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# Biodesizing Cotton Fabric using Amylase Enzyme Produced from Raw Cotton Fabric Waste Fermented by *Aspergillus Niger*

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**Abstract:** Biodesizing of woven fabric is needed to improve the absorption properties of the fabric. Biodesizing was carried out using amylase which hydrolyzed starch on woven fabrics. Commercial amylase production is limited. The amylase can be produced from *Aspergillus niger* fermentation on starch-containing substrates. The potential substrate for the fermentation process is raw cotton fabrics waste, which contains high starch and has not been utilized. Raw cotton fabrics waste cleaned and crushed. *Aspergillus niger* was inoculated on the substrate of raw cotton fabrics waste for 7 days at room temperature. Crude enzyme extracts were tested for amylase enzyme activites using the DNS method at temperatures of 30, 50, 70, and 90$^{o}$C and pH 3, 5, 7, 9. The amylase enzyme produced was used for biodesizing of raw cotton fabrics at 70$^{o}$C, pH 7, for 10, 30, 50 and 70 minutes. Biodesizing fabrics is carried out by weight reduction, absorbency, and fabric surface morphology. Enzyme activity test showed that the amylase worked effectively at 70$^{o}$C and pH 7. Crude enzyme hydrolize starch effectively at 70 minutes which result in 7.24% of weight reduction, 10 seconds absorption value, and smoother morphological surfaces.

**Keywords:** amylase enzyme; *Aspergillus niger;* biodesizing; raw cotton fabrics waste
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## 1. Introduction

During the weaving process the warp yarn are exposed to considerable mechanical strain. In order to prevent breaking, they are usually reinforced by sizing with a gelatinous substance [1]. About 75% sizing agents used consist of starch and its derivatives. Starch has been used as sizing components for cotton, being readily available, relatively cheap, and based on natural, sustainable materials. Pretreatment process in textile is carried out to remove impurities before dyeing and finishing process. Desizing as one of pretreatment process in which the size applied to the warp yarn before weaving is removed to enhance the penetration of dyes and chemicals in the next wet processing [2]. Desizing methods are available in various types, they are alkali desizing, oxidative desizing, acidic desizing, and enzymatic desizing. Enzymatic desizing is the most widely practiced method of desizing starch. Enzyme which is used in desizing process is amylase. Amylase is a hydrolytic enzyme which catalyses the breakdown of dietary starch into smaller oligosaccharides, dextrin and maltose [1], [3]. Users believe that amylase more effective method to remove starch without damaging to the fabrics because it work in specific substance. The process of removing starch by the amylase is called biodesizing process. Environmental an economic benefits of enzymatic desizing are avoiding chemical fiber damage, increasing biodegradability of effluent, and less handling of aggressing chemical which harmfull to the environment [1]. While the amylase enzyme is still very limited and the price is relatively expensive. This enzyme cannot be produced domestically. Therefore, efforts are needed to solve this problem, so that the availability of enzymes can fulfill market share needs.

Enzymes can be obtained from various sources including from the growth period of wheat (malt), the remains of animal pancreas, plants and microorganisms [4]. Microorganisms are considered the most economical compared to other sources, because they can be produced through fermentation techniques in a lower cost with less time and space requirement, and because of their high consistency, process modification and optimization can be done very easily [5]. The microorganisms that are widely used to produce enzymes are mold such as Aspergillus. One of the Aspergillus species that has the potential to produce amylase enzymes is *Aspergillus niger*. *Aspergillus niger* accounts for almost 95% of the commercial production of amylase and other enzymes [6].

*Aspergillus niger* is a mold from genus of Aspergillus, family of Moniliaceae, order Hyphomycetales, and division Deuteromycota [7]. The mold can grow optimally at temperatures of 35-37 0C, with minimum temperature of 6-8 $^{o}$C and maximum temperature of 45-47 $^{o}$C. It has large conidial carrier head that is packed densely, round and black, black-brown or purple-brown. The Konidian is large and contains pigments. Most strains in this group have skleeotia which is gray to black. Some strains are used in the production of citric acid, gluconate acid and enzymes [8].

