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Fungi biodiversity during biorefinery of oil palm waste

RORO KESUMANINGWATI[1,2,♥], RUDY AGUNG NUGROHO[3], SURYA DARMA[2], FAHRUNSYAH[2], KRISHNA PURNAWAN CANDRA[1,4], ESTI HANDAYANI HARDI[5], SURIA DARMA[2]

[1]Doctoral Program of Environmental Science, Universitas Mulawarman. Jl. Sambaliung, Samarinda 75123, East Kalimantan, Indonesia
[2]Department of Agroecotechnology, Faculty of Agriculture, Universitas Mulawarman. Jl. Paser Balengkong, Samarinda 75123, East Kalimantan, Indonesia. Tel.: +62-541-2083337, ♥email: roro_kusumaningwati@faperta.unmul.ac.id
[3]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Mulawarman. Jl. Barong Tongkok No. 4, Samarinda 75123, East Kalimantan, Indonesia
[4]Department of Agricultural Product Technology, Faculty of Agriculture, Universitas Mulawarman. Jl Tanah Grogot, Samarinda 75123, East Kalimantan, Indonesia
[5]Department Aquaculture, Faculty of Fisheries and Marine Science, Universitas Mulawarman. Jl. Gunung Tabur, Samarinda 75123, East Kalimantan, Indonesia

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Abstract. *Kesumaningwati R, Nugroho RA, Darma S, Fahrunsyah, Candra KP, Hardi EH, Darma S. 2024. Fungi biodiversity during biorefinery of oil palm waste. Biodiversitas 25: 3596-3607.* The rapidly growing palm oil industry faces significant waste management challenges that necessitate improvements in the biorefinery processes. This study aimed to determine the diversity of fungi during the biorefinery process of oil palm waste. An optimization study was conducted utilizing Response Surface Methodology (RSM) based on the Box-Behnken experimental design. We examined four factors: the bioactivator, Oil Palm Empty Fruit Bunches (OPEFB), Palm Oil Mill Effluent (POME), and the composting time in a 3 kg biorefinery system. The results revealed 16 and 21 types of fungi at the genus and species levels, respectively. The biodiversity indices, specifically the Simpson (D) and Shannon (H'), reflected shifts in fungal diversity throughout the biorefinery process. The indices at the genus level peaked in the middle of the biorefinery (day 24.5, indicating the highest diversity) with low (D=0.085-0.154) and medium (H'=1.992-2.671) values. On day 7, the compost temperature was 34.98°C, declining to 29.50 and 28.82°C on days 24.5 and 42, respectively. Aspergillaceae and Rhizopodaceae were the two dominant families involved in the composting process, with *Aspergillus*, *Fusarium*, and *Rhizopus* being the predominant genera. At the species level, *Aspergillus flavus* Link, *Aspergillus fumigatus* Fresen., *Aspergillus niger* Tiegh., and *Rhizopus* sp. were fully engaged during the composting process. OPEFB concentration and composting time significantly influenced the compost C/N ratio, as did the interaction between composting time and the other two factors (EM4 volume and OPEFB). The optimal composting conditions were determined to be: EM4 volume of 15 mL, OPEFB at 30.895%, POME at 59.989%, and a composting time of seven days. The resulting compost exhibited a C/N ratio of 19.084, moisture content of 49.999%, C-organic content of 72.595%, N-total of 3.791%, and K-total of 5.867%, with a desirability of 77.6%.

Keywords: Composting, fungi biodiversity, Oil Palm Empty Fruit Bunches, Palm Oil Mill Effluent, Response Surface Methodology

INTRODUCTION

The diversity of microorganisms in organic fertilizer biorefineries is intricately linked to composting. Composting within the biorefinery process enables the production of biological fertilizers, providing a viable alternative for waste recovery challenges (Rocha et al. 2024). Biorefinery is a waste management technology that benefits the environment by reducing greenhouse gas emissions and pathogen sources (Chojnacka 2023). This is a sustainable method for converting biomass resources to valuable organic fertilizers (Adetunji et al. 2023; Rathour et al. 2023).

Organic fertilizers are a critical source of microorganisms essential for decomposing organic material and plant nutrients. Applying organic fertilizers affects the microbial diversity and promotes soil health by increasing microbial activity in the nutritional cycle (Lazcano et al. 2021; Prasedya et al. 2022). The production of organic fertilizers through biorefinery processes offers a solution to waste recovery (Rocha et al. 2024), as biorefineries are environmentally efficient waste management technologies (Chojnacka 2023). Biorefineries associated with microorganisms are strategies for the sustainable utilization of biomass (Adetunji et al. 2023; Rathour et al. 2023) and to add value to biorefinery products such as palm oil waste (Duan et al. 2020; Gaur et al. 2024).

Accurate methodologies are essential for palm oil waste biorefineries to assess the influence of various factors. RSM optimizes research outcomes through statistical analysis of composting data to design laboratory-scale experiments. Researchers employ the RSM statistical method to conduct complex variable testing on natural logarithms. The fungal biodiversity index is determined through the Shannon Index (H') (Mulya et al. 2021), Simpson's dominance index (Pratiknyo and Setyowati 2020), Margalef Index (species richness) (Wardhana et al. 2022), and the Shannon Evenness index. An evenness index of 0.26<E<0.50 indicates low evenness, while an index in the range of 0.51<E<0.75 indicates a relatively even distribution. An evenness index of 0.76<E<0.95

suggests almost even distribution, and that of $0.96<E<1.00$ indicates even distribution.

The composting pathway converts polymers containing sugar, cellulose, and hemicellulose into various products (Sharma et al. 2021; Rodionova et al. 2022). Composting palm coconut residue increases raw protein content and softens fiber (Azman et al. 2023). The composition of the organic materials, the use of bioactivators like EM4, and the duration of composting all influence the composting process. Microorganisms such as the *Lactobacillus* sp., *Saccharomyces* spp., *Actinomycetes*, and cellulose-degrading fungi are found in EM4 and other bioactivators. They help maintain the balance of carbon and nitrogen. The duration of composting also affects the process due to the high fiber content (Sembiring et al. 2021; Nurcahyanti et al. 2024), which typically requires approximately six months for decomposition (Heryadi et al. 2021). Identifying key factors influencing the composting of palm oil waste is critical for developing effective composting methods. This study employed RSM to assess the effects of various factors, including bioactivator concentration (EM4), waste composition, and composting time, resulting in compost that meets the Indonesian National Standard for compost from organic domestic waste (SNI 19-7030-2004) (BSN 2004).

