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Full Length Research Paper

Bioconversion of empty fruit bunches (EFB) and palm oil mill effluent (POME) into compost using Trichoderma virens

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This study shows the performance of *Trichoderma virens* as an activator for conversion of empty fruit bunches (EFB) and palm oil mill effluent (POME) into compost. EFB and POME are two abundant wastes produced by oil palm industries which keep accumulating. Since there is no proper way to dispose these wastes, a potential way is to turn them into value-added product which is compost. However, normal composting will take about 4 to 6 months and additional pure fungi on compost can reduce the time to only 21 to 45 days. It also promotes plant growth and fight plant diseases. *T. virens* is one of the potential fungus activator and the enzyme production by this specific fungus has been studied. Biodegradation of EFB and POME supplemented with *T. virens* and organic N (chicken manure) gave significant changes as compared to EFB and POME alone. Application of *T. virens* resulted in higher xylanase and cellulase activities which lead to rapid degradation of cellulose and hemicelluloses. Compost with *T. virens* has higher xylanase activity on day 36 which is 4.43 µmol/(min.mg) as compared to the control which has 3.48 µmol/(min.mg). The cellulase activity is 13.214 FPU/mg and 11.314 FPU/mg for compost with *T. virens* and compost without bioinoculant on day 36, respectively. The N, P, K content of compost with *T. virens* increased significantly after maturation which is 1.304, 0.5034 and 0.645%, respectively. This result shows that *T. virens* played a great role by shortening the composting period of EFB and POME while producing nutrient-enriched compost.

Key words: Empty fruit bunches (EFB), palm oil mill effluent (POME), bioconversion, *Trichoderma virens.*

INTRODUCTION

In Malaysia, palm oil industries generate many liquid and solid wastes, especially the palm oil mill effluent (POME) and empty fruit bunch (EFB) which keeps accumulating. If there is no proper way to manage these wastes, it will contribute to land pollution and increase the waste treatment cost. One potential way to turn the wastes into value-added product is by converting it into compost through microorganism degradation. This conversion could solve several problems including reducing the high load of waste produced by palm oil industry. It can also create a market for biofertilizer which have proven to be more effective and cheaper than chemical fertilizer. The industry can generate profit through the waste and the application of biofertilizer in agriculture would treat the soil diseases resulting from extensive use of chemical fertilizer by returning its biodiversity and fertility.

Conventionally, natural composting takes four to five months to reach maturity. However, composting process can be shortened to three to four weeks only by mixing the organic material with certain microorganisms. Composting process can be accelerated to one month by inoculation of cellulolytic fungi such as *Aspergillus* and

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Abbreviations: EFB, Empty fruit bunch; **POME,** palm oil mill effluent.

Table 1. Lignocellulosic content of mixed composition of raw materials with *T. virens*.

Parameter (%)	Day 0	Day 22	Percentage reduction until day 22 (%)	Day 36 (After maturation)	Total percentage reduction (%)
Cellulose	52.581	31.26	21.321	26.771	25.81
Hemicellulose	25.456	19.532	5.924	17.897	7.559
Lignin	19.052	10.006	9.046	5.598	13.454

All values are mean of two replicates.

Trichoderma (Biswas and Narayanasamy, 2002). Therefore, fungi from Trichoderma spp. which has been proven to effectively degrade the organic matter into minerals can be utilized for this purpose.

Plant growth promoting fungi (PGPF), *Trichoderma* spp. is believed to confer benefits to plants not only by promoting the growth but also defending them from infection (Pandya and Saraf, 2010). *Trichoderma virens* inoculated in composted chicken manure and rye cover crops provided significant weed control (Héraux, 2005). There is also another technology which has been developed by Cuevas (1997), and it is the development of the windrow type of composting, and the main modernization is by using pure cultures of *Trichoderma harzianum*. Besides, a research done by Gaind and Nain (2007) shows that incorporation of paddy straw in soil in conjunction with *Trichoderma reesei* can improve soil biochemical properties. It was also reported by Pandya and Saraf (2010) that genus *Trichoderma* is an important biocontrol agent of several soil borne phytopathogens.

MATERIALS AND METHODS

The substrates used in this study were EFB and POME, obtained from the palm oil plantation of Persatuan Peladang Negeri Johor in Kahang, Johor. Chicken dung was obtained from animal farm in Ayer Baloi district, Johor. All raw materials were stored at 4°C for immediate use.

