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The Effect of $CaCO_3$ and $MgSO_4$ Fillers on the Characteristics of Biofoam Made from Oil Palm Leaf Substrate with Inoculums of *Rhizopus* sp. and *Neurospora sitophila*

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Abstract*— Biofoam is a biodegradable material expected to replace the role and function of styrofoam. The oil palm (*Elaeis guineensis* Jacq) industry has generated much biomass waste, including oil palm leaves. Biofoam based on the mycelium of the fungi *Rhizopus* sp. and *Neurospora sitophila* has the potential as an innovative alternative technology to replace polystyrene. The purpose of this research is to obtain the characteristics of biofoam that uses oil palm leaf fibers as a substrate with *Rhizopus* sp. and *N. sitophila* inoculums, as well as $CaCO_3$ and $MgSO_4$ fillers. The method used in this research is experimental in the laboratory. The first factor is the type of filler used, which includes variations of $CaCO_3$ and $MgSO_4$ at a 5% concentration. The second factor is the species of commercial inoculants used: Rhizopus sp. and *N. sitophila* at a 25% concentration. The solid fermentation study was conducted in polypropylene molds for 7 days at room temperature. The characteristic parameters observed were the number of fungal colonies, morphological analysis with a Keyence digital microscope, water absorption test, biodegradability test, and compressive strength test. The study results showed that the mycelium of *Rhizopus* sp. could grow well on the oil palm leaf substrate with a 7-day incubation period at room temperature (27°C), resulting in 12.1 x 10^3 CFU/g. The mycelium of *N. sitophila* could not grow on the oil palm leaf substrate within the 7-day incubation period. Characterization and morphological analysis tests showed that the biofoam quality closest to the standard was the formulation of *Rhizopus* sp. biofoam with the addition of $CaCO_3$ and $MgSO_4$. Both formulations met the water absorption and biodegradability standards but did not meet the compressive strength standards based on (SNI) 7188.7:2016.

***Keywords*— Biofoam; *Rhizopus* sp; *Neurospora sitophila*; filler ($CaCO_3$, $MgSO_4$); oil palm leaf fiber.**

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I. INTRODUCTION

Expanded polystyrene (EPS) is a plastic component in styrofoam. It is widely utilized as a packaging material due to its versatility and cell structure, which provides low density, high impact resistance, and high thermal insulation. However, environmental concerns arise from its non-biodegradability [1]. Styrofoam also contains styrene, a carcinogenic chemical that can leach into hot packaged foods. Burning Styrofoam waste at temperatures below 1000°C produces harmful, carcinogenic dioxins [2].

Environmental issues from the disposal of non-biodegradable materials have spurred research into using biodegradable materials for commercial packaging [3]. Biofoam, a biodegradable material, is expected to replace Styrofoam. The substrate generally used is lignocellulose derived from agricultural biomass [4]. Agricultural waste materials are promising raw materials for various products. Studies show that combining fibers with different reinforcing materials, such as natural fibers, can produce polymer materials with enhanced properties [5].

Palm oil leaf biomass waste can be used as a natural raw material and fiber source to strengthen biofoam substrates. Like palm leaves, agricultural waste often accumulates in palm oil processing factories. With increasing oil palm production and plantation areas, the volume of waste also increases. Palm oil waste includes empty bunches, shells, shoots, and leaves, which are thick and fibrous and contain up to 46.75% crude fiber [6].

Microorganisms such as fungi serve as natural adhesives for biofoam, producing mycelium that binds the biofoam. *Rhizopus* sp. and *Neurospora sitophila* are safe, commonly

found fungi used in packaging production for reinforcement. These fungi form mycelium consisting of long hyphae threads. *Rhizopus* is also used to produce enzymes like cellulase, protease, phytase, amylase, and pectinase [7], while *N. sitophila* produces lignocellulolytic enzymes such as lignase, cellulase, amylase, glucoamylase, and phytase [8].

