

## Production of Cellulase from Oil Palm Biomass as Substrate by Solid State Bioconversion

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**Abstract:** Solid state bioconversion (SSB) of lignocellulosic material oil palm biomass (OPB) generated from palm oil industries as waste was conducted by evaluating the enzyme production through filamentous fungus in lab-scale experiment. OPB in the form of empty fruit bunches (EFB) was used as the solid substrate and treated with the fungus *Trichoderma harzianum* to produce cellulase. The results presented in this study revealed that the higher cellulase activity of 0.0413 unit was achieved at the day 3 of fermentation. While the optimum study indicated the enzyme production of 0.0433 unit with moisture content of 50%, 0.0413 unit with 5% v/w of inoculum size and 0.0413 unit with co-substrate concentration of 2% (w/w) at days 9, 9 and 12 of fungal treatment, respectively. The parameters glucosamine and reducing sugar were observed to evaluate the growth and substrate utilization in the experiment.

**Key words:** Cellulase, Oil Palm Biomass, Solid State Bioconversion, *Trichoderma harzianum*

### INTRODUCTION

The oil palm industries in Malaysia are producing a huge potential of exploitation of non-oil palm biomass that are about 90 million tonnes of lignocellulosic biomass each year of which about 40 million tonnes are the form empty fruit bunches (EFB), oil palm trunks (OPT) and oil palm fronds (OPF) [1]. At present, the biomass is either left at plantation to provide organic nutrients to oil palm trees or burned illegally [2] or used as solid fuel in the boiler to generate steam or electricity at the mills.

The production of enzyme from OPB by microbial treatment taking advantage of the presence cellulose in OPB. The fungi *Trichoderma spp.* are the most reported to degrade cellulose and other compounds in cell wall by producing cellulase enzyme [3,4]. However, the production of cellulase enzyme from oil palm biomass was conducted by using solid state bioconversion (SSB) system. The attraction of SSB comes from its simplicity and its closeness to the natural way of life for many microorganisms, low capital costs for equipment, high volumetric productivity, decreased operational costs and as an alternative in preventing the environmental pollution[5].

Limited researches have been undertaken in order to provide a better utilization of oil palm wastes for value-added product applications [6]. Based on these current trend of treating the waste, therefore, this study was inspired to develop indigenous environmental friendly processes for the production of cellulase enzyme by microbial treatment of OPB in solid state

bioconversion. This study offers an alternative for a better utilization of oil palm wastes for value added product besides achieving zero waste strategies at plantation. In fact, cellulase enzymes possess good promise of application in production of ethanol, single cell protein, bioremediation, wastewater treatment and textile industry.

### MATERIALS AND METHODS

**Sample Collection:** The substrate used for this work was empty fruit bunches (EFB) as the part of oil palm biomass is cheap and readily available sources of lignocellulosics. The EFB was collected from oil palm industry (Seri Ulu Langat Palm Oil Mill Sdn. Bhd.), Dengkil, Selangor, Malaysia. The sample was collected as crushed of EFB with few centimeter of fibrous length and stored into autoclavable plastic bags in cold room at 4°C. Meanwhile, Wheat flour was used as the co-substrate and additional carbon source. The previous study reported it enhances the enzyme production [7].

**Fungal Strain and Inoculum Preparation:** Culture of *Trichoderma harzianum* (SCahmT105) obtained from the lab stocks was maintained on potato dextrose agar (PDA) plates and incubated at 32°C for seven days. After that, the cultured plates (4 plates) were washed with 100 mL of sterile distilled water. The surface was gently rubbed with a sterilized hockey stick and the mycelial suspension was transferred into sterilized 250 mL of conical flask by filtration for the use of final inoculum after measuring its concentration

( $2 \times 10^5$  spores/mL). The inoculum was kept in the refrigerator at 4°C [8]. Mineral solutions were prepared according to the method of Eriksson and Vallander [9].

**Treatment Procedures and Analysis:** The EFB were thoroughly washed to make them dust free and then dried [10]. Delignification was carried out by soaking EFB in 1% (w/v) NaOH for several hours and steamed as such 120°C for 1h. After alkali treatment, the material was washed completely free of alkali and dried. Subsequently, it was cut to 5-10 mm [11]. Treatment was carried out in 500 mL Erlenmeyer flask incorporating the fermentation medium contained 28% (w/w) of EFB, 2% (w/w) of wheat flour, 5% (v/w) of inoculum, 35% (v/w) of distilled water and 30% (v/w) of mineral solution. The samples were incubated at 32°C for 15 days. The sampling was done at the interval of 3, 6, 9, 12 and 15 days of treatment. The fermented sample for each sampling was mixed with 100 mL distilled water by shaking in a rotary shaker for 2 hours at room temperature ( $30 \pm 2^\circ\text{C}$ ) to extract the products and the extracted mixture was filtered and collected for the analysis such as enzyme activity assay, reducing sugar and glucosamine estimation.

