DEGRADATION OF OIL PALM EMPTY FRUIT BUNCH (EFB) USING COMPOST AND PALM OIL MILL EFFLUENT (POME) AS INOCULUM

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Abstract - Empty fruit bunch (EFB) and palm oil mill effluent (POME) were the major waste produced from oil palm mills in Malaysia. In this study, the physicochemical characteristic of POME, compost and EFB has been analyzed. Besides, the condition of composting process of EFB related to medium and inoculum type has been studies. As a result, the composting conditions with POME as a medium and compost as inoculum was the best condition. This condition was able to efficiently degrade the EFB by 20% of the weight loss within a month. However, the enzyme activity for this composting condition was really slow which believed that give an effect on slowing down the composting process. Therefore, further study can be done to optimize this condition for higher enzyme activity and weight loss.

Keywords - Empty Fruit Bunch, Compost, Solid State Fermentation, Palm Oil Mill Effluent.

I. INTRODUCTION

Malaysia is the second largest producers of palm oil in the world after Indonesia(MPOB 2017). Up to 2016, the total area of the plantation is about 5.74 million Ha with the total production 17,320,000 tonnes of palm oil(MPOB 2017). This in turn generates huge amount of oil palm derived waste such as oil palm empty fruit bunch (EFB), palm kernel shell (PKS), mesocarp fruit fibres (MF) and palm oil mill effluent (POME) (Damen and Faaij 2018). 23% (w/w) of EFB was generate per every fresh fruit bunch and around 60 tonnes per hour mill based within 10,000 Ha plantation. (Damen and Faaij 2018; Abdullah and Sulaiman 2013). While, 2.5-3.0 tonnes of POME per tonne of produced crude oil was generated duringsterilization and pressing of the fruits (Madaki and Seng 2013).

EFB and POME create the largest residual fractions from palm oil mills. Currently, EFB and POME has been used as raw materials for co-composting and this practice have been done in some of oil palm mills in Malaysia. The resulting compost was used at the oil palm plantation. POME was undergo treatment in anaerobic digester before used for composting. POME contains many soluble chemical materials that are detrimental to the environment. However, the proper use of POME in the land environment has been reconfirmed would directly improve soil fertility (Madaki and Seng 2013).

The usage of organic waste such as EFB and POEM as fertilizer have the ability to increase crop productivity and improve soil health while reducing waste disposable problem (Gaind and Nain 2007) The composting cycle in oil palm mill was compete in 3 months. However, the mature compost is still in fibrous which indicate incomplete degradation of EFB. The traditional composting of EFB and POME takes months. This is because EFB mainly composed of lignocellulose materials. Hydrolytic enzyme are needed to degrade the lignocellulose materials into monomeric sugar. According Siddiquee et al. (2017), the degradation can be prevented due to the robust lignin layer that act as natural barrier.

Siddiquee et al. (2017) reported that solid-state fermentation (SSF) as the best method to produce biocompost. The chosen of microorganisms that can adapt to the SSF condition is crucial. The organism must be able to produce large amount of enzyme to degrade lignocellulose materials. Normally, there are two types of SSF based on microorganism, which are pure culture SSF, or indigenous SSF. However, there are not much paper reported on the indigenous SSF for degradation of lignocellulosic materials to produce biocompost.

Therefore, by conducting some comprehensive and efficient strategies on the fermentation process using indigenous microorganism from POME and mature compost, potential and economical biocompost can be produce.

In the present study, mature compost and POME taken from mill was used as medium and inoculum for degradation of EFB using solid state fermentation. The production medium and the inoculum type has been screened. This study have five models of solid state fermentation with each of it is different based on medium and inoculum source.

II. DETAILS EXPERIMENTAL

2.1. Collection of samples

The EFB, compost and POME sample were taken from one of oil palm mill in Johor.

The compost samples were taken at week 12 of the composting process. While, POME samples were taken from the effluent pipe of anaerobic digester. These samples were used for physicochemical analysis and solidstate fermentation study. These samples were stored at 4°C for any further analysis.