MATERIALS AND METHODS

Materials

Palm oil (*Elaeis guineensis* Jacq.) waste (Oil Palm Empty Fruit Bunch (OPEFB), Palm Oil Mill Effluent (POME), and Palm Kernel Meal (PKM)) was obtained from the oil palm company PT. Multi Jayantara Abadi in Senipah Village, Tanjung Harapan Sub-district, Paser District. Bioactivator (EM4) and molasses were purchased from the local market in Samarinda. Chemicals including KCl, indicator PP, NaOH, NaF, H_2O_2, HCl, $Na_4P_2O_7$, P dye reagent solution, Bray and Kurt I extractor, ammonium acetate, NaCl, $K_2Cr_2O_7$, H_2SO_4, and indicator Conway were provided by Happy Laboratorium Sukses Bersama, Semarang, Indonesia.

Experimental design and data analysis

To achieve the Indonesian National Standard of C/N for compost (SNI 19-7030-2004), which requires a C/N ratio of 10-20 (BSN 2004), an optimization study was conducted to determine the fungal biodiversity involved in the palm oil waste composting process. The biodiversity index parameters observed included the Shannon diversity index (Mulya et al. 2021), Simpson's dominance index (Pratiknyo and Setyowati 2020), Margalef index (Wardhana et al. 2022), and Evenness index (E) (Dewi et al. 2023).

$$\text{Shannon Index } (H') = -\sum_{i=1}^{S}\left[\frac{n_i}{N}\ln\frac{n_i}{N}\right]$$

Where: S: the total number of species in a sample/community; n_i: the number of individuals of the i-th species, where i: 1, 2, ..., S; N: the total number of individuals in a sample (community); p_i: the proportion of the i-th species (frequency or relative abundance, n_i/N); the relative abundance represents the dominance of a species ($Di = 100.\ p_i$). $\sum p_i = 1$

With criteria $H' \leq 1$ for low, $1<H'<3$ for medium, and $H' \geq 3$ for high diversity.

$$\text{Simpson Index (D)} = \sum_{i=1}^{S} pi^2$$

Where: S: the total number of species in a sample/ community; p_i: proportion of species i to total species

With criteria 0-0.30 for low, 0.31-0.60 for medium, and 0.61-1.00 for high diversity.

$$\text{Margalef Index } (D_{mg}) = \frac{S-1}{\ln(N)}$$

Where: S: the number of species observed; N: the total number of individuals observed

With criteria $D_{mg}<2.5$ for low, $2.5>D_{mg}>4$ for medium, and $D_{mg}>4$ for high level of richness

$$\text{Evenness index (E)} = \left[\frac{H'}{\ln S}\right]$$

Where: H': is the diversity index; S: the number of species observed

We simulated the biorefinery process of palm oil waste using RSM using Box-Behnken Design with four factors: EM4 volume, chopped OPEFB, POME, and composting time. Each sample weighed 3 kg and consisted of EM4 (5-15 mL), OPEFB (0-50%), and POME (0-60%). PKM was added to achieve the total sample weight of 3 kg. The composting process was carried out for 7, 24.5, and 42 days. The physical and chemical characteristics observed during the biorefinery process included C, N, P, K, pH, moisture content, and resulting compost temperature. The data were analyzed using RSM (Design Expert version 13).

Experimental procedure

The palm oil mill waste used in this experiment included OPEFB, POME, and PKM. The OPEFB was cut to a size of ±5 cm and composted outdoors at room temperature. The EM4 starter was prepared by mixing EM4 and molasses in a 1:2 ratio. 5 mL, 10 mL, and 15 mL EM4 was diluted to 1 L with water according to the treatment dosage. The solution was stored in a closed container for four days at room temperature until the microorganisms became active and ready for use.

Analysis procedure

Fungi growth media preparation

Potato Dextrose Agar (PDA) media was prepared by dissolving 24 g of PDA in 600 mL of distilled water. The solution was sterilized in an autoclave at 121°C for 15 min at a pressure of 1 atm. After sterilization, the PDA media was cooled to 56°C, and 100 ppm of chloramphenicol was added to prevent bacterial contamination. The media was then approximately 10 mL was poured into petri dishes.

Fungi identification and population determination

1 g sample was placed into 10 mL of distilled water. The solution was then diluted to 10^{-2} to 10^{-5}. 1 mL of the diluted sample was poured into a petri dish containing PDA media and incubated at 30°C for 72 h to analyze the total fungal population. Fungal identification was performed using a guidebook by Samson (1984).

RESULTS AND DISCUSSION

Fungal diversity during composting of oil palm waste

Fresh palm oil waste material exhibited the following chemical characteristics: OPEFB with a pH of 6.15 and C/N ratio of 51.55, POME with a pH of 6.20 and C/N ratio of 14.01, and PKM with a pH of 5.12 and C/N ratio of 26.94. Biorefineries typically start at temperatures between 28.2°C and 35°C. Throughout the composting of palm waste over a period of 7 days, the temperature increased to 54.2°C, peaking on the fifth day. In the composting of palm oil waste over 24.5 days, the temperature reached 50.7°C, with the increase occurring until the fourth day. For the composting of palm oil waste over 42 days, the temperature peaked at 47.3°C, again increasing until the fourth day.

During the composting process, the diversity of fungi exhibited significant changes (Table 1). The dominant fungi from the beginning to the end of composting belonged to the Aspergillaceae and Rhizopodaceae families. Fungi from the families Hypocreaceae, Lichtheimiaceae, and Pyriculariaceae were present only at the beginning, while Peronosporaceae appeared at both the beginning and end of the process. Some fungi, such as those from the families Glomerellaceae, Pythiaceae, Xylariaceae, Thermoascaceae, and Trichocomaceae were present only during the third and fourth weeks.

At the peak of fungal diversity (day 24.5), a total of 12 families and genera were identified, along with 16 species in the composting of palm oil waste. The family, Aspergillaceae accounted for the highest percentage at 41.17%, with the genus, *Aspergillus* comprising 38.23%. At the species level, *Aspergillus flavus* Link represented 14.71%, *Aspergillus fumigatus* Fresen. 11.76%, and *Rhizopus* sp. 14.71%. The composting process is initiated by the activity of fungi such as *Aspergillus,* which provide essential metabolic substances for other microbes, thereby increasing the diversity of microbial communities and generating heat to commence the composting process (Dong et al. 2024). *Aspergillus* and *Penicillium* are fungi capable of secreting substrate-degrading enzymes, significantly influencing the decomposition of organic matter and cellulose (Wu et al. 2024).