**Figure 1.** Morphology of *Aspergillus niger [9]*



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*Aspergillus niger* productivity to grow and synthesize products in an environment is influenced by several factors, including pH, temperature, inoculum size, incubation period, carbon, and nitrogen sources [10].

One of the most nutrients for *Aspergillus niger* for its growth is carbohydrate. Many carbohydrate sources are found in rice, corn, cassava, groundnut oil wastes, wheat [11], [12]. One of the results of this processed product is made into flour without reducing the levels of carbohydrates contained in it. Carbon sources are usually simple sugars, such as dextrose. However, for certain purposes complex carbohydrates can be used as a source of carbon, for example cellulose. Although in small amounts, nutrients such as sodium, potassium, calcium, phosphorus, magnesium, iron, manganese, copper, zinc, chlorine and cobalt can be needed by the organism, so the culture medium must also contain these nutrients in small quantities.

Raw cotton fabrics waste is a raw fabrics made from cellulose that has not been processed so that it has not been contaminated with chemicals. In the weaving process, warp need sizing process in order to increase the strength of the warp. Sizing of warp is using natural starch (starch) with the percentage of starch following the amount of tetal warp. The high carbohydrate content (cellulose and starch) in the raw cotton fabrics waste is similar to the content found in rice bran. The use of rice bran as a solid substrate for the growth of *Aspergillus niger* has been widely investigated to produce amylase enzymes. Singh, S., Sharma, S., Kaur, C., Dutt, D. (2013) reporting that *Aspergillus niger* grows optimally on rice bran substrate and produces amylase enzyme [4].

The temperature will affect the growth rate of the *Aspergillus niger* mold, at the optimal temperature the growth of the mushroom colonies in converting the substrate into a product will be more increased and effective. Karri, et al (2014) states that the optimum media temperature of *Aspergillus niger* mold on rice bran substrate with the highest enzyme activity value is 30$^{o}$C [13].

The optimum pH is needed to produce the enzyme by the *Aspergillus niger*. During the fermentation process, pH of the media tends to change by various factors, changes in the pH of the environment will affect the metabolic process of *Aspergillus niger* as a result of optimum pH growth of *Aspergillus niger* mushroom media at pH 7 [14].

According to Karri et al (2014) said that the maximum production of amylase enzyme by *Aspergillus niger* mold was obtained after 6 days [13] Different fermentation time was states by Singh et al (2008) who reported that the highest enzyme activity from fermented *Aspergillus niger* fungi occurred at 7 days fermentation time [15].

Amylase enzymes has different characteristics. Sukandar (2009) in Jayanti (2011) stated that enzyme activity will be influenced by several factors, including the concentration of enzymes and substrates, temperature, pH, and inhibitors [16]. This factor will affect the enzyme in producing the product. At the optimum temperature, the collision between the enzyme and the substrate is very effective, so that the



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formation of the enzyme-substrate complex is easier and the product formed increases. The optimum temperature of the amylase enzyme generally ranges from temperatures of 40-70$^{o}$C [17], and the optimum enzyme concentration derived from bacteria or mol for desizing process is 0.5-1 g / L [2]. Singh et al (2008) stated that the temperature of the amylase enzyme from the *Aspergillus niger* for desizing process was optimum at a temperature of 70$^{o}$C [15]. The optimum pH condition requires an enzyme to activate all enzymes in binding to the substrate [18]. The optimum pH of the amylase enzyme is at pH 7 [1].

