



# BIO-DELIGNIFICATION OF OIL PALM EMPTY FRUIT BUNCH OF USING *Trichoderma Viride* AND *ESCHERICHIA COLI*

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## ABSTRACT

As one of the countries with the largest palm oil production in the world, Indonesia has extensive oil palm plantations. The production of palm oil in Indonesia continues to grow from year to year. Huge palm oil processing will also produce an enormous amount of palm oil waste. If this solid waste is not handled properly, it can lead to a buildup of oil palm waste. One of the wastes of the palm oil industry is the oil palm empty fruit bunch. Delignification becomes one of the solutions in the handling of the oil palm empty fruit bunch. In the research, the influences of the delignification time, solid-liquid ratio, and the concentration of microorganisms on the lignin removal were studied. Lignin and cellulose content of the samples were analyzed using Van Soest method. The experimental results indicated that the lignin content of the biomass before bio-delignification was 32.83%, and after treated with *Trichoderma viride* the lignin content decreased to 13.76% (achieved at 1:30 solid-liquid ratio and 6 weeks fermentation time), while with *Escherichia coli* the lignin content drop to 12.48% (1:50 solid-liquid ratio and 6 weeks fermentation time). The combination between *Trichoderma viride* and *Escherichia coli* reduced the lignin content to 18.57% within 6 weeks of fermentation time. The FTIR analysis shows that the functional group of phenol and aromatic compounds were detected. This result indicates that the lignin component still presents in the bio-delignified oil palm empty fruit bunch.

**Keywords:** delignification process, biomass, lignocellulosic material.

## 1. INTRODUCTION

One of the largest commodities in Indonesia is palm oil, and due to this reason, the palm plantation continues to grow every year. The high amount of palm oil production, it will produce a huge amount of solid wastes, and one of them is oil palm empty fruit bunch. This solid waste contains 35% of cellulose, 25% hemicellulose, 25% lignin, and the rest are other components (Martinez *et al.*, 2016). The high content of cellulose in the oil palm empty fruit bunch can be utilized as a new source of cattle fodder. However, the presence of lignin in oil palm empty fruit bunch will make some trouble to cattle due to the difficulty of the digestion process in the cattle stomach. Therefore, the reduction or removal of lignin content is required before it can be used as the alternative source of cattle fodder.

The delignification of lignocellulosic biomass can be carried out using various kinds of methods such as physical pretreatment, chemical pretreatment, thermophysical and chemical pretreatment, and biological pretreatment processes (Putro *et al.*, 2016). The physical pretreatment process usually requires a significant amount of energy for the size reduction of lignocellulosic biomaterial, while the chemical pretreatment process usually produces a substantial amount of hazardous waste after the delignification process. Thermophysical and chemical pretreatment process requires high temperature and pressure to degrade the lignin. Therefore it is considered as a high-energy process. Biological pretreatment is considered as cheap pretreatment process because it only requires microorganisms to degrade the lignin in the lignocellulosic biomass. However, this process is time-consuming.

The use of fungi and bacteria simultaneously in the process of delignification of lignocellulosic material is

still limited. In this study, we utilized *Trichoderma viride* (fungi) and *Escherichia coli* simultaneously to degrade the lignin content in oil palm empty fruit bunch. The effects of the delignification time, solid-liquid ratio, and the concentration of microorganisms on the lignin removal were studied

## 2. MATERIALS AND METHODS

### 2.1 Materials

Oil palm empty fruit bunch was obtained from oil palm plantation in West Sumatra. Before use, the biomass was repeatedly washed using tap water to remove soil, and other impurities and sun dried. Potato dextrose broth (PDB), nutrient broth (NB), Cetyl Trimethyl Ammonium Bromide (CTAB), acetone, and sulphuric acid were purchased from Merck, Germany.

### 2.2 Raw material preparation

Oil palm empty fruit bunch was ground until its particle size + 30 mesh. Subsequently, the moisture content of powder oil palm empty fruit bunch was measured using moisture analyzer (Ohaus MB200). If the moisture content > 12 %, the powder was dried in a circulated oven (Memmert) at 50°C for 24 h. Cellulose, hemicellulose, and lignin content were determined according to Van Soest method (Van Soest, 1994).