Chemical composition of materials

The chemical composition of the EFB, POME and chicken dung (small amount of additional raw material which is the nitrogen source) were analyzed. Nitrogen, phosphorus and potassium content were analysed using the Spectroquant Kit by Merck.

Inoculation of fungi

The fungal strain of *T. virens* was procured from Universiti Kebangsaan Malaysia (UKM). The fungal culture was maintained by subculturing it on potato dextrose agar and keeping it at room temperature for 7 days.

Lignocellulosic content

Lignocellulosic content was analysed using Datta's method (1981) with 3 stages of reflux. One gram of sample was inserted into thimbles and refluxed for 2 h with 150 ml distilled water at 100°C. Then, the sample was oven-dried for 24 h. Next, the sample was refluxed for two more hours in 150 ml H_2SO_4 at 100°C. After the second reflux, the sample was oven-dried for 24 h. The sample was treated with 10 ml of 72% (v/v) H_2SO_4 at room temperature for 2 h. It was diluted to 0.5 M H_2SO_4 and was refluxed at 100°C for 2 h. After the third reflux, the sample was oven-dried and weighed.

Xylanase assay

Xylanase activity was measured using US Army Natick Research & Development Laboratories method (Ghose and Bisaria 1987). Enzyme was extracted with citrate buffer of pH 4.8. Then, 1.5 g sample of enzyme filtrate was incubated with p-nitrophenyl-beta-D-xyloparanoside and volume was added to 1.5 ml with 0.05 M citrate buffer. All tubes were incubated at 50°C for 30 min. Three milliliters of di-nitrosalicylic acid was added to the sample and then the sample was boiled in a water bath. Xylanase activity was measured in terms of µmol xylose produced/min/mg.

Cellulase assay

Cellulase activity was tested using the method of National Renewable Energy Laboratory (Adney and Baker, 2008). Enzyme was first extracted with citrate buffer at pH 4.8. Next, 1.5 g sample of enzyme filtrate was incubated with filter paper. Citrate buffer (0.05 M) was added to the sample to make upto 1.5 ml of the total volume. All tubes were incubated at 50°C for 30 min. Three milliliters of di-nitrosalicylic acid was added and the sample was boiled in a water bath. Cellulase activity was measured in terms of filter paper unit per mg (FPU/mg).

RESULTS AND DISCUSSION

Lignocellulosic content

The lignocellulosic content of mixed composition of EFB, POME and chicken dung are presented in Tables 1 and 2 for both compost with *T. virens* and compost without bioinoculant, respectively. On day 22, the percentage reduction of all three components was significantly high for compost with *T. virens* as compared to compost without bioinoculants. The reduction of cellulose content was 21.321 and 2.691% for compost with *T. virens* and compost without bioinoculant, respectively. The reduction of hemicellulose content is 5.924% for compost with *T. virens* and 1.358% and for compost without bioinoculant. The reduction of lignin content for compost with *T. virens* and compost without bioinoculant is 9.046 and 2.069%,

Table 2. Lignocellulosic content of mixed composition of raw materials without bioinoculants.

Parameter (%)	Day 0	Day 22	Percentage reduction until day 22 (%)	Day 43	Day 64	Day 85	Day 92 (after maturation)	Total percentage reduction (%)
Cellulose	52.581	49.89	2.691	42.756	32.875	28.907	25.075	23.674
Hemicellulose	25.456	24.098	1.358	24.578	20.065	18.08	17.964	7.376
Lignin	19.052	16.983	2.069	14.002	12.85	9.006	7.502	10.046

All values are mean of two replicates.

