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Isolation and Characterization of Cellulase Enzyme from Sago Bettle Larvae

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Abstract: Sago beetle larvae are larvae that consume cellulose and convert it into simple compounds with the help of cellulase enzymes. The enzyme is produced by bacteria found in the larvae's intestines. This study aims to characterize the cellulase enzyme from R8W bacteria, which is a cellulolytic bacterium and derived from sago beetle larvae. The characterization in this research included determination of the optimum temperature, the optimum pH, and the optimum substrate concentration of the enzyme. The methods in this study consisted of the production of enzymes; characterization of cellulase enzyme by DNS method; and measurement of cellulase enzyme activity on natural substrates. The results showed that the cellulase enzyme R8W bacterial isolates from beetle larvae were in optimum conditions respectively at a temperature of 50 °C (enzyme activity of 0.070 U/mL), pH 8 (enzyme activity of 0.069 U/mL), substrate concentration of 2% (enzyme activity of 0.063 U/mL); cellulase enzyme activity of R8W bacterial isolates from sago beetle larvae on rice husk cellulose as a natural substrate was 0.103 U/mL. The characteristics of the cellulase enzyme of R8W bacterial isolate from sago beetle larvae had an optimum temperature of 50 °C, an optimum pH of 8, and an optimum substrate concentration of 2%.

Keywords: cellulase enzyme, cellulolytic bacteria, characterization, DNS method, sago bettle larvae

Abstrak: *Larva kumbang sagu merupakan larva yang mengkonsumsi selulosa dan mengubahnya menjadi senyawa sederhana dengan bantuan enzim selulase. Enzim ini diproduksi oleh bakteri yang ditemukan di usus larva. Penelitian ini bertujuan untuk mengkarakterisasi enzim selulase dari isolat R8W yang merupakan bakteri selulolitik dan berasal dari larva kumbang sagu. Karakterisasi dalam penelitian ini meliputi penentuan suhu optimum, pH optimum, dan konsentrasi substrat optimum enzim. Metode dalam penelitian ini terdiri dari produksi enzim; karakterisasi enzim selulase dengan metode DNS; dan pengukuran aktivitas enzim selulase pada substrat alami. Hasil penelitian menunjukkan bahwa isolat bakteri enzim selulase R8W dari larva kumbang berada pada kondisi optimum masing-masing pada suhu 50 °C (aktivitas enzim 0,070 U/mL), pH 8 (aktivitas enzim 0,069 U/mL), konsentrasi substrat 2% (aktivitas enzim 0,063 U/mL); Aktivitas enzim selulase isolat bakteri R_8W larva kumbang sagu pada selulosa sekam padi sebagai substrat alami adalah 0,103 U/mL. Karakteristik enzim selulase isolat bakteri R8W dari larva kumbang sagu memiliki suhu optimum 50 °C, pH optimum 8, dan konsentrasi substrat optimum 2%. Dengan demikian, enzim selulase dari isolat bakteri R8W mampu mendegradasi substrat dengan konsentrasi 2% secara optimal pada suhu 50°C dan pH 8.*

Kata kunci: *bakteri selulolitik, enzim selulase, karakterisasi, larva kumbang sagu, metode DNS*

INTRODUCTION

The sago beetle larvae originate from the coconut red beetle growing on sago palms. Sago plants that have been cut down produce sago palm trunks which are the habitat for sago beetles. The larvae of the red coconut beetle are known as sago caterpillars (Rhynchophorus ferrugineus) (Ariani *et al*. 2018) (Figure 1).

Besides having an amylase enzyme working at 70°C; pH 4; and substrate concentration of 1.25% (Baharuddin *et al*. 2022), sago caterpillar larvae (*Rhynchophorus ferrugineus*) consume cellulose by converting cellulose into simple compounds with the help of cellulase enzymes in the larvae's digestion. The cellulase enzymes are obtained from bacteria or fungi that have the potential to produce enzymes. Intestinal microorganisms are one of the components of the body containing cellulolytic bacteria. Wahyuningsih (2020) reported that cellulolytic bacteria could be isolated from the intestines of sago beetle larvae. The results obtained five isolates, namely R_1W, R_4W, R_8W, R_9W, and $R_{10}W$. Isolates

Figure 1. The sago beetle larvae

of R8W has cellulase enzyme activity at 5,1425 × 10^{-2} U/mL.