### 2.3 Determination of the kinetic of growth of *Trichoderma viride* and *Escherichia coli*

The identification of the kinetics of growth of both microorganisms was conducted using turbidimetry method. The determination of the kinetics of growth was used to determine starter incubation time of both microorganisms before being utilized for the



delignification process. At the stage of determination of the kinetics of growth, *Trichoderma viride* and *Escherichia coli* were cultured in PDB and NB media, respectively, and then turbidity measurements were taken from both media at any given time using a turbidimeter (Hanna HI-83414). When the increment of the value of the turbidity was insignificant, the turbidity measurement was stopped, this phenomenon indicates that the microorganism was in the stationary phase.

#### 2.4 Bio-delignification using *Trichoderma viride* and *Escherichia coli*

The procedure for bio-delignification process of oil palm empty fruit bunch using *Trichoderma viride* and *Escherichia coli* is as follow: 3 gram of oil palm empty fruit bunch was added into a 500 mL of Erlenmeyer flask, and subsequently 81 mL of sterile water and microorganism starter (*Trichoderma viride* or *Escherichia coli*) were added to the flask and incubated at room temperature at various fermentation time (1, 2, 4, 5, and 6 weeks).

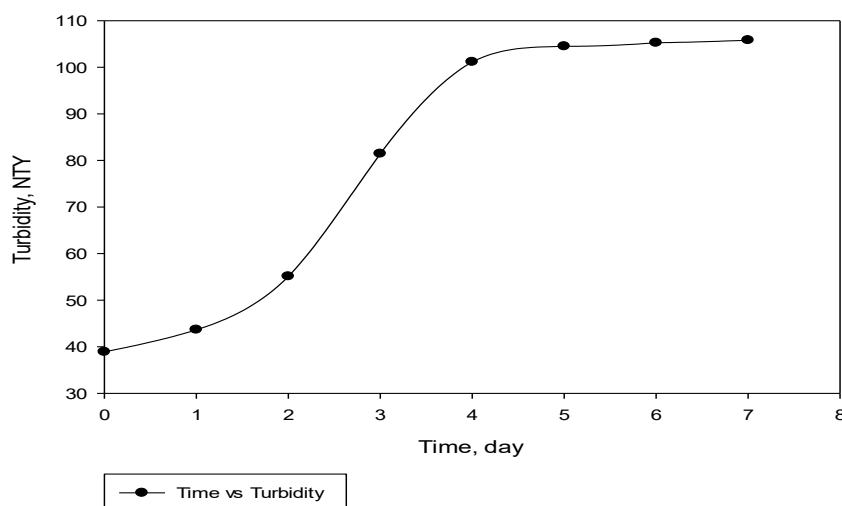
#### 2.5 Bio-delignification using mix culture of *Trichoderma viride* and *Escherichia coli*

The bio-delignification process of oil palm empty fruit bunch using mix culture of *Trichoderma viride* and *Escherichia coli* was conducted using the similar procedure with pure strain bio-delignification process. The difference is in the addition of starter, the ratio between *Trichoderma viride* and *Escherichia coli* was 1:1, 1:2, and 2:1.

### 3. RESULTS AND DISCUSSIONS

#### 3.1 The kinetic of growth of *Trichoderma viride* and *Escherichia coli*

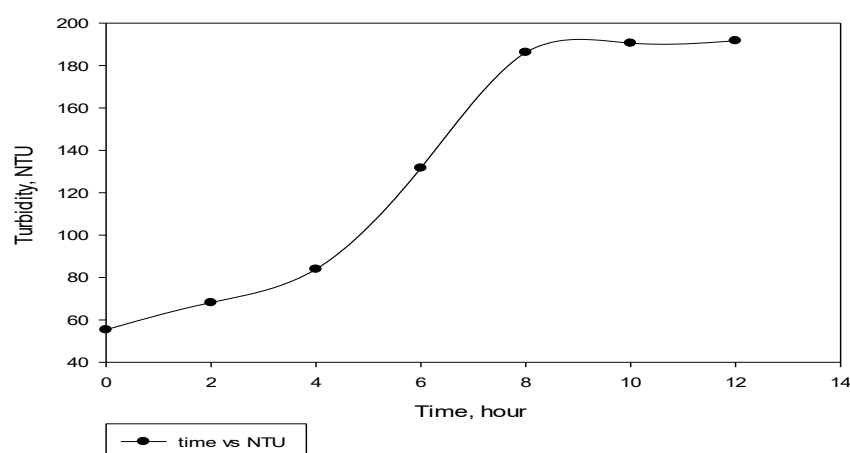
In the growth kinetics of *Trichoderma viride*, the lag phase occurs until the second day after the incubation process. In this phase, the *Trichoderma viride* grew slowly as microorganisms attempt to adapt to their environment. After the second day, *Trichoderma viride* begun to enter the exponential phase. Over the 4<sup>th</sup> day, *Trichoderma viride* entered the stationary phase, indicating the cell growth starts to slow down, and some cells die due to the depletion of nutrient content (Figure-1).



