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STUDY ON THE OPTIMIZATION OF TEMPERATURE AND pH OF AMYLASE ENZYME FROM BACTERIA THRU A NARRATIVE REVIEW

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ABSTRACT

Amylase enzyme is a type of enzyme that plays an important role in various industrial fields. The amylase enzyme is used to hydrolyze starch into simpler carbohydrate molecules, namely dextrin, maltose and glucose. Industries that use amylase include the paper industry, detergent industry, textile industry, food industry and the pharmaceutical sector such as medicine. The aim of this research is to determine the effect of optimum temperature and optimum pH of the amylase enzyme from several bacterial sources. This research was carried out using a narrative review method related to the influence of temperature and the influence of the pH of the amylase enzyme from bacteria. From the results of the literature study, it can be seen that there is an influence on the optimum temperature and optimum pH of the amylase enzyme from several bacteria, namely obtaining 22% amylase activity at a temperature of 60°C with the bacteria Bacillus sp. PA-05, thermo-alkaliphile bacteria, and Bacillus sp bacteria, followed by 14% at temperatures of 50°C, 40°C, 37°C, and 30°C with Arthrobacter arylaitensis LBF-003, Geobacillus sp. DS3 bacteria, Bacillus amyloliquefaciens bacteria, Pseudomonas stutzeri ISL B5 bacteria, Bacillus megaterium bacteria, Bacillus subtilis BI19 bacteria, Arthrobacter arylaitensis bacteria, and Azospirillum sp. JG3 bacteria, and followed by 7% at temperatures of 70°C, 55°C, and 45°C with R_2M larvae bacteria, Bacillus sp. RSAII-1b, and BSF amylolytic bacteria. At the optimum pH, 50% is at pH 7 with Arthrobacter arylaitensis bacteria, Bacillus sp. PA-05 bacteria, Azospirillum sp. JG3, BSF amylolytic bacteria, Bacillus amyloliquefaciens bacteria, Bacillus sp. bacteria and Geobacillus sp. DS3 bacteria, followed by 7% at pH 9, pH 8.5, pH 8, pH 7.5, pH 6, pH 5 and pH 4 with Arthrobacter arylaitensis LBF-003 bacteria, thermo-alkaliphile bacteria, Bacillus subtilis BI19, Pseudomonas stutzeri ISL B5 bacteria, Bacillus sp. RSAII-1b, Bacillus megaterium bacteria and R_2M larvae bacteria.

Keywords: *Amylase Enzyme, Microbial, Optimization, pH, Temperature.*

INTRODUCTION

Amylase is a hydrolase enzyme that hydrolyzes starch into maltose and glucose. The amylase enzyme is an extracellular enzyme that randomly breaks the α-1,4 bonds from the interior of the starch molecule and produces lower molecular weight products such as maltose and glucose (Lily, 2012; Melliawati, 2006). Amylase enzymes have high commercial value, so they are widely used in various industrial fields such as the starch, alcohol, paper, textile, food, and pharmaceutical industries (Sebayang, 2005; Karri et al., 2014). In the food industry, amylase enzymes are utilized for the hydrolysis of starch, and the hydrolysis products can be used for the production of glucose syrup with a relatively high sweetness level, as well as for making bread and baby food (Marsiti et al., 2012).

Amylase enzymes are in high demand in the textile industry, starch hydrolysis, food production (beer, bread, syrup, artificial sweeteners, animal feed industry), ethanol production, detergents, pharmaceuticals, and enzyme supplements. In its working process, the amylase enzyme has the ability to break the glycosidic bonds present in starch. The hydrolysis products are simpler molecules such as glucose, maltose, dextrin, and other organic acids (Palmer & Bonner 2007). The enzyme α-amylase (EC.3.2.11) is known by the names 1,4-α-D-glucanohydrolase and glycogenase (Bernfeld 1955). This enzyme works by randomly breaking the α-1,4-glycosidic bonds in starch, especially in the long chains, resulting in maltotriose, maltose from the amylose polymer in starch, and producing glucose and a small amount of dextrin from the amylopectin polymer that makes up starch. Because of its property of breaking glycosidic bonds randomly, this enzyme works faster compared to other amylases such as β-amylase (Soeka et al., 2015).