2.2. Physicochemical characterization

Scanning Electron Microscope (SEM) was used to observe the surface morphology EFB. EFB were send to Advance Membrane Technology Research Centre (AMTEC) for microscopic analysis. The EFB was coated with gold plated before observed using SEM. Analysis of BET was done to explain the surface area of the EFB. The samples were send to the Faculty Sciences, UTM for the analysis.

Elemental composition in EFB, POME and compost were analysed using ICPMS and ICPOES machines. The samples were send to University industrial research laboratory (UIRL) for the analysis.

2.3. Biodegradation of EFB using solid state fermentation

POME and matured compost were used directly as inoculum. 10% (v/v) of POME or compost were inoculated into the substrate. Before inoculation,POME and compose was undergone heat shock for 10 mins at 80 ºC. The SSF was done using 800 µm size of EFB. The EFB were moistening with right volume ofproduction medium and 10% (w/w) inoculum to generate final moisture level of 65%. All flasks were incubated at room temperature for 30 days and the production medium was added once every 10 days to moisturize the EFB. Lastly, the fermented substrate was collected every 5 days for enzyme analysis and weight loss analysis. The evaluated parameters were the production medium and the inoculum type. Thus, 5 model of solid state fermentation were set-up based with different medium and inoculum type used. The fermentation models was shows in Table 1.

Table 1: Different screening model in term of inoculum and medium for best conditions compost production

Model	Substrate	Inoculum	Media
1	EFB	Mature compost	Mandel's basal medium
2		POME	Mandel's basal medium
3		Mature compost	Non-sterile POME
4		Mature compost	Non-sterile POME supernatant
5		POME	100% filtered seawater

III. RESULTS AND DISCUSSION

3.1. Physicochemical characterization

Scanning electron microscopy (SEM) was done analyzed further the structural and morphological of the EFB. The analyzed EFB were the one after and before autoclaving to see the effect of autoclaving to the structural of the EFB. Before autoclaved, silica was detected on the surface of the EFB and only few micro pores.

After autoclaved, several micro pores were observed on the surface of EFB due to the autohydrolysis process during the autoclaving (Gonçalves et al. 2015). This micro pores resulting from the removal of the silica by the autoclaving process. The silica was shown in the Figure 1A under yellow circle. The presence of micro pores indicates higher surface area of cellulose for enzyme attack (Castoldi et al. 2014). The surface area of EFB after autoclave was higher than before autoclave EFB. This was proven using Brunauer-Emmett-Teller where the EFB after autoclaved have 2.593 m^2/g surface area while the EFB before autoclave have 2.096 m^2/g. In addition, the total area in pore for EFB after autoclave is 0.606 m^2/g while the EFB before autoclave is 0.098 m^2/g.

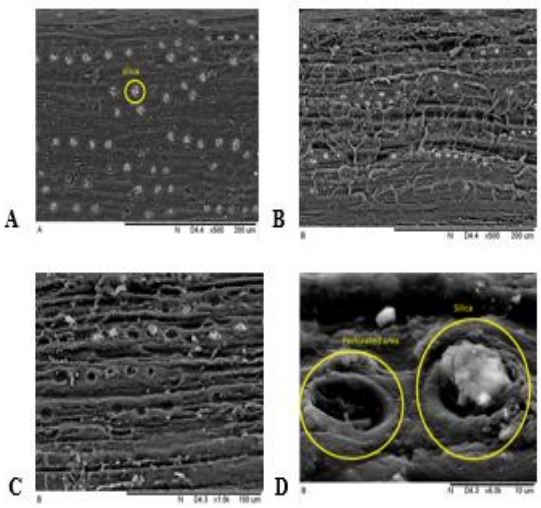


Figure 1: Images of EFB by SEM (a) before autoclave under 500 x magnification (b) after autoclave under 500 x magnification (c) after autoclave under 1000 x magnification (d) removal of silica after autoclave under 6000 x magnification