A combination of factors, including EM4, Organic Material from Palm Oil Waste (OPEFB and POME), and composting duration, led to variations in fungal diversity. The highest fungal presence was observed under the conditions: EM4 at 10 mL, OPEFB at 25%, POME at 60%, and composting time of 24.5 days. Conversely, the lowest fungal population was found in the first run under the conditions: EM4 at 10 mL, OPEFB at 25%, POME at 0%, and a composting time of 42 days (Table S1).

The fungal diversity exhibited variations during the composting periods of 7, 24.5, and 42 days. Environmental variables such as temperature, raw materials, and humidity (Wu et al. 2020; Aguilar-Paredes et al. 2023), as well as organic carbon content, influenced the observed diversity. *Rhizopus* sp., *Phytophthora* sp., *Cireinella* sp., and *A. flavus* were the dominant species on day 7 of the composting process. In contrast, during the longer composting period of 24.5 days, the dominant species included *A. fumigatus, A. flavus, Rhizopus* sp., and *Aureobasidium* sp. After 42 days, the fungal diversity in each treatment decreased, compared to the midpoint (24.5 days), with *A. flavus*, *A. fumigatus*, and *A. niger*. being the most prevalent species. Robledo-Mahón et al. (2020) reported that the *Aspergillus* genus remains substantially unchanged during composting. *Aspergillus* is a fungus that breaks down lignocellulose (Duan et al. 2020) and promotes compost maturity (Lu et al. 2022).

The *Aspergillus* became dominant between 24.5 and 42 days of composting. According to Bakar et al. (2022), the process involves utilizing *A. flavus* to ferment palm oil waste. Certain fungal species, including *A. niger* and *A. fumigatus*, release various enzymes into the surrounding environment (Wang et al. 2020). *A. niger* produces the enzyme phytase (Chuang et al. 2020), which breaks down phytate. *Penicillium, Trichoderma*, and *Mucor*, belonging to the Ascomycota and Basidiomycota groups, play crucial roles in composting due to their involvement in breaking down lignocellulose (Qiao et al. 2021; Zhao et al. 2022). These fungi can utilize diverse carbon sources and thrive in extreme conditions, such as high temperatures. Both psychological stress and malnutrition can reach significant levels (Mili and Tayung 2024). *Mucor* and *Trichoderma* also have protective and enhancing effects on plant growth (Shi et al. 2024).

The Shannon diversity index (H’) and the Margalef richness index (D_{mg}) are more accurately related to the abundance of fungal types during the composting process (the shifting of fungal types) at the family and genus levels, but not for the Simpson (D) and Evenness (E) indices (Table 2). Conversely, at the species level, the Simpson and Shannon indices are suitable for association. These indices increase throughout the composting process and decrease after reaching the optimum point, indicating the highest diversity. This observation aligns with the findings of Zhang et al. (2022), which showed that the Shannon diversity index increased and then decreased along with the composting rate. Generally, composting time affects the value of the species diversity index. The species diversity index decreases with compost maturity. A higher diversity value (H’) indicates greater species diversity and ecosystem stability, along with a higher wealth index (Dewi et al. 2023). Microscopic photos of the fungal types involved in the composting process of palm oil waste are presented in Figure 1.

