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Operating Conditions Effects Onenzyme Activity: Case Enzyme Protease

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Abstract
The Proteases an enzyme added to detergents to degrade the protein spots origin.Their action is manifested through its activity the middle of washing clothes. This activity depends on the operating conditions. In this article, the effects of temperature and pH of the reaction and the substrate concentration and time of washing medium on the enzyme activity were studied. There action mechanism has been shown. The activity measurements were made by absorption spectrometry.

I. Introduction

The enzyme is a molecule (protein) biocatalysts (biocatalyst) used to degrade protein origin tasks by fragmenting into smaller molecules for easy removal by detergents, and lowers the energy of activation of a chemical reaction and to speed up to millions of times. Enzymes are designated by the"-ase" suffix. Like any catalyst, acting at low concentrations and they are found in tactat the end of reaction[1].

Since 1960, the enzymes are used in the laundry products composition. At first they acted exclusively proteases, which remove stains caused by proteins, such as those contained in the egg, milk, grass and blood. In recent years, new types of enzymes have appeared on the market, in particular amylases and lipases. [2].

The enzymehas a role of catalyzing a chemical reaction and degrade proteins. The chemical components of the enzyme are: C, H, N and O. The hydrolyzed, partial and complete respectively produce peptides and amino acids.Their Activity Depends On Its Spatial Structure [3].

Towalski [4](1987),Reilly, PJ [5] (1984), and Starace(1980) studied the enzyme powder. They found that the parameters such as temperature, substrate concentration, enzyme concentration, pH and the dissolution time.

In this article, we will discuss the influence of parameters and operating conditions on the activity of the protease enzyme.

II. The Oretical Bases

Proteases are enzymes that attack proteins.They Consist of the amino acid units linked by peptide bonds. Proteins are specific substrates where chemical and biochemical reactions involved are performed without consuming enzyme[7]. Protease is composed of amine nitrogen with many Atoms, but also has an alcohol function in the center of the molecule. The Role of protease to degrade proteins, activate the detergent and remove blood stains, egg, milk, etc.

Unit of measurement of protease: It is difficult to measure the amount of enzyme in units of mass or molar concentration, the measured enzyme activity is defined in terms of reaction rate. The international unit Gu (glycose unit) is the amount of enzyme required to convert1micromol of substrate-product in one minute at as pecified temperature and pH (egpH7.0and25°C) and under conditions saturation of the substrate. Gu= 1micromol substrate consumed/ min[1].

Measurement ofthe enzymatic activity: The specific activity of an enzyme is the catalytic activity per unit weight of protein expressed in Gu/mg of solid enzyme. Kone lab Aqua 20 is a device gives the measurement result on enzymatic activity of enzyme solution Gu/ ml.It is given by the following empirical formula:%EnzymeX=(Gu/ml) /270
Where: X is the enzyme activity from the meter reading. It is expressed inGu/ ml.

Rateof enzymatic reaction: Michael is model which is based on the formation of enzyme-substrate complex (ES) according to the following equations:

$$\mathrm{E} + \mathrm{S} \underset{\mathrm{k_{-1}}}{\overset{\mathrm{k_1}}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{\mathrm{k_2}} \mathrm{E} + \mathrm{P} \quad (1)$$

The rate of reaction, V is equal to the constant k3 speed multiplied by the concentration of the reaction product [ES], either:

$$V = k_3[ES] \quad (2)$$

The rate of formation of product ES:

$$k_1[E][S] \quad (3)$$

Rate product decomposition ES :

$$(k_2 + k_3)[ES] \quad (4)$$

In steady state conditions, the rate of decomposition of the product is equal to that of the training:

$$k_1[E][S] = (k_2 + k_3)[ES] (5)$$

By rearrangement, we obtain:

$$[ES] = \frac{[E][S]}{\frac{(k_2+k_3)}{k_1}} (6)$$

The Michaelis constant, Km is:

$$k_m = \frac{(k_2+k_3)}{k_1} (7)$$

Fromwhere :$$[ES] = \frac{[E][S]}{k_m} (8)$$

More, $$[S] = [S]_{total} \text{ if} [E] \ll [S]. (9)$$

$$[E] = [E]_{total} - [ES] (10)$$

There fore,

$$[ES] = ([E]_{total} - [ES])[S]/k_m (11)$$

After rearrangement, we obtain:

$$[ES] = [E]_{total} \frac{[S]}{[S]+k_m} (12)$$

Thus, expression of the speed of the reaction is:

$$V = k_3[E]_{total} \frac{[S]}{[S]+k_m} (13)$$

The maximum rate is reached when all enzyme sites are saturated with substrate. That is to say, when: $$[S] \gg k_m (14)$$

Therefore, the ratio $\frac{[S]}{[S]+k_m}$ goes to 1.