Cellulase is composed of three main enzymes working synergistically in the degradation process of cellulose. These three enzymes include endoglucanase, exoglucanase or cellobiohydrolase and β-glucosidase (Thomas *et al*. 2018). Endoglucanase functions to randomly break β-(1,4) glycoside bonds in the amorphous cellulose structure to form a cellulose molecule with a simple structure. Exoglucanase plays a role in the process of severing two glucose units from the ends of reducing and non-reducing sugars to form cellobiose molecules. β-glucosidase functions to hydrolyze cellobiose into glucose (Behera *et al*. 2017).

The working activity of a cellulase enzyme in hydrolyzing cellulose is influenced by several factors such as temperature, pH, and substrate. The cellulase enzyme acts in forming a specific activity. The specific activity of cellulase enzymes describes the level of purity of an enzyme. The higher the specific activity of the enzyme, the higher the purity of it. The specific activity of the enzyme is the amount of substrate broken down by the enzyme. Cellulase enzyme activity is determined based on the amount of glucose produced from the breakdown of cellulose by the enzymes. The temperature factor affects the contact of the enzyme with the substrate. Enzymes are a series of amino acids whose working system is strongly influenced by environmental temperature. The pH and the substrate factors have a role in activating the active site of the enzyme in the hydrolysis process. The optimum pH is used to activate the active site of the enzyme binding to the substrate. An enzyme-substrate complex occurs when the affinity of the enzyme is equal to the number of substrate molecules. Cellulase enzyme activity increases with accreting substrate concentration.

The working of the enzyme activity is affected of temperature, pH, and substrate concentration. These factors are its characteristic of the enzyme. The influence of the optimum pH, temperature, and substrate concentration will maintain the stability of the enzyme work. Enzyme stability is needed in the use of enzymes in various fields, one of which is in the industrial sector. Enzymes are served in the industrial sector to produce higher quality products (Gao *et al*. 2018). Hafid *et al*. (2017) reported, Enzymatic hydrolysis was carried out to produce glucose which can be further fermented into bioethanol.

Based on the above background, the stability of enzymes in the hydrolysis process is influenced by various factors including temperature, pH, and substrate concentration. Therefore, a study was conducted to characterize the cellulase enzyme isolates R_8W bacteria from sago beetle larvae. This study aims to determine the optimal condition of the cellulase enzyme in degrading the substrate. Futhermore, the stability of enzymes in the hydrolysis process is influenced by various factors including temperature, pH, and substrate concentration. In this study, these factors were used to characterize the cellulase enzyme R8W bacterial isolate from sago beetle larvae. This study aims to determine the optimal condition of the cellulase enzyme in degrading its substrate.

MATERIALS AND METHOD

Materials

$R8_W$ bacterial isolate obtained from the larvae of the sago beetle (Rhynchophorus) in Tulak Tallu Village, Sabbang District, North Luwu Regency, South Sulawesi (Wahyuningsih, 2020). In addition, several materials were used, including distilled water; alcohol p.a (Merck, Jerman); acetate buffer; phosphate buffer; Tris-HCl buffer; yeast extract (Merck, Jerman); glucose (Merck, Jerman); potassium nitrate (Merck, Jerman); carboxymethyl cellulose p.a; magnesium sulfate (Merck, Jerman); monopotassium phosphate (Merck, Jerman); sodium potassium tatrate ($KNaC_4H_4O_6.4H_2O$) p.a, nutrient agar (NA), nutrient broth (NB), sodium hydroxide; sodium hypochlorite; sodium sulfite. Spectrophotometer UV-Vis *Geneys* 20 (Varian, Amerika Serikat); centrifugator Z 366 K (*Hermele*, Jerman), *Shaker MASQ 7000* (Thermo Scientific, Amerika Serika), incubator *hareus* (Thermo Scientific, Jerman), oven GmbH (*Memmert*, Jerman), autoclave *yx-280 D* (*GEA*, Jerman*), laminar air flow Isocide* 14644-1 (Esco, Singapura), *magnetic stirrer* (*Health*, Amerika Serikat), *sieve shaker AS* 200 (*Retsch*, Amerika Serikat), vortex mixer wizard (Velp Scientifica, Italia), analytical scale *ABS* (*Kern*, Jerman), waterbath *SH-31* (*Maspio*n, Indonesia).