The use of foodstuffs such as rice as a medium for producing enzymes from microorganisms less effective, because of the high costs. Alternative materials that can be utilized as a growth medium of mold to produce enzymes also mostly contained in raw cotton woven fabric waste originating from the textile industry. In the textile industry, raw cotton woven fabric is a raw fabric that has not been processed yet. Utilization of raw woven fabric in some textile industries is not considered very well, in fact in one of the textile industries in the Cimahi area and some textile industries in West Java, has not been done properly. Fabrics in low grade and large defect left until they are weathered and dusty in the warehouse even though the amount of waste produced is not so much. Raw cotton fabric contain (94% cellulose) (1.3% protein) (1.2% pectin) (0.6% wax) (1.2% ash) (1.7% pigment and other substances) [19]. it also contain starch which is intentionally added for the purposes of the weaving process. The starch will coat the surface of the thread so that the surface becomes slippery, flexible, will not break easily due to friction. In general, starch used for the weaving process consists of natural starch, synthetic and modified so that the use of starch types needs to be adjusted to the type of fiber used. Raw cotton woven fabric is natural fiber so that the covenant process is carried out using natural starch. Starch are complex carbohydrates produced by cassava in the form of tapioca flour. The high levels of cellulose found in raw cotton woven fabric waste in addition high levels of carbohydrates found in starch made the raw cotton woven fabric as of the most needed elements of the *Aspergillus niger* to grow and reproduce.

Raw cotton woven fabric which has a high carbohydrate content will produce high glucose as well as for the growth nutrition of *Aspergillus niger* fungi in producing amylase enzymes. Waste of raw cotton woven fabric potential to be used as solid media in the fermentation process of *Aspergillus niger* in producing amylase enzymes. High cellulose and starch (amylose & amylopectin) in the raw cotton fabric waste assumed to be used as a solid medium for molds in producing amylase enzymes for desizing process. If the waste of raw cotton woven is used as a solid substrate for fungal growth media, it can increase the utilization of raw cotton fabric waste that has not been treated properly.

Amylase effective to hydrolise many kind of starch including commercial starch, yam, maize, cassava, and sweet potato [20], [21]. Chinnamal (2013) produce amylase from microbial then used to remove starch from raw cotton. Amylase has good



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activities in almost after desizing analysis including residual starch, iodine test, shrinkage, fabric weight , tensile strength and absorbency [22].

Solid state fermentation as a fungal growth medium was chosen because it can represent the condition of the substrate used which is insoluble in water and does not contain free water but is sufficient to contain water for microbial purposes. The media functions as a source of carbon, nitrogen and energy sources. In addition, it is believed to reduce production costs, simple techniques, low energy requirements and easy product recovery.

## 2. Materials and Methods

The research was conducted on a laboratory scale at Politeknik STTT Bandung.

### 2.1. *Material*

Microbial culture of *Aspergillus niger* was obtained from the Microbiology Laboratory of the Faculty of Chemical Engineering, Institut Teknologi Bandung. Raw cotton woven fabric waste substrates was obtained from PT Bratatex, Jl. Mahar Martanegara Cimahi. Also other chemicals as follow: $KH_2PO_4$ (p.a.), $NaNO_3$ (p.a.), $MgSO_4$ (p.a.) and glucose, starch, buffer pH, DNS

### 2.2. *Methods*

#### 2.2.1. Material characterization

The research begins with the initial characterization of raw cotton fabric waste which will be used as a substrate in the fermentation process by *Aspergillus niger*.

#### 2.2.2. Production of Amylase

Production of amylase enzymes use solid fermentation method. Raw cotton woven fabric waste crushed into small pieces. Microbial culture of *Aspergillus niger* was inoculated on 8 grams of raw cotton waste medium by adding 1 ml of inoculant and then incubated at $30^oC$ for 7 days. Additional nutrients such as $KH_2PO_4$, $NaNO_3$ and $MgSO_4$ are given to be able to meet the nutritional needs that can be accepted by the fungus *Aspergillus niger* in addition to nutrient intake from its own growth media. The extraction of amylase enzyme was carried out by adding 100 mL of distilled water to the fermentation substrate and shaken at 150 rpm for 10 minutes at room temperature. Next, centrifuged at 3,000 rpm for 20 minutes. The supernatant obtained was used as a crude enzyme extract.



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(a) (b) (c)

**Figure 2.** Preparation process (a) *Aspergillus niger* culture (b) raw cotton fabric waste after crushed (c) sterilization

2.2.3. Enzyme activities

The amylase enzyme activity was tested using the DNS method at temperatures of 30, 50, 70, and 90$^o$C and pH 3, 5, 7, 9 [20].

