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Research Article

Activity of Enzyme in Escherichia Coli Through Molecular Techniques

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Abstract: Enzymes are protein biomolecules that act as catalysts in various biochemical reactions, both in biological systems and industrial applications. The uniqueness of enzymes lies in their ability to accelerate the rate of reactions with a high degree of specificity towards certain substrates without undergoing permanent changes in their chemical structure. Enzyme activity is strongly influenced by various environmental factors, such as temperature, pH, substrate concentration, and the presence of inhibitors or activators. Therefore, quantitative testing of enzyme activity is an important step in understanding the characteristics of enzymes and their applications in various fields. Escherichia coli produces Extended-Spectrum B-Lactamase Enzyme (ESBL) and plays a role in damaging the structure of beta lactam antibiotics so that the antibiotics cannot kill bacteria. Bacteria that produce ESBLs need to be watched out for because ESBLs are produced by genes located on plasmids, which can easily be transferred to other bacteria, and often also carry resistance genes to other antibiotics. Objective: to accurately measure the activity or concentration of enzymes in samples of Escherichia coli bacteria and understand the influence of variables such as substrate concentration on the reaction rate. Method: spectrophotometry through enzyme extraction, making a standard curve and testing enzyme activity against variations in substrate concentration. Results: samples with concentrations of 0.1 and 0.3 showed good and appropriate absorbance results. However, in the sample 0.5 ; 0.7 ; and 1.0 indicates an absorbance number that is slightly higher than it should be. Conclusion: the enzyme in Escherichia coli bacteria has good activity at sample concentrations of 0.1 and 0.3.

Keywords: Activity, Concentration, Enzyme, Escherichia Coli, Spectrophotometry

1. Introduction

Enzymes are protein biomolecules that act as catalysts in various biochemical reactions, both in biological systems and industrial applications. The uniqueness of enzymes lies in their ability to accelerate the rate of reactions with a high degree of specificity towards certain substrates without undergoing permanent changes in their chemical structure. Enzyme activity is strongly influenced by various environmental factors, such as temperature, pH, substrate concentration, and the presence of inhibitors or activators. Therefore, quantitative testing of enzyme activity is an important step in understanding the characteristics of enzymes and their applications in various fields (Dewi and Prihatini, 2021).

The quantitative enzyme test aims to accurately measure the activity or concentration of enzymes in a sample. The data generated from this testing has great significance, both in basic research such as enzyme kinetics studies, to practical applications in the medical, food and pharmaceutical fields. Methods often used for these quantitative measurements, such as spectrophotometry, provide precise results and allow in-depth analysis of enzyme performance under certain conditions (Istia'nah et al, 2021).

Theory Enzymes are protein macromolecules that act as biocatalysts. Enzymes will increase the speed of chemical reactions. This biokalis can be found in every living creature. Amylase is one of the enzymes that is widely studied by scientists. The amylase enzyme works

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to degrade polysaccharides and convert them into short chain oligosaccharides. Enzyme action is influenced by several factors, namely substrate and enzyme concentration, temperature and inhibitors (Putri, et al, 2023).

One factor that influences the speed of enzyme catalysis is concentration. The graph of the relationship between substrate concentration and initial speed is shown in Figure 2.1. Based on the graph, it can be observed that the enzyme speed increases as the substrate concentration increases. At a certain point, the enzyme speed will tend to remain constant with increasing substrate concentration. This situation shows that the enzyme has been saturated in binding to the substrate where the enzyme is at its maximum speed, which is called Vmax. Another parameter used in determining other kinetics is Km. Km is defined as the concentration of a certain substrate when the enzyme reaches half its maximum speed (Putra, et al, 2021).

Escherichia coli is a gram-negative bacteria that can produce the enzyme penicillin G acylase. E.coli can be found in two different habitats, namely primary habitat (host) and secondary habitat (external environment) (Advinda and Fariani, 2022).

The primary habitat of Escherichia coli is the intestines of vertebrates, while the secondary habitat of Escherichia coli is water and soil. and vertebrate animal phases. The differences between primary habitat and secondary habitat include that in the primary habitat the environmental temperature is constant, anaerobic, nutrients vary, competition, while in the secondary habitat the temperature varies, aerobic, nutrients do not vary, competition is low. E. coli that lives in primary habitats is able to adapt to its environment when compared to E. coli that lives in secondary habitats, so that the Escherichia coli populations in primary habitats and secondary habitats are genetically different (Megahati, 2011).

Extended-spectrum β-lactamases (ESBL) are enzymes produced in plasmids of Gram-negative bacteria from the Enterobacteriaceae group that already have resistance to β-lactam antibiotics. The most commonly known ESBL-producing bacteria are Escherichia coli (E. coli) and Klebsiella pneumoniae (K. pneumonia) and are often considered the main causes of urinary tract infections (UTIs), pneumonia and sepsis. CTX-M β-lactamase is the most common ESBL enzyme recovered from human bacterial isolates, while subtype variations depend on geographic region. ESBL-producing bacteria are nosocomial pathogens and are increasingly being found as infectious agents in the community. The incidence of ESBL-producing bacteria has spread widely in the field of veterinary medicine, for example as a cause of mastitis in dairy cows since 2000 (Effendi, 2020).