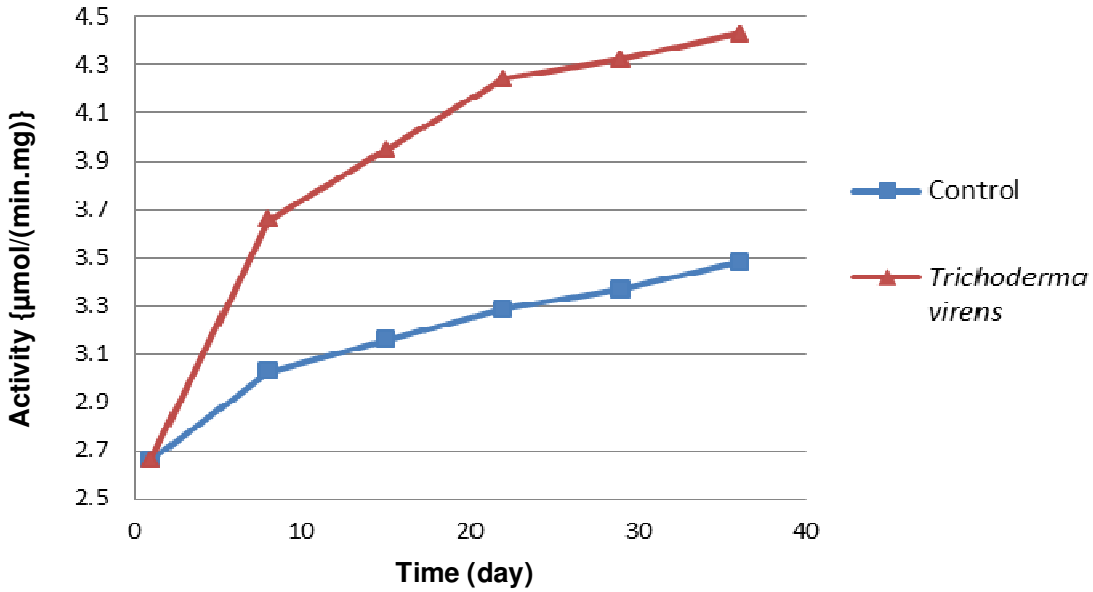

Figure 1. Xylanase activity versus time of composting with *T. virens* and composting without bioinoculant.

respectively. High percentage in compost with *T. virens* indicates that the component is efficiently degraded. This finding is similar to that of Singh and Sharma (2002) who reported a rapid degradation of wheat straw with the use of fungi as compared to compost without bioinoculant which has slower degradation.

After maturation on day 36, the total percentage reduction of lignocellulosic for compost with *T. virens* is 25.81, 7.559 and 13.454% for cellulose, hemicellulose and lignin, respectively. However, the compost without bioinoculant takes up to 92 days to reach maturity with the total percentage reductions of lignocellulosic at 23.674, 7.376 and 10.046% for cellulose, hemicellulose and lignin, respectively. It was observed that all three organic components of cellulose, hemicelluloses and lignin decreased significantly for both composts. However, the compost with *T. virens* matured on day 36, while compost without bioinoculant matured on day 92. This shows that *T. virens* reduced the maturation time by 60.9% as compared to compost without bioinoculant. Therefore, addition of *T. virens* as accelerating agent affects the speed of composting and maturation time (Haddadin et al., 2009).

Enzyme assay

Enzyme assay is one of the characteristics that respond more quickly as compared to organic matter composition in the compost. 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 without bioinoculant (control) and compost with *T. virens*. It shows that both composts have increasing xylanase activity. However, compost with *T. virens* has higher activity with the increment of 27.32609% on day 8 and up to 39.97576% on day 36. This is due to the existence of *T. virens* which produces xylanase. Compost without bioinoculant only approaches 12.21415% increment on day 8 and increased to 23.61561% on day 36. Highest xylanase activity for compost without bioinoculant was 3.48 µmol/(min.mg) which is on day 36. The highest cellulase activity is for compost mixture with *T. virens* on day 36 which is 4.32 µmol/(min.mg). *Trichoderma* spp. is definitely known to produce enzyme with high xylanolytic activity (Wong and Saddler, 2002).