**Figure-1.** The growth kinetics of *Trichoderma viride*.

In the growth kinetics of *Escherichia coli* (Figure-2), the lag phase occurred until 4 hours of incubation. The exponential phase took place at a range of

time 4 to 8 hour. *Escherichia coli* entered the stationary phase after 8 hours as shown in Figure-2, at stationary phase the growth of *Escherichia coli* relatively constant.

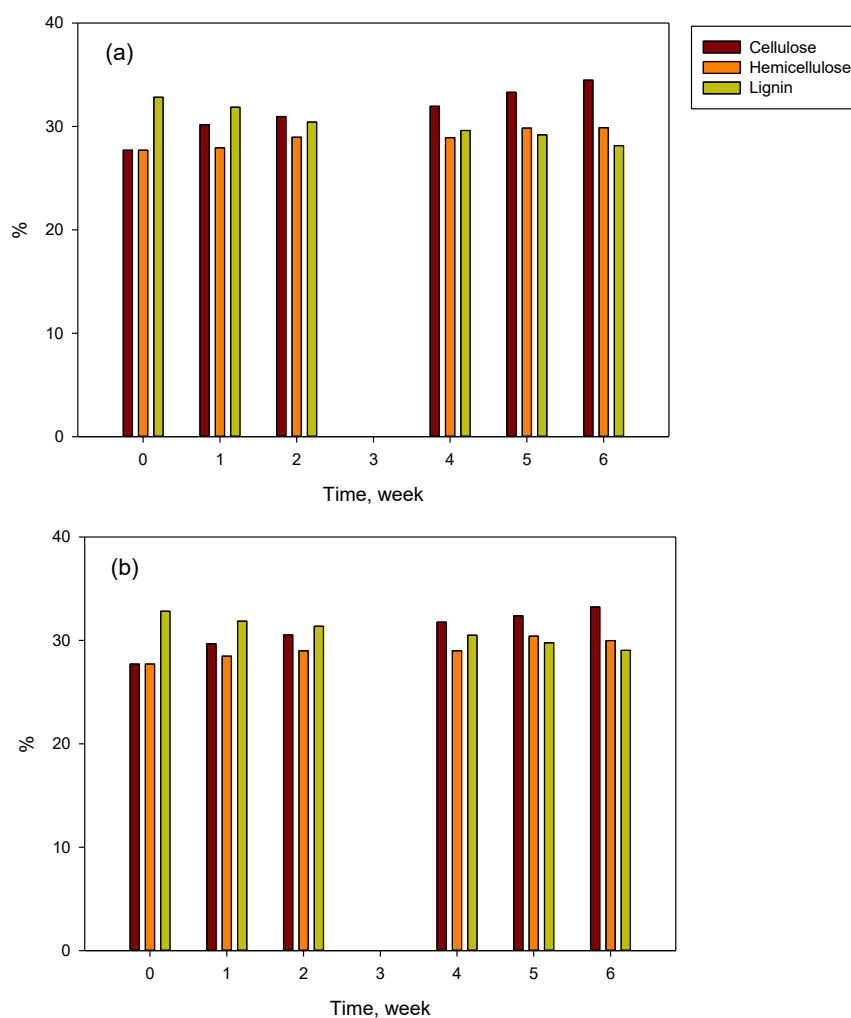


**Figure-2.** The growth kinetics of *Escherichia coli*.

### 3.2 Bio-delignification using *Trichoderma viride*

The composition of cellulose, hemicellulose, and lignin in oil palm empty fruit bunch were 27.71%,

27.71%, and 32.83%, respectively. The removal of lignin by *Trichoderma viride* at a various ratio of solid: liquid can be seen in Figure-3.



