

Effects of Xylanase and Cellulase Production during Composting of EFB and POME using Fungi

Dayana Amira R., Roshanida A.R., Rosli M.I.

Abstract—Empty Fruit Bunches (EFB) and Palm Oil Mill Effluent (POME) are two main wastes from oil palm industries which contain rich lignocellulose. Degradation of EFB and POME by microorganisms will produce hydrolytic enzyme which will degrade cellulose and hemicellulose during composting process. However, normal composting takes about four to six months to reach maturity. Hence, application of fungi into compost can shorten the period of composting. This study identifies the effect of xylanase and cellulase produced by *Aspergillus niger* and *Trichoderma virens* on composting process using EFB and POME. The degradation of EFB and POME indicates the lignocellulolytic capacity of *Aspergillus niger* and *Trichoderma virens* with more than 7% decrease in hemicellulose and more than 25% decrease in cellulose for both inoculated compost. Inoculation of *Aspergillus niger* and *Trichoderma virens* also increased the enzyme activities during the composting period compared to the control compost by 21% for both xylanase and cellulase. Rapid rise in the activities of cellulase and xylanase was observed by *Aspergillus niger* with the highest activities of 14.41 FPU/mg and 3.89 IU/mg, respectively. Increased activities of cellulase and xylanase also occurred in inoculation of *Trichoderma virens* with the highest activities obtained at 13.21 FPU/mg and 4.43 IU/mg, respectively. Therefore, it is evident that the inoculation of fungi can increase the enzyme activities hence effectively degrading the EFB and POME.

Keywords—EFB, cellulase, POME, xylanase

I. INTRODUCTION

IN Malaysia, palm oil industry produces tonnes of liquid and solid waste particularly the palm oil mill effluent (POME) and empty fruit bunch (EFB). One potential way in using these wastes is by converting it into compost. Nowadays, composting is becoming vital in management of wastes through recycling and reducing the mass and volume of the waste [1]. Natural composting period takes four to five months to reach maturity but it can be reduced to only three to four weeks by applying suitable microorganisms.

Oil palm waste, especially EFB is rich in lignocellulose [2] which can be used to produce lignocellulolytic enzymes, xylanase and cellulase [3]. Lignocellulosic materials like EFB

are made of cellulose, hemicellulose and lignin [4]. For composting, the lignocellulosic material must be converted into simpler compound which will be easily uptaken by the plant. The bioconversion of lignocellulose requires the action of multiple enzymes such as cellulase and xylanase. During composting, enzyme plays an important role in degrading the complex compound into simpler compound.

Lignocellulolytic enzymes are produced by fungal fermentation in solid substrate systems [5]. Lignocellulolytic fungi produce enzymes mainly cellulase, hemicellulase, ligninase and pectinase [6]. Cellulase is involved in degradation of cellulose [7, 2] while xylanase and related debranching enzymes produced by filamentous fungi will help in hydrolysis of hemicellulose [8]. Cellulase and xylanase produced will help in degradation hence will lead to faster maturation time.

The aim of this study is to evaluate cellulases and xylanases produced by *Aspergillus niger* and *Trichoderma virens* using Empty Fruit Bunches and Palm Oil Mill Effluent as substrates, and to study its capacity in degrading a natural lignocellulosic substrate.

II. MATERIALS AND METHODS

A. Raw Materials

EFB and POME were kindly provided by Persatuan Peladang Negeri Johor. Chicken manure was obtained from animal farm in Ayer Baloi district, Johor. These materials were stored in cold room for immediate use. Raw materials were autoclaved at 121°C for 20 minutes. After autoclaving, the raw materials were mixed with composition; 25 g of EFB, 30 g of POME and 5 g of chicken manure.

B. Inoculation of Fungi

The strains of *Aspergillus niger* and *Trichoderma virens* were used in this work. Stock cultures were maintained on potato dextrose agar slants. Spores were washed from a 7-day agar slant culture with 10 ml sterile 1% Tween-80 solution, and 2 ml aliquots (10^8 spores/ml) were added to each 250 ml shake flask containing 100 ml potato dextrose broth. The inoculated flasks were incubated at 30°C and 200 rpm for 48 hours.