Amylase enzymes are enzymes capable of hydrolyzing starch and producing glucose. Amylase enzymes can be produced by all living organisms to catalyze biochemical reactions, allowing these reactions to occur more quickly with a catalyst. Amylase enzymes for industrial needs are extracted from various types of microorganisms, as microorganisms produce enzymes that can be utilized by humans in a highly variable quantity and type. In addition, microorganisms can be cultured to obtain the produced enzymes [(Ningsih et al., 2012); (Istia'nah et al., 2020)].

RESEARCH METHOD

This research uses a narrative review type. The narrative review type aims to identify and summarize previously published articles, avoid research duplication, and seek new fields of study that have not yet been researched (Ferrari, 2015).

RESULTS AND DISCUSSION

In this study, a total of 1,707 articles were obtained, including 35 from PubMed, 182 from Semantic Scholar, and 1,490 from Google Scholar. The search for articles used the keywords enzyme amylase, temperature, pH, microbial, and optimization in English, while in Indonesian, the keywords used were enzim amilase, suhu, pH, mikroba, and optimasi. After the article search process, the articles were selected based on topic relevance, resulting in 189 articles.

The articles were re-selected by reviewing them in their entirety, yielding 14 articles that matched the topic, consisting of 5 English-language journals and 9 Indonesian-language journals. The collected articles consist of 7 articles on Bacillus bacteria and 7 articles on other topics. The analyzed articles are presented in tabular form.

Table 1: Article that has been analyzed

No	Article Title	*Author*/ Year	Bacterial Source	Strain and Isolat	Optimum Temperature	Amylase Activity	Optimum pH	Amylase Activity
1.	Characteristics of the amylase enzyme from the bacterium Bacillus megaterium at variations in temperature, pH, and substrate concentration (Semantic Scholar) (Sinta 3/S3)	(Istia'nah *et al.,* 2020)	*Bacillus megateriu m*		Temperature 37°C	1,279 U/mL	pH 5,0	1,241 U/mL
2.	Optimization of amylase enzyme production from Jakarta marine bacteria (Arthrobacter arilaitensis) (Google	(Purnawan *et al.,* 2015)	*Arthrobacter arilaitensis*		Temperature 30°C	2,4 U/mL	pH 7	2,1 U/mL

	Scholar) (Sinta 2/S2)							
3.	Partial purification and characterization of amylase from the marine bacterium Arthrobacter arilaitensis LBF-003 (Google Scholar) (Sinta 2/S2)	(Rahmasari *et al.,* 2016)	*Arthrobacter arilaitensis* LBF-003	Strain LBF-003	Temperature 50°C	0,037 U/mL	pH 9	0,025 U/mL
4.	The potential of Bacillus sp. PA-05 as an obligate thermophile for amylase production (Semantic Scholar)	(Agustien *et al.,* 2013)	*Bacillus sp.* PA-05	Strain PA-05	Temperature 60°C	2,4 U/mL	pH 7	2,8 U/mL
5.	Optimization of amylase activity from thermo-alkaliphilic bacteria (Google Scholar)	(Indriati *et al.,* 2015)	Bakteri termo-alkalifil		Temperature 60°C	48,92 U/mL	pH 8,5	39,27 U/mL
6.	The production of dextrin from cassava starch using amylase catalyst derived from the fractionation of Azospirillum sp. JG3 (Semantic Scholar) (Sinta 1/S1)	(Zusfahair *et al.,* 2012)	*Azospirillum* sp. JG3	Strain JG3	Temperature 30°C	11,934 U/mL	pH 7	11,980 U/mL
7.	Amylase activity of amylolytic bacteria from black soldier fly larvae (Hermetia illucens) (Semantic	(Kresnawaty *et al.,* 2019)	Bakteri amilolitik BSF		Temperature 45°C	0,039 U/mL	pH 7	0,039 U/mL