The working principle of a UV-Vis (Ultra Violet-Visible) spectrophotometer is based on light absorption, where atoms and molecules interact with light. The combination of Ultraviolet and visible spectrophotometric principles is called Ultraviolet-visible (UV-Vis) spectrophotometer. UV and visible sources are two different light sources used in this instrument. UV-Vis spectrophotometry is based on the Lambert-Beer law. If monochromatic light passes through a compound, some of the light will be absorbed, some will be reflected and some will be emitted. The rotating mirror on the inside of the spectrophotometer will divide the light beam from the light source into two (Sembiring et al, 2019). The wavelength in the ultraviolet region is 180 nm−380 nm, while in the visible region it is 380 nm−780 nm (Ahriani et al, 2021).

The general aim of this research is to accurately measure the activity or concentration of enzymes in samples of Escherichia coli bacteria and understand the influence of variables such as substrate concentration on the reaction rate using the UV-vis spectrophotometric method through enzyme extraction, creating a standard curve and testing enzyme activity against variations in substrate concentration. The specific objectives of this research are to understand standard curve absorbance data, determine enzyme activity against variations in substrate concentration and determine enzyme speed values.

2. Materials and Research Methods

2.1. Materials

The tools used in this research were drop pipettes, beakers, measuring flasks, analytical scales, measuring pipettes, reaction tube racks, test tubes, cuvettes and spectrophotometers. The materials used in this research were distilled water, iodine reagent, bacterial culture, NB media (Nutrient Broth, starch, Ecsherichia coli bacterial culture.

2.2. Research Methods

Enzyme extraction. One dose of Ecsherichia coli bacteria was taken and put into 25 mL of NB media. Bacterial cultures were incubated in a shaker incubator for 18 hours at 37°C. The culture was centrifuged at 5000 rpm for 10 minutes. The filtrate obtained is a crude enzyme extract.

Creation of a Standard Curve. The first step in making a standard curve is to make starch solutions with different concentrations. The concentration variations used are 0; 0.1; 0.3; 0.5; 0.7 and 1.0 mg/mL. The starch solution was put into six different test tubes, each containing 8 mL. Add distilled water and 1 mL of iodine reagent to the starch solution as shown in Table 3.3 The reaction tube is incubated at 37°C for 10 minutes. The absorbance of the sample was measured at a wavelength of 590 nm using a UV-Vis spectrophotometer.

Test enzyme activity against variations in substrate concentration. Starch solutions are made in various concentrations, namely 0; 0.1; 0.3; 0.5; 0.7 and 1.0 mg/mL. The starch was put into six different tubes, each containing 8 mL. Then, water and 1 mL of enzyme crude extract were added to the starch solution as shown in Table 3.3.2. The mixture was incubated at 37 ℃ for 10 minutes. Then, 1 mL of iodine reagent was added. After that, the test tube is immersed in cold water. Measure the absorbance of the sample at a wavelength of 590 nm using a Uv-Vis spectrophotometer.

3. Results and Discussion

3.1. Research Result

Calculation of NB Media (Nutrient Broth) 13 grams = 1,000 mL make 25 mL = (13 × 25) : 1000 = 0,325 grams. Put it in a 25 mL beaker and heat it to 145°C, then add distilled water and E. Coli bacteria with a hose. Then, let it cool, centrifuge at 3,000 rpm for 20 minutes.

Enzyme Activity Test: The crude extract of the enzyme that has been centrifuged is put into 1 mL test tubes each in a total of 6 tubes and the extra crude enzyme is added to the respective starch solution, then absorbed at a wavelength of 590 nm. Then the four concentrations determined above (2.5; 7.5; 12.5; 17.5) were dissolved respectively into a 25 mL volumetric flask using distilled water to the lower meniscus boundary line. After that, put 8 mL of each starch solution into a test tube and then add 1 mL of distilled water and 1 mL of iodine to each solution. Next, incubate the 4 samples at 37℃ for 10 minutes. Finally test using a spectrometer.

