Jurnal Teknologi

Full paper

Yield Performance and Biological Efficiency of Empty Fruit Bunch (EFB) and Palm Pressed Fibre (PPF) as Substrates for the Cultivation of *Pleurotus Ostreatus*

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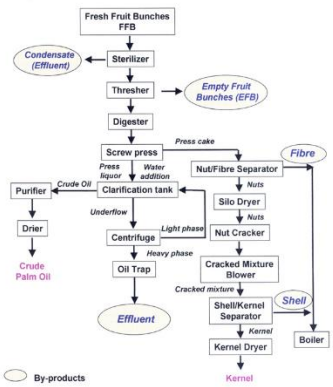
Article history

Received :22 October 2012
Received in revised form :
2 August 2013
Accepted :15 August 2013

Graphical abstract



Abstract

This research was undertaken to evaluate the performance yield and biological efficiency of empty fruit bunch (EFB) and shredded palm pressed fibers (PPF) as an alternative substrate which is widely available for the cultivation of *Pleurotus ostreatus.* Currently rubber tree sawdust (RS) is used which is of limited supply. In this study, five different substrates were prepared either alone or in combinations in the ratio of 1:1 with rubber tree sawdust. These substrates were supplemented with fixed ratio of rice bran and limestone to increase the yield of *Pleurotus ostreatus*. Shredded palm pressed fiber (PPF) and rubber sawdust (RS) in the ratio of 1:1 took 52 days for the mycelium to fully colonize the substrate as compared to 49 days for rubber saw dust alone. The performance yield and biological efficiency observed for the substrates comprising of 50% PPF and 50% rubber tree sawdust and 50% EFB and 50% rubber tree sawdust(RS) are 2.325 kg with a biological efficiency of 232.5% and 1.380 kg with a biological efficiency of 138%, respectively. The results obtained show that both shredded palm pressed fiber (PPF) and empty fruit bunch (EFB) show great potential as substrate for the cultivation of *Pleurotus ostreatus* when incorporated with rubber tree sawdust (RS).

Keywords: *Pleurotus ostreatus*; palm biomass; substrates; palm pressed fiber; palm empty fruit bunch

Abstrak

Kajian ini telah dijalankan untuk menilai hasil prestasi dan kecekapan biologi tandan kosong kelapa sawit (EFB) dan sabut kelapa sawit tertekan yang telah dicincang (PPF) sebagai substrak alternative yang boleh diperolehi dengan meluas untuk penanaman *Pleurotus ostreatus* yang kini menggunakan habuk kayu pokok getah(RS) yang bekalannya amat terhad. Dalam kajian ini, lima substrak yang berbeza telah disediakan sama ada bersendirian atau dalam gabungan nisbah 1:1 dengan habuk kayu pokok getah. Substrak-substrak ini telah ditambah dengan dedak padi dan batu kapur dalam nisbah yang ditetapkan untuk meningkat hasil *Pleurotus ostreatus.* Sabut kelapa sawit tertekan yang telah dicincang (PPF) dan getah habuk kayu dalam nisbah 1:1 mengambil masa 52 hari untuk miselium memenuhi substrat berbanding 49 hari bagi habuk kayu pokok getah. Hasil prestasi dan kecekapan biologi yang diperolehi bagi substrat yang terdiri daripada 50% PPF dan 50% habuk kayu pokok getah dan 50% EFB dan 50% habuk kayu pokok getah adalah 2.325 kg dengan kecekapan biologi 232.5% dan 1.380 kg dengan kecekapan biologi 138 %, masing-masing. Keputusan yang diperolehi menunjukkan bahawa kedua-dua sabut kelapa sawit tertekan yang telah dicincang (PPF) dan tandan buah kosong kelapa sawit (EFB) mempunyai potensi yang tinggi sebagai substrak bagi penanaman *Pleurotus ostreatus* apabila digabungkan dengan habuk kayu pokok getah.