Cellulase activity assay was carried out by the method suggested by Mandel *et al.* [12]. The unit measured is referring to unit of "mmoles glucose equivalent per minute in 60 minutes". Determination of glucosamine as the growth indicator was based on the method suggested in Zheng and Shetty [13]. Reducing sugar was measured according to dinitrosalicylic acid (DNS) method suggested by Miller [14]. The data were the average of three replications.

The traditional method of optimization 'one-factor-at-a-time' technique was used in this study. This method is determined by varying one factor while keeping the other factors at a constant level. The effect of inoculum size, co-substrate dosage and total moisture content were studied.

## RESULTS AND DISCUSSION

**Production of Cellulase by Solid State Bioconversion:** The production of cellulase was carried out in solid state bioconversion system using oil palm empty fruit bunches (EFB) as solid substrate. Figure 1 shows the cellulase activity was successfully detected. The highest cellulase activity of 0.0413 unit was appeared at day 3 of fermentation.

From the treatment by *Aspergillus flavus* Linn Isolate NSPR 101, Ojumu *et al.* [15] obtained the best result from sawdust with an enzyme activity value of 0.0743 IU/mL while bagasse and corncob gave 0.0573 IU/mL and 0.0502 IU/mL, respectively.

Figure 2 shows glucosamine concentration and reducing sugar released during 15 days of fermentation. Glucosamine indicated the fungal growth in the bioconversion process as increasing trend (batch growth

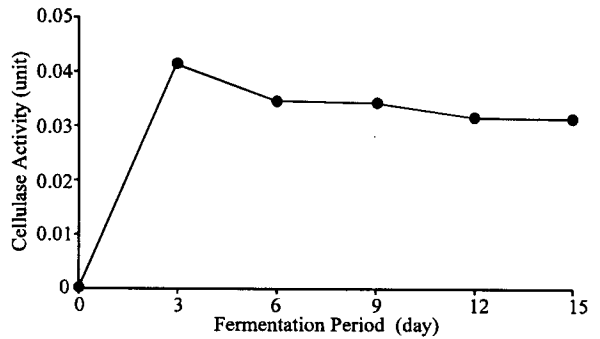


Fig. 1: Production of Cellulase from Oil Palm Empty Fruit Bunches by *T. harzianum* in Solid State Bioconversion.

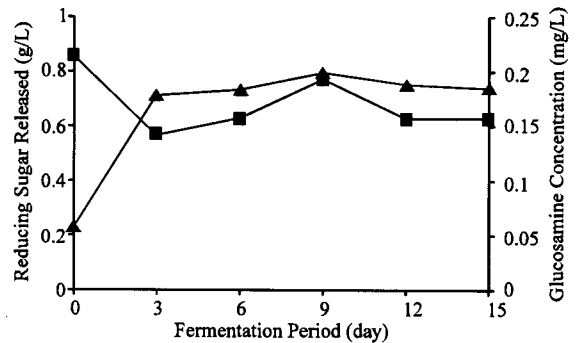


Fig. 2: Determination of Glucosamine Concentration (▲) and Reducing Sugar (■) for 15 Days of Fermentation

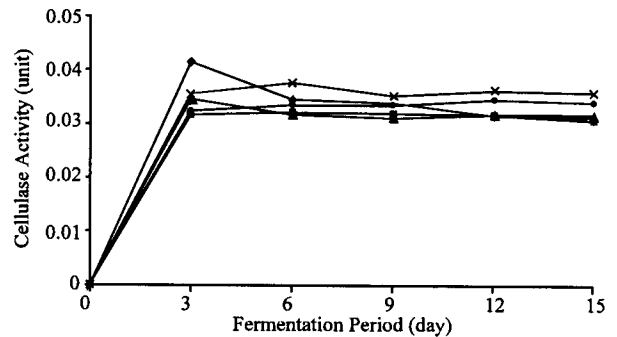


Fig 3: Effect of Inoculum Size on Cellulase Production. 5% Inoculum (◆), 10% Inoculum (■), 15% Inoculum (▲), 20% Inoculum (X) and 25% Inoculum (●)

curve) while reducing sugar showed substrate conversion to simple sugar as the decreasing trend. The decreasing value of glucosamine and reducing sugar started at day 3 and 6, respectively of fermentation. This result is correlated to the enzyme activity, as the enzyme start decreasing at day 3, biomass also decreasing. Reducing sugar yield is not a linear function of the quantity of enzyme in the assay mixture, as discussed by Ghose [16], twice the amount of