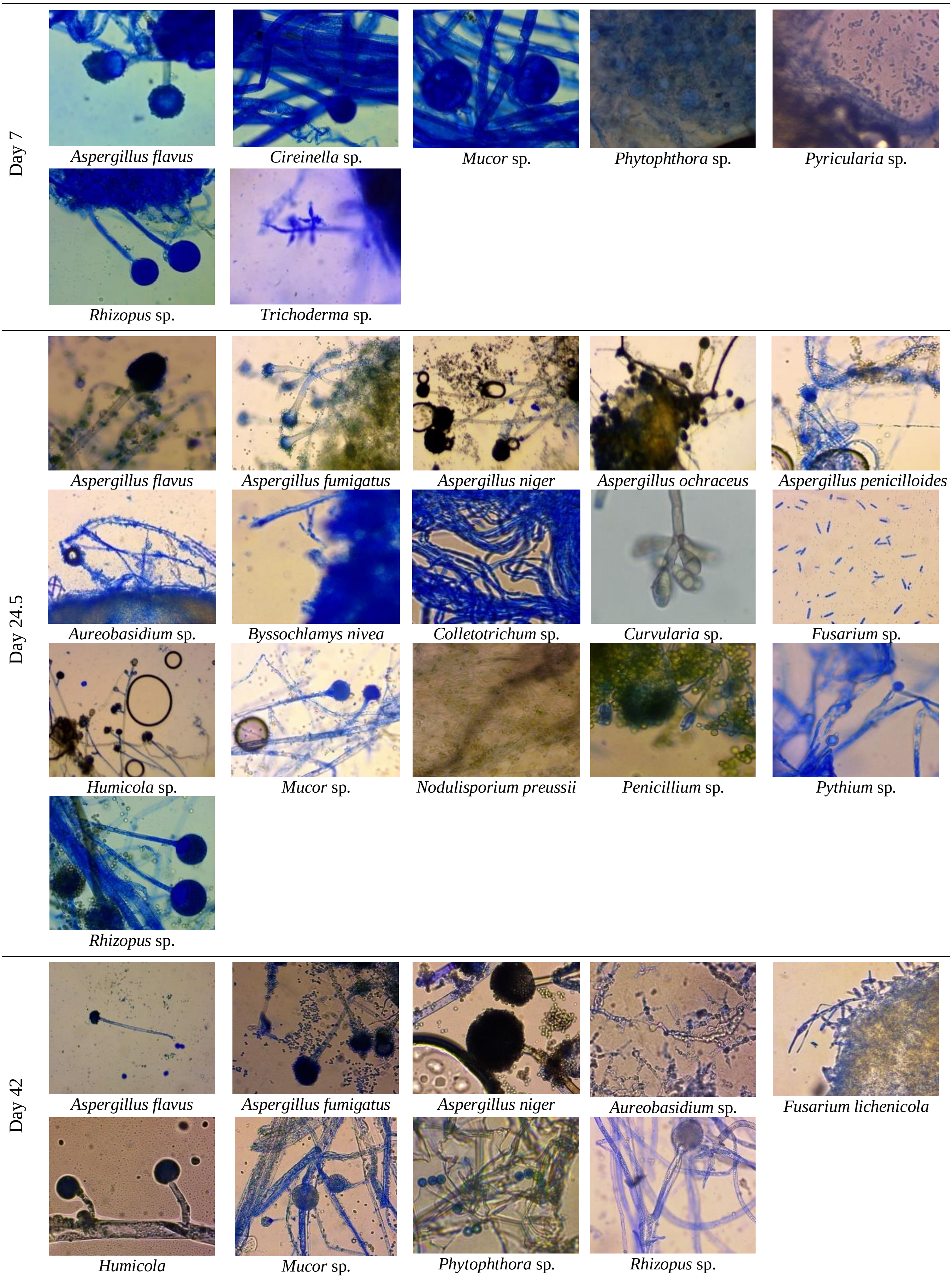

Figure 1. Visualization of fungi involved in composting palm oil waste

Table 1. The abundance (%) and the shifting of fungal diversity during composting of palm oil waste at 7-42 days

No.	Taxonomy level/name	Composting time (day); [Temperature (°C)]		
		7th; [34.98]	**24.5; [29.50]**	**42nd; [28.82]**
Family				
1	Aspergillaceae	23.52	41.17	60.00
2	Chaetomiaceae		5.88	2.86
3	Glomerellaceae		5.88	
4	Hypocreaceae	11.76		
5	Lichtheimiaceae	11.76		
6	Mucoraceae		5.88	11.43
7	Nectriaceae	5.88	2.94	5.71
8	Peronosporaceae	17.65		2.86
9	Pleosporaceae		2.94	
10	Pyriculariaceae	5.88		
11	Pythiaceae		2.94	
12	Rhizopodaceae	23.53	14.71	11.43
13	Saccotheciaceae		8.82	5.71
14	Thermoascaceae		2.94	
15	Trichocomaceae		2.94	
16	Xylariaceae		2.94	
Sum of family		7	12	7
Genus				
1	*Aspergillus*	23.52	38.23	60
2	*Aureobasidium*		8.82	5.71
3	*Circinella*	11.76		
4	*Colletotrichum*		5.88	
5	*Curvularia*		2.94	
6	*Fusarium*	5.88	2.94	5.71
7	*Humicola*		5.88	2.86
8	*Mucor*		5.88	11.43
9	*Nodulisporium*		2.94	
10	*Paecilomyces*		2.94	
11	*Penicillium*		5.88	
12	*Phytophthora*	17.65		2.86
13	*Pyricularia*	5.88		
14	*Pythium*		2.94	
15	*Rhizopus*	23.53	14.71	11.43
16	*Trichoderma*	11.76		
Sum of genus		7	12	7
Species				
1	*Aspergillus flavus* Link	11.76	14.71	20
2	*Aspergillus fumigatus* Fresen.	5.88	11.76	20
3	*Aspergillus niger* Tiegh.	5.88	2.94	20
4	*Aspergillus ochraceoroseus*		5.88	
5	*Aspergillus penicilloides*		2.94	
6	*Aureobasidium* sp.		8.82	5.71
7	*Byssochlamys nivea*		2.94	
8	*Circinella* sp.	11.76		
9	*Colletotrichum* sp.		5.88	
10	*Curvularia* sp.		2.94	
11	*Fusarium lichenicola*	5.88		5.71
12	*Fusarium* sp.		2.94	
13	*Humicola* sp.		5.88	2.86
14	*Mucor* sp.		5.88	11.43
15	*Nodulisporium ochraceum*		2.94	
16	*Penicillium* sp.		5.88	
17	*Phytophthora* sp.	17.65		2.86
18	*Pythium* sp.		2.94	
19	*Pyricularia* sp.	5.88		
20	*Rhizopus* sp.	23.53	14.71	11.43
21	*Trichoderma* sp.	11.76		
Sum of species		9	16	9

Note: EM4 starter of 5-15 mL was mixed with the composted material at the beginning of the composting process. Rows shaded in grey indicate the fungi involved from day 7 until the end of the composting process on day 42

Table S1. Effect of bio activator, OPEFB, POME and composting time on compost characteristics