Kata kunci: *Pleurotus ostreatus;* biojisim kelapa sawit; substrak; sabut kelapa sawit tertekan; tandan buah kosong kelapa sawit

1.0 INTRODUCTION

Malaysia is the world's largest exporter of palm oil, accounting to 11% of the world's oil and fats production and 45% of the palm oil market share, with export earnings from all palm oil products amounting to Ringgit Malaysia (RM) 65.19 billions in 2008. In that year alone, 4.49 million hectares of land in Malaysia are under oil palm cultivation yielding 17.73 million tones of palm oil (CPO) and 2.13 million tones of palm kernel oil (PKO) [1]. 87.75 million tones of fresh fruit bunches (FFB) were processed in 2008 by 410 palm oil mills. In the processing of fresh fruit bunches to extract palm oil and palm kernel oil as shown Figure 1, the industry generates an estimate of 65.5 million tonnes of waste yearly in the form of palm empty fruit bunches (EFB), palm

kernel shell (PKS), palm pressed fibers (PPF) and palm oil mill effluents (POME) [3], besides the field biomasses (palm fronds and oil palm trunks during replanting) from the plantation. The characteristics and quantities of these biomasses produced per tonne of fresh fruit bunch processed in the mill are shown in Table 1. Table 2 shows the types, the estimated quantities and percent utilization of palm biomass in 1998. In practice the palm kernel shell and palm pressed fibers are used as fuels to generate steam and electricity for the milling process *in situ*. However, there is still surplus fiber and shell available which creates an accumulation problem. The empty fruit bunches are either incinerated for its ash (potassium content of 30%) or composted with digested POME which serve as a very good fertilizer or applied to the field in the form of mulch to control weeds, prevent erosion and as soil conditioner. Due to the cost of labor, transportation and distribution, its usage as mulching is becoming very expensive resulting in excessive piles of EFB being left either next to the mill or on the plantation edges. These practices create environmental pollution problems as incineration and boiler emit gases with particulates such as tar and soot droplets of 20-100 microns and a dust load of about 3000 to 4000 mg/nm [5] and indiscriminate dumping of EFB causes additional methane emission into the atmosphere. At present, amid efforts to reduce waste and the growing environmental concerns, a new inexpensive usage for these wastes ought to be looked into. As these biomasses from palm oil mills contain cellulose, hemicelluloses and lignin, they are suitable renewable raw material for bioconversion into value added products as they are abundant and easily accessible.

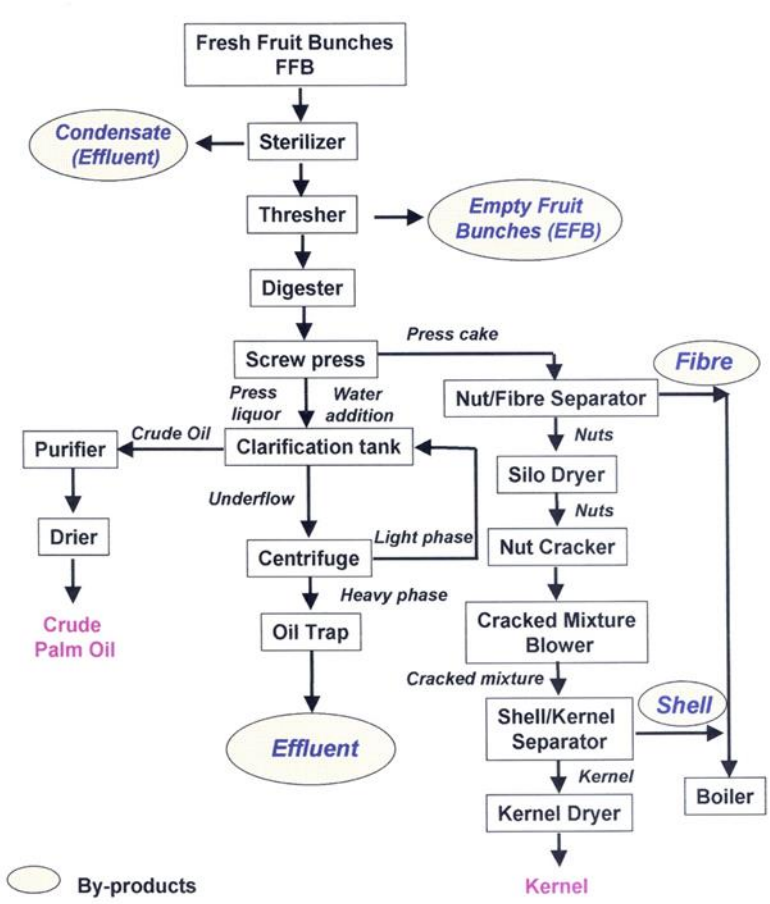


Figure 1 Flow diagram of a palm oil mill [2]