Figure 2 shows the cellulase activity versus time of

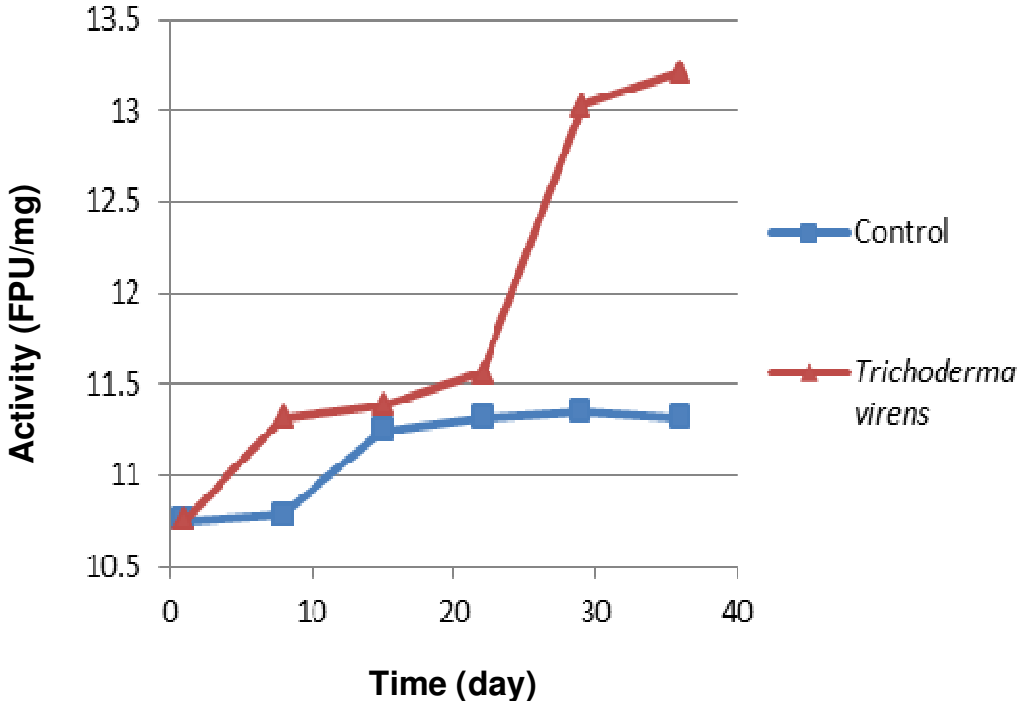

Figure 2: Cellulase activity versus time of composting with *T. virens* and composting without bioinoculant.

compost without bioinoculant (control) and compost with *T. virens.* It shows that both composts have increasing cellulase activity. However, it is obvious that *T. virens* have higher activity for the total of 36 days of composting. The increase of compost with *T. virens* is 4.94% on day 8 and up to 18.61% on day 36. When compared with compost without bioinoculant, the increase was observed to be 0.29% on day 8 and up to 4.94% on day 36. The highest xylanase activity for compost without bioinoculant is 13.214 FPU/mg which is on day 36. The highest activity of cellulase is for compost with *T. virens* which is 11.314 FPU/mg on day 36.

Both xylanase and cellulase activity of compost with *T. virens* show increasing trend towards the maturity of compost and this is in agreement with Gaind and Nain (2007) which used *Aspergillus awamori* and *T. reesei*. Compost with *T. virens* has high enzyme activities for both xylanase and cellulase which indicates that the compost was effectively degraded. This is due to the existence of fungal which can produce digestive enzyme such as xylanase and cellulose.

Macronutrients content

Tables 3 and 4 show the percentage of nitrogen, phosphorus and potassium content of both composts at before and after maturation. Chemical analysis shows that compost with *T. virens* has higher macronutrients content as compared to compost without bioinoculant. High total nitrogen content of compost with *T. virens* might be due to enhanced decomposition of organic matter by the fungi (Pramanik et al., 2007). High total nitrogen is also governed by the initial nitrogen content of the raw materials. Besides, the increasing concentration on nitrate-nitrogen (NO_3-N) at the end of the composting process increases the total nitrogen content. However, ammonium-nitrogen (NH_4-N) is high in the early stage of composting but decrease gradually during maturation of the compost (Young et al., 2005).

Phosphorus content is also higher with regards to maturation for both compost and this is because phosphorus is not lost by volatilization or lixiviation during the composting process. The concentration of phosphorus increases as composting proceeds (Young et al., 2005). Similarly, potassium is high for both compost as compared to initial value since PO_4^- ions from humic colloids are released into the system (Pramanik et al., 2007). For compost with *T. virens*, there was higher percentage of phosphorus and potassium due to the presence of fungal which plays an important role in increasing the P and K content during the process.

Table 5 shows the N, P, K percentage of compost from different raw materials and microorganism studied by different researchers. The nitrogen percentage in this study (1.304%) was observed to be in the range of other studies which is 0.98 to 2%. Phosphorus and potassium percentage in this study is observed to be lower as com-

Table 3. Composition of compost at initial stage.