The maximum rate is:

$$V_{max} = k_3[E]_{total} (15)$$

Michaelisrate is given by the following formula:

$$V = V_{max} \frac{[S]}{[S]+k_m} (16)$$

III. Experimental Procedure

To measure the protease activity in the detergent powder, we used the Konelab Aqua 20. This system is a system analyzer for continuous random loading biochemical analysis and serum electrolytes. It canperform 200 operations per houranalysis.

The analysis method commonly used for this activity by Unilever is based on the action of proteases on the methyl casein which is a protein consisting of several amino acids linked together by peptide bonds. The NH2 groups formed from the action of proteases on casein methyl, react with a color indicator trinitrobenzene sulfonic acid giving a yellow complex which evolves with time according to the enzymatic activity. The mechanism is clarified by the following reactions:

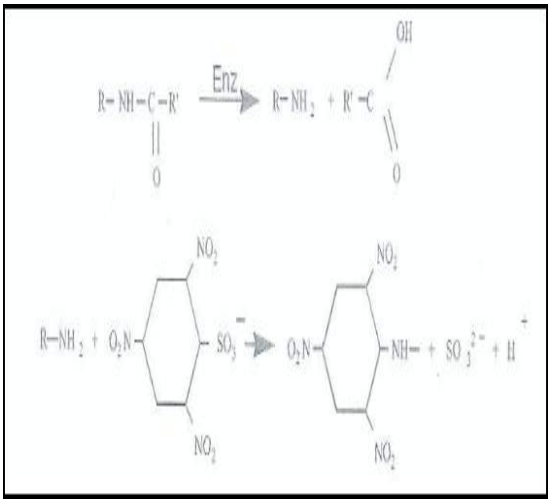

Figure-1: ... Mechanism of the chemical reaction to measure the activity of the protease enzyme.

Inside the device, as shown in the following figure, a light beam passes through a cuvette containing the sample and reagents for measuring the absorbance.

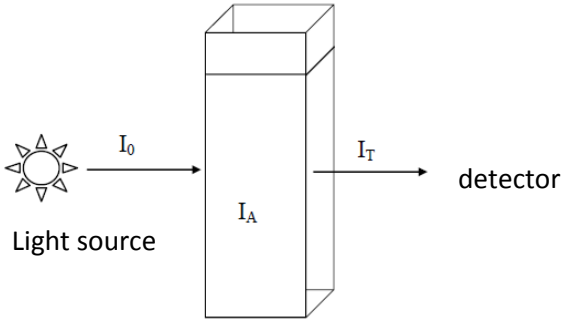

Figure-2: .... Measure the absorbance of the product of the chemical reaction.

The enzymatic activity is automatically calculated with reference to a calibration curve where the door absorbance versus concentration.

The enzyme concentration is given by the ratio :

$$enzymeconcentration = Activity / enzymaticactivity. (17)$$

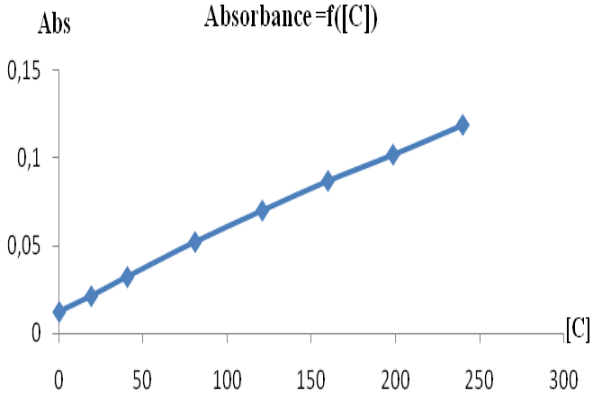

Figure-3: ... Curve calibration device Konelab aqua 20 [8].