Table 1 Characteristics and amounts of Lignocellulosic resources produced based on per tonne of Fresh Fruit Bunch (FFB) processed [2]

Biomass	Wet Wt. (%)	Moisture Content (%)	Dried Weight (kg)	Physical Characteristics
Palm Pressed Fiber (PPF)/Mesocarp Fiber	13.5	42	78	Fibrous, short strands
Palm Kernel Shell (PKS)	5.5	7	51	Irregular, broken
Empty Fruit Bunches (EFB)	22.0	65	77	Fibrous, bulky
Palm Oil Mill Effluent (POME)	70.0	95	5	Dark viscous liquid

Pleurotus ostreatus also known as "oyster mushroom" is the second most cultivated edible mushroom worldwide after *Agaricus bisporus* [6]. It is rich in protein, chitin, minerals, and vitamins and has all the essential amino acids that the human body needs. *Pleurotus spp*. also possesses various medicinal properties such as antitumor, antigenotoxic, anti-inflammatory, antiviral, antioxidant, antimicrobial, antihypertensive, antiplatelet-aggregating, antihyperglycaemic, antimicrobial, hypocholestrolaemic besides being immunomodulatory [7].

Pleurotus ostreatus is a fungus that can be cultivated on various lignocellulosic substrates. This capability of the oyster mushroom is due to the presence of its extracellular lignocellulolytic enzymes, also named fibrolytic enzymes, including xylanases, cellulases, and laccases [8] which help it to degrade the lignocellulose substrate turning it into an energy source for the fungi. Any agricultural waste that contains cellulose and lignin is a possible substrate for growing this fungus. A lot of research has been done on the cultivations of *Pleurotus* mushroom using various lignocellulosic wastes [9-20]. About 90 different kinds of agricultural wastes worldwide are studied as oyster mushroom substrates [21]. Stajic´ *et al.* [22] described that the lignocellulolytic enzymes production by *Pleurotus ostreatus* depends strongly on the strain, substrate composition and physical properties such as the crystalline or amorphous nature, accessible area, surface area, porosity and mainly particle size [23–24] and conditions of cultivation.

Table 2 Types, estimated quantity and % utilization of oil palm biomass in Malaysia (1998) [4]

Biomass	Quantity produced (million tones)	Quantity utilized (million tones)	% Utilized	Method of Utilization
Pruned fronds	27.2	25.83	95	Mulch
Trunk and fronds (at replanting)	1.38	1.10	80	Mulch
Palm Pressed Fiber (PPF)	3.56	3.20	90	Fuel
Palm Kernel Shell (PKS)	2.41	2.17	90	Fuel
POME	1.43	0.5	35	Nutrient source & organic fertilizer
Empty Fruit Bunch (EFB)	3.38	2.20	65	Mulch & burned ash

Pleurotus ostreatus cultivation is simple and cheap requiring only live steam pasteurization. Its growth time is shorter, need minimum environmental controls and is not often attacked by diseases and pests [25]. In addition, the spent mushroom substrate (SMS) can be used for mushroom re-cultivation, as an organic fertilizer and also be utilized as cattle feed since cellulose and lignin are decomposed and protein is re-synthesized during the cultivation giving SMS enough digestible nutrient [26].

Presently, in Malaysia, the commercial cultivation of *Pleurotus ostreatus* utilizes sawdust from rubber tree as the base medium. Rubber tree sawdust (RS) has a uniform size structure making it suitable for plastic bag cultivation plus its structure facilitates the enrichment of the substrate [27]. However, due to the low availability of rubber tree, it has become a serious problem to the mushrooms grower. Thus a new alternative substrate ought to be looked into to overcome the shortage of sawdust from rubber trees. Table 3 and Table 4 show the chemical and elemental compositions of rubber wood, respectively.

