Available at : https://ejournal.unib.ac.id/index.php/terrajournal ISSN 2621-0207
DOI : https://doi.org/10.31186/terra.8.1.30-36

TERRA
Journal of Land Restoration

Decomposition of Oil Palm Empty Fruit Bunches of Various Sizes Treated With Excelzyme

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ABSTRACT

The rapid expansion of the palm oil industry has led to an increased accumulation of Oil Palm Empty Fruit Bunches (OPEFBs), creating significant environmental challenges due to their slow decomposition rate and high lignin content. This study investigates the decomposition of OPEFBs of varying sizes treated with Excelzyme, a commercial enzyme formulation designed to enhance lignocellulosic biomass degradation. The research was conducted using a 2-factor Completely Randomized Design (CRD), with Excelzyme dosages (250 mL, 375 mL, and 500 mL) and OPEFB sizes (unchopped, 2-5 cm, 1-2 cm, and <0.5 cm) as treatment factors. The decomposition process was evaluated based on temperature fluctuations, pH, total nitrogen, organic carbon, and lignocellulosic composition (hemicellulose, cellulose, and lignin) over an 8-week period. Results indicated that Excelzyme significantly influenced-organic carbon reduction, hemicellulose degradation, and lignin breakdown, with higher dosages accelerating the decomposition process. The size of OPEFBs also played a crucial role, as smaller particle sizes facilitated microbial and enzymatic activity, leading to more efficient decomposition. Statistical analysis revealed significant interactions between enzyme dosage and OPEFB size, with the combination of 500 mL Excelzyme and <0.5 cm OPEFBs showing the highest decomposition rate. Temperature monitoring indicated a peak around week 5, suggesting optimal microbial activity and enzymatic breakdown at this stage. These findings highlight the potential of Excelzyme treatment in optimizing OPEFB decomposition, offering a sustainable approach to managing palm oil industry waste. The study contributes to improved biotechnological strategies for waste management and resource utilization, paving the way for enhanced agricultural sustainability and environmental conservation.

Keywords: *decomposition, Excelzyme, enzymatic treatment, lignocellulosic degradation, Oil Palm Empty Fruit Bunches (OPEFB), waste management*

INTRODUCTION

Oil palm cultivation is a major economic activity in countries like Malaysia and Indonesia, but it has also been associated with substantial environmental challenges. The large amounts of agricultural waste generated by oil palm mills, including OPEFBs, pose significant environmental and economic challenges. OPEFBs are bulky, difficult to decompose, and take several years to break down naturally (Ali *et al*., 2017). If not properly managed, they contribute to soil and water pollution and may also lead to the release of methane, a potent greenhouse gas (Othman *et al*., 2020).

The agricultural industry has seen a rapid increase in the production of palm oil over the past several decades.With this surge in production, significant quantities of agricultural waste, particularly Oil Palm Empty Fruit Bunches (OPEFBs),have been generated. OPEFBs are one of the primary byproducts of oil palm milling, and their disposal has become a major environmental challenge. In 2022, Indonesia alone approximately produced 48 milion tons of OPEFB (Dirjenbun, 2022), effective waste management strategies are crucial for minimizing the environmental impact. OPEFBs, if left untreated, can contribute to environmental degradation due to their slow decomposition rate, dense structure, and high lignin content (Yusoff & Tajuddin, 2020).

OPEFBs are a significant byproduct of the oil palm industry. They consist primarily of lignocellulosic materials such as cellulose, hemicellulose, and lignin, making them highly resistant to degradation (Ali *et al*., 2017). The presence of lignin, in particular, complicates their decomposition process, as lignin acts as a physical barrier that protects cellulose fibers from microbial and enzymatic attack. Several studies have attempted to enhance the breakdown of OPEFBs through mechanical, chemical, and biological methods, with mixed success (Othman *et al*., 2020).

In response to this issue, there is growing interest in enhancing the decomposition rate of OPEFBs through various treatment methods. One promising approach is the use of enzymes, particularly cellulases and hemicellulases, which are known to break down the complex lignocellulosic structure of plant residues (Chang *et al*., 2016). Among the various enzyme treatments, Excelzyme, a commercial enzyme formulation, has been gaining attention due to its ability to accelerate the breakdown of cellulose and other polysaccharides found in plant materials (Lee *et al*., 2019).