2.2.4. Biodesizing

The results of the enzyme activity test were used as parameters of the biodesizing process. The amylase enzyme produced was used for the biodesizing process raw cotton fabric at 70$^0$C, pH 7, for 10, 30, 50, and 70 minutes.

2.2.5. After Desizing Characterization

Biodesizing fabric is carried out by weight reduction test, absorbency test, and fabric surface morphology test.

## 3. Results

Raw cotton fabric waste used in this study contains 7.16% of starch. *Aspergillus niger* is fermented on a substrate that comes from the waste of raw cotton fabric waste that has been cut into small pieces to form a slightly broken fiber. Substrate visualization of raw cotton woven fabric waste before and after fermentation with *Aspergillus niger* can be seen in Figure 3.

(a) (b)

**Figure 3.** Substrate visualization (a) before fermentation (b) after fermentation



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Solid state fermentation produce amylase enzyme which can be seen in Figure 4. The enzyme tested for its activity on temperature and pH so that optimum conditions were used for the biodesizing process.

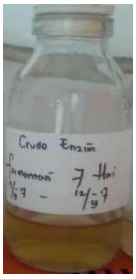

**Figure 4.** Crude Amylase Enzyme

3.1. *Enzyme Activity*

Enzyme activity is the ability of enzymes to work in converting substrates into products (reducing sugars). The amylase enzyme obtained from fungal microorganisms has different characteristics. This is because the enzyme amylase in carrying out its catalytic activity is influenced by several factors including temperature and pH.

3.1.1. Effect of temperature on enzyme activity

The test results of amylase enzyme activity against temperature can be seen in Figure 5.

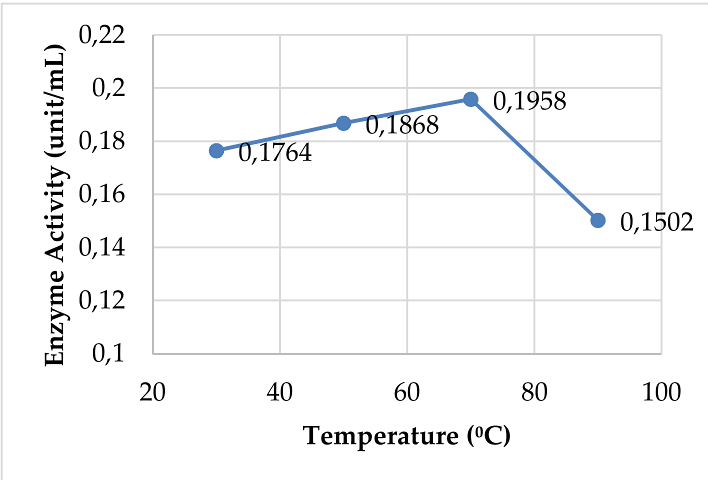


**Figure 5**. Amylase enzyme activity against temperature

3.1.2. Effect of pH on enzyme activity

The test results of amylase enzyme activity against pH can be seen in Figure 6.



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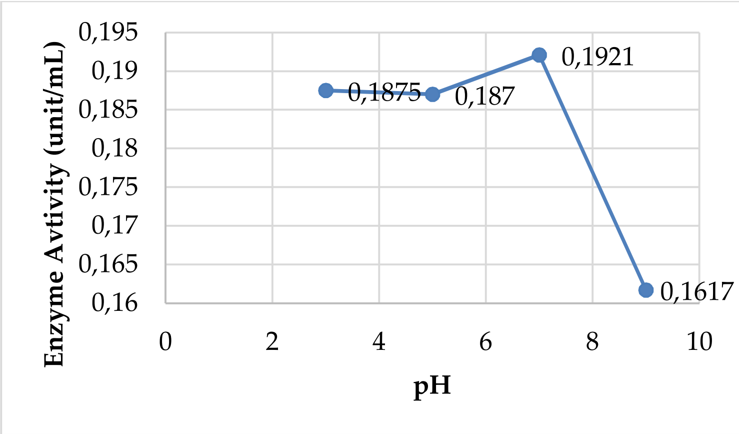


**Figure 6**. Amylase enzyme activity against pH

3.2. *Biodesizing of raw cotton fabric using amylase enzyme from raw cotton fabric waste fermented by Aspergillus niger*