Table 3 Chemical composition of rubber tree wood [28]

	% Dry matter
Hemicellulose and cell wall	29.0
Lignin	28.0
Cellulose	39.0
Ash	4.0

Table 4 Elemental composition of rubber tree [29]

Elements	Wt. %, dry basis
C	46.04
H	6.15
N	0.32
	1.68-0.2 [29]
S	0.0
O	46.56
Cl	0.03
Ash	0.89

Mushroom requires carbon, nitrogen and other inorganic compounds for its nutritional sources. The carbon sources suitable for mycelia growth are starch, glucose, fructose, maltose, amnnose, sucrose, pectin, cellulose and lignin whereas the nitrogenous sources are peptone, corn steep liquor, soybean cake powder, ammonium sulphate, asparagines, serine, alanine and glycine [30]. The typical chemical composition of different types of oil palm biomass is shown in Table 5 whereas Table 6 shows the elemental composition in EFB, PPF and palm kernel shell. Both PPF and EFB contain 47.2% carbon and 1.4% nitrogen, and 48.4% carbon and 0.2% nitrogen, respectively as shown in Table 6. This means that the PPF and EFB have the potential to be used as substrates for the *Pleurotus* cultivation.

Table 5 Typical chemical composition of different oil palm biomass [31]

Biomass (% Dry matter)	EFB	Trunk	Frond	PPF	PKS
Cellulose	39.0	59.0	42.0	21.0	6.0
Hemicellulose	22.0	10.0	21.0	16.0	36.0
Lignin	29.0	11.0	23.0	43.0	36.0
Glucose	0.43	0.65	0.47	0.23	0.07
Xylose	0.26	0.12	0.24	0.18	0.4

Table 6 Chemical composition on dry basis of palm oil waste [32]

Element	Empty Fruit Bunch (%)	Fiber (%)	Shell (%)
H	6.3	6.0	6.3
C	48.8	47.2	52.4
S	0.2	0.3	0.2
N	0.2	1.4	0.6
O	36.7	36.7	37.3
Ash	7.3	8.4	3.2

2.0 MATERIALS AND METHODS

Fresh EFB and PPF were obtained from Kilang Sawit Kulai, Felda Taib Andak, Johor, Malaysia. The fresh empty fruit bunches were cut into smaller pieces, dried and then shredded using a shredder whereas fresh palm pressed fibers were only shredded. RS was obtained from a rubber tree processing mill. Five different substrates were prepared as shown in Table 7 with a total of fifteen replicates prepared for each substrate. The preparation of substrate involved mixing of the main material with the addition of 5% rice bran. The substrates were mixed thoroughly until no lumps of rice bran were found. Then, 2% calcium carbonate was added to the mixture. The calcium carbonate neutralizes the acidity of the substrate. The mixture was again stirred until no calcium carbonate was visible. Water (80% of the total weight of mixture) was then added to increase the moisture content. The substrates were mixed again until all the water was absorbed. 1kg of each substrate was then placed in a polyethylene bag. The bags of substrate were then compressed and closed with PVC-necks, which were covered with cotton plugs and wrapped with papers to prevent the entry of insects.

Table 7 Composition of substrate

Substrate	Composition of substrate
EFB	100% EFB
EFB + RS	50% EFB + 50% rubber tree sawdust
PPF	100% PPF
PPF + RS	50% PPF + 50% rubber tree sawdust
RS	100% rubber tree sawdust

All the substrates were placed on steel shelves in an upright position and sterilized at 100°C for about 8 hours in the sterilization chamber. After sterilization, the bags were left to cool and then inoculated with 10g of spawn. The substrates were subsequently placed vertically in a spawn running room maintained at 25°C and relative humidity at 85%. This was done by periodically spraying water on the floor of the room. The substrates were left in a hut covered with nets specifically designed so as to allow only 70% sunrays for mycelium to grow throughout the bags. The number of days for the completion of mycelium growth to fully colonize the substrate was recorded. At the end of the colonization period, the bags were rearranged horizontally. After 70 days, the upper parts of the bags were unfolded to induce fruiting for the first cropping. A tiny pinhead will be seen on the surface of the substrates and these will grow into full size mushrooms within a day or two. Water was sprayed in the form of fine mist to maintain the moisture and lower the temperature. The fruit bodies were ready for picking just when the periphery of caps started turning upwards. This will be evident as small crinkles appeared on the side of the pileus (cap). The bags that had already been cropped were closed for another 10 days until the next harvest. In total, 4 harvests/flushes were collected. The total weight of the fruiting bodies was recorded for all 4 flushes.