Run	Factors				Response (C/N)		Water content		Phosphorous		Potassium		Temperature		pH		TPM	
	EM4 (mL)	OPEFB (%)	POME (%)	TC (days)	A	P	A	P	A	P	A	P	A	P	A	P	A	P
1	15	25	0	24.5	8.38	9.73	47.44	43.32	1.84	2.98	6.48	5.75	29.1	29.20	6.76	6.67	158.00	198.84
2	15	50	30	24.5	9.39	8.74	55.64	59.17	1.19	2.93	7.23	6.33	28.8	29.03	6.78	7.11	226.00	216.73
3	10	25	30	24.5	7.66	8.23	64.26	49.93	1.79	3.18	6.24	5.29	28.5	29.29	6.96	6.82	207.00	200.67
4	10	25	60	7.0	18.24	17.06	59.36	49.30	1.74	1.87	4.89	5.11	35.2	35.08	6.03	6.05	178.00	190.55
5	10	25	30	24.5	7.51	8.23	44.56	49.93	2.04	3.18	6.15	5.29	28.8	29.29	6.79	6.82	156.00	200.67
6	10	0	0	24.5	7.02	6.53	25.12	34.08	4.82	5.44	4.61	4.71	29.3	29.76	5.85	6.09	222.00	200.23
7	10	0	60	24.5	6.50	5.50	51.74	43.42	3.78	4.68	5.09	5.12	29.8	29.89	6.23	6.56	224.00	237.48
8	10	25	30	24.5	8.19	8.23	49.52	49.93	2.85	3.18	5.70	5.29	29.5	29.29	6.86	6.82	178.00	200.67
9	10	25	30	24.5	7.58	8.23	51.43	49.93	4.33	3.18	6.07	5.29	30.0	29.29	6.78	6.82	231.00	200.67
10	5	25	30	42.0	9.98	10.51	64.52	57.17	0.82	2.18	3.96	5.00	29.8	29.68	6.71	7.14	173.00	156.53
11	10	0	30	7.0	10.78	13.41	33.62	33.45	2.36	2.99	3.81	4.53	35.0	35.22	5.12	4.95	268.00	242.82
12	10	25	0	42.0	9.51	8.95	49.45	50.56	1.53	1.72	5.50	5.46	28.8	28.70	7.14	7.10	90.00	99.92
13	10	0	30	42.0	7.48	8.84	29.62	44.05	2.40	2.04	5.39	5.29	29.8	29.57	6.68	6.56	174.00	177.32
14	5	25	60	24.5	7.41	8.42	59.49	*56.54*	4.89	*3.61*	6.10	*4.82*	29.4	*29.32*	*7.36*	*7.31*	*313.00*	*260.13*
15	15	0	30	24.5	6.82	5.66	35.28	36.82	5.56	4.99	5.68	5.58	30.2	30.03	6.05	6.11	305.00	306.24
16	10	25	30	24.5	10.16	8.23	60.09	49.93	4.47	3.18	5.75	5.29	29.5	29.29	6.69	6.82	237.00	200.67
17	10	50	30	42.0	7.99	8.02	65.77	66.41	1.83	1.27	5.56	6.04	28.6	28.47	7.34	7.38	192.00	202.31
18	10	50	30	7.0	20.28	21.31	45.68	55.81	2.05	2.47	4.52	5.28	35.2	35.52	5.87	5.86	234.00	215.81
19	10	25	60	42.0	12.06	11.21	63.29	59.90	1.02	1.52	6.37	5.87	28.6	29.28	7.55	7.29	213.00	238.05
20	10	50	50	24.5	9.45	9.23	45.67	64.22	5.07	3.63	5.92	5.80	29.8	29.32	7.47	7.34	220.00	240.39
21	5	25	30	7.0	16.01	15.87	36.63	46.56	2.95	3.69	5.60	4.24	34.4	34.63	5.42	5.61	303.00	327.53
22	10	25	0	7.0	21.57	20.68	42.11	39.95	3.71	3.53	4.84	4.70	36.5	35.60	4.96	5.20	229.00	226.42
23	10	50	0	24.5	9.49	9.85	56.70	56.44	6.20	4.96	4.52	5.46	29.4	29.45	7.08	6.90	207.00	185.35
24	5	50	30	24.5	9.26	8.71	65.60	63.04	4.82	5.91	4.36	5.00	29.7	29.71	7.15	7.09	297.00	315.40
25	15	25	60	24.5	5.81	7.96	52.07	52.67	4.32	4.88	4.06	6.16	30.1	29.79	6.96	6.99	292.00	282.46
26	5	25	0	24.5	7.80	8.01	62.47	47.19	8.05	7.36	4.06	4.42	29.5	29.84	6.77	6.59	244.00	241.50
27	15	25	30	7.0	21.10	19.93	46.89	42.69	3.45	1.71	5.14	5.57	35.7	35.95	5.73	5.45	177.00	185.86
28	15	25	30	42.0	8.21	7.71	55.27	53.30	2.19	1.06	6.58	6.33	28.3	28.20	7.11	7.06	310.00	277.86
29	5	0	30	24.5	5.50	4.44	27.09	*40.68*	6.34	*5.12*	3.09	*4.25*	29.9	*29.52*	*6.68*	*6.35*	*199.00*	*227.90*

Note: TC: Time Composting; A: Actual; P: Predicted; TPM: Total Population of Microorganism

Table 2. The fungal biodiversity profile during palm oil waste composting

Index biodiversity	Age of composting (days)		
	7	**24.5**	**42**
Family level			
D	0.176 (low)	0.223 (low)	0.394 (medium)
H	1.824 (medium)	1.991 (medium)	1.333 (medium)
D_{mg}	2.118 (low)	3.432 (medium)	1.688 (low)
E	0.937 (almost evenly distributed)	0.776 (almost evenly distributed)	0.685 (relatively even)
Genus level			
D	0.176 (low)	0.201 (low)	0.394 (medium)
H	1.824 (medium)	2.058 (medium)	1.333 (medium)
D_{mg}	2.118 (low)	3.342 (medium)	1.688 (low)
E	0.937 (almost evenly distributed)	0.802 (almost evenly distributed)	0.685 (relatively even)
Species level			
D	0.142 (low)	0.154 (low)	0.085 (low)
H	2.069 (medium)	2.671 (medium)	1.992 (medium)
D_{mg}	2.824 (medium)	2.250 (low)	4.821 (high)
E	0.941 (almost evenly distributed)	0.906 (almost evenly distributed)	0.924 (almost evenly distributed)

Note: D (Simpson dominance index): 0.00-0.30 (low), 0.31-0.60 (medium), 0.61-1.00 (high); H' (Shannon diversity index): ≤ 1 (low), 1 < H' < 3 (medium), ≥ 3 (high); Dmg (level of richness): < 2.5 (low), 2.5 > Dmg > 4 (medium), > 4 (high); E (Evenness index): 0.26-0.50 (less even), 0.51-0.75 (relatively even), 0.76-0.95 (almost evenly distributed), 0.96-1.00 (evenly distributed)

Only a few fungi, specifically *Penicillium* sp., are present in this composting process and provide significant advantages. Acid and alkaline phosphatases facilitate the crucial function of phosphorus solubilization from organic materials, and also act as biostimulants (Chorolque et al. 2022). The increase in fungal diversity is also related to nutrient availability and the transformation of organic matter in the soil (Kracmarova et al. 2023). The Fusarium fungus is present in the composting process of palm oil waste, contributing to its pathogenic nature. Robledo-Mahón et al. (2020) reported that concentrations range from 0.1-5%. However, the population declines as the compost matures. Elevated temperatures are crucial for eradicating pathogenic microorganisms throughout composting (Qiao et al. 2021).

Effect of bioactivator, OPEFB, POME, and composting time

The effects of bioactivator, OPEFB, POME, and composting time on the C/N ratio, P, and K content of the compost during the biorefinery process are shown in Table 3. The palm oil waste composting process effectively reduced the C/N ratio to 5.50-21.10 from the initial values of 51.55, 26.94, and 14.01 for OPEFB, PKM, and POME, respectively. This decrease in the C/N ratio is lower than that observed in a previous study by Dini and Afriani (2022), which reported C/N ratios of 24.51-25.90. Physicochemical factors, including nitrogen and organic carbon in the C/N ratio, play a crucial role in microbial communities, influencing microbial reproduction and energy production during the composting process. Therefore, the C/N ratio serves as an indicator of compost maturity (Xiao et al. 2024). The use of EM4 in composting also affects carbon sequestration, thereby accelerating the reduction of the C/N ratio (Zhang et al. 2024). Additionally, water content significantly impacts the composting process, particularly in terms of nutrient availability for microbial metabolism and physiological activity. The ideal water content for composting is between 40% and 60-65%.