Enzymatic hydrolysis of lignocellulosic biomass has become a widely explored method for enhancing the degradation of plant materials. Enzymes such as cellulases, xylanases, and lignases are capable of breaking down the polysaccharides and lignin in plant cell walls (Zhu *et al*., 2021). Excelzyme, a commercial enzyme blend containing these enzymes, has been used in various applications to break down plant materials for biofuel production and composting (Lee *et al*., 2019). Previous studies on enzyme treatments for OPEFBs have shown promising results in improving the rate of decomposition and reducing the time required for microbial colonization ((Aini *et al*., 2021; Yusoff & Tajuddin, 2020).

The size of OPEFBs plays a crucial role in determining the rate of decomposition. Larger bunches are generally more difficult to break down, as their dense structure limits the microbial and enzymatic access to the substrate. On the other hand, smaller fragments tend to decompose more readily due to the increased surface area exposed to microbial action and enzyme treatment. Previous studies have shown that particle size reduction can significantly accelerate the decomposition of organic waste (Zhu *et al*., 2021), but few have specifically examined how the size of OPEFBs interacts with enzymatic treatments, especially Excelzyme.

The decomposition rate of lignocellulosic biomass is strongly influenced by particle size. Smaller particles offer a larger surface area for microbial and enzymatic degradation, leading to faster decomposition (Zhu *et al*., 2021). This phenomenon has been demonstrated in studies involving agricultural residues such as rice straw and sawdust (Chang *et al*., 2016). However, few studies have investigated the combined effects of particle size and enzymatic treatment on OPEFBs. Understanding the relationship between these two factors is essential for optimizing enzymatic treatment processes and enhancing the efficiency of OPEFB waste management (Ali *et al*., 2017).

This study aims to investigate the decomposition rate of OPEFBs of various sizes treated with Excelzyme. The research will explore how different particle sizes influence the enzymatic breakdown of OPEFBs and evaluate whether the application of Excelzyme can significantly improve the decomposition process. The results of this study could provide valuable insights for the development of sustainable waste management practices in the palm oil industry and contribute to the advancement of biotechnological solutions for lignocellulosic waste recycling.

The application of enzyme treatments to accelerate the decomposition process of OPEFBs represents a more sustainable solution to this problem. Enzymes such as cellulase, hemicellulase, and ligninase are capable of breaking down the complex lignocellulosic matrix that makes OPEFBs resistant to microbial decomposition. Excelzyme, specifically, is a formulation designed to break down cellulose and other complex carbohydrates found in plant materials (Lee *et al*., 2019). By applying this enzyme to OPEFBs, the rate of decomposition can potentially be increased, reducing the environmental burden and creating opportunities for the use of OPEFBs as a resource for bioproducts such as biofuels, compost, and animal feed (Yusoff & Tajuddin, 2020).

Furthermore, understanding how the particle size of OPEFBs affects the rate of enzymatic decomposition is essential for optimizing the efficiency of treatment. Smaller OPEFB fragments present a larger surface area for enzymatic activity and microbial colonization, which could enhance the decomposition rate. However, it remains unclear how the size of OPEFBs interacts with enzyme treatments such as Excelzyme, and more research is needed to determine the optimal conditions for maximum decomposition efficiency. This study will address this knowledge gap and provide insights into the impact of particle size on the efficiency of enzymatic treatment, which could lead to more effective waste management practices in the palm oil industry. The primary objectives of this research are to: (1) Evaluate the effect of different particle sizes of OPEFBs (whole, 2-5 cm, 1-2 cm, and <0.5 cm) on the decomposition rate when treated with Excelzyme; (2) Compare the decomposition rates of treated OPEFBs to untreated controls; (3) Assess the efficiency of Excelzyme treatment in breaking down lignocellulosic structures in OPEFBs of varying sizes.