3.0 RESULT AND DISCUSSIONS

In this research, *Pleurotus ostreatus* was cultivated on either fine palm pressed fiber (PPF), palm empty fruit bunch (EFB) alone or in combinations with rubber tree sawdust (RS). These substrates were supplemented with fixed ratio of rice bran and calcium carbonate.

The various results obtained from the research are presented in Table 8 and Table 9. All results were obtained in 3 replicates and data were expressed as means. Substrate EFB did not yield any result as all replicates failed to complete spawn running (mycelia fully colonized substrate) as shown in Table 8. The first flush was harvested 5 days after the bags were opened, while the second flush was harvested in about 15 days after the first harvest. The third and fourth flushes were obtained in similar manner. For substrates PPF and PPF + RS, six and three replicates, respectively, were identified to be contaminated. The production of *Pleurotus ostreatus* from substrate EFB + RS and RS, on the other hand, showed no sign of contamination.

Table 8 Days for completion of spawn running for different substrates

Substrate	Days for completion of spawn running
EFB	-
EFB + RS	60
PPF	56
PPF + RS	52
RS	49

The total weights of all the fruiting bodies harvested in all four flushes were weighed as total yield of *Pleurotus ostreatus*. The biological efficiency (BE) expressing the yield of fresh fruit bodies per g dry substrate was calculated according to the following equation as was given by Chang *et al.* [33]:

$$\text{Biological efficiency (\%)} = \frac{\text{Fresh weight of mushroom}}{\text{dry weight of substrate}} \times 100 \quad (1)$$

The time taken for complete mycelia colonization of the substrates depends on the substrate used. All the replicates for substrate RS have no difficulty for the mycelium to fully colonize the bags. Table 8 shows substrate RS also requires the shortest time of 49 days to complete the spawn running due to the easy digestion and fast decomposition of RS. The second shortest time is taken by substrates PPF + RS (52 days) followed by substrate PPF (56 days). Substrate EFB + RS took the longest time (60 days) for the mycelium to fully colonize the bag.

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Table 9 Weight, total yield and biological efficiency of different substrates

Substrate	Average yield per flush (g)				Total yield in 4 flushes (g)	Biological efficiency (%)
	1	2	3	4		
EFB	-	-	-	-	-	-
EFB + RS	870	193	249	68	1380	138.0
PPF	415	95	70	10	590	59.0
PPF + RS	1360	510	340	115	2325	232.5
RS	1465	430	330	100	2325	232.5

Different substrates caused a difference in yield performance and biological efficiency as shown in Table 9. The yields were highest in the first flush ranging from 70% for Substrate PPF to 58% for substrate PPF + RS with 64% for the control substrate, substrate RS. More than 90% of the yield was obtained in the first three flushes in all the substrates with the exception of substrate EFB. Both substrates PPF + RS and RS produced the highest total yield and biological efficiency of 2325 g and 232.5 %, respectively, followed by substrate EFB + RS. Substrate PPF produces the lowest total yield of 590 g. This result is in agreement with the findings of Shah *et al.* [16] and Onuoha *et al.* [17], which shows that the best biological efficiency is obtained from RS.

Table 10 C/N and cellulose: lignin ratios of Empty Fruit Bunch, Palm Pressed Fibre and Rubber Tree Sawdust

Waste	C (% Dry weight)	N (% Dry weight)	Cellulose (% dry weight)	Lignin (% dry weight)	Cellulose: lignin ratio[a]	C: N ratio[a]
EFB	48.8	0.2	39.0	29.0	1.34	244
PPF	47.2	1.4	21.0	43.0	0.49	34
RS	46.04	0.32	39.0	28.0	1.39	144

[a] composition of non-amended raw materials

The cellulose: lignin ratios of both the empty fruit bunch (EFB) and the rubber tree sawdust (RS) are similar whereas the carbon:nitrogen (C: N) ratio of EFB is much higher than that of RS as indicated by Table 10. Dundar and Yildiz [11] and Yildiz and Karakaplan [13] findings state that different nitrogen content and C: N ratio of the substrates used for cultivation *of the Pleurotus spp.* affect the yield performance. According to Zadrazil F. [34], *pleurotus spp*. grows well on substrates of low nitrogen content of which the C: N ratio is high. This does not compare favorably with the result of this study in which Substrate EFB failed to complete spawn running even though its C: N ratio is the highest compared to the other substrates.