Parameter	N (%)	P (%)	K (%)
Compost (EFB, POME and chicken dung)	0.913	0.370	0.603

Table 4. Composition of the compost.

Parameter	N (%)	P (%)	K (%)
Compost with *T. virens* (day 36)	1.304	0.543	0.645
Compost without bioinoculant (day 92)	0.961	0.412	0.633

Table 5. Nitrogen, phosphorus and potassium content in the compost.

Materials/microorganism	N (%)	P (%)	K (%)	Reference
Wheat straw + *T. harzianum* + *A. niger* + *Azotobacter chroococcum*	0.98	0.19	0.55	Singh and Sharma (2002)
Olive pomace + *T. harzianum* + *Phanerochaete chrysosporium*	1.6	1.1	19.8	Haddadin et al. (2009)
Rice straw + weeds + chicken manure + *T. harzianum*	2.0	2.9	1.9	Virginia (1997)
Rice straw + okara + vinasse + buffalo manure + rock phosphate	1.94	1.95	0.953	Rashad et al. (2010)
empty fruit bunches + palm oil mill effluent + chicken dung + *T. virens*	1.304	0.543	0.645	This study

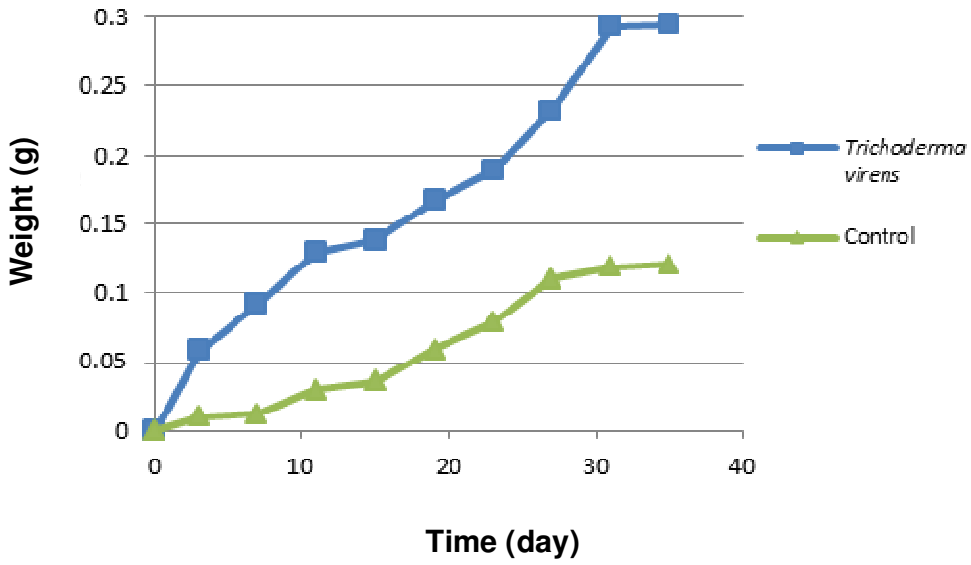

Figure 3. Microbial biomass of compost with *T. virens* and compost without bioinoculant.

compared to most of the other studies, which might be due to several factors such as composition and type of raw materials and mixed microorganism. Therefore, an optimization study will be further carried out in order to improve the percentage of N, P and K.

Microbial biomass

Figure 3 shows the microbial biomass of compost with *T. virens* and compost without bioinoculant. An increasing number of *T. virens* cell was observed during the composting period. *T. virens* spread and grow vigorously by producing many cells in order to secrete digestive enzyme and degrade the lignocellulosic component. The increasing trend of the cell is parallel with the increase of the enzyme activity, hence will also make it to be a good biocontrol agent when it is applied to soil. *Trichoderma* spp. are high colonizers of their habitat and easily utilizes the substrate and secreting digestive enzymes.

Conclusion

Composting of EFB and POME with *T. virens* shortened the composting period to 60.9% as compared to compost without bioinoculant. Xylanase and cellulase production was observed to be high in compost with *T. virens* hence, lead to rapid degradation of both hemicelluloses and cellulose, respectively. *T. virens* also contributes towards the higher percentage of nitrogen, phosphorus and potassium content. The outcome of this study is beneficial in converting the waste into value-added products in a short time with additional good impacts on the plant as well. Application of compost with *T. virens* to plant is expected to have benefits and therefore thorough studies should be done further.

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