C. Lignocellulosic Content

Lignocellulosic content was analysed using Datta's Method [9] with 3 stages of reflux. 1 g of sample was inserted into

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thimbles and refluxed for 2 hour with distilled water at 100°C. Then, the sample was oven-dried for one day. The sample was then refluxed again for 2 more hours in 0.5 M H₂SO₄ at 100°C. After the second reflux, the sample was oven-dried for one day. The sample was treated with 10 ml of 72% (v/v) H₂SO₄ at room temperature for 2 hours. It was then diluted to 0.5 M H₂SO₄ and underwent final reflux at 100°C for 2 hours. After the third reflux, the sample was oven-dried and weighed.

D. Xylanase Assay

Xylanase activity was determined using US Army Natick Research & Development Laboratories method [10]. p-nitrophenyl-beta-D-xyloparanoside was added to 0.5 g sample in 0.05 M citrate phosphate buffer (pH 4.8) and incubated at 50°C for 60 minutes. 3 mL of di-nitrosalicylic acid was added and the sample was boiled in a water bath for 5 minutes. OD was measured under 540 wavelengths. Xylanase activity was measured in terms of µmol xylose produced/min/mL.

E. Cellulase Assay

Cellulase activity was assayed using method from National Renewable Energy Laboratory [11]. Enzyme was extracted with citrate buffer at pH 4.8. 0.5 g sample of compost, rolled filter paper strip (1.0 x 6.0 cm) and 1.5 mL 0.05 M citrate buffer were added together. The resulting solution was incubated at 50°C for 60 minutes. The reaction was stopped by adding 3 mL of dinitrosalicylic acid (DNS). All tubes were boiled in the water bath for 5 minutes. OD550 was measured using Spectrophotometer. Cellulase activity was measured in terms of filter paper unit per mL (FPU/mL).

F. Microbial Biomass

The fungal biomass was analysed using dry weight cell method [12]. 1 g of sample was dried to constant mass at 105 °C for 24 h. The difference was calculated in weight and the dry weight was expressed in g of fungal/g of sample.

III. RESULTS AND DISCUSSIONS

A. Decomposition of Lignocellulose

Lignocellulosic content is expected to indicate the potential of fungi [13] in empty fruit bunches (EFB) and palm oil mill effluent (POME). The cellulosic content of mixed composition of EFB, POME, and chicken manure is reported in Table 1 for all compost with and without bioinoculants. It was observed that both organic components of cellulose and hemicellulose

decreased significantly for all composts. However, compost with *Trichoderma virens* matured the fastest which is on day 36, followed by compost with *Aspergillus niger* which matured on day 43. Compost without bioinoculant matured on day 92.

On day 22, the reductions of all cellulose and hemicellulose components are significantly high for both composts with bioinoculants compared to compost without bioinoculants. The highest reduction of cellulose and hemicellulose content is 21.321% and 5.924%, respectively, for composts with *Trichoderma virens*. However, the reductions of the two components are slightly the same for composts with *Aspergillus niger* where cellulose reduction is 20.505% and hemicellulose reduction is 5.058%. Compared to compost without bioinoculant, cellulose reduction is only 2.691% and 1.358% reduction for hemicellulose on day 22. It can clearly be noticed that the difference between the lignocellulose reduction for compost with bioinoculant and compost without bioinoculant is certainly huge and this indicates that the fungi effectively degrading the materials. The major difference is due to the application of fungi to compost and this finding is similar with Singh and Sharma [13] who reported a fast degradation of wheat straw with the application of fungi compared to composting without bioinoculant which has slower degradation.

After maturation on day 36, the total percentage reduction for compost with *Trichoderma virens* is 25.81% and 7.559% for cellulose and hemicellulose, respectively. Total reduction of lignocellulose content after maturation is nearly the same for all compost. However, the time taken for the compost to reach maturity is different due to the existence of different fungi which helps the degradation of each component. As for compost without bioinoculant, it reaches maturation on day 92 where the total percentage reduction is 23.674% and 7.376% for cellulose and hemicellulose, respectively.

The degradation of EFB and POME indicates the lignocellulolytic capacity of *Aspergillus niger* and *Trichoderma virens* with more than 7% decrease in hemicellulose and more than 25% decrease in cellulose for both inoculated compost. The composting period for compost without bioinoculant is almost three times longer than compost with *Trichoderma virens* and compost with *Aspergillus niger*. It can clearly be noticed that inoculation of fungi can degrade cellulose and hemicellulose hence speed up the composting process.