The EFB, compost and POME were analysed for its chemical component before the biodegradation process. Table 2 showsthe chemical component of the EFB, supernatant POME, autoclaved supernatant of POME and compost. Percentage of carbon in EFB was 45.42% (w/w) which was the highest among all

samples. High amount of carbon and low amount of nitrogen cause high C:N ratio. High C:N ratio that present in EFB make its suitable for fungal growth and metabolism (Zhang and Elser 2017).The C:N ratio is important because it effect the residue decomposition and also nitrogen cycling in the soil. However, nitorgen also play an important role in fermentation process because nucleic acids, proteins and enzyme are made up of nitrogen that responsible for microbial growth. The most abundant metal element found in EFB fibres was calcium, which 31.72 ppm. While, potassium was the most abundant in supernatant POME, autoclaved supernatant POME and compost with 150.91 ppm, 159.74 ppm and 341.14 ppm, respectively.High potassium content makes POME a suitable production medium in solid state fermentation for compost production.

This is because, a good compost must contain high amount of potassium for plant growth. Most agricultural soil contain total potassium from 4 to 25g per kg and less then 1% of total potassium available to plants (He et al. 2001). Thus, this compost can be a great source of potassium for crops.

Table 2: Elemental analysis of EFB,POME and compost collected from oil palm mill

Elements	Unit	Concentration			
		OPEFB	Supernatant POME	Supernatant POME (Autoclaved)	Mature compost
Carbon (C)	% (w/w)	45.42	0.45	0.11	8.96
Nitrogen (N)	% (w/w)	0.45	0.60	0.51	0.76
Hydrogen (H)	% (w/w)	4.67	10.80	10.85	7.89
Sulphur (S)	% (w/w)	0.169	0.09	0.05	0.17
Calcium (Ca)	ppm	31.72	32.38	17.52	51.94
Copper (Cu)	ppm	0	0.08	0	2.26
Iron (Fe)	ppm	5.03	14.10	0.99	71.30
Potassium (K)	ppm	5.11	150.91	159.74	341.14
Magnesium (Mg)	ppm	8.80	24.58	22.81	28.99
Manganese (Mn)	ppm	0.02	0.54	0	9.49
Sodium (Na)	ppm	1.43	4.15	2.35	6.51
Silicon (Si)	ppm	18.79	12.39	6.96	6.58
Zinc (Zn)	ppm	0.20	0.16	0	4.11
Phosphorus	ppb	13.90	97.47	60.98	20.73

3.2. Biodegradation of EFB using solid state fermentation

It was found in this study that the different microorganisms and selection of different production medium in SSF can improve the lignocellulosic degradation, but usually not for all enzymes. The changes in the activity of the lignocellulolytic enzymes were compared for all 5 models as shown in Figure 2 and Figure 3. Cellulase and xylanase are important to hydrolysed cellulose into simple sugar such as glucose, cellobiose and fructose. While, xylanase is important for production of simple sugar such as arabinose, maltose, cylose and galactose. Based on Figure 2A, endoglucanase activity in all models were really low. Model 1 and model 2 shows slightly high in the activity of endoglucanase at day 25 with the maximum activity of 0.0017 U/g. For exoglucanase activity, Model 1 shows high activity at day 15 with the activity was 7.4 U/g and the activity start to rise again at day 30 with the activity was 4.9 U/g. While in model 2, the activity was really low and it start to increase at day 30 with the concentration of 8.6 U/g as shown in the Figure 2B. Model 1 and model 2 were also having high activity of beta-glucosidase. The activity of beta-glucosidase start to increase since day 5 for model 2 and day 10 for model 1 and having the maximum concentration of 31 U/g and 18 U/g, respectively as shown in the Figure 2C. Besides, model 1 and model 2 were also observed high activity of xylanase as shown in Figure

2D. Xylanase activity in model 1 was started at day 5 and increased up to the concentration of 52 U/g. same as model 2 which started to increase the concentration on xylanase at day 10 and have been increase up to 32.8 U/g. However, model 3, model 4 and model 5 shows a very little activities of cellulase and xylanase. Using POME and sea water as a medium resulting in the low enxyme activity and these were probably because of the inhibitors presence in the POME and sea water that inteferes with the compost or microbial community in the compost or vise versa. In this case, mandel medium shows to be a really good medium for the production of cellulase and xylanase. This has been proven by the previous study which the mandels basal medium has been used for the production of cellulase and xylanase (Ang et al. 2013; Singhania et al. 2010; Oberoi et al. 2010).