The calibration curve is an affine equation: Abs = a* [C] + b.
where: b = 0.012 et a = 4.7 10^{-4}.

Measuring the rate of reaction between the protease enzyme and the substrate

To do this, we used hydrogen peroxide as substrate and turnip juice as enzyme. The rate of the chemical reaction is the time of washing. A mixture was made to check the speed of the reaction. This mixture is the result of grinding of 20 grams of turnip in mortar and 20 ml of distilled water. After filtration, the filtrate was placed in a flask, to which was added dropwise hydrogen peroxide. And for every drop, it counts the number of air bubbles released from the filtrate. We used the number of air bubbles appear depending on the substrate concentration. This manipulation is repeated for different concentrations of substrate.

Measurement of the influence of temperature on enzyme activity

Enzymes are extremely temperature sensitive. They operate in an optimal temperature range. Above and below this optimum, the enzymatic activity is slowed. To demonstrate this sensitivity, we prepared enzyme solution 200Gu/ml activity. The temperature of the solution is fixed and the corresponding enzymatic activity was measured.

Measurement of the influence of pH on enzyme activity

Enzyme activity is strongly influenced by the pH of the reaction medium, each enzyme has a maximum activity at a given pH, it has an optimal pH (Towalsky, 1987). To do this, samples of seven solutions of different pH were prepared. Then, the enzyme activity was measured for each sample.

Measurement of the influence of the time of dissolution

The enzyme activity depends on the stirring time and with optimum corresponding to the boundary from which the activity decreases due to denaturation of the enzyme. To do this, we prepared a solution of enzyme maximum activity equal to approximately 160Gu/ml. The solution is placed on a stirrer. Measures changes in activity are performed each 5 minutes.

IV. RESULTS AND DISCUSSION

III-1-Effect of substrate concentration on the rate of the enzymatic reaction

The curve is divided into three parts:

The first between 0 and 0.0018 mol / L portion is characterized by the complete absence of air bubbles. The second part is in the range [0.0022 to 0.0067 mol / L]. The number of bubblesincreaseslogarithmically.

The third part starts from the concentration 0.0089 mol / L. The number of bubbles generated by units of time becomes constant and equal to the value 44 bubbles / min.

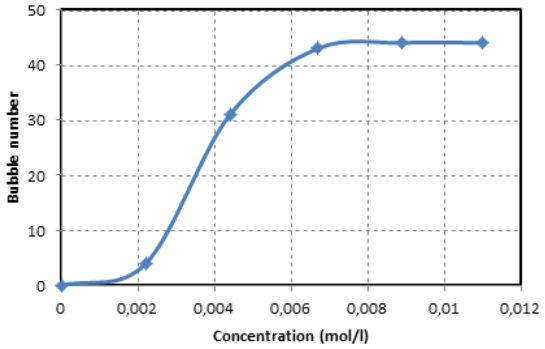

Figure-4: Concentration effect on the number of released bubbles [8]

Therefore, the speed of the reaction increases with the concentration of the substrate to a certain limit value when it becomes constant. The limit value corresponds to the saturation of the enzyme. These results are explained by the model of Michaelis.

INFLUENCE OF TEMPERATURE ON THE ENZYMATIC activity

The test results of the influence of temperature on enzyme activity are shown in the figure below.

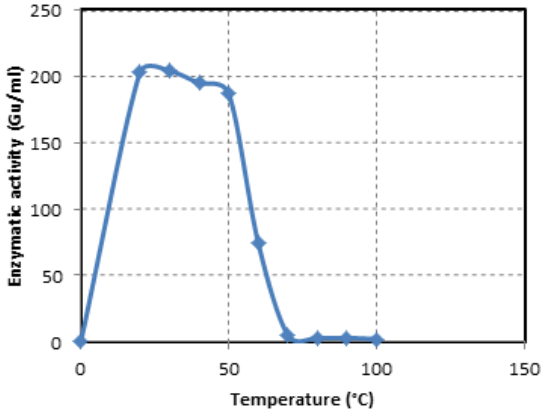

Figure-5: Effect of temperature on enzyme activity [8]

The curve is divided into three zones: the first is characterized by the growth of the enzyme activity in function of the temperature rise from 0 to 20 ° C. the second zone starts at a temperature of 20 ° C and end at 50 ° C. It is the seat of a constant enzyme activity.Beyond 50 ° C, the activity drop gradually according to the increase in temperature. It is almost zero at 70 ° C; this is the third area.