Cellulase enzyme production from microbial isolates of sago beetle larva

About 5×10^{-7} sel/mL of R_8W bacterial isolates of sago beetle larvae were taken from agar media. The isolates were transferred to the inoculum medium aseptically. Then, the inoculum was homogenized with a shaker at room temperature and at a speed of 180 rpm for 24 hours (Azizah 2017).

Inoculum media was inserted into the production medium as much as 75 mL. Then, it was homogenized with a shaker at room temperature and a speed of 180 rpm for 48 hours. The mixture was centrifuged at a speed of 4500 rpm and a temperature of 4 °C for 10 minutes in a cold centrifuge. The supernatant was taken as a crude extract of the cellulase enzyme.

Preparation of glucose standard solution

Glucose standard solution was prepared with 1 mg/mL main glucose solution. This solution was prepared with 0.025 g of glucose.

Characterization of cellulase enzyme crude extract

Determination of optimum temperature

A mixture of 1% CMC and a pH 7 phosphate buffer solution of 2 mL each was put into five test tubes. The mixture was added with 2 mL of crude extract of cellulase enzyme. Then the mixture was incubated at different temperatures (30-70) °C for 15 minutes. Next, 2 mL of DNS reagent was added to the mixture and was heated in boiling water for 10 minutes. The mixture was then cooled for 10 minutes. Then the absorbance of the mixture was measured on a UV-Vis spectrophotometer with a wavelength (λ) of 540 nm (Novitasari & Putri 2016).

Determination of optimum pH

One percent of CMC solution about 2 mL was put into five tubes. The solution in these tubes was added 2 mL of buffer solution with the variation of pH (acetate buffer pH 5; phosphate buffer pH 6, 7 and 8; Tris-HCl buffer pH 9). Furthermore, the mixture was added to the crude extract. Measurement of enzyme activity was carried out by DNS method. The DNS (Dinitrosalicylic Acid) method is used to measure reducing sugar in solution. In principle, reducing sugar oxidizes aldehyde or ketone groups, while DNS is reduced to a red-orange compound (3-amino-5-nitrosalicylic acid). This reaction is accelerated by heating and base (NaOH). The intensity of the color formed is measured by a spectrophotometer at 540 nm, then compared with a standard curve to calculate the sugar concentration. This method is simple, sensitive, and suitable for the analysis of reducing sugar, although less specific.

Detemination of optimum substrate concentration

About 2 mL of crude enzyme extract was reacted with 2 mL of CMC substrate with various concentrations of 0.5%, 1%, 1.5%, 2% and 2.5%. The mixture was added with 2 mL of phosphate buffer pH 7. Measurement of enzyme activity was carried out by DNS method.

Determination of cellulase enzyme crude extract activity to nature substrate

Rice husks were dried in the sun. After that, they were crushed. Then, the delignification process was carried out using 10% of NaOH for 90 minutes at 55 °C. The results were filtered and the residue was soaked with 8% NaOCl for 1 hour using a magnetic stirrer. The precipitate was washed with distilled water until the its pH was neutral (the test utilized litmus paper). Then, the precipitate was dried in an oven at 50 °C for 7 hours (Pramana *et al*. 2016).

Rice husk cellulose powder was dissolved with optimum substrate concentration. The addition of 2 mL of optimum pH buffer and 2 mL of crude extract enzyme was carried out on the cellulose substrate in a 100 mL beaker. The mixture was incubated for 15 minutes at the optimum temperature (Baharuddin 2016).