Temperature significantly affects the composting process. Temperature data (Figure S1-S4) indicate that the composting phase is marked by a temperature rise, while the ripening process involves a reduction in temperature, ultimately stabilizing at a level close to the surrounding room temperature, approximately 25°C (Wu et al. 2024). The temperature correlates with microbial activity during various life cycle stages, including lag, log, and stationary phases (Kanong and Sakulrat 2022). At the end of the composting process, a consistent temperature (Zhao et al. 2024) indicates a decrease in the rate of organic matter breakdown (Alkarimiah and Suja 2020).

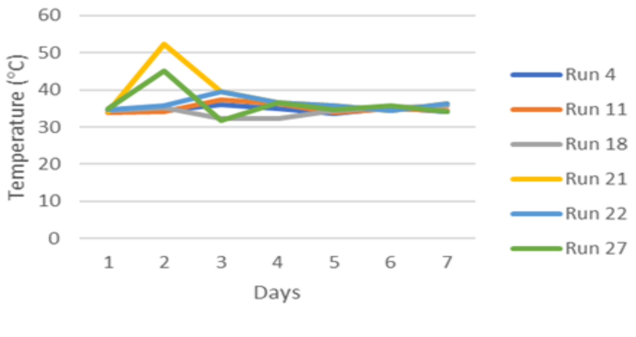

Figure S1. Temperature profile at 7 days of oil palm waste composting

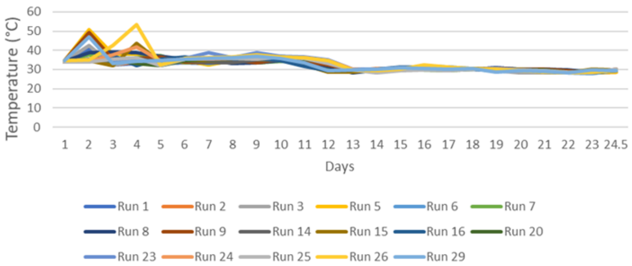

Figure S2. Temperature profile at 24.5 days of oil palm waste composting

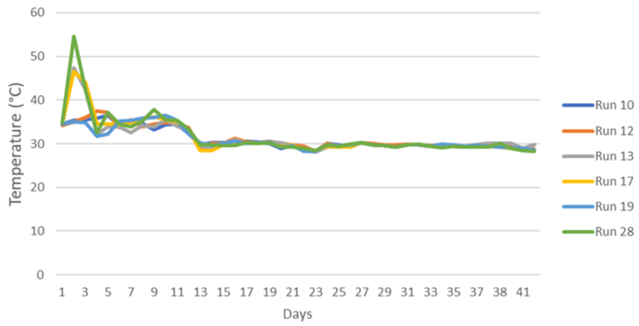

Figure S3. Temperature profile at 42 days of oil palm waste composting

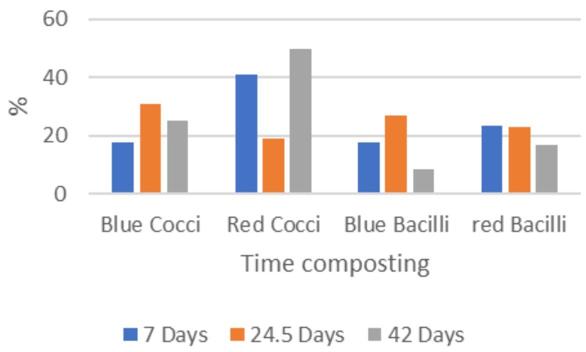

Figure S4. Bacteria in oil palm composting

The four factors are correlated with the C/N ratio and compost temperature as a quadratic model, each with a significance of $p<0.0001$. The OPEFB (B, $p=0.0008$), composting time (D, $p<0.0001$; D^2, $p<0.0001$), and the interaction between EM4 volume (A) and D ($p=0.0364$), B and D ($p=0.0090$), and C and D ($p=1.47$) significantly affected the C/N ratio of the resulting compost. Conversely, only composting time (D, $p<0.0001$; D^2, $p<0.0001$) and the interaction AD ($p=0.0206$) significantly affected compost temperature (Table 4).

According to ANOVA on the regression (equation model) for the responses of C/N, moisture content, potassium, temperature, pH, and total microbial population, the model was statistically significant with $p<0.05$, except for the phosphorus response, which was not statistically significant ($p>0.05$) (Table 4 and Table S2). Among all the responses tested, C/N is closely related to the characteristics of compost, significantly affecting compost quality, which must meet the standard SNI 19-7030-2004, where the C/N ratio should be between 10-20 (BSN, 2004). To produce C/N values within the minimum and maximum ranges, we can adjust the parameters affecting the composting process according to the RSM method. Figure 2 illustrates the influence of EM4 concentration, material composition, and fermentation time on the C/N ratio of compost under minimum and maximum conditions.

Table 3. Effects of bio activator, OPEFB, POME, and composting time on C/N compost

Run	Factors				Response (C/N)	
	EM4 (mL)	EFB (%)	POME (%)	Composting time (days)	Actual	Predicted
1	15	25	0	24.5	8.38	9.73
2	15	50	30	24.5	9.39	8.74
3	10	25	30	24.5	7.66	8.23
4	10	25	60	7.0	18.24	17.06
5	10	25	30	24.5	7.51	8.23
6	10	0	0	24.5	7.02	6.53
7	10	0	60	24.5	6.50	5.50
8	10	25	30	24.5	8.19	8.23
9	10	25	30	24.5	7.58	8.23
10	5	25	30	42.0	9.98	10.51
11	10	0	30	7.0	10.78	13.41
12	10	25	0	42.0	9.51	8.95
13	10	0	30	42.0	7.48	8.84
14	5	25	60	24.5	7.41	8.42
15	15	0	30	24.5	6.82	5.66
16	10	25	30	24.5	10.16	8.23
17	10	50	30	42.0	7.99	8.02
18	10	50	30	7.0	20.28	21.31
19	10	25	60	42.0	12.06	11.21
20	10	50	50	24.5	9.45	9.23
21	5	25	30	7.0	16.01	15.87
22	10	25	0	7.0	21.57	20.68
23	10	50	0	24.5	9.49	9.85
24	5	50	30	24.5	9.26	8.71
25	15	25	60	24.5	5.81	7.96
26	5	25	0	24.5	7.80	8.01
27	15	25	30	7.0	21.10	19.93
28	15	25	30	42.0	8.21	7.71
29	5	0	30	24.5	5.50	4.44