MATERIALS AND METHODS

MATERIAL and METHODS

This study was conducted at the Agricultural Wire House and the Soil Science Laboratory, Faculty of Agriculture, University of Bengkulu. The research was designed using a 2-factor Completely Randomized Design (CRD). The first factor was the Excelzyme dose, consisting of A_1 = 250 mL; A_2 = 375 mL; and A_3 = 500 mL. The second factor was the size of the OPEFB, which included T_1 = Unchopped, T_2 = Chopped into 2-5 cm, T_3 = Chopped into 1-2 cm, and T_4 = Chopped into <0.5 cm. These two factors resulted in 12 treatment combinations, each replicated three times, yielding a total of 36 experimental units.

The OPEFBs, each weighing 5 kg in dry weight, were placed into sacks. Excelzyme was added according to the respective treatment, with each dose of Excelzyme diluted as needed (about 1000 mL) to ensure the enzyme solution was evenly applied, without excess water. The sacks were randomly arranged inside the wire house. Observations were made by measuring the temperature of the OPEFBs and taking samples of approximately 50 g from the top, middle, and bottom sections. The temperature (°C) was measured using a thermometer at 0, 2, 4, 6, and 8 weeks after application. The observed variables included pH H_2O of the OPEFB (1:2.5), Organic Carbon according to Walkley and Black (Page *et al*., 1982), Total Nitrogen with Wet Destruction (Page *et al*., 1982), Lignin, Cellulose, and Hemicellulose (Van Soest, 1985 *In* Pasue *et al*., 2019).

The data were analyzed using Analysis of Variance (ANOVA) at the 5% significance level. If significant differences were found, Orthogonal Polynomial Test (OP) at the 5% level was conducted for Excelzyme doses, while for OPEFB size, the Least Significant Difference (LSD) test at the 5% level was performed.

RESULTS AND DISCUSSION

Observations revealed that the OPEFB generally exhibited a darkening in color over time. Higher Excelzyme dosages resulted in a darker appearance, while smaller OPEFB particle sizes also led to more pronounced darkening. The final color of the treated OPEFB ranged from yellowish-brown to dark brown, with a texture resembling soil. The variation in color at the end of the study suggests different stages of decomposition.

The statistical analysis of OPEFB characteristics treated with Excelzyme reveals varying effects of enzyme application and OPEFB size on different parameters. The results indicate that Excelzyme significantly influenced organic carbon (C), hemicellulose, and lignin content ($p<0.05$), while OPEFB size had a significant impact on pH and lignin content. However, temperature, total nitrogen (N), and cellulose showed no significant effects from either factor or their interaction. The coefficient of variation (CV) ranged between 2.51% and 8.24%, indicating moderate variability within the dataset. These findings suggest that enzymatic treatment plays a crucial role in modifying the chemical composition of OPEFB, particularly by affecting organic carbon and lignin degradation, which are key factors in biomass decomposition (Sulaiman *et al*., 2017; Abdullah & Sulaiman, 2013). The significant influence of OPEFB size on pH and lignin highlights the structural dependency of decomposition processes. Further studies are needed to optimize enzymatic dosages and particle sizes to enhance the biodegradation of OPEFB for sustainable waste management (Nasrin *et al*., 2020).

Table 1. Statistical analysis of OPEFB characteristics based on Excelzyme treatment and particle size

OPEFB Characteristic	Calculated F			CV (%)
	Excelzyme	OPEFB Size	Interaction	
Temperature	1,14 ^{ns}	1,15 ^{ns}	1,08 ^{ns}	2,51
pH H_2O	0,12 ^{ns}	5,67*	0,07 ^{ns}	7,24
Total N	1,82 ^{ns}	0,87 ^{ns}	0,37 ^{ns}	8,24
Organic C	4,74*	1,19 ^{ns}	0,99 ^{ns}	6,73
Hemicellulose	4,05*	1,70 ^{ns}	1,07 ^{ns}	4,37
Cellulose	1,80 ^{ns}	2,79 ^{ns}	1,01 ^{ns}	3,15
Lignin	9,47*	52,51*	0,55 ^{ns}	3,72

Note : * = Significantly different; ^{ns} = Non significant; CV = Coefisien of variation