Biofoam is vulnerable to degradation without fillers to strengthen its structure. Calcium carbonate ($CaCO_3$) is a commonly used filler [9]. $MgSO_4$ can absorb air, enhancing the hydrophilic properties of biofoam and increasing its strength by adding stiffness to the material structure [10]. Additionally, $MgSO_4$ is a nutrient source for microorganisms in biofoam, aiding the material's biodegradation process.

Given this background, this research aims to create biofoam using an oil palm leaf substrate, *Rhizopus* sp. and *N. sitophila* fungi inoculum, and $CaCO_3$ and $MgSO_4$ fillers. The resulting biofoam will be characterized for water absorption, biodegradability, and compressive strength. This research hopes to develop biofoam as a solution to the health and environmental hazards posed by Styrofoam packaging.

II. Materials and Method

A. Literature Review

One alternative solution to reducing Styrofoam usage is to replace it with biofoam. Biofoam, a biodegradable material, is anticipated to fulfill the role and function of Styrofoam [11]. Mycelium-based biofoam has the potential to serve as an alternative to petroleum polymer-based foam by utilizing fungal mycelium and lignocellulose as a matrix and substrate [12]. Palm oil leaf fiber, strengthened by fungal mycelium, is one such biodegradable material with significant potential as a raw material for biofoam [6].

Neurospora sitophila is a proteolytic fungus commonly found in soil, rice, and fermented products. It produces enzymes such as amylase, glucoamylase, and phytase, which serve as energy and carbon sources [8]. *N. sitophila* can also produce cellulase enzymes, specifically extracellular endoglucanase, from various agricultural waste in the packaging paper industry. This indicates that *N. sitophila* can be an effective inoculum in biofoam production [13].

Indarti et al. [14] investigated biofoam production using the fungus *Rhizopus* sp. The medium was made by mixing 500 g of coconut fiber, 200 ml of water, 32 g of wheat flour, and 26 g of tempeh yeast. Other media variations aimed at optimizing the growth of *Rhizopus* sp. reduced water to 35 ml, wheat flour to 25 g, and tempeh yeast to 13 g. The resulting biofoam was molded into a cup shape by placing the mixture into two polypropylene plastic cups. The best formulation consisted of 200 g coconut fiber, 35 ml water, 25 g wheat flour, and 13 g tempeh yeast. However, the bio-foam produced was not as strong as typical Styrofoam, necessitating additional fiber-binding materials.

Rodhibilah et al. [15] researched creating biofoam with *Rhizopus* sp. The medium consisted of autoclaved sugar cane waste mixed with flour, $CaCO_3$, *Rhizopus* sp. inoculum, and distilled water in a mass ratio of 5:2:5:12 (% of the sugarcane fiber used). This research explored various substrate compositions, including fiber size (20, 40, and 60 mesh) and types of flour (soybean, rice, and sago flour), as well as incubation temperature variations (29°C and 35°C). The optimum formulation for *Rhizopus* sp. mycelial growth consisted of bagasse, 25% soy flour, 5% $CaCO_3$, and distilled water at an incubation temperature of 29°C. The mycelia grew well and evenly throughout the substrate with an average length of ± 1.3 cm.

In addition to $CaCO_3$, several inorganic compounds, such as $MgSO_4$, K_2HPO_4, $ZnSO_4$, and $CuSO_4$, show potential as reinforcing materials for biofoam [15]. This research used $CaCO_3$ and $MgSO_4$, which can enhance biofoam strength by adding stiffness to the material structure [10]. $CaCO_3$ increases the mechanical strength and stiffness of biofoam, making it more durable and resistant to deformation [16]. The calcium and magnesium content in $CaCO_3$ and $MgSO_4$ are micronutrients for fungal mycelium growth [10].

The bio-foam standard includes a water absorption capacity of 26.12%, a biodegradation level of 100% over 60 days, and a compressive strength of 1.3-1.39 MPa (SNI 7188.7:2016). Morphological test standards, based on research by Bruscato et al. [17], show that mushroom mycelium-based bio foam exhibits elongated hyphae morphology that is well distributed into substrate particles and gaps.