Measurement of enzyme activity was carried out by DNS method. Cellulase activity in the hydrolysis process is based on the amount of reducing sugar. One unit of enzyme activity is defined as the amount of enzyme that produces 1 mole of glucose per minute (U/mL) which is expressed in the equation:

$$AE = \frac{[glu]}{Mr\ glu} x \frac{F_p}{V_{enz}} x \frac{V_{subs}}{t} x\ 1000 \mu mol\ mmol^{-1}$$

Which [*glu*] is concentration of glucose; *Mr glu* is relative mass of glucose; F_p is dilution factor; V_{enz} is enzyme volume; $V_{substrate}$ is substrate volume; t is time of incubation.

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RESULT AND DISSCUSION

Optimum temperature of crude extract cellulase enzyme

Based on the test results, the optimum temperature of the crude extract of the cellulase enzyme from bacterial isolates of sago beetle larvae was obtained at a temperature of 50 °C using pH 7 and and 1% of CMC substrate. The result can be seen in Table 1.

Cellulase enzymes work well at optimum conditions. The optimum conditions for enzymes to form enzyme complexes with substrates are affected by temperature. Temperature affects the rate of catalyst in an enzymatic reaction. When the optimum temperature is reached, the reaction rate increases due to the increase in kinetic energy. An increase in kinetic energy will increase the chances of the formation of an enzyme-substrate complex (Putri & Wardani 2018).

Changes in temperature can increase and decrease enzyme activity. If the enzyme exceeds the optimum temperature limited then the enzyme activity

decreases. The decrease in enzyme activity due to temperatures above the optimum temperature can damage the protein structure in the enzyme.

Figure 2 showed that the temperature of 50 °C crude extract of the cellulase enzyme worked optimally with an activity of 0.070 UmL^{-1}. Basically, the enzymes produced by a microbe work optimally at a certain temperature. Cellulase enzymes generally have optimum activity at a temperature of 20 °C to 50 °C. Enzymes that work at these temperature conditions are enzymes from the mesozyme group. Kusumaningrum et al. (2019) stated that enzymes that work at 50-80 °C are thermozyme or thermostable enzymes. Based on this, the bacterial isolate R_8W cellulase enzyme was suspected to belong to the mesozyme group.

Optimum pH of crude extract cellulase enzyme

Enzyme is a protein that has biochemical activity. Enzymes also act as catalysts in a reaction. Cellulase enzymes require certain pH conditions in enzymatic reactions. The optimum pH of cellulase enzyme from bacterial isolates of sago beetle larvae was pH 8 at 37 °C and at 1% of CMC substrate. The result can be seen in Table 2.

Changes in pH conditions will affect the effectiveness of the active site of the enzyme. The active site of the enzyme plays an important role in the formation of the enzyme-substrate complex (Irawati 2016). Changes in pH also affect the level of enzyme activity. High enzyme activity was determined based on the active group of the enzyme side chain. This active group functions as a catalytic site in binding the substrate. Changes in pH affect the ionization process of amino acid side groups in enzymes. Enzymes work optimally under certain conditions that can increase their activity. In this study, the effect of pH on cellulase enzyme activity of R8W bacterial isolates can be seen in Figure 3.

Table 1. Effect of temperature variations on cellulase enzyme activity

	T (ºC)	A	[glucose] (mg/mL)	EA (U/mL) ± SE
enzyme activity	30	0.296	0.133	0.050±0.004
	40	0.322	0.143	0.053±0.002
	50	0.432	0.189	0.070±0.002
	60	0,422	0.185	0.068±0.003
	70	0,357	0.155	0.058±0.004

Notes *:T* is temperature; *A* is absorbance; *[glucose]* is concentration of glucose; *EA* is enzyme activity