	Scholar) (Sinta 2/S2)							
8.	Characteristics of the amylase enzyme from the bacterium Bacillus amyloliquefaciens (Google Scholar)	(Ningsih *et al.,* 2012)	*Bacillus amyloliquefaciens*		Temperature 40°C	0,0483 U/mL	pH 7	1,4986 U/mL
9.	Characterization of amylase enzyme from R_2M bacterial isolate of sago beetle larvae from North Luwu (Google Scholar)	(Baharuddin *et al.,* 2022)	Bakteri R_2M larva kumbang sagu	Isolat R_2M	Temperature 70°C	0,116 U/mL	pH 4	0,049 U/mL
10.	Optimization of the medium for the production of extracellular amylase by the *Pseudomonas stutzeri* ISL B5 isolated from municipal solid waste (*Pubmed*)	(Dutta *et al.,* 2016)	*Pseudomonas stutzeri* ISL B5	Strain ISL B5	Temperature 40°C	2,63 U/mL	pH 7,5	2,59 U/mL
11.	Molecular identification of a newly isolated *Bacillus subtilis* BI19 and optimization of production conditions for enhanced production of extracellular amylase (*Pubmed*)	(Dash *et al.,* 2015)	*Bacillus subtilis* BI19	Strain BI19	Temperature 37°C	7,25 U/mL	pH 8,0.	7,77 U/mL
12.	Production optimization and characterizati	(Arfah *et al.,* 2015)	*Bacillus sp* RSAII-1b	Strain RSAII-1b	Temperature 55°C.	0,1647 U/mL	pH 6,0	0,1637 U/mL

	on of amylase enzyme isolated from termofil *Bacteria Bacilus* sp RSAII-1b from lejja hot spring south sulawesi (*Google Scholar*)							
13.	Amylase production by *Bacillus* sp. using cassava as substrate (*Google Scholar*)	(Senthilkumar *et al.,* 2012)	*Bacillus* sp.		Temperature 60°C	3,9 U/mL	pH 7	3,8 U/mL
14.	Purification and characterization of thermostable alpha-amylase from *Geobacillus* sp. DS3 from Sikidang Crater, Central Java, Indonesia (*Google Scholar*)	(Widiana *et al.,* 2022)	*Geobacillus* sp. DS3	Strain DS3	Temperature 50°C	1,00 U/mL	pH 7	1,40 U/mL

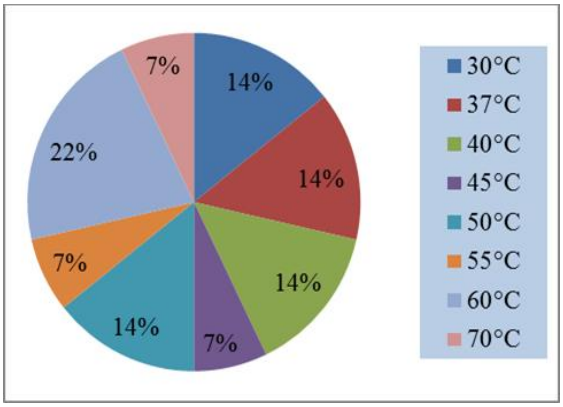

Figure 1. Percentage Variation of Optimal Temperature for Amylase Enzyme in Bacteria