With this foundational knowledge, there is potential to apply the mycelium of *Rhizopus* sp. and *N. sitophila* on an oil palm leaf fiber substrate for biofoam packaging. The research aims to develop a biofoam formulation incorporating various amounts of $CaCO_3$ and $MgSO_4$ fillers, and inoculum of *Rhizopus* sp. and *N. sitophila*. Water will be added to maintain moist conditions for fungal growth. The resulting product will be characterized using a Keyence digital microscope, water absorption test, biodegradation test, and physical-mechanical test (compressive strength), with the goal of producing a safe, economical, and environmentally friendly biofoam as a Styrofoam alternative.

B. Research Methods

In this research, oil palm leaf fiber formulation and *Rhizopus* sp inoculum were carried out. and *N. sitophila*. as well as measurements of *Rhizopus* sp mycelium. and *N. sitophila* produced. Next, the characteristics of the biofoam formulation that has been produced are tested. The first stage was formulated to grow *Rhizopus* sp. and *N. sitophila* in oil palm leaf fiber biomass. This research was carried out experimentally in the laboratory.

TABLE I
FACTORIAL DESIGN 2X2

	(A0B0)	
	A1 ($CaCO_3$ 5%)	**A2 ($MgSO_4$ 5%)**
B1 (*Rhizopus* sp.)	A1B1	A2B1
B2 (*N. sitophila*)	A1B2	A2B2

The first treatment is a type of material-strengthening inorganic compound (a), which consists of 2 levels. The first level is a1 = $CaCO_3$ inorganic compound 5%, and the second is a2 = $MgSO_4$ inorganic compound 5%. The second treatment is a type of fungal inoculum, (b) which consists of 2 levels. b1 = 25% *Rhizopus* sp inoculum concentration and b2 = 25% *N. sitophila* inoculum concentration. a0r0 is styrofoam packaging as a comparison in testing water absorption, compressive strength, and degradability.

The combination of factors a and b produces 4 biofoam formulations, which are characterized by the following formulation code names:

- a1b1 = Oil palm leaf substrate with 5% $CaCO_3$ filler and 25% *Rhizopus* sp inoculum.
- a1b2 = Oil palm leaf substrates with 5% $CaCO_3$ filler and 25% *N. sitophila* inoculum.
- a2b1 = Oil palm leaf substrates with 5% $MgSO_4$ filler and 25% *Rhizopus* sp inoculum.
- a2b2 = Oil palm leaf substrates with 5% $MgSO_4$ filler and 25% *N. sitophila* inoculum.

Next, a protein source, specifically 25% soy flour, and 12% distilled water are added to regulate humidity. The biofoam is then formed using a packaging mold and incubated in a mushroom incubator made from a dark-colored plastic container at room temperature (27ºC) for 7 days. Mycelial growth on the biofoam is observed on incubation days 3, 5, and 7. Four types of analysis are conducted as test parameters: morphological analysis with a Keyence digital microscope, water absorption test, decomposition test, and physical mechanical test (compressive strength). Data from measurements of biofoam characteristics (water absorption capacity, biodegradable test, and compressive strength test) are analyzed using SPSS (Statistical Package for Social Science) version 25 with the Independent Sample T-Test method, which tests for differences between the averages of independent samples.

1) Preparation of Raw Materials. Palm oil leaves originating from post-harvest waste are ground using a grinder, then the fiber is filtered using a mesh sieve, to obtain the required fiber mesh size (±20 mesh). Fibers are cleaned by soaking them in water to remove dirt and dust. After cleaning, the ground leaves were wrapped in heat-resistant plastic and then dried in an autoclave at 121°C at 1 atm pressure for 15 minutes. *Rhizopus* sp. inoculum, as material containing fungal cultures, was obtained from Raprima inoculum produced by LIPI, which predominantly contains *Rhizopus oligosporus* [18].