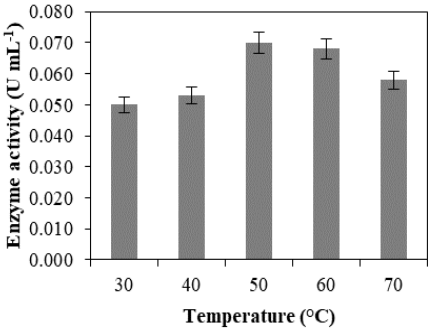

Figure 2. Correlation between temperature and enzyme activity

Tabel 2. Effect of pH variations on cellulase enzyme activity

pH	A	[glucose] (mg/mL)	EA (U/mL) ± E
5	0.144	0.071	0.026±0.001
6	0.275	0.115	0.042±0.005
7	0.285	0.128	0.047±0.001
8	0.426	0.186	0.069±0.005
9	0.384	0.169	0.063±0.007

Notes *: A* is absorbance; *[glucose]* is concentration of glucose; *EA* is enzyme activity

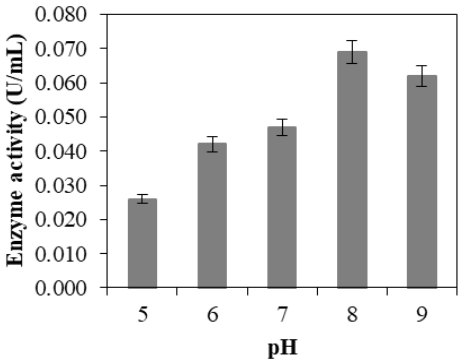

Figure 3. Correlation between pH and enzyme activity

Enzyme activity increases as the pH value increases. Cellulase enzyme activity was optimal at pH 8 with an enzyme activity value of 0.069 U/mL and enzyme activity decreased at pH 9. The pH value above the optimum pH will decrease the enzyme activity. An inappropriate change in pH will cause the enzyme denaturation process to occur (Prima *et al*. 2015). The pH value above the optimum pH also causes the enzyme conformation to change. The change occurs due to the breaking of bonds in the enzyme protein that maintains the tertiary structure (Hamzah 2018).

Enzyme denaturation will affect the high or low power of the enzyme catalyst. Enzymes have an active group that is negatively and positively charged. When it reaches the optimum pH, the activity will increase because a balance between the two charges of the active group of the enzyme occurs. The charge of the active group of the enzyme in an acidic environment tends to be positive and in an alkaline environment it tends to be negative so that the value of the enzyme activity decreases (Dini & Munifah 2014).

Each enzyme has an optimum pH to reach its maximum activity. The active site of the enzyme is only active in the optimum pH range. In the curve above the optimum pH area for the cellulase enzyme from the bacterial isolate R_8W was at pH 7 to pH 9 and the optimum was at pH 8. This is in line with the research of Alam *et al*. (2013), the cellulase enzyme activity of thermophilic bacteria isolated from agricultural compost was optimal at pH 8 with an enzyme activity of 1,623 U/mL. Irawati (2016) obtained pH 8 as the optimal pH were cellulase enzymes from Bacillus Circulans with an enzyme activity value of 0.021 U/mL, cellulase enzymes were isolated from the desert (Sonia & Kusnadi 2015). The results of this study are different from those of Purkan *et al*. (2015), the activity of the cellulase enzyme derived from Aspergillus niger is pH 4, the cellulase enzyme derived from seaweed waste is pH 5 (Dini & Munifah 2014).

The addition of an acid or base will result in a change in the amino acid. Changes in amino acids make enzyme activity decrease. Novitasari & Putri (2016) informed that enzyme solutions under acidic and high alkaline conditions formed zwitter ions. This condition is characterized by a decrease in enzyme activity due to the absence of the active site of the enzyme that will bind to the substrate.

Optimum concentration substrate of cellulase enzyme of R_8W bacterial isolate from sago battle larvae

The optimum substrate concentration of cellulase enzyme from bacterial isolates of sago beetle larvae was 2% using conditions of 37°C and pH 7. The complete results can be seen in Table 3.