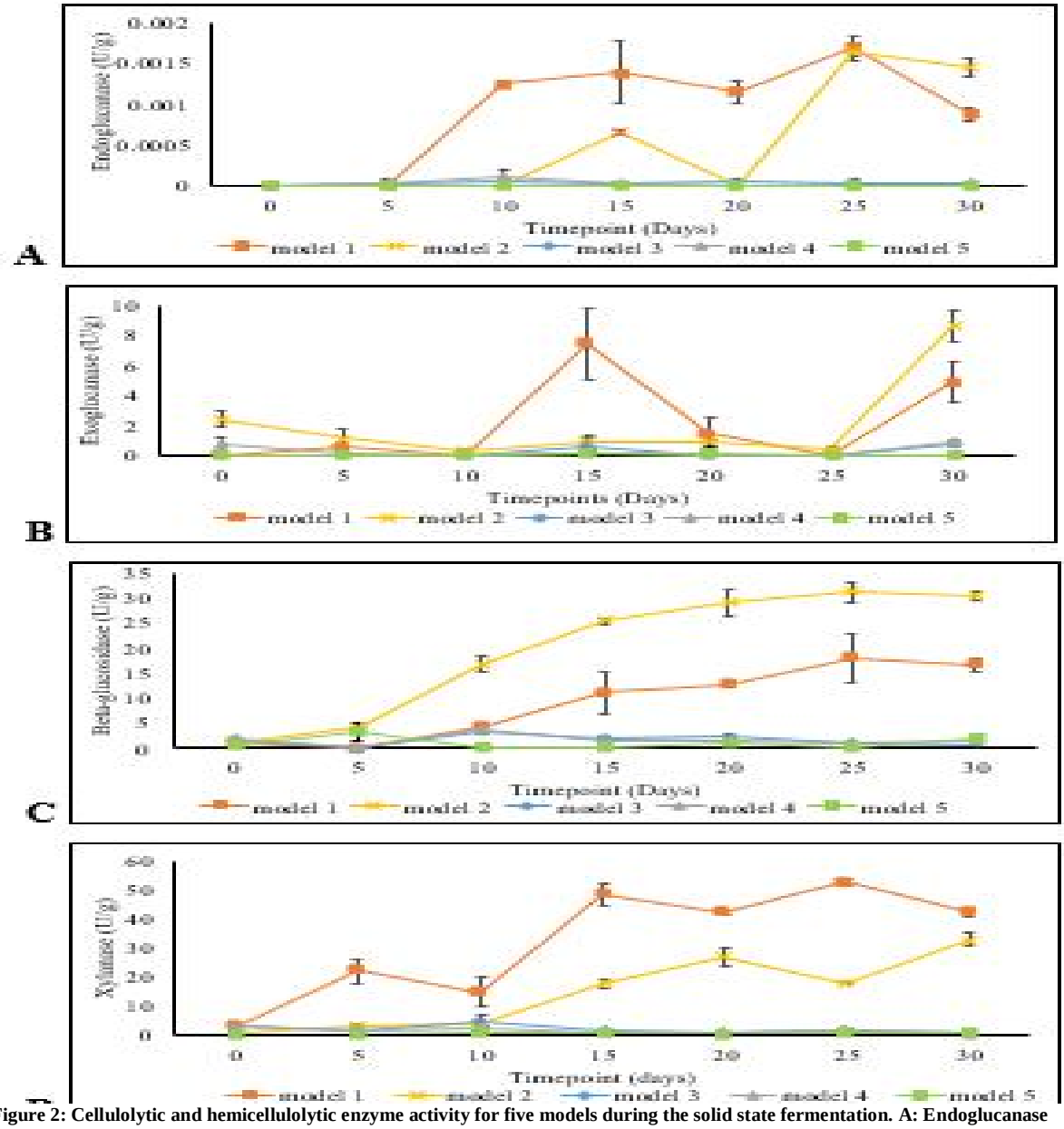


Figure 2: Cellulolytic and hemicellulolytic enzyme activity for five models during the solid state fermentation. A: Endoglucanase activity; B: Exoglucanase extivity; C: Beta-glucosidase activity; D: Xylanase activity