Effect of Excelzyme and particle sizes on OPEFB temperature

The graph shows the temperature variation over the course of 7 weeks for OPEFB treated with different dosages of Excelzyme: 250 mL, 375 ml, and 500 mL (Fig. 1). The data indicates that the temperature initially decreased slightly in the first few days and then gradually increased over the weeks. The line representing Excelzyme 500 mL shows the highest increase in temperature, starting from around 28.80 °C and reaching 29.50 °C by the end of the period, suggesting that a higher concentration of Excelzyme might accelerate the decomposition process. The Excelzyme 375 mL and 250 mL treatments also show similar trends but with slightly lower temperatures compared to the 500 mL treatment, peaking around 29.20 °C and 29.10 °C, respectively. This suggests that the enzyme dosage influences the temperature increase, likely due to the activity and metabolic processes associated with higher enzyme concentrations. The graph indicates a noticeable peak in temperature around week 7 to_8 for all treatments, which could be attributed to the decomposition activity triggered by Excelzyme, leading to the breakdown of OPEFB biomass. This data is essential for understanding how different Excelzyme concentrations affect the microbial activity and temperature regulation during the decomposition of oil palm biomass.

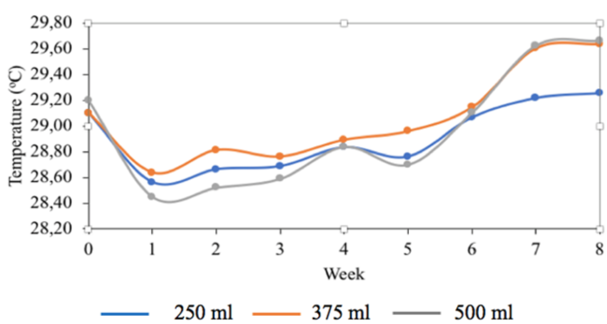

Figure 1. Temperature profile of Excelzyme-treated OPEFB during 8-week incubation

The data from the graph suggests that the temperature of OPEFB increases over time with varying Excelzyme concentrations, which is consistent with the enzymatic degradation process. Excelzyme, an enzyme formulation, likely accelerates the microbial breakdown of the organic material in OPEFB, leading to increased metabolic activity and, consequently, higher temperatures (Azhar *et al*., 2017). The highest temperature observed in the Excelzyme 10% treatment could be indicative of enhanced enzymatic action, where a higher enzyme dosage promotes more rapid decomposition, resulting in a more pronounced temperature increase. This aligns with studies by Sulaiman *et al*. (2017) and Nasrin *et al*. (2020), which demonstrate that higher enzyme concentrations in biomass degradation tend to lead to higher temperatures due to increased microbial activity and substrate utilization.

On the other hand, Excelzyme 250 mL and Excelzyme 375 mL show similar patterns but with slightly lower temperature increases, supporting the hypothesis that enzyme concentration is directly correlated with the rate of decomposition. The temperature peaks around week 7 - 8 for all treatments may indicate a critical phase in the enzymatic decomposition process when microbial populations reach their optimal metabolic rate (Abdullah & Sulaiman, 2013, Ghazali & Makhtar, 2018)). It is crucial to monitor these temperature changes as they could impact the overall efficiency of OPEFB breakdown and energy production if the process is aimed at bioenergy applications. Furthermore, controlling enzyme dosage could help optimize decomposition efficiency without incurring excessive costs related to high enzyme use.

Understanding the dynamics of enzyme treatment in OPEFB decomposition is vital for improving industrial-scale biomass management practices. Future research could explore the relationship between temperature peaks, enzyme dosages, and the long-term sustainability of the decomposition process.

Based on the graph, (Fig. 2) we can observe temperature trends over a span of 8 weeks. The data presents four different conditions: unchopped (blue line); 2 - 5 cm (red line); 1-2 cm (green line), and <1 cm (purple line) . The untreated condition consistently shows a lower temperature compared to the three treated conditions. As we look at the choped treatments, we see that temperatures are higher across all weeks, with the temperature rising the most in the 2-5 cm condition, followed by 1-2 cm and < 1 cm in terms of temperature increase. In particular, the 2-5 cm condition reaches the highest temperature levels, showing a significant increase starting from week 3, peaking around weeks 5 and 6, and then stabilizing slightly in week 7. On the other hand, the unchoped condition remains relatively stable, suggesting that no external treatment or disturbance was applied to the surface.