2) Biofoam formulation. The cleaned oil palm leaf fibers, which are dried in an autoclave at 121°C for 15 minutes to sterilize them. The formulation aims to grow *Rhizopus* sp. And *N. sitophila* in these palm oil leaf fibers. The first factor in this formulation is the type of inorganic compound used to reinforce the material. Two compounds are tested: 5% calcium carbonate ($CaCO_3$) (a1) and 5% magnesium sulfate ($MgSO_4$) (a2). The second factor is the type of fungal inoculum used, either 25% *Rhizopus* sp. (r1) or 25% *Neurospora sitophila* (r2). These combinations aim to determine the optimal conditions for biofoam production.

Styrofoam packaging is also used as a comparison for characteristic tests to benchmark the biofoam's performance. In preparing the biofoam formulation, distilled water is added to maintain the necessary moisture levels for fungal growth, along with 25% soy flour as a protein source to support the mycelial growth of *Rhizopus* sp. and *N. sitophila*.

The formulation mixture is then molded into two polypropylene packaging molds. These molds are wrapped to maintain humidity and incubated at room temperature (27ºC) for one week, providing the optimal environment for fungal colonization and biofoam formation [19]. After the incubation period, the biofoam is dried in an oven at 60ºC for two days to halt mycelium growth, ensuring the biofoam's stability and durability. This meticulous process aims to create a biofoam that can effectively replace conventional Styrofoam packaging, offering a more sustainable and environmentally friendly alternative.

3) Observation of the Growth of Rhizopus sp. and N. sitophila. After a 7-day incubation period, 1 gram of the biofoam is weighed and then crushed using a mortar with 9 ml of sterile distilled water added to homogenize the mixture. The homogenized sample is placed into a sterile test tube and further homogenized using a vortex mixer. From this mixture, 1 ml of the sample is taken and added to 9 ml of physiological NaCl solution to create a 10^{-1} dilution. This graded dilution process continues up to a 10^{-4} dilution. Next, 0.1 ml of each dilution is inoculated onto petri dishes containing Potato Dextrose Agar (PDA) media and incubated at 25°C. Colony growth on the surface of the media is observed every 24 hours for 7 days using the Total Plate Count (TPC) method [18]. This method allows for the quantification and analysis of fungal colony growth, providing insights into the effectiveness and proliferation of *Rhizopus* sp. and *N. sitophila* within the biofoam matrix.

4) Morphological Analysis. After drying, the biofoam is examined under a Keyence digital microscope at magnifications ranging from 250X to 500X. The purpose of this morphological analysis is to assess the distribution of the mycelium within the biofoam matrix, determining whether it is evenly spread throughout the material [20]. This step is crucial for ensuring the structural integrity and uniformity of the biofoam, which directly impacts its performance as a sustainable packaging material.

5) The Water Absorption Test is essential for assessing how much water the biofoam absorbs after immersion, providing insights into its suitability as a packaging material compared to Styrofoam. Consumers often prioritize packaging with specific properties, including water resistance. Biofoam is considered compliant with SNI standards if its maximum water absorption does not exceed 26.12%. To conduct this test, biofoam samples measuring 2.5 cm × 5 cm are first weighed to obtain their initial weight (M0). Each sample is then immersed in water for one minute before being removed and gently dried with a tissue to eliminate surface water. Subsequently, the sample is re-weighed to determine its final weight (Mt). The amount of water absorbed by the biofoam is calculated using the equation:

$$\text{Water Absorbance (\%)} = (\text{mo-mi})/\text{mo} \times 100\% \quad (1)$$

This test methodology enables a quantitative assessment of the biofoam's water absorption capacity, crucial for evaluating its performance relative to traditional packaging materials like Styrofoam. [21].

6) Biodegradability testing is conducted to assess the ability of biofoam to naturally degrade. Biofoam is considered compliant with SNI standards if it biodegrades entirely within 60 days. This test involves burying the biofoam samples in soil. 2% liquid EM4 fertilizer is added to the substrate soil to ensure soil fertility. EM4 is a micromixed culture consisting of Lactobacillus, Actinomyces, Streptomyces, yeast fungi,

and photosynthetic bacteria that collaboratively degrade organic matter. Organic material containing EM4 molecules undergoes decomposition through aerobic and anaerobic fermentation facilitated by these bacteria. The bacteria break down biodegradable foam by enzymatically breaking polymer chains into monomers.