Substrate is an important factor in the mechanism of enzyme action. Enzyme activity is determined by the number of substrates that can be hydrolyzed. High substrate concentration will increase the rate of enzyme catalyst to the maximum speed. When the maximum speed is reached, the excess substrate concentration does not increase the reaction rate. Excessive substrate concentration results in the saturation of the catalytic site of the enzyme. This is caused by the absence of the active site of the enzyme that binds to the substrate to form an enzyme-substrate complex (Oktavia *et al*. 2014). Enzyme activity is strongly influenced by the balanced amount of substrate. In this study, cellulase enzyme activity which is influenced by variations in substrate concentration can be seen in Figure 4.

The enzyme-substrate complex is formed by contacting the enzyme with the substrate at the active site of the enzyme. High substrate concentrations will increase enzyme activity and will decrease at certain concentrations (Prastika 2018). The decrease in enzyme activity at a certain concentration was caused by the active site of the enzyme bound to the substrate. The low substrate concentrations only form a small amount of enzyme complex, while high substrate concentrations cause the active site of the enzyme to bind more to form a substrate enzyme complex. Based on the above curve, the enzyme activity increased with increasing substrate concentration. At a concentration of 0.5-2% an increase in enzyme activity. This is as reported by (Irawati 2016), the substrate concentration is proportional to the enzyme activity, at low concentrations the substrate enzyme complex

Tabel 3. Effect of substrate concentration variations on cellulase enzyme activity

[substrate] (%)	A	[glucose] (mg/mL)	EA (U/mL) ± SE
0.5	0.193	0.091	0.034±0.001
1.0	0.226	0.104	0.039±0.001
1.5	0.286	0.129	0.048±0.002
2.0	0.387	0.170	0.063±0.004
2.5	0.361	0.160	0.059±0.006

Notes: *[substrate]* is concentration of substrate; *A* is absorbance; *[glucose]* is concentration of glucose; *EA* is enzyme activity

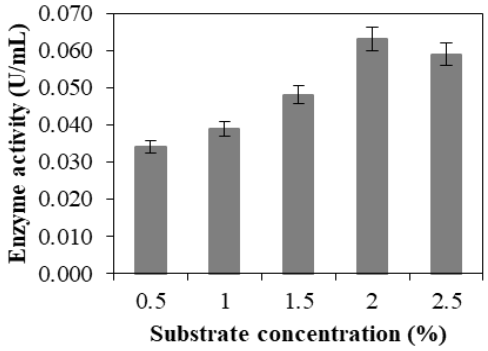

Figure 4. Correlation between substrate concentration and enzyme activity

produces less reducing sugar so that the enzyme activity is low.

The addition of substrate above 2% decreased enzyme activity due to the high amount of glucose produced. Novitasari & Putri (2016) stated that the high amount of glucose products resulting from cellulose hydrolysis will stick to the allosteric side of the enzyme and become an inhibition of glucose products so that a decrease in enzyme activity occurs.

The cellulase enzyme activity of the R_8W isolate was optimal at a substrate concentration of 2% with a cellulase enzyme activity value of 0.063 U/mL. Pertiwi (2017) reported that the cellulase enzyme activity of the yeast *Candida utilis* is optimal at a substrate concentration of 2% with an enzyme activity value of 0.033 U/mL. This condition is different from the results of research by Novitasari & Putri (2016), the measurement of cellulase enzyme activity from *Lactobacillus plantarum* at various substrate concentrations resulted in optimal enzyme activity at a substrate concentration of 1.5% with cellulase enzyme activity of 0.060 U/mL. Another study also measured the effect of optimum substrate concentration on cellulase enzyme activity of *Bacillus circulans*. The optimal concentration was obtained at a concentration of 2.5% with a cellulase activity of 0.022 U/mL. Differences in enzyme activity from the effect of optimum substrate concentration are caused by differences in the characteristics of the cellulase enzyme produced by a microorganism (Irawati 2016).