As seen in Figure 1, the variation in the optimal temperature for amylase enzymes in bacteria is categorized into several optimal temperature ranges, which are between 30°C and 70°C with percentages from 7% to 22%. The lowest optimal temperature category is at 30°C with a percentage of 14% for the bacteria Arthrobacter arilaitensis and Azospirillum sp. JG3. The next optimal temperature category is 37°C with a percentage of 14% for the bacteria Bacillus megaterium and Bacillus subtilis BI19. The next optimal temperature category is 40°C with a percentage of 14% for the bacteria Bacillus amyloliquefaciens and Pseudomonas stutzeri ISL B5. The next

optimal temperature category is 45°C with a percentage of 7% for the amylolytic bacteria BSF. The next optimal temperature category is 50°C with a percentage of 14% for the bacteria Arthrobacter arilaitensis LBF-003 and Geobacillus sp. DS3. The next optimal temperature category is 55°C with a percentage of 7% for the bacteria Bacillus sp. RSAII-1b. The next optimal temperature category is 60°C with a percentage of 22% for the bacteria Bacillus sp. PA-05, thermophilic-alkaliphilic bacteria, and Bacillus sp. The highest optimal temperature category is at 70°C with a percentage of 7% for the bacteria R_2M larvae of the sago beetle (Table 1).

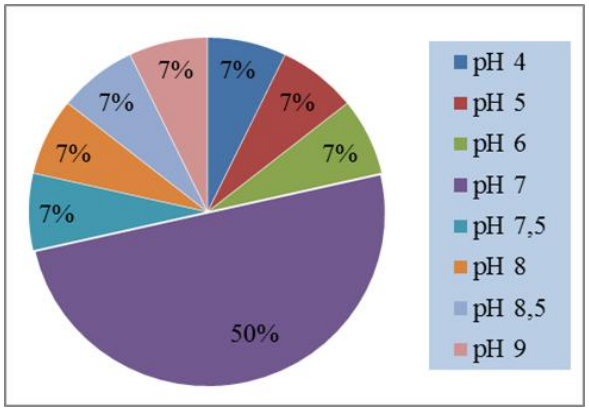

Figure 2. Percentage Variation of pH Optimization of Amylase Enzyme in Bacteria

As seen in Figure 2, the variation of pH for amylase enzyme optimization in bacteria is categorized into several different optimum pH categories, ranging from pH 4 to pH 9 with percentages from 7% to 50%. The lowest optimum pH category is at pH 4 with a percentage of 7% for the R_2M sago beetle larvae bacteria, the next optimum pH category is pH 5 with a percentage of 7% for Bacillus megaterium bacteria, the next optimum pH category is pH 6 with a percentage of 7% for Bacillus sp. RSAII-1b bacteria, the next optimum pH category is pH 7 with a percentage of 50% for Arthrobacter arilaitensis, Bacillus sp. PA-05, Azospirillum sp. JG3, amylolytic BSF bacteria, Bacillus amyloliquefaciens, Bacillus sp., and Geobacillus sp. DS3 bacteria, the next pH category is pH 7.5 with a percentage of 7% for Pseudomonas stutzeri ISL B5 bacteria, the next optimum pH category is pH 8 with a percentage of 7% for Bacillus subtilis BI19 bacteria, the next pH category is pH 8.5 with a percentage of 7% for thermo-alkaliphilic bacteria, and the highest optimum pH category is at pH 9 with a percentage of 7% for Arthrobacter arilaitensis LBF-003 bacteria (Table 1).

Based on the results of the article collection that have undergone selection, the optimum temperature and pH of amylase enzymes from bacteria in each journal can be seen in Table 1.

Temperature greatly affects enzyme activity because enzymes are chains of amino acids whose functioning is closely related to environmental temperature (Tarigan et al., 2015). At the optimum temperature, the reaction between the substrate and the enzyme is very effective, thereby increasing the product yield (Istia'nah et al., 2020).