Biodesizing on raw cotton fabric is done by varying the contact time of 10, 30, 50, and 70 minutes. Data analysis was obtained from the results of weight reduction test, fabric absorption test, and fabric surface morphology on the results of the biodesizing process by the amylase enzyme as follows :

3.2.1. Weight reduction of fabrics

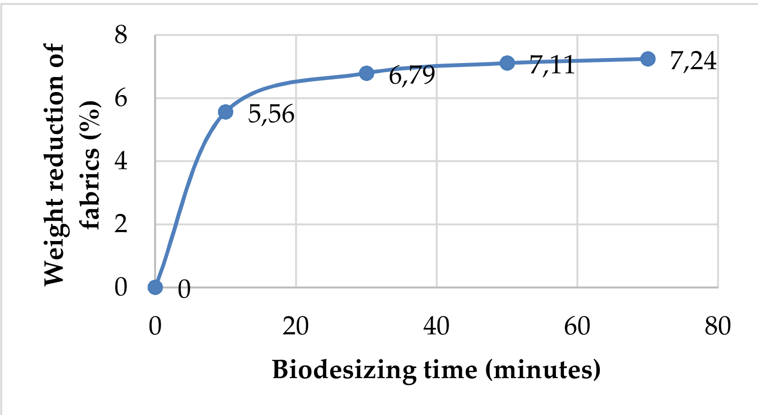


**Figure 7.** Biodesizing time against weight reduction of fabrics

3.2.2. Fabric absorption



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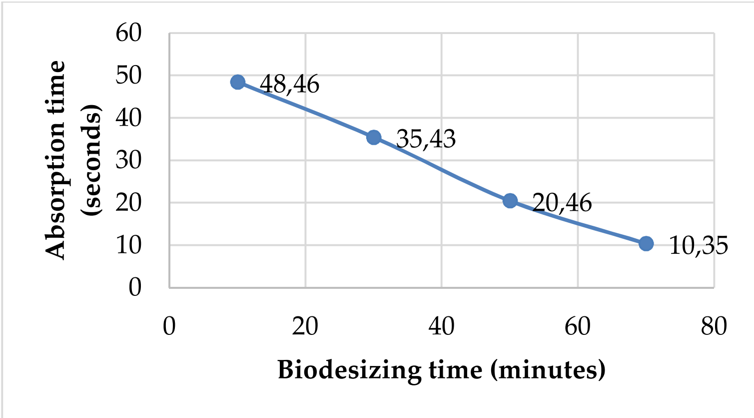
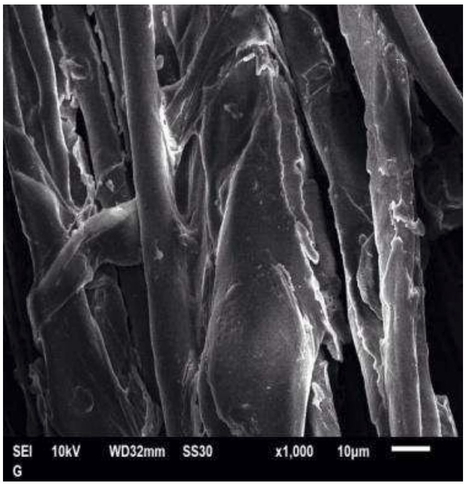
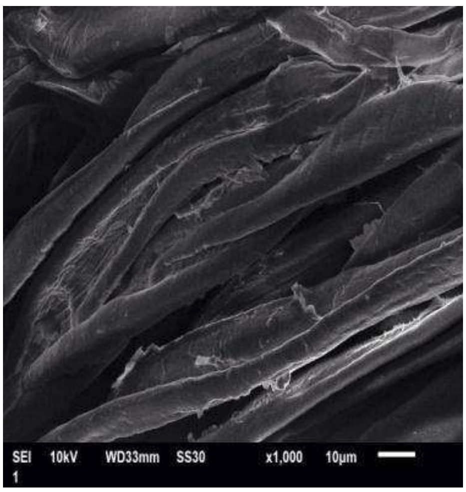