In this test, biofoam samples measuring 2.5 cm × 5 cm are initially cut, placed in a desiccator, and weighed as the initial weight (W0). These samples are then buried 20 cm deep in soil-filled boxes and left for 14 days. After incubation, samples are cleaned of any soil residues, re-weighed, and recorded as the final weight (Wi). Subsequently, the biodegradation ability of the samples, expressed as the percentage of weight lost, is calculated using the following equation [21]:

$$\text{Biodegradability (\%)} = (wo\text{-}wi)/wo \times 100\% \quad (2)$$

This method allows for the quantitative evaluation of how effectively the biofoam breaks down in a soil environment under controlled conditions, ensuring it meets environmentally sustainable criteria set by regulatory standards.

7) Compressive test. In the compressive strength test using a Shimadzu AG-IS autograph 10 kN universal testing machine (UTM), both biofoam and Styrofoam are prepared with dimensions of 14 cm in length and 3 cm in width. The test involves placing the sample on the UTM platform and applying a load directly above the center of the sample. The load is gradually increased in a controlled manner until the sample fractures or breaks. The maximum load at which the sample fails is recorded as the compressive strength of the material [21].

This test is crucial for determining the maximum load capacity that biofoam and Styrofoam can withstand before structural failure. The results provide critical insights into the mechanical strength of both materials and facilitate comparison of biofoam's performance as a potentially more environmentally friendly alternative to Styrofoam.

III. Results and Discussion

A. Growth of Fungal Mycelium on Biofoam

The growth of *Rhizopus* sp. and *N. sitophila* mycelium on the biofoam was halted by drying in an oven at 60°C for 48 hours. The evenly grown mycelium that covers the surface of the biofoam will proceed to the characterization stage. Direct observations of the biofoam can be viewed in Fig. 1. *Neurospora sitophila* and *Rhizopus* sp. are two fungal species commonly found in natural environments, yet they exhibit differing abilities to thrive on oil palm leaves [22]. This variance can be attributed to the composition of oil palm leaves, particularly the presence of lignin, cellulose, and hemicellulose [23].

Oil palm leaves contain approximately 16.9% lignin [24], which contributes to their resilience against microbial degradation. *N. sitophila* is capable of breaking down lignin under optimal conditions, typically at a temperature of 32°C and pH 4.2 [25]. However, in certain studies, *Neurospora* encountered challenges in growing on oil palm leaves, likely due to less favorable conditions for lignin-degrading enzyme production [26]. Nevertheless, other microorganisms, especially those within ligninolytic fungal groups such as Phanerochaete and Trametes genera, possess effective lignin-degrading capabilities. These fungi produce enzymes like peroxidases and laccases, pivotal in cleaving lignin's chemical bonds [27].

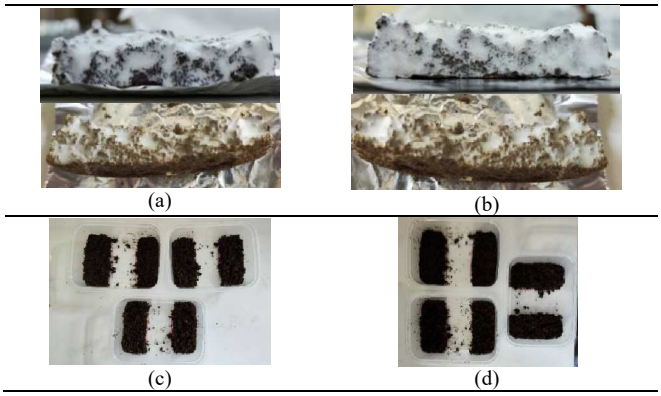

Fig. 1 Mycelium on Biofoam: (a) *Rhizopus* sp. with $CaCO_3$, (b) *Rhizopus* sp. with $MgSO_4$, (c) *N. sitophila* with $CaCO_3$, (d) *N. sitophila* with $MgSO_4$