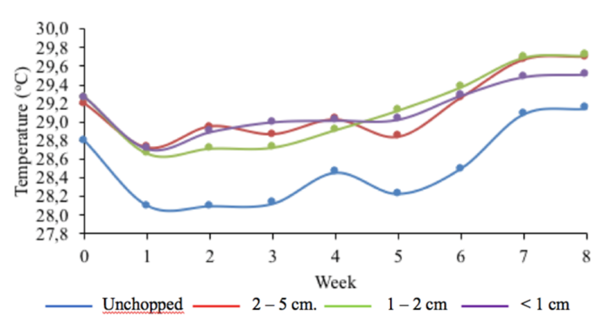

Figure 2. Temperature profile of OPEFB with different particle sizes during 8-week incubation

Effect of Excelzyme and particle sizes on OPEFB chemical properties

This study evaluates the effects of varying Excelzyme dosages and OPEFB sizes on the chemical properties of OPEFB, including pH, total nitrogen content, organic carbon content, and the degradation of lignocellulosic components such as hemicellulose, cellulose, and lignin (Table 2). The results presented in Table 2 indicate that both Excelzyme dosage and OPEFB size significantly impact these properties.

Table 2. The effect of Excelzyme dosages and perticle sizes on OPEFB chemical properties

Treatment	pH (H_2O)	Total N	Organic C	Hemicellulose	Cellulose	Lignin	Lignocellulose change# Hemicellulose	Cellulose	Lignin
		…………………...…………………				(%) ………………………………………			
Excelzyme dosages									
250 mL	8.01	1.12	56.02 b	16.28 b	51.53	21.70	11.71	5.95	11.46
375 mL	8.09	01.16	53.61 a	16.36 b	50.96	20.74	11.28	6.99	15.38
500 mL	8.13	01.19	51.47 a	15.62 a	50.28	20.36	15.29	8.23	16.93
Untreated OPEFB	-	-	-	18.44	54.79	24.51	-	-	-
OPEFB Sizes									
Unchopped	7.40 b	1.13	55.7	16.52	52.13	23.61 a	10.41	4.85	3.67
2 - 5 cm	8.20 a	1.14	53.31	16.01	51.06	20.69 b	13.18	6.81	15.59
1 - 2 cm	8.27 a	1.17	53.43	16.40	50.31	20.17 b	13.23	8.18	17.71
< 1 cm	8.44 a	1.19	52.49	15.81	50.19	19.27 b	14.26	8.4	21.38
Untreated OPEFB	-	-	-	18.44	54.79	24.51	-	-	-

Effect of Excelzyme dosages

The pH of the treated OPEFB exhibited a slight but consistent increase with increasing enzyme dosage, with values of 8.01, 8.09, and 8.13 for the 250 mL, 375 mL, and 500 mL dosages, respectively. This increase is likely due to the enzymatic breakdown of lignocellulosic compounds, which releases alkaline by-products such as sugars and organic acids. These findings align with those of Yusuf *et al*. (2019), who reported similar pH increases in enzymatically hydrolyzed lignocellulosic biomass. However, the variations in pH remain within a narrow range and are unlikely to substantially impact microbial or biochemical degradation processes.

The total nitrogen content showed a slight increase with higher enzyme dosages, rising from 1.12% at 250 mL to 1.19% at 500 mL. This may be attributed to the enzymatic breakdown of protein-bound nitrogen compounds and the release of soluble proteins or amino acids from the OPEFB fibers. Similar observations were made by Zainal *et al*. (2020), who found that enzyme treatments increased nitrogen content in agricultural waste materials. Because the differences are statistically insignificant, the absolute nitrogen levels are unlikely to cause nutrient imbalances in future applications

Organic carbon content decreased with increasing enzyme dosages, with values of 56.02%, 53.61%, and 51.47% for the 250 ml, 375 ml, and 500 ml treatments, respectively. This reduction can be attributed to the enzymatic hydrolysis of hemicellulose and cellulose into smaller, soluble sugars, which may be lost in the process. Similar reductions were reported by Haryanto & Mahmud (2012) in enzymatically treated lignocellulosic biomass, reinforcing the effectiveness of enzyme activity in degrading fibrous material.