Table 1 shows several types of bacteria with different optimum temperatures and pH levels for amylase enzymes; these bacteria can be classified into Gram-positive and Gram-negative bacteria. Gram-positive bacteria are bacteria that absorb the crystal violet dye, which is blue-purple in color. Their cell walls are partially composed of peptidoglycan, making the cell walls rigid, and they also consist of polysaccharides and teichoic acids. The structure of their cell walls is thick, around 15-80 nm, single-layered, more susceptible to penicillin, and their cell shapes are round, rod, or filamentous. They are chemoorganoheterotrophic, motile, aerobic, and some groups form endospores while others do not. Examples of Gram-positive bacteria in the article include Bacillus megaterium, Arthrobacter arilaitensis, Arthrobacter arilaitensis LBF-003, thermo-alkaliphilic bacteria, Bacillus sp. PA-05, Bacillus sp. RSAII-1b, Bacillus sp., Bacillus amyloliquefaciens, Bacillus subtilis BI19, and Geobacillus sp. DS3. Meanwhile, Gram-negative bacteria are those that absorb the red dye safranin, contain less peptidoglycan and have a high lipid content, do not have teichoic acid, possess a double plasma membrane covered by a permeable outer membrane, have a thin cell wall structure of 10-15 nm, are more resistant to antibiotics, are round, oval, and comma-shaped, are phototrophic, motile, non-motile, aerobic, anaerobic, and do not form endospores. Examples of Gram-negative bacteria in the article include Azospirillum sp. JG3, amylolytic BSF bacteria (Proteus sp.), and Pseudomonas stutzeri ISL B5 (Rini et al., 2020).

The optimum temperature of amylase also varies (Table 1), and the differences in the optimum temperature of the amylase enzyme can be attributed to the characteristics of the bacteria producing it, where generally mesophilic bacteria will produce enzymes that are also mesophilic, and thermophilic bacteria will produce enzymes that are also thermophilic. Based on the range of optimum growth temperatures, bacteria can be classified into three groups: psychrophilic, mesophilic, and thermophilic. Psychrophilic bacteria are extremophilic organisms that can grow at low temperatures, living in cold temperatures ranging from -5°C to 30°C, and can thrive best in the optimal temperature range of 10°C-20°C. Mesophilic bacteria are organisms that grow best at moderate temperatures, neither too hot nor too cold, and can live at temperatures ranging from 10°C to 45°C, thriving at optimal temperatures of 20°C-40°C. Thermophilic bacteria are organisms that thrive at high temperatures, living well at temperatures ranging from 25°C to 80°C, and can grow at optimal temperatures of 50°C-80°C (Rini et al., 2020).

The optimum temperature for amylase enzymes from various bacteria varies, as seen from the range of optimum temperatures. For mesophilic bacteria, examples include Bacillus megaterium at 37°C with an activity of 1.279 U/mL, Arthrobacter arilaitensis at 30°C with an activity of 2.4 U/mL, Bacillus subtilis BI19 at 37°C with an activity of 7.25 U/mL, Bacillus amyloliquefaciens at 40°C with an activity of 0.0483 U/mL, Azospirillum sp. JG3 at 30°C with an activity of 11.934 U/mL, Pseudomonas stutzeri ISL B5 at 40°C with an activity of 2.63 U/mL, and amylolytic BSF bacteria at 45°C with an activity of 0.039 U/mL. For thermophilic bacteria, examples include Arthrobacter arilaitensis LBF-003 at 50°C with an activity of 0.037 U/mL, Bacillus sp. PA-05 at 60°C with an activity of 2.4 U/mL, thermo-alkaliphilic bacteria at 60°C with an activity of 48.92 U/mL, R_2M larvae of sago beetles at 70°C with an activity of 0.116 U/mL, Bacillus sp RSAII-1b at 55°C with an activity of 0.1647 U/mL, Bacillus sp at 60°C with an activity of 3.9 U/mL, and Geobacilus sp. DS3 at 50°C with an activity of 1.00 U/mL. In psychrophilic bacteria, there are no articles discussing low or cold temperatures.