In addition to lignin, oil palm leaves contain about 27.9% cellulose [24], comprising glucose chains linked by β-glycosidic bonds [28]. *Rhizopus* sp. produces cellulase enzymes—endocellulase, exo-cellulase, and β-glucosidase—that facilitate the breakdown of cellulose into glucose molecules [29]. Environmental factors such as temperature, humidity, pH, and nutrient availability significantly influence the activity and efficiency of these cellulase enzymes during cellulose degradation [30]. *Rhizopus* sp., adapted to the environmental conditions of oil palm leaves, exhibits efficient cellulose degradation [31].

Hemicellulose, another substantial component in oil palm leaves at approximately 21.1% [24], possesses a more intricate structure compared to cellulose [32]. Fungi like *Rhizopus* sp. produce hemicellulose enzymes—such as xylanase, mannanase, and galactanase—that break down hemicellulose into simple sugars like glucose, xylose, and mannose [33], [34]. These enzymes act synergistically to dismantle the chemical bonds within hemicellulose, providing sugars that serve as essential energy and nutrient sources for fungal growth and reproduction [35].

B. Fungal Population Growth

Fungal population growth was calculated using the Total Plate Count (TPC) method and observed every 24 hours for 168 hours (7 days). Mycelium growth calculations were carried out on the fungus *Rhizopus* sp., and the fungus *N. sitophila*. Observation profile of mycelial length growth of *Rhizopus* sp. and *N. sitophila* can be seen in Figure 2 below.

The results from Table 1 and Figure 2 illustrate the total plate count (TPC) calculations for *Rhizopus* sp., revealing colony growth in formulations with the addition of $CaCO_3$ and $MgSO_4$. By day 7, colony counts reached 12.1 x 10^3 CFU/g and 9.3 x 10^3 CFU/g, respectively, with the highest increase observed in the $CaCO_3$ supplemented formulation. Calcium carbonate serves as a pH regulator in the growth media, crucial for optimal fungal development, as most fungi thrive under near neutral to alkaline pH conditions [36]. These findings align with prior studies, such as Surbakti et al. [37],

where *Rhizopus* sp. exhibited colony growth reaching 10.23 CFU/g by the third day of incubation in soybean media.

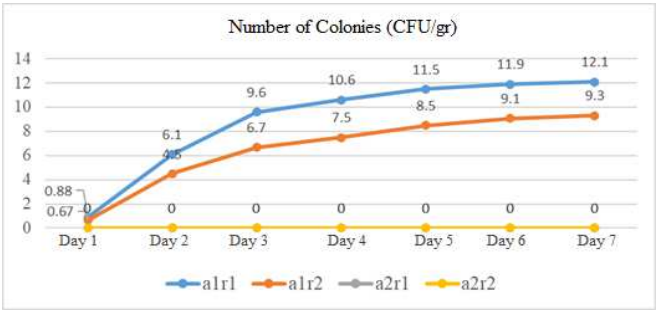

Fig. 2 Graph of increase in fungal colonies for 7 days in 4 Biofoam formulations. a1r1: *Rhizopus* sp and $CaCO_3$ 5%; a2r1: *Rhizopus* sp. and $MgSO_4$ 5%; a1r2: *N. sitophila* and $CaCO_3$ 5%; a2r2: *N. sitophila* and $MgSO_4$ 5%

Figure 2 depicts the growth curve of *Rhizopus* sp., characterized by two distinct phases. The exponential phase spans from day 1 to day 5, marked by a significant increase in colony numbers [38]. The subsequent stationary phase, occurring from days 5 to 7, shows a less pronounced increase in colony count, indicating nutrient depletion or other limiting factors [39]. Interestingly, no lag phase or death phase was observed in the fungal growth during this study. This absence can be attributed to the inoculum's adaptation to the oil palm leaf substrate, facilitating immediate growth initiation [38]. Additionally, the ongoing exponential growth phase throughout the observation period precluded the emergence of a death phase, highlighting sustained fungal activity and vitality [40].