Lignolytic enzymes observed in this study were lignin peroxidase, managnese peroxidase and laccase as shown in the Figure 3. In this study, manganese peroxidase activity produced in model 4 with the maximum concentration of 0.15 U/g at day 10. Model 3 also shows some manganese activity at day 20 with the concentration of 0.07 U/g. The lignin peroxidase activity was observed in all model except model 5. The lignin peroxidase activity start rised at day 5 for model 2 and day 10 for model 1, model 3 and model 4 with the concentration of 0.94 U/g, 1.2 U/g, 1.4 U/g, 1.4 U/g respectively. In this present study, lignin peroxidase activity is higher at first 10 days of composting process and only after that the cellulolytic activity start to rised at day 15. However, all models shows almost no activity of laccase.

Delignification is important to allow the degradation of cellulose and hemicellulose below the lignin layer. The increase of lignin peroxidase will increase the reducing sugar production at day 10 and day 15 of the fermentation. This indicate that the lignin of EFB have been hydrolyzed by the lignin peroxidase which strongly bonded with cellulose and hemicellulose.The breakdown of lignin will exposing the cellulose and hemicellulose which can be easily attack by the microorganims for the degradation into simple sugar.

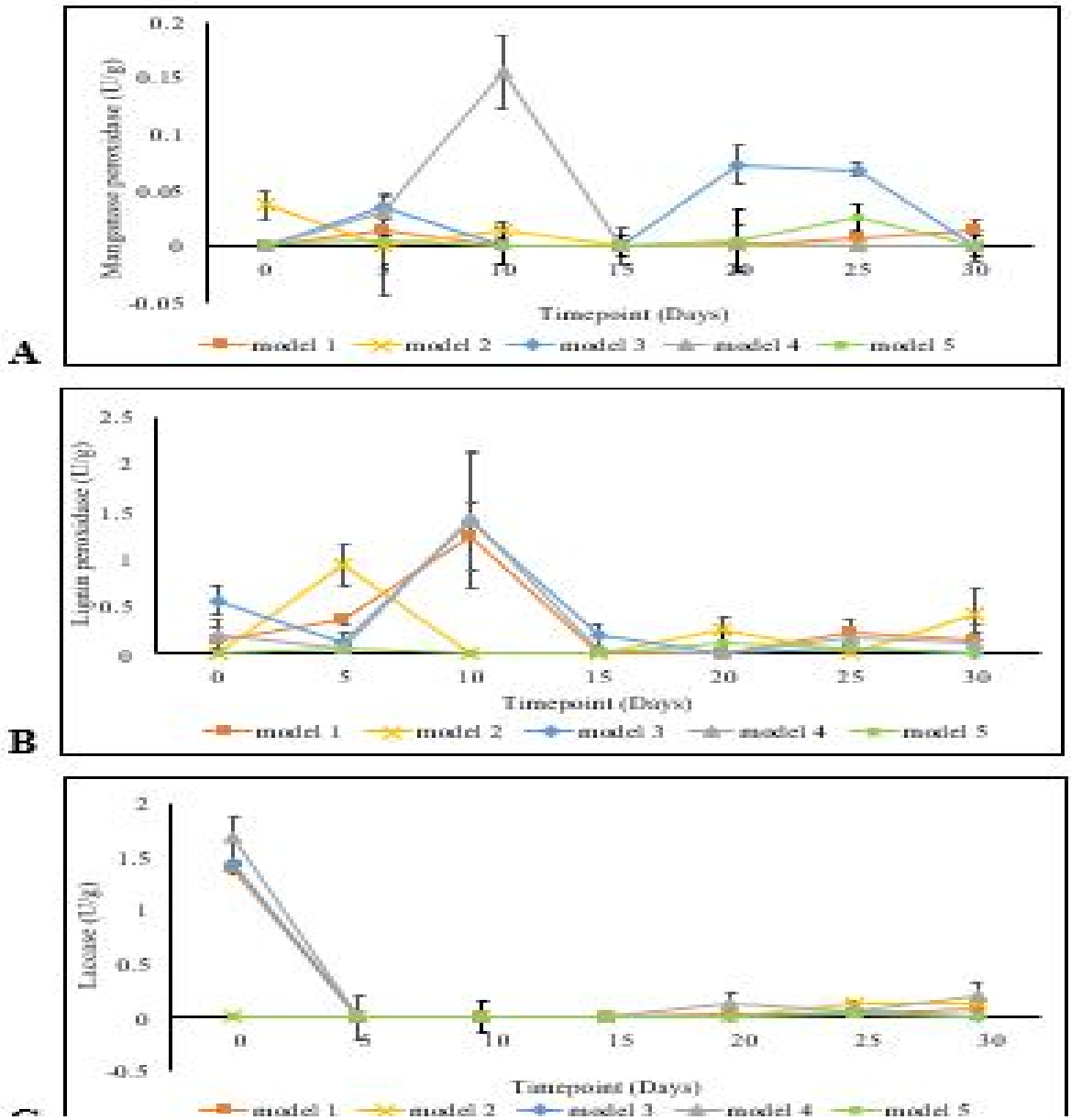