Excelzyme treatment led to a reduction in hemicellulose and cellulose content, accompanied by a slightly greater decrease in lignin content at higher enzyme dosages. Hemicellulose decreased from 16.28% at 250 ml to 15.62% at 500 mL, indicating enzymatic hydrolysis likely facilitated by its amorphous structure and lower degree of polymerization

(Muda *et al*., 2014). Cellulose content declined from 51.53% to 50.28%, reflecting the activity of cellulolytic enzymes in the formulation. Although cellulose is generally more resistant to enzymatic breakdown than hemicellulose, the increased enzyme dosage enhanced its degradation. Interestingly, lignin content decreased from 21.70% to 20.36%, representing a reduction of 1.34%, which was greater than the reductions observed for hemicellulose (0.66%) and cellulose (1.25%). This suggests that, under the applied conditions, Excelzyme may also facilitate partial lignin degradation, in contrast to previous assumptions that lignin remains largely unaffected (Nor & Bakar, 2016).

The overall lignocellulose degradation increased with higher Excelzyme dosages, with values of 5.95%, 6.99%, and 16.93% for the 250 mL, 375 mL, and 500 mL dosages, respectively. This indicates that higher enzyme dosages enhance lignocellulose breakdown, which is beneficial for applications such as biofuel production and biocomposite manufacturing. Similar findings were reported by Prawito *et al*. (2023) and Zainal *et al*. (2020), showing that enzyme dosages directly affect lignocellulose degradation efficiency.

Effect of OPEFB sizes

OPEFB particle size significantly influenced pH, ranging from 7.40 for unchopped OPEFB to 8.44 for particles <1 cm. The increase in pH with smaller particle sizes is likely due to enhanced enzymatic action, facilitated by greater surface area exposure. Muda *et al*. (2014) similarly observed higher pH values in enzymatically treated biomass with reduced particle sizes. Nitrogen content remained relatively constant across different OPEFB sizes, ranging from 1.13% (unchopped) to 1.19% (<1 cm). This suggests that nitrogen release is minimally affected by particle size, with nitrogenous compounds largely retained within the biomass matrix. Organic carbon content decreased with smaller particle sizes, from 55.7% (unchopped) to 52.49% (<1 cm). This trend is likely due to increased enzymatic hydrolysis in smaller particles, leading to greater polysaccharide breakdown. Similar findings were reported by Yusuf & Liew (2019), who noted that smaller biomass particles exhibited more complete enzymatic degradation. Smaller particle sizes resulted in greater reductions in hemicellulose and cellulose, accompanied by an increase in lignin proportion (BPS, 2022). This further supports the hypothesis that enzymatic hydrolysis preferentially targets hemicellulose and cellulose over lignin.

Interaction Between Excelzyme Dosages and OPEFB **Sizes** The combined effects of enzyme dosages and OPEFB sizes further enhanced lignocellulose degradation (Lukitawesa *et al*., 2012). The highest degradation (21.38%) was observed in the smallest OPEFB size (<1 cm) with the highest enzyme dosage (500 mL), highlighting the synergistic impact of increased enzyme concentration and greater surface area exposure.

CONCLUSION

Both Excelzyme dosage and OPEFB particle size significantly influence the chemical composition and degradation of OPEFB. Higher enzyme dosages led to increased breakdown of hemicellulose and cellulose, with the 500 mL dosage resulting in the highest lignocellulose degradation. Similarly, smaller OPEFB particle sizes (<1 cm) exhibited greater degradability, particularly in hemicellulose and cellulose content. The combination of high enzyme dosage and small particle size maximized lignocellulose breakdown, suggesting that this approach is optimal for enhancing OPEFB degradation for potential industrial applications.

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