Table 4. Type, intercept, and coefficient of regression (equation model) for bioactivator (A), palm oil waste (B and C), and composting time (D) for improving the biorefinery process and compost quality

Source	C/N (Quadratic, *p*<0.0001)	
	Coefficient	*p*-value
Intercept	8.23	
A-Vol EM4 (mL)	0.3125	0.4773
B-OPEFB (%)	1.84*	0.0008
C-POME (%)	-0.3397	0.4550
D-Time (days)	-4.40*	<0.0001<0.0001
AB	-0.2975	0.6942
AC	-0.5450	0.4743
AD	-1.73*	0.0364
BC	0.1759	0.8317
BD	-2.25*	0.0090
CD	1.47	0.0673
A^2	-0.3355	0.5716
B^2	-1.01	0.1077
C^2	0.6326	0.3141
D^2	5.61*	< 0.0001
Lack of Fit		0.2526

Note: Data were analyzed by ANOVA. Coefficients followed by * indicate significant effects (p<0.05) on the C/N of compost

Optimization

The optimization conditions for the composting process of palm oil waste were based on the criteria presented in Table 5. The primary optimization criterion is the C/N ratio, which serves as a key parameter in determining compost maturity (SNI 19-7030-2004, with a target range of 10-20). Preliminary data indicate that the C/N ratios for fresh palm oil waste, specifically OPEFB, POME, and PKM are 51.55, 14.01, and 26.94, respectively. This study yielded an optimal C/N ratio of 19.084, demonstrating compliance with the compost maturity criteria according to the Indonesian national standards. The composting process also significantly reduced the C/N levels for EFB, POME, and PKM by 71%, 58.9%, and by 82.6%, respectively. These results indicate that the composting of palm oil waste over 42 days is highly effective for producing organic fertilizers.

Optimization criteria in Table 5 were utilized to yield 100 solutions with a desirability of 37.7-77.6%. The optimal conditions for the palm oil waste composting process involved an EM4 starter of 14.9999 mL, OPEFB at 30.8953%, POME at 59.9887%, and a composting time of 7 days. These composting conditions produced compost with physical and chemical characteristics of C/N 19.084, moisture content 49.999%, organic C 72.595%, total N 3.791%, and total K 5.867% (Table 6). The desirability of these composting conditions is 77.6%. These findings provide crucial information for formulating waste management strategies to expedite the processing of palm oil waste and mitigate its accumulation. Reducing composting time will positively impact the mitigation of negative effects associated with palm oil waste. Composting is more effective at reducing the adverse impacts of using palm biomass as mulch, as it minimizes contaminants, breeding grounds for pests and pathogens, and unpleasant odors (Awoh et al. 2023).

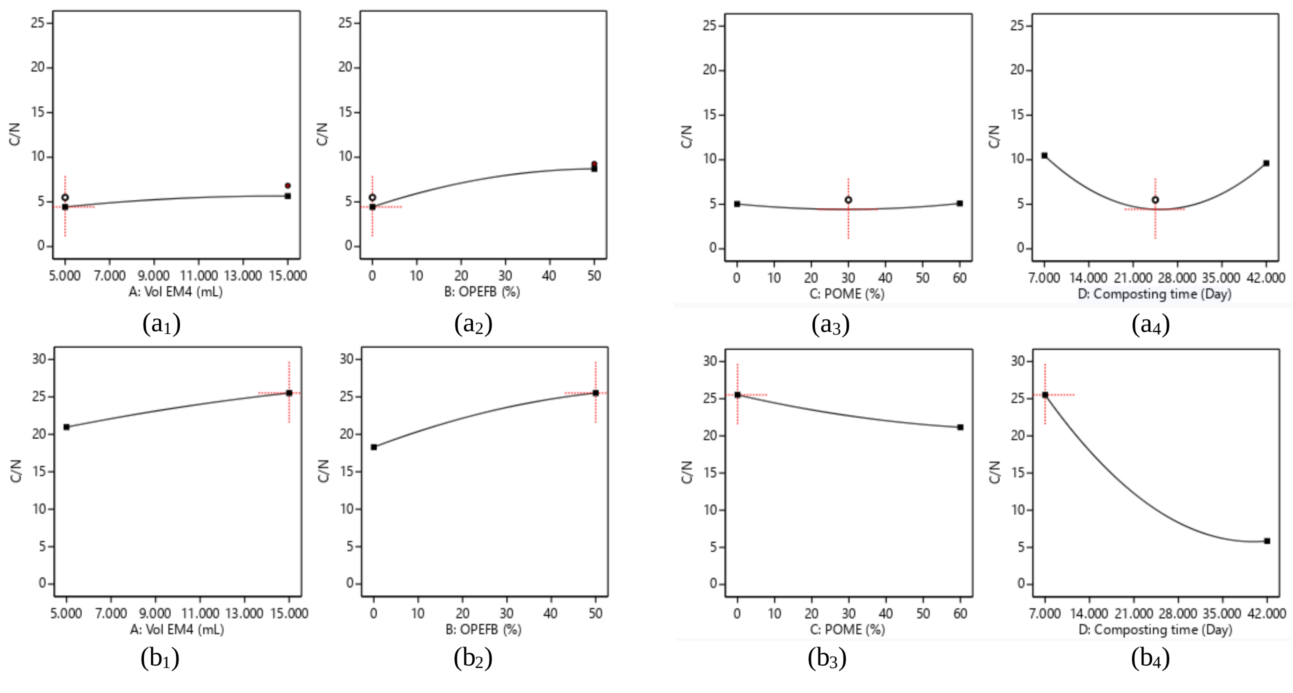

Figure 2. Effect of EM4, OPEFB, POME, and composting time on C/N of compost. Condition for C/N min (a1-a4) and C/N max (b1-b4)