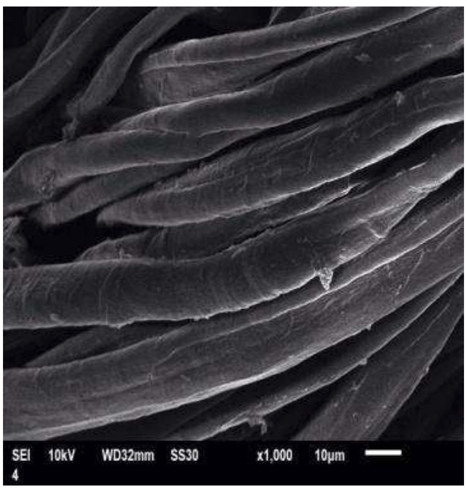

**Figure 8.** Biodesizing time against absorption time

3.2.3. Fabric Surface Morphology




(a) undesized




(b) biodesizing 10 minutes (c) biodesizing 70 minutes

**Figure 9.** The Fabric Surface Morphology using Scanning Electron Microscope



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## 4. Discussion

Production of amylase enzyme through the fermentation of raw cotton woven fabric waste with *Aspergillus niger* had been done. The substrate changes during the fermentation period. Initially, the white substrate becomes surrounded by black spots around the substrate. This shows that the substrate becomes the growth medium of *Aspergillus niger*. During the growth period, *Aspergillus niger* produces black pigments which are a sign of the existence of *Aspergillus niger* [23]. This can be seen in Figure 3. After the fermentation process is complete, harvesting the enzyme is carried out to produce a crude enzyme as shown in Figure 4.

### 4.1. *Enzyme activity*

#### 4.1.1. Enzyme Activity Against Temperature

Based on Figure 5, enzyme activities increased at a temperature of 30°C to 50°C and optimum at a temperature of 70°C with an enzyme activities value of 0.1958 Unit / mL. This condition shows an increase in kinetic energy or an increase in the effectiveness of enzymes that will facilitate the formation of substrate enzyme complexes, so that the products are more [18]. At a temperature of 90°C the enzyme activity decreased with the enzyme activity value of 0.1502 Unit / mL this occurs because the enzyme undergoes denaturation.

#### 4.1.2. Enzyme Activity Against Temperature

Based on Figure 6, the value of enzyme activities have increased with increasing value of acidity. Optimum enzyme activity was at pH 7 with an enzyme activity value of 0.1921 Unit / mL and decreasing at pH 9 with an enzyme activity value of 0.1627 Unit / mL. Decrease in the effectiveness of the amylase enzyme is caused by damage to the amylase enzyme at high pH. pH is one of the important factors that must be considered. This is because, that an enzyme is a protein molecule, its protein molecule stability is greatly influenced by the acidity of the environment, in certain pH conditions the protein molecules of the enzyme will be damaged (denatured).

### 4.2. *Biodesizing of raw cotton fabric using amylase enzyme from raw cotton fabric waste fermented by Aspergillus niger*

Biodesizing is the process of starch removal using the amylase enzyme as the stage of application of the product synthesized by the *Aspergillus niger* mold from the solid state fermentation process of raw cotton woven fabric on textile materials in the form of cotton woven fabric which still contains starch. The amylase enzyme will specifically degrade starch on the surface of the fiber by hydrolyzing the insoluble chain of starch molecules into water-soluble glucose. Enzyme characteristics are influenced by several factors including temperature, pH, and contact time [1].