The optimum temperature for amylase enzymes varies, with some at 30°C and others at 70°C, due to the characteristics of the bacteria producing them and their environment. The 30°C optimum amylase is classified as mesophilic bacteria that thrive at moderate temperatures, such as in marine environments or soil-dwelling bacteria. In contrast, the 70°C optimum amylase is classified as thermophilic bacteria that can grow well at high temperatures, such as in hot spring environments. The optimum temperature is related to the enzyme activity that increases with the rise in temperature until it reaches its optimum. This occurs due to the increase in kinetic energy, which enhances the chances of collisions between the enzyme and substrate to react and form the enzyme-substrate complex, thereby increasing the product yield. A rise in temperature beyond the optimum causes a decrease in activity, as the enzyme undergoes denaturation due to high heat. High temperatures will break secondary bonds such as hydrogen bonds that maintain the enzyme at its natural temperature, causing the secondary and tertiary structures of the enzyme to partially degrade, followed by a decrease in activity (Zusfahair et al., 2012).

The variation in the optimum pH value of the amylase enzyme is caused by the characteristics of the bacteria producing it or the type of bacteria and its environment. Generally, bacteria live in environments with a neutral pH value (6.6-7.5), but each bacterium has a minimum pH, an optimum pH, and a maximum pH. Based on the range of optimum pH values for their growth, bacteria are classified into three groups: acidophilic bacteria, neutrophilic bacteria, and alkaliphilic bacteria. Acidophilic bacteria are groups of bacteria that thrive in acidic pH, ranging from pH 2.0-5.0. Neutrophilic bacteria are groups of bacteria that thrive in neutral pH, ranging from pH 5.5-8.0. Alkaliphilic bacteria are groups of bacteria that thrive in alkaline pH, ranging from pH 8.5-9.5 (Rini et al., 2020).

The review results showed that the optimum pH of amylase enzymes from various bacteria differed, with acidophilic bacteria such as Bacillus megaterium and R_2M larvae of the sago beetle. Neutrophilic bacteria such as Arthrobacter arilaitensis, Bacillus sp. PA-05, Azospirillum sp. JG3, amylolytic BSF bacteria, Bacillus amyloliquefaciens, Pseudomonas stutzeri ISL B5, Bacillus subtilis BI19, Bacillus sp RSAII-1b, Bacillus sp, and Geobacilus sp. DS3. In contrast, examples of alkaliphilic bacteria are Arthrobacter arilaitensis LBF-003 and thermo-alkaliphilic bacteria.
The optimum pH value is related to its activity because enzyme activity will be optimal if there is a balance between its charges, and the enzyme will denature if it is at a pH far from its optimum pH, due to structural changes in the enzyme that will affect enzyme activity (Bollag, 1996).

The results of amylase enzyme activity influenced by temperature varied, ranging from 0.037 U/mL to 48.92 U/mL, and the activity of amylase influenced by pH ranged from 0.025 U/mL to 39.27 U/mL. This is because the substrate concentration in the bacteria varies. If the substrate used is extracted from natural materials such as cassava peel, the activity results are low. This is because the substrate has not yet been completely separated

from its matrix, or the substrate still contains other components, such as cassava peel, which may also contain cellulose that cannot be hydrolyzed by amylase. If the substrate used is pure, then the enzyme activity results are high. Enzymes work very selectively and sensitively, so they function more optimally when using pure substrates (Kresnawaty et al., 2019). The enzyme activity at the optimum temperature and pH of bacteria varies, and the differences in enzyme activity among these bacteria are influenced by environmental factors. Changes occurring in the environment can result in alterations in the morphological and physiological properties of microorganisms (Zuraidah et al., 2020).

Enzyme activity is the ability of an enzyme to convert a certain number of moles of substrate into product per unit of time (Ramadhan et al., 2021). The specific activity of an enzyme is a measure of the enzyme's purity, and its value increases during the purification of an enzyme, reaching a maximum and remaining constant when the enzyme is in its pure state. The specific activity of an enzyme is defined as the number of enzyme units per milligram of protein (Lehninger, 1982). Meanwhile, the total enzyme activity is the enzyme extract activity per unit volume multiplied by the total volume of the protein solution (Siregar, 2011). The factors that influence enzyme activity include: Temperature, enzymes have a specific temperature that causes their activity to reach an optimum. When the temperature increases to the optimum temperature, the reaction rate will rise because the kinetic energy increases. The increase in temperature will also increase the frequency of collisions between enzyme molecules and substrates, making the enzyme active. An increase in temperature beyond the optimum temperature can cause the enzyme to denature and deactivate its catalytic activity. Temperatures exceeding the optimum limit will cause the substrate to change its conformation, preventing it from entering the active site of the enzyme. This will result in decreased enzyme activity because the enzyme-substrate complex does not form, leading to a low enzyme concentration (Tazkiah et al., 2017).