Microorganisms that produce cellulase enzymes are bacteria and yeasts. The enzymes produced by microorganisms have different activities. This activity is caused by differences in the amino acids that make up the enzyme. As reported by Sukharnikov *et al*. (2012), the amino acids that make up the cellulase enzyme from the bacterium Hahella chejuensis are tryptophan, glutamine, aspartic acid, serine, threonine, phenylalanine, lysine, tyrosine, aspargarin, histidine, arginine, glutamic acid. while the constituent components of cellulase amino acids from yeast Aspergillus terreus are methionine, glutamic acid, threonine, serine, glycine, phenylalanine, leucine, valine, alanine, arginine, proline, cysteine, aspartic acid, proline, cysteine, histidine, lysine, tyrosine, and tryptophan (Mirzaakhmedov *et al*. 2007).

Cellulase enzyme activity is the number of substrates that can be hydrolyzed. The substrate will bind to the active site of the enzyme. Enzymes provide sites capable of binding protons to amino, carboxy and other functional groups (Irawati 2016). The substrate is bound to the active site of the enzyme through hydrogen bonds, hydrophobic interactions, and covalent interactions. The residue on the active site of the enzyme acts as a proton donor or acceptor (Nauli 2014).

Cellulase Enzyme Activity on Rice Husk Cellulose Substrate

The cellulose used was derived from delignified rice husk waste. The activity of cellulase enzymes in hydrolyzing cellulose from rice husks at a temperature of 50 °C, pH 8, and a substrate concentration of 2% was 0.103 U/mL.

The production of cellulase enzymes requires an inducer compound in the form of a substrate for microorganisms. Rice husk contains three main components, namely cellulose by 32.67%, hemicellulose by 31.68% and lignin by 18.81% (Maruf & Damajanti 2020). The high lignin content in rice husk inhibits the substrate hydrolysis process by enzymes so that delignification needs to be carried out. Delignification is the process of breaking the lignin bonds that bind cellulose and hemicellulose. Delignification aims to break down the structure of lignocellulose so that cellulose is easily hydrolyzed by enzymes. Delignification can be done chemically using an alkali or an acid solution (Novia *et al*. 2017). Rice husks were delignified using 10% sodium hydroxide (NaOH) and 8% sodium hypochlorite (NaOCl), 10% sodium hydroxide (NaOH) to destroy the lignin structure in rice husks.

Rice husks added with NaOH in the heating process will produce a black precipitate which indicates the presence of lignin in rice husks (Umaningrum *et al*. 2018). The remaining lignin content in rice husks was delignified again using 8% NaOCl. As reported by Setyaningsih *et al*. (2020), the use of 8% NaOCl in the bleaching process resulted in perfect white rice husk cellulose which indicates the absence of lignin left in the delignification process. Damage to the lignin structure will make it easier for microorganisms to hydrolyze substrates for growth and enzyme production.

Measurement of cellulase enzyme activity using rice husk cellulose at optimum pH and temperature resulted in enzyme activity of 0.103 U/mL. The magnitude of the enzyme activity was caused by the delignification of the lignin structure of the rice husks so that the hydrolysis process of cellulose increased and the conditions used were the optimal conditions of the measurement results with CMC substrate.

Baharuddin et al. (2014) stated that the lower the cellulose content on the substrate, the smaller the substrate enzyme complex formed. The use of cellulose as a natural substrate was able to increase the activity of the cellulase enzyme from R_8W isolate.

Purkan *et al*. (2015) stated cellulase enzyme activity with the addition of rice husk substrate obtained enzyme activity of 0.251 U/mL. Baharuddin *et al*. (2014) stated that cellulase enzyme activity is optimal in the use of rice straw cellulose as a substrate. The enzyme activity is 0.0435 U/mL in CC4 isolates and 0.0245 U/mL in CC2 isolates. Febryanti *et al*. (2021) reported that cellulose enzyme activity from oil palm empty fruits bunch is 0.1308 U/mL in isolate S_{10}.

CONCLUSION

The cellulase enzyme R_8W bacterial isolate from sago beetle larvae worked optimally at a temperature of 50 °C, pH 8, and a substrate concentration of 2%. Cellulase enzyme activity of R_8W bacterial isolate from sago beetle larvae on rice husk cellulose substrate was 0.103 U/mL

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