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4.2.1. Weight reduction of fabrics

A weight reduction test was carried out to find out how much starch was lost on the fabric after 10, 30, 50, and 70 minutes using the amylase enzyme. The weight reduction test results can be seen in Figure 7. Processing time affects the percent weight reduction value. Weight reduction of fabric that biodesized at 70 minutes higher than the other fabrics. It means that the longer the processing time the more starch is removed. Increasing the processing time the more enzymes that hydrolyze the substrate. This amylase enzyme will hydrolyze the amylose and amylopectin starch chains on the surface of the fiber along with the increase in processing time. At that time, the amylase enzyme effectively attacked the starch polymer chains so that starch can dissolve into a simpler form (glucose, maltose) so that the starch material will decrease and cause the weight reduction percentage value to increase. Enzyme molecules are catalysts that are very efficient in accelerating the conversion of substrate to final products. One single enzyme molecule can make as many as one thousand substrate molecules per second. This fact also explains that the enzyme molecule is not consumed or changes during the reaction process, meaning the length of contact or reaction between the enzyme and the substrate determines the effectiveness of the enzyme's work. In addition, the increasing effectiveness of the enzyme activity in degrading starch on the surface of the fiber is also supported by the process conditions besides the length of time, temperature and pH the process is also very influential for enzymes in degrading starch that coats the fabric, if the pH value and temperature are low or height will cause a denaturation process which results in a decrease in the value of enzyme activity.

4.2.2. Fabric Absorption

The absorption test determines the ability of fabrics to water absorption. The result was absorption time value. The ability to absorb cloth is influenced by the content of components in the fabric. Fabrics that still contain a lot of starch will have poor absorption capacity. Good fabric absorption can be obtained by removing components that can block the absorption process. The results of the absorbency test can be seen in Figure 8.

The results of absorption test of the fabric shows that the longer the time of the biodesizing process, the shorter the absorption time of the drops test results. That shows that the starch attached to the surface of the fiber is partially dissolved which means that the enzyme effectively degrades insoluble starch to dissolve in water so that the fiber pores are more open so it is effective for absorbing water. However, the absorption time obtained from the results of this test has not been very effective, the shortest absorption time only occurs in 10 seconds, because other components such as other fiber impurities have not been removed. The processing time affects the percent weight reduction value.



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4.2.3. Fabric Surface Morphology

Fabric surface morphological test was carried out using Scanning Electron Microscope (SEM). This test was carried out to determine the appearance of the fiber surface resulting from the biodesizing process using enzymes (biodesizing) with variations in the time of biodesizing process 10, 30, 50, and 70 minutes. The surface image of the fiber on the surface testing of this material is carried out on blank cloth to see the fabric which is actually still coated with starch compared to the fabric which was tested at 10 and 70 minute process time variations in order to see the performance of the amylase enzyme in degrading the substrate at the shortest time and the longest time. Image of fiber surface image as a result of testing the surface morphology of the material can be seen in Figure 9.

From Figure 9, it is clear that the surface image of cotton fiber treated when the longest starch removal process or for 70 minutes biodesizing process produce cleanest surface image of the fabric. Figure (C) shows that there is no starch lining the fiber. Different things are shown on the fabric in Figure (B) with the treatment of the starch removal process for 10 minutes, it can be seen that the starch still covers the surface of the fiber. However, the same process has been carried out using the amylase enzyme. It happens that, the activity of the amylase enzyme in hydrolyzing the substrate is influenced by the processing time. The longer the time, the effectiveness of the amylase enzyme to attack the starch polymer chains that are on the surface of the fiber is increasing so that starch can dissolve into a simpler form (glucose, maltose) so that the starch material in the fiber will decrease. During the 10 minute process the contact time of the enzyme in binding to the substrate is not optimum so that even though the starch removal process has been carried out, when the contact time of the reaction between enzymes is less effective the starch removal process will not be effective because the enzyme will be very active binding the substrate as contact time increases

## 5. Conclusions

The raw cotton woven fabric waste can be used as a solid substrate for the amylase enzyme production using fermentation process by the *Aspergillus niger* fungus with starch content on the substrate of 7.16%. The amylase enzyme produced from the solid state fermentation process of the raw cotton woven fabric waste by the *Aspergillus niger* effectively removes the starch contained in the cotton fabrics. The longer the processing time, the greater the weight reduction value, the shorter the absorbency time decreases and the surface morphology of the material is cleaner. The best value for weight reduction, absorption, and surface morphology was found in 70 minutes with a weight reduction percentage of 7.24%, 10 seconds absorption test and the cleanest surface morphology among other fabrics. Raw cotton fabric waste can be used as an alternative growth medium for *Aspergillus niger*

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