**Figure-3.** The effect of fermentation time on the composition of cellulose, hemicellulose, and lignin on the delignified oil palm empty fruit bunch using *Trichoderma viride*. Solid-liquid ratio of (a) 1:30, and (b) 1:50



From Figure-3 it can be seen that the lignin content in oil palm empty fruit bunch decreases with the increase of fermentation time. According to Lakshmanan and Sadasivan (2016), *Trichoderma viride* produces the lacase enzyme, and this lacase enzyme is involved in the delignification process. This enzyme is synthesized and secreted when the nutrient content, carbon or nitrogen elements, are in the limited environment (Wesenberg *et al.*, 2003). The decrease of lignin content in the first and second week of fermentation is not significant. This indicates that the production of an enzyme by the fungi is not in substantial amount, resulting a small amount of lignin could be degraded. Until the sixth week, the decrease of lignin levels at 1:30 ratio from 32.83% to 28.15%, and at 1:50 ratio from 32.83% to 29.03%. The highest percentage of delignification achieved at 1:30 ratio is 13.76%, while the ratio of 1:50 is 11.47%.

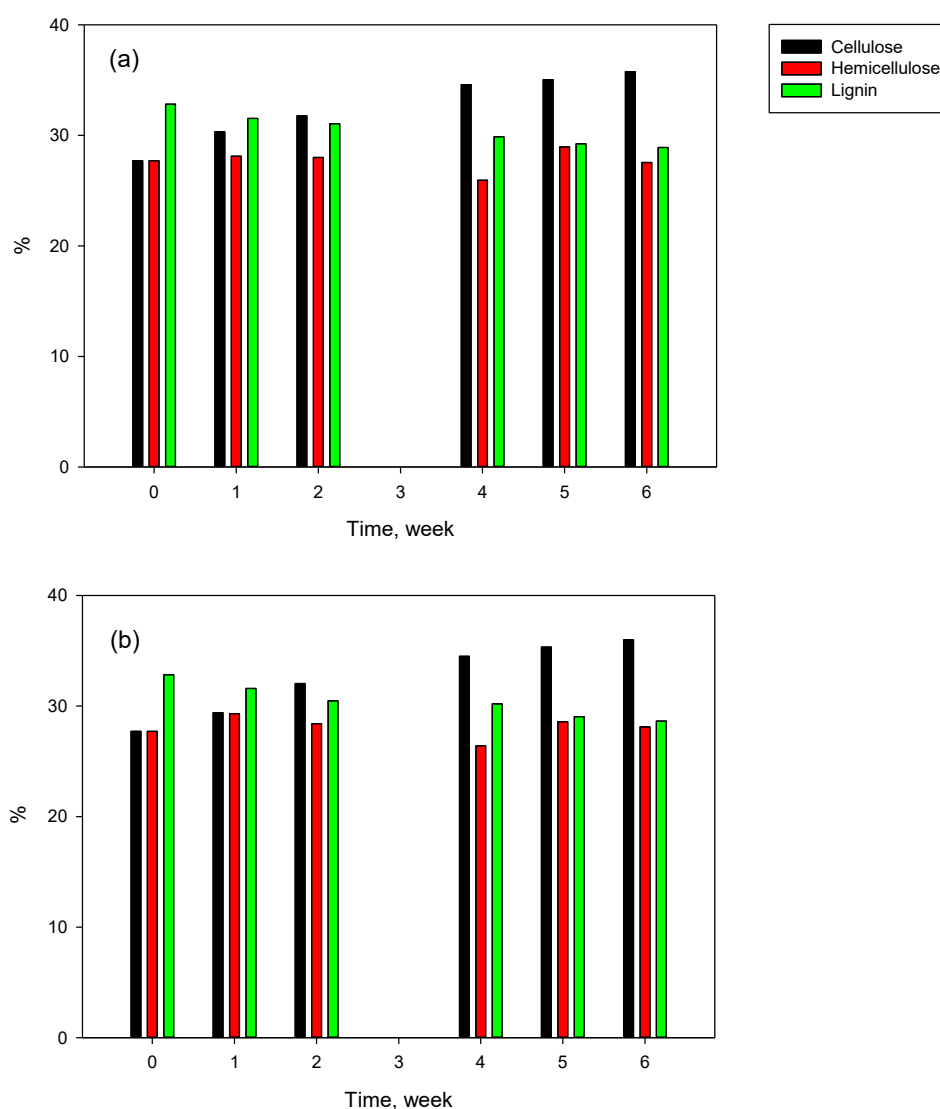
The delignification results using *Trichoderma viride* indicate that the substrate with a solid-liquid ratio of 1: 30 gave higher removal of lignin than 1:50 ratio. At the solid-liquid ratio Of 1:30, the amount of added nutrient was less than the substrate with a solid-liquid ratio of 1:50. Microorganisms will consume nutrients from the media first, and the lacase enzyme is secreted when the microorganisms are in a nutrient-poor environment (Wesenberg *et al.*, 2003). Since the amount of nutrients added is less on the substrate with a solid-liquid ratio of 1:30, the fungus will consume the nutrients faster, and the production of lacase enzymes is quicker than the solid-liquid ratio of 1:50.