Therefore, the increase in temperature increased the rate of denaturation of the enzyme, which is accompanied by a decrease in the catalytic activity of the enzyme.

Influence of pH on enzyme activity

The test results are summarized in the following curve.

When the medium is acidic, the activity increases as a function of pH for growth. This increase in enzyme activity reached a maximum of 32 Gu / ml at a pH equal to 7. Then decreases gradually. Therefore, the optimum pH is close to the value 7.

PH occurs in two different ways: either by modifying the secondary or tertiary structure of the enzyme,or by modifying the electrical charges of radicals of amino acids in the active site.

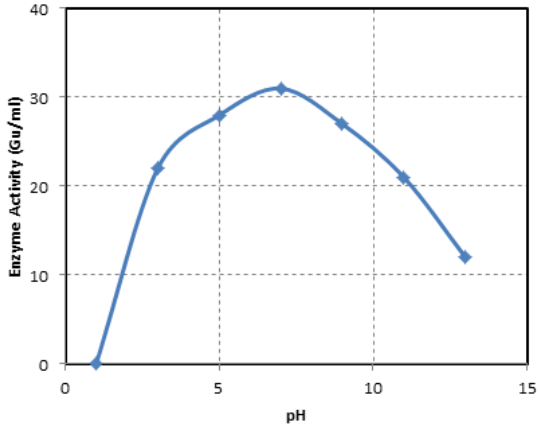

Figure-6: Effect of pH on enzyme activity [8]

Effect of time of dissolution

The following figure is a graphical representation of the change of the enzymatic activity in function of the stirring time.

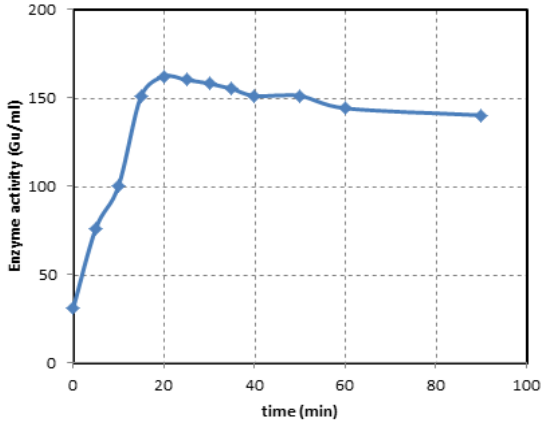

Figure-7: Effect of dissolution time on the activity of the enzyme [8].

The curve shows that the enzyme activity is maximal for a stirring time of 20 minutes. The maximum enzyme activity is 160 Gu / ml. If the stirring time is greater than 20 min then the enzyme activity gradually fall. This drop is explained by the onset of degradation of the enzyme. In practice, the enzymatic activity is measured after a mixing time equal to at least 20 minutes.

V. CONCLUSION

The presence of the protease enzyme in the detergent is to enhance the efficiency of cleaning and separation of all types of soil. This cleaning efficiency depends primarily on the properties of the enzyme. Protease has properties of protein breakdown. This property has been demonstrated through testing with the enzyme in blood and eggs. The effectiveness of the enzyme is translated by its activity. However, it should be noted that the effectiveness of the enzyme depends on the operating conditions. Through the tests we have shown that the substrate concentration affects the activity of the enzyme. For higher substrate concentration 0.0089 mol / L, the activity remains constant.

The temperature is an important factor in cleaning by detergents rich enzyme. The activity of the enzyme increases with temperature to a maximum value.Cettevaleurmaximaleestatteinteetresteconstante dansthe temperature range of [20 ° C; 50 ° C]. Beyond this range the activity decreases due to change in the nature of the enzyme.

The pH of the medium plays a very important role on the effectiveness of cleaning detergents rich enzyme. Tests have shown that the enzyme activity increases as a function of the pH increase to a maximum and then decreases. The maximum pH is approximately equal to 7. To a basic pH, the activity decreases due to enzyme denaturation.

The dissolution time of the enzyme by agitation is a key parameter in the effective use of the protease enzyme. The enzyme activity reached its maximum at a stirring time of 20 min. Beyond this value the enzyme may lose its effectiveness by denaturation.

VI. Acknowledgments

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