A. Mycelium Morphology Analysis

Mycelium morphology analysis was carried out using a Keyence microscope to see the morphological structure and determine the distribution of the mycelium. The results of microscopic observations of biofoam with a Keyence VHX-7000 digital microscope with 250x and 500x magnification can be seen from Figure 3 below:

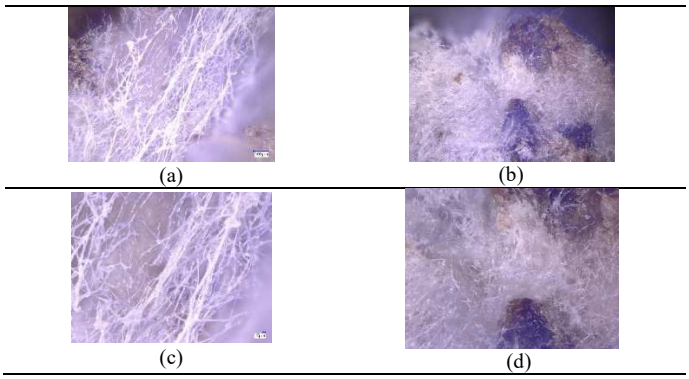

Fig. 3 Morphology of Biofoam Mycelium. (a) Rhizopus sp. and $CaCO_3$ (250x); (b) Rhizopus sp. and $MgSO_4$ (250x); (c) Rhizopus sp. and $CaCO_3$ (500x); (d) Rhizopus sp. and $MgSO_4$ (500x)

Figure 3 at 250× magnification reveals the morphological analysis of *Rhizopus* sp. mycelium, demonstrating robust growth that effectively binds to the fibers across the entire surface of biofoam containing $CaCO_3$ filler. In contrast, biofoam with $MgSO_4$ filler shows a less uniform distribution of *Rhizopus* sp. mycelium. Further observations at 500× magnification on both biofoam formulations depict elongated mycelial structures resembling white threads, indicative of vigorous growth penetrating and filling substrate interstices [17]. Fungi propagate on substrates through hyphal filaments, forming interconnected networks known as mycelium [41]. They derive nutrients by enzymatically degrading substrates, thereby increasing biomass. Some fungi grow externally, forming a dense or smooth coating termed 'fungal skin'. Mycelium responds dynamically to internal damage by regenerating, reinforcing, and reconnecting adjacent hyphal branches, crucial characteristics for developing mycelium-based materials [14].

B. Water Absorbance Test

The Water Absorption Test is essential for evaluating how much water biofoam absorbs after immersion. This test determines if the biodegradable foam matches the water resistance properties of styrofoam, which consumers prioritize in plastic packaging. Biofoam meets SNI standards if it absorbs a maximum of 26.12% water. The test outcomes for biofoam, reinforced with *Rhizopus* sp. fungus mycelium, are detailed in Table 2. The test results of biofoam reinforced by the mycelia of the fungus *Rhizopus* sp., can be seen in Table 2.

TABLE II
WATER ABSORBANCE TEST RESULT

Formulae	Water Absorbance ± SD
Rhizopus sp. and $CaCO_3$ 5%	20,26% ± 1,37
Rhizopus sp. and $MgSO_4$ 5%	21,02% ± 0,51
Styrofoam	12.27% ± 0.97

Each formulation has 20.26% and 21.02% water absorption capacities, while styrofoam absorbs water at 12.27%. The water absorption test results for biofoam meet standards, as per (SNI) 7188.7:2016, where the maximum allowable water absorption for biofoam is 26.12%. The water absorption capacity of biofoam is influenced by the mycelium that grows on the formulation, covering the entire surface of the biofoam, thus preventing water from quickly penetrating the oil palm leaf fibers. The surface of *Rhizopus* sp. mycelium exhibits hydrophobic properties [42].