Figure 3: Lignolytic enzyme activity for five models during the solid state fermentation. A: Manganase peroxidase activity; B: Lignin peroxidase activity; C: Laccase activity

The degradation of lignocellulose biomass was estimated by the weight loss of the EFB throughout the composting process.

The percentage of weight reduction of EFB was shows in the Figure 4. Model 1 until model 4 having almost similar reduction percentage which were 20.5%, 19.6%, 20.7% and 20.1% respectively. However, model 5 shows really low reduction of EFB event after the prolong fermentation which give the final reduction of 6.8%. Thus, this conclude that model 5 having really slow degradation of EFB. Model 1 until model 4 can be a great condition for the degradation of EFB. Model 3 was considered as the best model because it is able to degrade the EFB as much as model 1 and model 2 event the enzyme production was not at optimum level. Another advantage of model 3 was this model was considered as a cost effective condition since it is only using waste, which was POME as a medium and compost as inoculum. POME in this model not only act as medium and moisture agent but it also act as inoculum source since it contain abundant of potential microorganisms that help throughout the composting process.

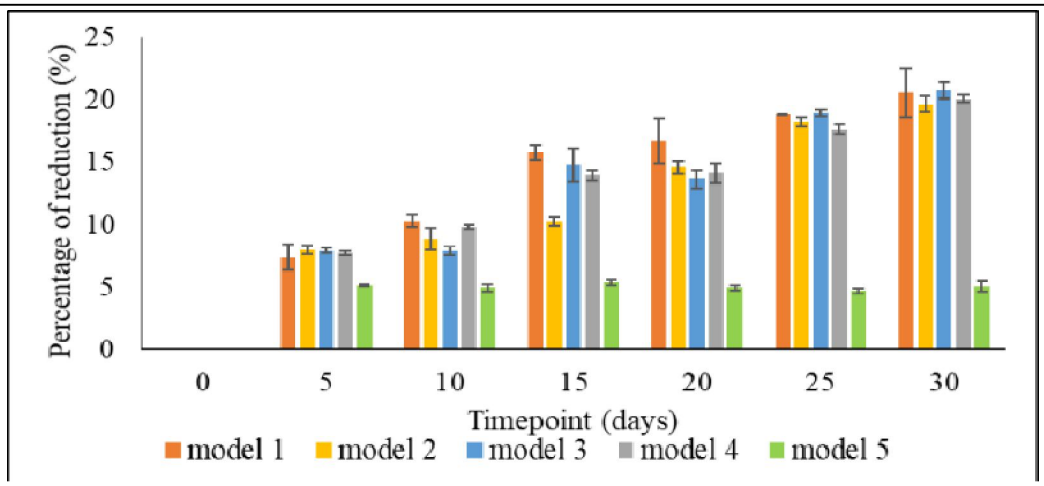


Figure 4: Percentage of the lignocellulose biomass (EFB) weight loss during the composting process