TABLE I
LIGNOCELLULOSIC CONTENT OF MIXED COMPOSITION OF RAW MATERIALS WITH *ASPERGILLUS NIGER*, *TRICHODERMA VIRENS* AND CONTROL

Parameter	Day 0	Day 22	Percentage reduction until day 22, %	After maturation	Total percentage reduction, %
Cellulose, %					
<i>Aspergillus niger</i>	52.581	32.076	20.505	25.921*	26.66*
<i>Trichoderma virens</i>	52.581	31.26	21.321	26.771**	25.81**
Control	52.581	49.89	2.691	25.075***	23.674***
Hemicellulose, %					
<i>Aspergillus niger</i>	25.456	20.398	5.058	17.201*	8.255*
<i>Trichoderma virens</i>	25.456	19.532	5.924	17.897**	7.559**
Control	25.456	24.098	1.358	17.964***	7.376***

All values are mean of two replicates.

* Matured on day 36 ** Matured on day 43 *** Matured on day 92

B. Changes of Enzyme Activities during Composting

Enzyme assay is one of the characteristics that respond more quickly compared to organic matter status in the compost [14] and it could also be used in characterizing the composting process [15]. Incorporation of EFB, POME, and chicken manure with inoculated fungi influences the enzyme activities in compost.

Figure 1 shows the xylanase activity versus time of compost with *Trichoderma virens*, compost with *Aspergillus niger*, and compost without bioinoculant (control). The trend shows a sharp increase in the activity of xylanase in the early stages of composting for both inoculated composts. This sharp increasing trend at initial stage is similar with Song and Wei's study [3] which uses *C. cellulans* in bagasse. However, the highest increment is in the compost with *Trichoderma virens*, with increment of 27.326% on day 8 and up to 39.976% on day 36. This finding is in agreement with Wong and Saddler [16] who stated that *Trichoderma* spp. produces enzyme with high xylanolytic activity. Inoculation of *Trichoderma virens* into the compost gives the highest activities with 4.43 IU/mg. High xylanase affect the maturation period where it will degrade hemicellulose and shorten the composting period. As in Table 1, compost with *Trichoderma virens* matured on day 36 and the hemicelluloses content was reduced nearly to 7%.

As for compost with *Aspergillus niger*, the increment on day 8 is 20.193% and up to 31.659% on day 36. This research indicates that xylanase activity is different with different strains [17]. Lowest increment of xylanase activity was observed for compost without bioinoculant where it has only approached 12.214% increment on day 8 and increased to 23.616% on day 36. This is probably because of no additional microorganism exists to help in secreting the xylanase enzyme.

Figure 2 shows the cellulase activity versus time of compost with *Trichoderma virens* compost with *Aspergillus niger*, and compost without bioinoculant (control). Cellulase activity shows a similar pattern to those observed for xylanase activity (Figure 1). This shows that all composts have increasing cellulase activity. However, it is obvious that *Aspergillus niger* has the highest activity (14.410 FPU/mg) for the whole 36 days of composting. *Aspergillus* species are the most efficient producer of β -glucosidase (cellulase enzyme) compared to *Trichoderma* spp. [18, 19] and it also has great capacity of secreting cellulose-degrading enzymes [7]. The increment of compost with *Aspergillus niger* is 5.681% on day 8 and up to 25.357% on day 36. Compared with compost without bioinoculant, the increment was observed to be at 0.291% on day 8 and up to 4.942% on day 36. As for compost with *Trichoderma virens*, the highest activity was on day 36 (13.214 FPU/mg) with the increment of 4.942% on day 8 and up to 18.605% on day 36. Similar pattern can be viewed for cellulase production by *Trichoderma viride* on bagasse [20]. Conversely, cellulase can be increased by using different compositions of raw materials [19] however this might affect the quality of the biofertilizer itself.

Inoculation of *Aspergillus niger* and *Trichoderma virens* has also increased the enzyme activities during the composting period compared to the control compost by 21% for both xylanase and cellulase. These results indicate that the

inoculations of *Trichoderma virens* and *Aspergillus niger* to composts have a more active effect on xylanase and cellulase activities. Other than the fungi itself, degradable organic compounds in raw materials might also stimulate the enzyme activity [21]. Selection of carbon source used in composting also affects the yield of enzyme [19]. However, both activities do not vary too much since the lignocellulosic source is the same. Cellulose and hemicellulose are both the main substrate for cellulase and xylanase production, respectively. Different substrate used will have different amount of enzyme produced.