The pH value will cause changes in the charge of the enzyme molecules, which can affect enzyme activity, either thru structural changes or changes in the charge of amino acid residues that function to bind the substrate. For example, an enzyme with a negative charge reacts with a substrate with a positive charge to form an enzyme-substrate complex, then at a low pH value, the enzyme will be protonated and lose its negative charge. The same thing happens at high pH; the substrate will ionize and lose its positive charge. The change in H^+ ions present in the enzyme solution affects the catalytic site and the conformation of the enzyme. Conditions of too low or too high pH cause changes in enzyme conformation, thereby reducing enzyme activity (Dali et al., 2013). A high concentration of enzymes results in an optimal ability of the enzymes to degrade substrates, leading to an increase in the produced products. Conversely, a low concentration of enzymes results in a less than optimal ability of the enzymes to degrade substrates, leading to a lower yield of products (Insani et al., 2013).

Substrate concentration affects the reaction rate. A substrate concentration that is too low can result in a low reaction rate, but the speed will increase with the increase in substrate concentration. At the maximum reaction, the enzyme becomes saturated with its substrate and cannot function quickly (Ramadhan et al., 2021). Activator is a compound or ion that can increase the rate of enzymatic reactions up to a certain amount to prevent the presence of inhibitors (Soeka, 2015). If the activator is too large, it will cause competition between free activators and the activator-substrate complex against the enzyme (Lestari et al., 2011).

Inhibitor, it has been explained that the mechanism of enzymes in a reaction is thru the formation of the enzyme-substrate complex. Therefore, inhibition or hindrance in a reaction that uses an enzyme as a catalyst can occur if the binding of the substrate to the enzyme is impeded. Molecules or ions that can inhibit a reaction are called inhibitors. The inhibition used by inhibitors can be in the form of reversible inhibition. Irreversible inhibition is caused by the destruction or modification of a functional group present in the enzyme molecule. Reversible inhibition, on the other hand, involves the binding of the inhibitor molecule without causing a conformational change in the enzyme. Allosteric inhibition, the formation of a bond between the enzyme and the inhibitor, affects the conformation of the enzyme, causing the active site to change shape. As a result, the binding of the substrate to the active site of the enzyme is inhibited (Ischak et al., 2017).

CONCLUSIONS

Based on the results of the literature review conducted (the number of journals reviewed is 14 journals), it can be concluded that the highest percentage of optimum temperature is 22%, found in Bacillus sp. and thermo-alkaliphilic bacteria at 60°C, followed by a percentage of 14% at 50°C, 40°C, 37°C, and 30°C with Arthrobacter arilaitensis, Geobacillus, Bacillus amyloliquefaciens, Pseudomonas stutzeri, Bacillus megaterium, Bacillus subtilis, and Azospirillum bacteria. The lowest percentage of optimum temperature is 7%, found at 70°C, 55°C, and 45°C with R_2M larvae, Bacillus sp., and BSF amylolytic bacteria. The highest percentage of optimum pH is 50%, found at pH 7 with Arthrobacter arilaitensis, Bacillus sp., Azospirillum, BSF amylolytic bacteria, Bacillus

amyloliquefaciens, and Geobacillus bacteria, followed by a percentage of 7% at pH 9, pH 8.5, pH 8, pH 7.5, pH 6, pH 5, and pH 4 with Arthrobacter arilaitensis, thermo-alkaliphilic bacteria, Bacillus subtilis, Pseudomonas stutzeri, Bacillus megaterium, and R_2M larvae bacteria.

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