Table S2. Type, intercept and coefficient of equation model for bio activator, oil palm waste and composting time for improving the biorefinery process and compost quality

Source	C/N (Quadratic, *p*<0.0001)		Water Content (linier, p 0,001)		Total of N		Total of P		Total of K		Temperature		pH		TPM	
	C	***p*-value**	**C**	***p*-value**	**C**	***p*-value**	**C**	***p*-value**	**C**	***p*-value**	**C**	***p*-value**	**C**	***p*-value**	**C**	***p*-value**
Intercept	8.23		49.9		3.65		3.17		5.28		29.2		6.82		200.6	
A-Vol EM4 (mL)	0.3125	0.4773	-1.934	0.4591	0.0166667	0.8668	-0.776667	0.0824	0.666667	0.0127	**-0.0416667**	0.7919	**-0.0583333**	0.4603	-5.08333	0.6118
B-OPEFB(%)	1.84*	0.0008	11.179	0.0002	-0.64659	< 0.0001	-0.321451	0.4536	0.375666	0.1423	-0.202111	0.2151	0.435013	< 0.0001	-0.503322	0.9599
C-POME(%)	-0.3397	0.4550	4.67266	0.0887	0.00277183	0.9783	-0.464055	0.2974	0.203976	0.4291	0.0178344	0.9128	0.26144	0.0053	25.5632	0.0242
D-Time (days)	-4.40*	< 0.0001	5.3025	0.0501	-0.2725	0.0106	-0.539167	0.2149	0.38	0.1378	-3.175	< 0.0001	0.783333	< 0.0001	-19.75	0.0633
AB	-0.2975	0.6942					-0.7125	0.3385			-0.3	0.2824	0.065	0.6328	-44.25	0.0206
AC	-0.5450	0.4743					1.41	0.0700			0.275	0.3228	-0.0975	0.4758	16.25	0.3542
AD	-1.73*	0.0364					0.2175	0.7667			-0.7	0.0206	0.0225	0.8681	65.75	0.0017
BC	0.1759	0.8317					**-0.0821653**	0.9184			**-0.0464969**	0.8766	**0.0268185**	0.8567	6.93948	0.7145
BD	-2.25*	0.0090					-0.065	0.9292			-0.35	0.2131	-0.0225	0.8681	13	0.4561
CD	1.47	0.0673					0.365	0.6196			0.275	0.3228	-0.165	0.2354	43.5	0.0225
A^2	-0.3355	0.5716					0.765581	0.1945			0.100745	0.6383	**-0.0142578**	0.8929	46.6404	0.0034
B^2	-1.01	0.1077					0.795905	0.1853			0.18508	0.4000	-0.144238	0.1941	19.2605	0.1749
C^2	0.6326	0.3141					0.764394	0.2143			0.151609	0.5007	0.0793279	0.4778	-1.57334	0.9112
D^2	5.61*	< 0.0001					-1.78067	0.0068			2.72575	< 0.0001	-0.491758	0.0003	-10.3596	0.4475
Lack of fit		0.2526		0.4514		0.5809		0.3873		0.0091		0.6996		0.0224		0.5732

Table 4. Optimization criteria

Factors	Goal	Limit (lower-upper)	Weights (lower-upper)	Importance (1-5)
Vol. EM4 (mL)	In range	5-15		3
Conc. OPEFB (%)	Maximized	0-50	1-1	5
Conc. POME (%)	Maximized	0-60	1-1	5
Composting Time (days)	In range	7-42	1-1	3
Response				
C/N	In range	10-20*	1-1	5
Moisture Content (%)	In range	25.12-50*	1-1	3
C Organic (%)	In range	9.8-32*	1-1	3
N Total (%)	In range	0.4-4.95*	1-1	3
P Total (%)	None			
K Total (%)	None			
Temperature (°C)	None			
pH	None			
Total Fungi (CFU/g)	None			

Note: *According to SNI 19-7030-2004, the Indonesian National Standard for Compost from Organic Domestic Waste (BSN 2024)

Table 5. Characteristics of the compost resulting from palm oil waste

Characteristics	Predicted mean	95% PI low	95% PI high
C/N	19.0837	14.2408	23.9265
Moisture content (%)	49.9993	29.0725	70.9261
C organic (%)	72.5947	53.0726	92.1132
N total (%)	3.79122	2.99118	4.59126
P total (%)	2.84703	-1.85026	7.54432
K total (%)	5.86664	3.85164	7.88167
Temperature (°C)	36.0793	34.3261	37.8325
pH	5.98257	5.1131	6.85205
Total population of microorganisms	171.694	60.8798	282.508

In conclusion, the fungal biodiversity during the biorefinery process was assessed at the family and genus levels, revealing 16 and 21 types at the genus and species level, respectively. The biodiversity indices, specifically Simpson (D) and Shannon (H'), aligned with the shifts in fungal diversity throughout the biorefinery process. The biodiversity index at the genus level peaked in the middle of the process (day 24.5), indicating low (D = 0.085-0.154) and medium (H' = 1.992-2.671) values, respectively. On day 7, the compost temperature was 34.98°C, declining to 29.50°C and 28.82°C on days 24.5 and 42, respectively. The dominant families fully involved in the composting process were Aspergillaceae and Rhizopodaceae, while the dominant genera included *Aspergillus*, *Fusarium*, and *Rhizopus*. At the species level, *A. flavus, A. fumigatus, A. niger*, and *Rhizopus* sp. were fully engaged. OPEFB concentration and composting time significantly affected the compost C/N ratio, as did the interaction between composting time and the other two factors (EM4 volume and OPEFB). The optimal composting conditions were determined to be an EM4 volume of 15 mL, OPEFB at 30.895%, POME at 59.989%, and a composting time of seven days. The resulting compost exhibited a C/N ratio of 19.084, moisture content of 49.999%, C-organic content of 72.595%, N-total of 3.791%, and K-total of 5.867%, with a desirability of 77.6%. This optimal biorefinery condition has the potential to mitigate the environmental challenges posed by palm oil waste, which contributes to greenhouse gas emissions (methane). The composting process effectively reduced the C/N ratios of OPEFB by 71%, POME by 58.9%, and PKM by 82.6%, demonstrating a promising alternative for converting palm oil waste into organic fertilizer.

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