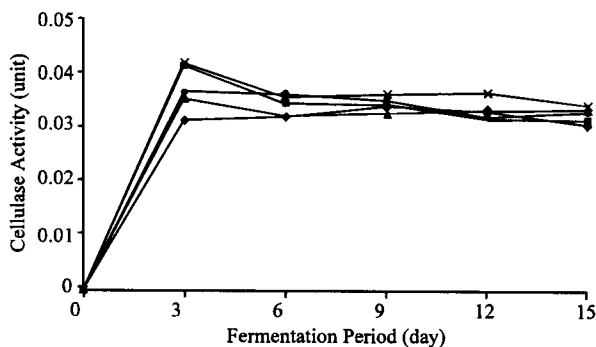


Fig. 4: Effect of Co-Substrate (Wheat Flour) on Cellulase Activity. 1% Wheat Flour (♦), 2% Wheat Flour (■), 3% Wheat Flour (▲), 4% Wheat Flour (●) and 5% Wheat Flour (X)

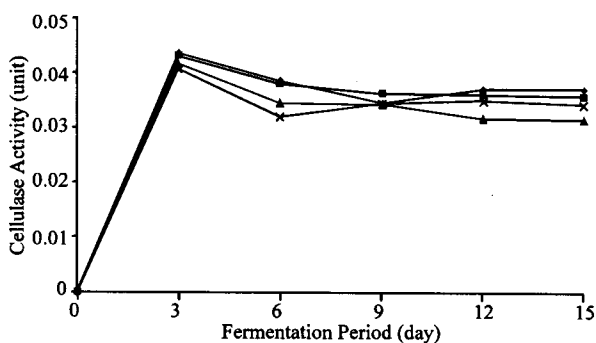


Fig. 5: Variation of Moisture Content on Cellulase Activity. 50% Moisture (♦), 60% Moisture (■), 70% Moisture (▲) and 80% Moisture (X)

enzyme would not be expected to yield twice the reducing sugar in equal time.

From the previous study, the depression in cellulase activity may be due to cumulative effect of cellobiose, a dimer of glucose which is known to inhibit both endoglucanase and glucosidase [17]. Hatakka [11] also suggested that delignification produces aromatic water-soluble products which can repress the cellulolytic action of the enzyme. However, as a lignocellulosic compound, pre-treatment of substrate is very crucial, especially in cellulose degradation for cellulase production. The ideal pretreatment would reduce the lignin content, reduce crystallinity of cellulose and increase surface area [18].

**Effect of Inoculum Size:** The effect of inoculum sizes 5-25% (v/w) on enzyme production is shown in Fig. 3. In general, cellulase activity increases with higher inoculum size, except in day 3 where 5% of inoculum size gave the highest cellulase level of 0.0413 unit for 15 days fermentation. The higher inoculum size (20%, 25%) gave higher value of cellulase compared to lower inoculum size (5%, 10%) after the day 3 of fermentation.

**Effect of Co-substrate (Wheat Flour):** The presence of additional carbon source such as wheat flour in fermentation medium can enhance the enzyme production [7]. Figure 4 shows the effect of co-substrate on the cellulase activity. In general, enzyme activity increased as the percentage of co-substrate increase. Fermentation medium containing 2% wheat flour had shown the highest cellulase activity of 0.0413 unit obtained at day 3.

**Effect of Moisture:** Moisture content is a critical factor on SSF processes because this variable has influence on growth and biosynthesis and secretion of enzyme. According to Lonsane *et al.* [19], lower moisture content causes reduction in solubility of nutrients of the substrate, low degree of swelling and high water tension. On the other hand, higher moisture levels can cause a reduction in enzyme yield due to steric hindrance of the growth of the producer strain by reduction in porosity (interparticle spaces) of the solid matrix, thus interfering oxygen transfer.

Based on the Fig. 5, the optimal moisture content in the solid substrate appears to be at 50%. Under this condition, a cellulase activity of 0.0433 unit was obtained. In other previous studies, Parsertsan *et al.* [20] obtained optimal initial moisture content of CMCase activities (23.8 U/g) at 50% for cellulase production from *Aspergillus niger* ATCC 6275 in palm oil mill wastes.

The study showed the cellulase activity of 0.0413 unit was appeared at day 3 of fermentation period in the solid state bioconversion process. The higher cellulase production was obtained with the optimum process condition at moisture content of 50%. Overall, the study provides that the lignocellulosic waste EFB has a good potential to be used as solid substrate in SSB system for production of cellulase using *T. harzianum*

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