CONCLUSIONS

Composting process of EFB using solid state fermentation was studied and major conclusion are as follow:

1. In all models, model 3 was considered as the most efficient and cost effective condition which only using compost and POME as the inoculum and medium. POME itself provide the inoculum in this model.
2. There is almost no different by using supernatant POME or whole crude POME for composting in term of biomass weight loss.
3. Delignification was happened in early stage of composting and followed by cellulose and hemicellulose degradation since lignolytic enzyme has loosen up the lignin layer and allow the enzyme and microorganisms to attack to the next layer.
4. Future study can be done to optimize the composting condition.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the funding support for this work provided by Ministry of Higher Education Malaysia and Biotechnology and Biological Sciences Research Council (BBSRC) United Kingdom under program of United Kingdom-Southeast Asia Newton Ungku Omar Fund (UK-SEA-NUOF) with project number 4B297 and BB/P027717/1.

REFERENCES

[1] Abdullah N, Sulaiman F (2013) The Oil Palm Wastes in Malaysia. doi:10.5772/55302

[2] Ang S, Shaza E, Adibah Y, Suraini A, Madihah M (2013) Production of cellulases and xylanase by Aspergillus fumigatus SK1 using untreated oil palm trunk through solid state fermentation. Process Biochemistry 48 (9):1293-1302

[3] Castoldi R, Bracht A, de Morais GR, Baesso ML, Correa RCG, Peralta RA, Moreira RdFPM, de Moraes MdLT, de Souza CGM, Peralta RM (2014) Biological pretreatment of Eucalyptus grandis sawdust with white-rot fungi: study of degradation patterns and saccharification kinetics. Chemical Engineering Journal 258:240-246

[4] Damen K, Faaij A (2018) Greenhouse gas balances of biomass import chains for "green" electricity production in the Netherlands.

[5] Gaind S, Nain L (2007) Chemical and biological properties of wheat soil in response to paddy straw incorporation and its biodegradation by fungal inoculants, vol 18. doi:10.1007/s10532-006-9082-6

[6] Gonçalves FA, Ruiz HA, dos Santos ES, Teixeira JA, de Macedo GR (2015) Bioethanol production from coconuts and cactus pretreated by autohydrolysis. Industrial Crops and Products 77:1-12

[7] He Z, Yang X, Kahn BA, Stoffella PJ, Calvert DV (2001) Plant nutrition benefits of phosphorus, potassium, calcium, magnesium, and micronutrients from compost utilization. Compost utilization in horticultural cropping systems:307-320

[8] Madaki YS, Seng L (2013) Palm oil mill effluent (POME) from Malaysia palm oil mills: Waste or resource, vol 2.

[9] MPOB (2017) Malaysian Oil Palm Industry Performance 2016 and Prospects for 2017. Malaysian Palm Oil Board (MPOB). Ministry of Plantation Industries & Commodities (MPIC).

[10] Oberoi HS, Chavan Y, Bansal S, Dhillon GS (2010) Production of cellulases through solid state fermentation using kinnow pulp as a major substrate. Food and Bioprocess Technology 3 (4):528-536

[11] Siddiquee S, Shafawati SN, Naher L (2017) Effective composting of empty fruit bunches using potential Trichoderma strains. Biotechnology Reports 13:1-7. doi:https://doi.org/10.1016/j.btre.2016.11.001

[12] Singhania RR, Sukumaran RK, Patel AK, Larroche C, Pandey A (2010) Advancement and comparative profiles in the production technologies using solid-state and submerged fermentation for microbial cellulases. Enzyme and Microbial Technology 46 (7):541-549. doi:https://doi.org/10.1016/j.enzmictec.2010.03.010

[13] Zhang J, Elser JJ (2017) Carbon:Nitrogen:Phosphorus Stoichiometry in Fungi: A Meta-Analysis. Frontiers in microbiology 8:1281-1281. doi:10.3389/fmicb.2017.01281

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