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# Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

*Journal by Innovative Scientific Information & Services Network*

**RESEARCH ARTICLE** BIOSCIENCE RESEARCH, 2023 20(2): 518-529. **OPEN ACCESS**

# Fabrication and Properties Evaluation of *Rhizopus oligosporus* Mycelium-Based Biofoam with Various Lignocellulosic Substrates

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Biofoam, a biodegradable foam, was developed with the purpose of replacing the use of Styrofoam, particularly as food containers. Fungal mycelia as well as polysaccharides and proteins can be used to create foam. The type of fungi, substrate composition, and incubation condition all have an impact on the fabrication of mycelia-based biofoam. Hence, in this research, the fabrication, and properties evaluation of *Rhizopus oligosporus* mycelia-based biofoam were carried out using various lignocellulosic (sugarcane trash (SCT), pineapple leaves (PL), and a mix of both with the inoculum concentration (20%, 25%, and 30%)). The substrate also included soybean starch, $CaCO_3$, and water (in a ratio of 5:2:15 %w/w of the lignocellulosic used). The results showed that the inoculum required for optimum mycelium growth on the SCT, PL, and mix substrates was 25%, 25%, and 30%, respectively. Biofoams (S2, P2, and SP3) have low moisture content and water absorption but high density and biodegradability compared with other biofoams. The biofoams obtained from this study also had a higher MOE and lower MOR than Styrofoam as the control.

**Keywords: Lignocellulosic, mycelium-based biofoam, pineapple leaf, *Rhizopus oligosporus*, sugarcane trash**

## INTRODUCTION

For the first time, Styrofoam was the first trademark brand for Dow Chemical's foam insulation which is made from polystyrene (PS), a petroleum-based material. Then, a foam material was developed from the conversion of PS into expanded polystyrene (EPS) the trademark "foamed polystyrene" or "EPS" can be replaced with "Styrofoam," because the name "Styrofoam" has become synonymous with all rigid foam products (Chandra et al., 2016). Styrofoam is a multifunctional material synthesized by the polymerization reaction of styrene (Chandra et al., 2016), (Farrelly & Shaw, 2017). The advantages of styrofoam such as being lightweight, water resistant, and inexpensive. Therefore, it is widely applied for single-use food services (cups, lunchboxes, and other containers), packaging materials, insulation, and decoration (Nukmal et al., 2018), (Zhang et al., 2018), (Rizal et al., 2020). The unreactive characteristic of Styrofoam makes it difficult to degrade by nature (non-biodegradable) and cost-consuming recycling process. Thus, they accumulated in landfills and waters, such as rivers or oceans, as litter. Moreover, the main precursors of Styrofoam are benzene and styrene, which have been classified as carcinogens, and these residues can present health risks for producers and consumers of Styrofoam (Chandra et al., 2016), (Farrelly & Shaw, 2017).

Biofoam or bio-degradable foam is a material produced by natural sources, such as starch (Sumardiono et al., 2021), cellulose (Adiyar et al., 2019), protein (Wu, 2017), and mycelium-based (Bruscato et al., 2019), (Islam et al., 2018), to substitute the use of Styrofoam. During the last few years, research and development of mycelia-based biofoam have been carried out widely due to the simple process and molded easily. In the manufacturing process, the fungi inoculum is grown on a substrate consisting of biomass, additional nutrients, fillers, and water as a moisture regulator. Teixeira et al (2018), developed a biocomposite from the fungi *Pleuratus ostreatus,*

*Pleurotus eryngii,* and *Pycnoporus sanguineus* using coconut powder and wheat bran as substrates, with 60-70% humidity. Bruscato et al. (2019), developed biofoam from the fungi *Pycnoporus sanguineus, Pleurotus albidus,* and *Lentinus velutinus* using a substrate consisting of sawdust, wheat bran, and $CaCO_3$, with 66% humidity. Nashiruddin et al. (2022), created *Pleurotus ostreatus* mycelium-based biofoam using various biomass substrates includingrice husk, sawdust, and sugarcane bagasse where the rice bran and $CaCO_3$, with rice husk being the best.

Generally, the development of mycelia-based biofoam uses macrofungi, while the use of microscopic fungi is limited. *Rhizopus* sp. is a microscopic fungus that belongs to the edible mushroom family and grows relatively quickly (around seven days). *Rhizopus* sp. inoculum can be obtained easily and inexpensively in the marketplace as "tempeh" inoculum. In the previous studies, *Rhizopus* sp. mycelia have been successfully grown on the substrates of bagasse, coconut coir (Indarti et al., 2021), and sugarcane trash (Rodhibilah et al., 2022); however, further characterization has not been carried out related to the product. Hence, in this research, the development of *Rhizopus oligosporus* mycelia-based biofoam was carried out using a variety of substrates composition that was sugarcane trash (SCT), pineapple leaves (PL), and a mix of both as well as analyzing its physical, chemical, and mechanical properties. The identification of tempeh inoculum was performed to determine the species of *Rhizopus* fungus contained therein, as well as the analysis of the chemical content of the substrate components.