Also, it is known that the level of moisture (water content) in the environment affects the metabolism of microorganisms in producing enzymes. The metabolic process of the formation of enzymes in fungi requires a lower level of moisture than bacteria (Subramaniyam and Vimala, 2012). Therefore, under higher humidity conditions, the formation of enzymes in fungi becomes less.

For cellulose, there is an increase in both substrates after delignification. In the solid-liquid ratio of 1:30, the cellulose level increased to 34.48% and for the solid-liquid ratio = 1:50, the cellulose level increased to 33.24%. Increased cellulose levels are due to the reduced percentage of lignin in the substrate due to delignification. The hemicellulose content in the substrate did not show any trend since the fungi other than degrading lignin will also consume carbohydrate (cellulose and hemicellulose) content in the substrate (Singh *et al.*, 2008). Hemicellulose has a shorter, amorphous-shaped molecular chain so that hemicellulose is more easily degraded than cellulose, and fungi will consume more accessible carbohydrates first, in this case, is hemicellulose.

### 3.3 Bio-delignification using *Escherichia coli*

Figure-4 depicts the influence of fermentation time on the composition of cellulose, hemicellulose, and lignin on the delignified oil palm empty fruit bunch using *Escherichia coli*.



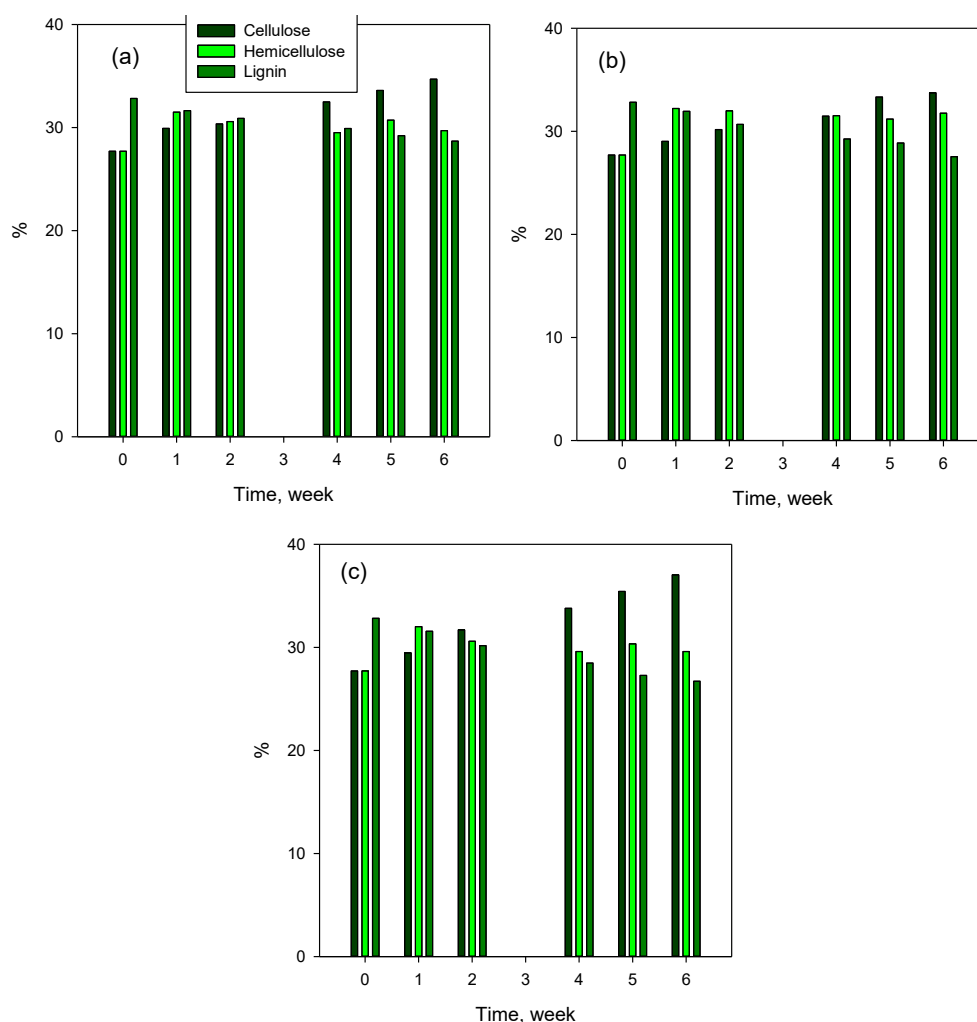
**Figure-4.** The effect of fermentation time on the composition of cellulose, hemicellulose, and lignin on the delignified oil palm empty fruit bunch using *Escherichia coli*. Solid-liquid ratio of (a) 1:30, and (b) 1:50