The inability of the mycelium of substrate EFB alone to fully colonize and subsequently produce fruit bodies might be attributed to the particle size of the substrate. Empty fruit bunch is observed to be much finer as compared to that of palm pressed fiber and rubber tree sawdust with rubber tree sawdust being the coarsest. Particles that are too small will result in the wet substrate becoming over compact and hence, reducing the porosity and aeration available. This in turn will suppress the fungal development. This finding is in agreement with that found by Zhang *et al.* [20]. The porosity of substrate EFB + RS is increased with the addition of the coarser rubber tree sawdust, thus promoting the delignification of the substrate.

Substrate PPF gives the lowest yield as compared to the other substrates (with the exception of substrate EFB). In contrast, Onuoha *et al.* [17] found oil palm fiber alone did not support mycelium growth and thus did not produce any fruit body. They attributed this to the presence of oil and the inability of the fungus to extract the cellulose entrapped in the fiber. Based on Table 10, it is believed that the low yield and overall BE of substrate PPF might be attributed to the low C/N and cellulose: lignin ratios of the PPF which is in agreement with the finding of Philippoussis *et al.* [19]. However, in the case of substrate PPF + RS which give the highest yield and biological efficiency indicates that higher yield and biological efficiency were due to the blending of rubber tree sawdust to the palm pressed fiber which helps increase the C/N and cellulose:lignin ratios of the combined substrate.

Nitrogen plays an important role in spawn running and in growth of fruit bodies. Hence for the growth and yield of *pleurotus ostreatus*, rice bran is added to provide the organic nitrogen needed. Both palm empty fruit bunch and fine palm pressed fiber are identified to contain nitrogen as shown in Table 6 with palm pressed fiber and palm empty fruit bunch having 1.4% and 0.2%, respectively. Since Substrate EFB contains the

least amount of nitrogen (0.2%) as compared to other substrates, it is possible that this amount together with the supplemented rice bran's nitrogen is insufficient for the growth of the mycelium and subsequent growth of *Pleurotus ostreatus* fruit bodies. When palm empty fruit bunch is combined with rubber tree sawdust, the total yield increases to 1380 g indicating that supplementation with a substrate high in nitrogen content contributes to higher yield. This finding is in agreement with Mane *et al*. [9], Dundar *et al* [11] and Patil *et al.* [18], which suggested that substrates rich in nitrogen might be a factor in increasing the mushroom yield.

Addition of rubber tree sawdust to both empty fruit bunch and palm pressed fiber significantly increased the yield performance and biological efficiency. This finding agreed well with that of Pathmashini *et al.* [14]. Critical examination of substrate packing density and substrate particle sizes need to be done on these substrates so as to have a better understanding on the mechanism of *Pleurotus ostreatus* fruiting in the future. Further research needs to look into the effect of the chemical composition of the substrate in terms of trace elements. It is also recommended that the composition of the mixture of palm pressed fiber and rubber tree sawdust to be more varied in order to determine the optimum mixture for the cultivation of the *Pleurotus ostreatus*.

■4.0 CONCLUSION

Both empty fruit bunch and palm pressed fiber are not very appropriate as sole substrate for the cultivation of *Pleurotus ostreatus* but have tremendous potentials when combined with rubber tree sawdust.

Using the biomass generated from the palm oil mills which is available throughout the year at little or no cost, it will not only solve the environmental pollution problem but it can also offer an economically promising way to convert low quality biomasses into a valuable high protein food. Plus the spent substrates can help further generate more income to the mushroom growers.

Acknowledgments

The authors would like to thank En. R. Azmi Buang and En. Mohd Zainuddin Buang for the use of their oyster mushroom farm at Kampung Sinaran Baru, Johor and help in running the experiments.

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