Table 1. Absorbance Results

Number	Sample	Absorbance Results	Information
1	**0,1**	**0,038**	**succeed**
2	**0,3**	**0,055**	**succeed**
3	**0,5**	**1,173**	**succeed**
4	**0,7**	**2,892**	**succeed**
5	**1,0**	**0,235**	**succeed**

Table 2. Standard Curve Absorbance Data

Tube Number	8 mL starch in x mg/mL	**Distilled Water (mL)**	**Iodine Reagent (mL)**	Absorbance 590 nm
1	0,0	9	1	0,00
2	0,1	1	1	0,120
3	0,3	1	1	0,618
4	0,5	1	1	1,294
5	0,7	1	1	2,186
6	1.0	1	1	3,00

From the standard curve absorbance data obtained, it is known that at dilutions of 0.1 and 0.3 the enzyme has the lowest activity and activity of 0.120 while the highest is 0.618. Based on these results it can be concluded that enzyme activity in Escherichia coli is influenced by substrate concentration. This is in accordance with research from Muliasari and Permatasari, 2022 and also Nurkhotimah and Yulianti, E, 2017; Pratantie, et al, 2021; Hasanah and Ilmi, 2020; Inayah, et al, 2022; Sumardi, et al, 2019; Kusumanimgrum, et al, 2019; Deviana and Rakhmawati, 2018; Yusron, et al, 202; Solahuddin, et al, 2021; Indrawati, et al, 2018; Simamora and Sukmawati, 2018; Gumelar and Fariyamto, 2020; Akuba and Pakaya, 2020; Deavina, et al, 2018; Vera, et al, 2023., Damira, et al, 2021 ; Fitriana and Asri, 2022; Sari and Supriyanti, 2016; Sulistiyono, et al, 2021; Simamora and Sukmawati, 2020; Sari and Indrayani, 2024; Amalia, et al, 2022 who said that the activity of the amylase enzyme is influenced by temperature and the amount of substrate.

Table 3. Enzyme Activity Test Data on Substrate Concentration Variations

Tube Number	8 mL starch in x mg/mL	Water (mL)	Enzyme Crude Extract (mL)	Iodine Reaget (mL)	Absorbance 590 nm
1	0,0	8	1	1	0,00
2	0,1	0	1	1	0,038
3	0,3	0	1	1	0,055
4	0,5	0	1	1	1,713
5	0,7	0	1	1	2,892
6	1.0	0	1	1	0,235

From the enzyme activity test data for varying concentrations obtained, it is known that at dilutions of 0.1 and 0.3 the enzyme has the lowest activity and activity of 0.038 while the highest is 0.055.

Table 4. Calculation of Enzyme Speed Values

Tube Number	[S] beginning (mg/L)	[S] end (mg/L)	[S] (mg/mL)	V (mg/mL.min)
1	0,0	0,00	0,00	0,00
2	0,1	0,120	-0,02	-0,002
3	0,3	0,618	-0,318	-0,0318
4	0,5	1,294	-0,794	-0,0794
5	0,7	2,186	-1,486	-0,1486
6	1.0	3,00	-2	-0,2

From the calculation data obtained for the enzyme speed values, it is known that at dilutions of 0.1 and 0.3 the enzyme has the lowest activity and activity of -0.002 while the highest is -0.0318. Based on these results, it can be concluded that enzyme activity in Escherichia coli is influenced by substrate concentration. This is in accordance with research from Muliasari and Permatasari, 2022 and also Nurkhotimah and Yulianti, E, 2017; Pratantie, et al, 2021; Hasanah and Ilmi, 2020; Inayah, et al, 2022; Sumardi, et al, 2019; Kusumanimgrum, et al, 2019; Deviana and Rakhmawati, 2018; Yusron, et al, 202; Solahuddin, et al, 2021; Indrawati, et al, 2018; Simamora and Sukmawati, 2018; Gumelar and Fariyamto, 2020; Akuba and Pakaya, 2020; Deavina, et al, 2018; Vera, et al, 2023., Damira, et al, 2021 ; Fitriana and Asri, 2022; Sari and Supriyanti, 2016; Sulistiyono, et al, 2021; Simamora and

Sukmawati, 2020; Sari and Indrayani, 2024; Amalia, et al, 2022 who said that the activity of the amylase enzyme is influenced by temperature and the amount of substrate.

4. Discussion

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The enzyme test using starch solution samples with different concentrations tested on a spectrophotometer showed that this research was successful. Samples 0.1 and 0.3 showed good and appropriate absorbance results. However, in the sample 0.5 ; 0.7 ; and 1.0 indicates an absorbance number that is slightly higher than it should be. This can be caused by several factors, namely when diluting the mother liquor the dosage was not appropriate. However, overall the enzyme test research using starch samples was successful.

5. Conclusion And Suggestions

The enzyme test using starch solution samples with different concentrations tested on a spectrophotometer showed that the research was successful. Samples 0.1 and 0.3 showed good and appropriate absorbance results. However, in the sample 0.5 ; 0.7 ; and 1.0 indicates an absorbance number that is slightly higher than it should be. This can be caused by several factors, namely when diluting the mother liquor the dosage was not appropriate. However, overall the enzyme test practicum using starch samples was successful. Enzyme activity in Escherichia coli is influenced by the amount of substrate. The advice given for further research is that when diluting the mother liquor, ensure that the dosage is appropriate and for further research, enzyme extracts from other bacteria can be used.

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