Similar behavior to *Trichoderma viride* was observed for delignification of oil palm empty fruit bunch with *Escherichia coli*. This bacterium also can degrade the lignin content in lignocellulosic material as seen in Figure 4. *Escherichia coli* produces dye-decolorizing peroxidase (DyP) enzyme. DyP enzyme is a peroxidase enzyme that can degrade lignin (Gonzalo *et al.*, 2016). The experimental results indicate that high solid to liquid ratio gave better lignin removal efficiency. At a solid-liquid ratio of 1:50, *Escherichia coli* degraded the lignin content from 32.83 % to 28.64 % within 6 weeks of fermentation time. For metabolic processes of enzyme production, bacteria require an environment with high moisture

content (Subramaniyam and Vimala, 2012). Therefore, on a substrate with a solid-liquid ratio of 1:50, the production of enzyme more significant than the solid-liquid ratio of 1:30. Since the amount of DyP enzyme was quite substantial, the delignification process also faster which gave higher lignin degradation.

### 3.4 Bio-delignification using mixture of *Escherichia coli* and *Trichoderma viride*

The bio-delignification performances of a mixture of *Escherichia coli* and *Trichoderma viride* on oil palm empty fruit bunch are shown in Figure-5.



**Figure-5.** The effect of fermentation time on the composition of cellulose, hemicellulose, and lignin on the delignified oil palm empty fruit bunch using mixture of *Trichoderma viride* and *Escherichia coli* with ratio of (a) 1:1, (b) 1:2, and (c) 2:1

Figure-5 shows that the highest lignin removal occurred in delignification with combination ratio of *Trichoderma viride*: *Escherichia coli* 2: 1. In this combination ratio, the lignin content of substrate originally 32.83% decreased to 26.72%. The combination between *Trichoderma viride* and *Escherichia coli* gave better delignification performance than single fungi or bacterium. The higher removal of lignin possibly due to a symbiotic relationship between *Trichoderma viride* and *Escherichia coli*.

#### 4. CONCLUSIONS

In this study, *Trichoderma viride* and *Escherichia coli* were used as the biological agents to remove lignin from lignocellulosic waste material (oil palm empty fruit bunch). The delignification process was conducted at different solid-liquid ratio and fermentation time. Both of those variables gave significant effect on the lignin removal. The lignin content in oil palm empty fruit bunch decreased with the increase of fermentation time. *Trichoderma viride* gave better lignin removal performance at a solid-liquid ratio of 1:30 while for

*Escherichia coli* the best removal performance was achieved at a solid-liquid ratio of 1:50. The combination of *Trichoderma viride* and *Escherichia coli* gave better removal efficiency than single fungus or bacterium.

#### ACKNOWLEDGEMENT

Financial support from Student Creativity Program - Research, Indonesia Ministry of Research, Technology, and Higher Education and the support from Widya Mandala Surabaya Catholic University Research Grant are acknowledged.

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