C. Biodegradability Test

The degradability test assesses the biofoam's response to environmental conditions over a specified period, determining the percentage of decomposition observed. This provides insights into the potential timeline for complete decomposition of biofoam in soil. The biodegradability of biofoam offers environmental benefits, as it naturally decomposes compared to conventional materials like styrofoam. Biofoam meets SNI standards if it achieves 100% decomposition within 60 days (SNI) 7188.7:2016). The test results for biofoam reinforced with *Rhizopus* sp. mycelium are presented in Table 3.

TABLE III
BIODEGRADABILITY TEST RESULT

Formulae	Biodegradability± SD
Rhizopus sp. and $CaCO_3$ 5%	28.31% ± 0.92
Rhizopus sp. and $MgSO_4$ 5%	41.04% ± 8.1
Styrofoam	1.85% ±0.36

These biofoam formulations achieved degradability values of 28.31% and 41.04% respectively, whereas styrofoam exhibited a water absorption capacity of 1.85%. According to the Indonesian National Standard ((SNI) 7188.7:2016), packaging materials should completely degrade (100%) within 60 days. By the 14th day, biofoam should ideally have degraded to at least 23.3% according to these standards. The study's findings demonstrate that all biofoam formulations met the degradability test criteria. This research underscores that mycelium-based materials can be effectively buried in soil after their useful life as packaging, decomposing within weeks. Moreover, these materials are cost-effective on a large scale and significantly more environmentally friendly compared to styrofoam, which poses more significant environmental risks.

D. Compressive Test

The compressive strength test was conducted to determine the maximum load that biofoam, used as protective packaging, can endure. According to the Indonesian National Standard ((SNI) 7188.7:2016), biofoam should withstand a pressure range of 1.3 – 1.39 MPa to be compliant. The test results for biofoam reinforced with *Rhizopus* sp. mycelia can be observed in the relevant data table (Table 4).

TABLE IV
COMPRESSIVE TEST RESULT

Formulae	Compressive± SD
Rhizopus sp. and $CaCO_3$ 5%	0.25 ± 0.07
Rhizopus sp. and $MgSO_4$ 5%	0.16 ± 0.05
Styrofoam	4.95 ± 0.71

Each formulation of biofoam displayed compressive strength values of 0.25 MPa and 0.16 MPa respectively, whereas styrofoam exhibited a significantly higher compressive strength of 4.95 MPa. The addition of $CaCO_3$ and $MgSO_4$ can influence the compressive strength of biofoam through several mechanisms [43]. $CaCO_3$ enhances biofoam strength by interacting with the polymer matrix, serving as a filler, and affecting pore structure. Conversely, $MgSO_4$ can impact structural strength and stability by interacting with the polymer matrix and altering chemical reactions [44]. These additives offer the potential to enhance the compressive strength of bio foam [45].

Compared to research by Lelivelt et al. [12], which focused on natural fiber-based materials using C. versicolor mycelia, their materials exhibited strengths and stiffness ranging from 2.6 to 9.4 MPa. This suggests that the current biofoam formulation using *Rhizopus* sp. mycelium does not meet the standards for biofoam packaging, as its compressive strength remains lower than that achieved by the fungal mycelia studied previously. Additional coatings or treatments can be considered in the final stages of mycelium-based material production to improve material properties [12]. Chitosan coating enhances biofoam's compressive strength by filling empty cavities in the dried chitosan gel. Chitosan possesses amine, primary hydroxyl, and secondary functional groups that enable strong hydrogen bond formation with the substrate [46].

IV. CONCLUSION

The mycelium of *Rhizopus* sp. demonstrates robust growth on oil palm leaf substrates within 7 days of incubation at room temperature (27°C), yielding 12.1 x 103 CFU/ml. In contrast, *N. sitophila* mycelium fails to grow on oil palm leaf substrates even after a 7-day incubation period, thereby preventing its progression to the characterization stage. Characterization tests and morphological analysis indicate that both formulations produce biofoam meeting water absorption standards and exhibiting biodegradability. However, they do not meet compressive strength standards as per (SNI) 7188.7:2016. To enhance the material's physical properties, efforts should focus on optimizing and incorporating coating materials during the final stages of biofoam production.

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