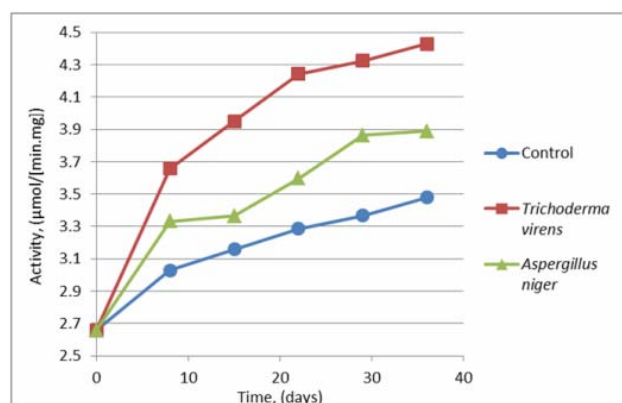


Fig. 1 Xylanase activity versus time of compost with *Trichoderma virens*, compost with *Aspergillus niger* and compost without bioinoculant(control)

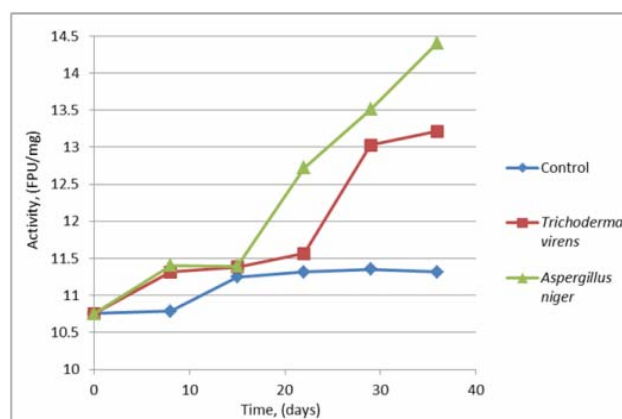


Fig. 2 Cellulase activity versus time of compost with *Trichoderma virens*, compost with *Aspergillus niger* and compost without bioinoculant(control)

C. Microbial Biomass

Figure 3 shows the microbial biomass of compost with *Trichoderma virens*, compost with *Aspergillus niger* and compost without bioinoculant. Increasing number of *Trichoderma virens* and *Aspergillus niger* cells were observed during the composting period. Both fungi spread and grow vigorously by producing many cells in order to secrete digestive enzyme and degrade the lignocellulosic component.

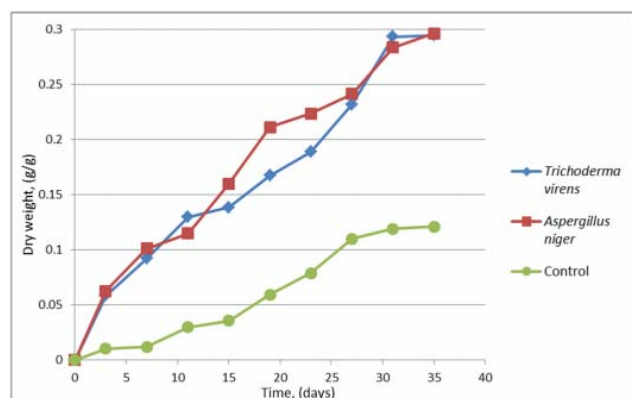


Fig. 3 Microbial biomass of compost with *Trichoderma virens*, compost with *Aspergillus niger* and compost without bioinoculant

Both composts with bioinoculant show an increasing trend. However, towards the end of the composting process, *Aspergillus niger* and *Trichoderma virens* started to show insignificant increment due to the decreasing substrate availability. For compost without bioinoculant, the trend is also increasing and this is probably due to the interference from microorganisms from the environment.

The increasing trend of the cell is parallel with the increasing enzyme activity hence also leads to a good biocontrol agent when it is applied to the soil. Fungi are highly colonizers of their habitat and easily utilize the substrate and secrete digestive enzyme. This existence of fungi in compost can surely accelerate the composting process and shorten the composting period.

IV. CONCLUSION

Inoculation of *Aspergillus niger* and *Trichoderma virens* with empty fruit bunch (EFB) and palm oil mill effluent (POME) accelerates the composting process. EFB and POME were effectively degraded from lignocellulose to simpler compound. The application of fungi for lignocellulose breakdown produces cellulase and xylanase which lead to efficient conversion of substrate into compost. These results also indicate the cellulolytic capacity of fungi and its potential use in the degradation of oil palm waste as well as recycling the waste for the use of compost. Therefore, the inoculation of fungi can increase the enzyme activities hence effectively degrade the EFB and POME.

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