs within a pocket of the enzyme called the active site. Enzyme catalyzed reactions are approximately 10^6 to 10^{12}times faster compared to uncatalyzed reactions.

Basically, the overall enzymatic reaction is composed of two elementary reactions, first in which enzyme (E) and substrate (S) combine to form an unstable enzyme substrate complex (ES) and second in which ES decomposed to products (P) and (E)

$$E+S \underset{K2}{\overset{K1}{\rightleftharpoons}} ES \xrightarrow{K3} E+P$$

For understanding the enzyme kinetics, Michaelis-menten equation and Lineweaver Burk-double reciprocal plot can be studied.For studying kinetics of enzyme reaction, various factors influence the rate of enzyme reactions and these are concentration of substrate, temperature and pH.

In an enzyme catalyzed reaction, velocity of reaction and enzyme concentration are directly proportional. Hence rate of reaction increases proportionally as the concentration of enzyme is increased, so the substrate concentration is in excess and hence the reaction is independent of substrate concentration. Then it is clear that any change in the amount of product formed over a particular time period will depend on the enzyme concentration. Till a threshold concentration, increase in rate of reaction is seen with increase in the substrate concentration.

At a particular temperature and pH and optimum temperature will significantly alter the enzyme activity. Enzyme reactions are extremely temperature sensitive and change in reaction temperature as small as 1 or 2°C may change the reaction rate by 10 to 20 %. At high temperature there is denaturation of proteins and hence inactivation of enzyme also high or low pH usually results in complete loss of activity for most enzymes.

In present research work, isolated microbial strains from soil samples found around Tulja-bhavani temple of Tuljapur applied for amylase and protease production are partially purified and produced enzymes are well studied by using enzyme kinetics. By analyzing various parameters, such as concentration of enzyme substrate concentration, Temperature, pH. Apply for comparative study of above mentioned kind of enzymes produced from microbial strain.

In present research work, we observe the study of enzyme reaction rates and how they change in response to changes in experimental parameters is known as kinetics, in which activity of the enzyme is depends on the substrate and environmental conditions such as pH, temperature and ionic strength.

II. MATERIALS AND METHODS

1) Partially purified Amylase (40% ammonium sulphate fraction)

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2) Partially purified protease (60 to 70% ammonium sulphate fraction)
3) Standard substrate for assay performance
   i) Maltose for amylase
   ii) Casein for protease
4) Buffer-0.1 M phosphate buffer.

1. **Effect of substrate concentration:-**
   To study the effect of substrate concentration , maltose for purified amylase and casein for purified protease.
   The different soluble concentrations (W/V) of maltose were used i.e. 0.2, 0.4, 0.6, 0.8, 1.0 & 0.5 % respectively for assaying the enzyme activity.
   The effects of substrate concentration on enzyme activity have a lot of implications which helps in understanding the characteristic of enzyme. Experimental procedure used optimal time and enzyme concentration to be used are kept constant, the amount of substrate added is varied and then enzyme concentration for varied substrate concentration is calculated.This shows that enzyme concentration is directly proportional to substrate concentration.
2. **Effect of incubation temperature**:-
   To study the effect of temperature for observing the activity of amylase and protease at different temperature from 10^{o} C TO 80^{o} C respectively and identified the optimum temperature for activity of amylase and protease.
   Like any chemical reaction, the velocity of an enzymic reaction also increases with temperature but only upto a point. A study of the response of reaction velocity to temperature help us to calculate a very important parameter namely the activation of energy of the reaction.
   Activation energy i.e. speed of enzyme catalysed reaction is calculated by using Arrhenius equation, as amylase is a soluble enzymes give a straight line.
3. **Effect of pH**:-
   The purified amylase and protease was incubated at different pH values and these are 2.0, 6.0, 7.0, 8.0, 9.0, 10.0 by a standard pH meter using a model Equiptonics EQ-621.
   The substrate binding to enzyme will be affected by pH of the medium. A number of assay tubes are kept with different controls and only varying parameter is the pH of buffer added to each tube. Measure the activity under different pH values, by this method optimal pH can be found out.

III. RESULT AND DISCUSSION

In nature occurrence of amylolytic and proteolytic bacteria are widespread and are able to perform their growth at different temperature, pH and also different carbon sources with different incubation period.

After purification, characterization of the enzyme protein is an important part in the study of the enzyme in response to chemical properties like optimum pH, optimum temperature, energy of activation and kinetic properties.

In this research work, extracellular amylase and protease are obtained from bacterial strains of pseudomonas aeruginosa and Bacillus sp. isolated from soil around Tulja-bhavani temple of Tuljapur. The results obtained in this work prove the ability of bacterial strain to produce extracellular amylase and protease. As large quantity of protein solution was required for repeated amylase assays performed by using 70% ammonium sulphate fraction.

The two bacterial strains separately incubated in different culture media in which amylase production is enhanced with the addition of starch in media. Alone starch provide the -c- source to the growth of respective microbial strain, whereas the protease production not depend on addition of -c- source in culture media but the growth is fulfill by additional nitrogen sources of culture media and these are in the form of peptic digest of animal tissue, beef extract, yeast extract and also Nacl.

The amylase production occurs early at 48 hrs. for 50°C whereas protease production follow 72 hrs at 37°C. this says that the pseudomonas aeruginosa respond the ingredients of culture media and perform for the growth but needs high temperature but at room temperature, protease perform the growth but takes large incubation time i.e. 72 hrs.

When we have to learn in the present research work, in response to an enzyme kinetics, the optimal condition for activity of partially purified amylase include pH is 6.0 and temperature is 40°C, whereas for protease it becomes pH is 8.0 and temperature is 37°C. This shows that amylase activity considerably decreased at low acidic as well as at high basic pH,but shows the activity nearer to neutral pH with optimum temperature is 40°C.

In present research work the results indicated that, the optimum incubation temperature for purified amylase and protease enzyme was 40°C, and 37°C and the velocity of an enzyme catalyzed reaction reached upto 523.01 units/ml^{-1} and 549.23 units/ml^{-1}

So the extracellular protease and amylase have very little difference with their activity as they are isolated from different microbial strain but both follow little difference in response to their enzyme catalyzed reaction.

IV. CONCLUSION

In an enzyme catalyzed reaction, velocity of enzyme reaction are altered by altering the concentration of other parameters such as pH, temperature & also substrate concentration. The Km value for amylase and protease is in between 10^{-5} to 10^{-2} moles, so it may however be noted that enzyme kinetics is not dependent on the concentration of enzyme but it alter by other parameters such as substrate concentration, pH and temperature.

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