## MATERIALS AND METHODS

### Materials

The raw materials used in this research are sugarcane trash, which is taken from PTPN X, and pineapple leaf, which is taken from a pineapple plantation in Ngancar, Kediri. The inoculum tempeh "Raprima", contained *Rhizopus* sp fungus, was obtained from a marketplace in Depok, while soybean starch was obtained from a marketplace in Bandung. Calcium carbonate ($CaCO_3$) (Merck, CAS 471341), potato dextrose agar (PDA) (Himedia, CAS 1933146), sodium chloride (NaCl) (Merck, CAS 7647145), and distilled water.

### Methods

#### Determination of *Rhizopus oligosporus* from tempeh Inoculum of "Raprima"

The current study of *Rhizopus oligosporus* fungi in tempeh inoculum was performed based on the method used by Utama et al. (2018), with slight modification. "Raprima" was inoculated into a 0.85% sodium chloride solution and vortexed for 1 minute (Thermo Scientific). The suspension was diluted to $10^{-5}$ g/ml before being poured into a petri dish containing PDA using the pour plate method. The petri dishes containing inoculate were incubated at 27 $^{o}$C for 3-7 days to obtain the fungal isolates. Purification of the fungal culture was accomplished by streaking each colony with a similar morphology on each PDA medium in the petri dish, which was then incubated at room temperature for 3-5 days (Hidayat, 2021). To determine the type of fungus, the pure isolates were identified macroscopically and microscopically. Macroscopic identification is based on fungal colony characteristics such as color, shape, and surface. Meanwhile, microscopic identification is based on the morphological characteristics of the fungal isolates observed using a light microscope and the slide culture method (Valencia & Meitiniarti, 2017).

#### Chemical content analysis of lignocellulosic source and soybean starch

The chemical content analysis of pineapple leaf consists of extractive (TAPPI, 1996), total lignin (acid-soluble lignin (ASL) and acid-insoluble lignin (AIL) (Sluiter et al., 2008), holocellulose (Wise et al., 1996), and α- cellulose (Rowell et al., 2012). While the analysis of carbon, hydrogen, and nitrogen content in soybean starch was determined by CHN analyzer (Leco CHN 628).

#### Fabrication of biofoam

The fabrication of mycelium-based biofoam refers to our previous research (Rodhibilah et al., 2022). PL and SCT were cleaned and dried using an oven at 105 $^{o}$C for 24 h followed by grounding with a grinder and then filtered using a 20-mesh sieve. The grounded PL and SCT were cleaned using water, then autoclaved at 121 $^{o}$C, for 15 min. After that it was mixed with soybean starch, $CaCO_3$, and distilled water with a mass ratio 5:2:12 (% w/w) of the lignocellulosic used. The *Rhizopus oligosporus* inoculum used various concentration of lignocellulosic at 20%, 25%, and 30%. The formulation for biofoam fabrication can be seen in Table 1. The mixture was stirred until evenly distributed, then molded and put into heat-resistant plastic. The substrate was incubated for 1 week at room temperature (29 $^{o}$C). After 1 week, the substrate, which has been overgrown by mycelia, is dried using an oven (60 $^{o}$C, 48 h) and ready to be characterized.

#### Characterization of biofoam

The biofoam characterizations included density analysis by measuring mass and sample volume (Ningrum et al., 2022), moisture content analysis using Moisture Analyzer (Shimadzu MOC63u), and water adsorption using the ABNT NBR NM ISO 535 (1999) and SNI 1969: 2008 methods (Hendrawati et al., 2019). Moreover, the biodegradability of biofoam was determined using a soil degradation method mixed with EM4 (Hendrawati et al., 2015).

**Table 1: Biofoam formulation**

| No. | Sample | Kind of Fibre | | Inoculum of *Rhizopus oligosporus* | | | Soybean starch | $CaCO_3$ | Distilled water |
|---|---|---|---|---|---|---|---|---|---|
| | | SCT | PL | 20% | 25% | 30% | | | |
| 1. | S1 | √ | | √ | | | √ | √ | √ |
| 2. | S2 | √ | | | √ | | √ | √ | √ |
| 3. | S3 | √ | | | | √ | √ | √ | √ |
| 4. | P1 | | √ | √ | | | √ | √ | √ |
| 5. | P2 | | √ | | √ | | √ | √ | √ |
| 6. | P3 | | √ | | | √ | √ | √ | √ |
| 7. | SP1 | √ | √ | √ | | | √ | √ | √ |
| 8. | SP2 | √ | √ | | √ | | √ | √ | √ |
| 9. | SP3 | √ | √ | | | √ | √ | √ | √ |

In this method, the biofoam (2.5 x 5 cm) was weighed (W0), then buried in a box (containing soil and 2% v/v EM4 as the liquid fertilizer) to a depth of 10 cm, and then stored for 14 days. After that, the samples were cleaned of soil residues and weighed for mass (W1). The ability of the sample to decompose or degrade is expressed in the percentage of reduced sample mass, which can be calculated using equation 1:

$$\text{reducing mass}\ (\%) = \frac{\text{wo}-\text{wi}}{\text{wo}} \text{ x } 100\% \qquad (1)$$

Mechanical analysis was carried out using the Universal Testing Machine (Shimadzu AG-IS autograph 10 KN). Briefly, the dimensions of the biofoam including length, width, and thickness were measured using a micrometer and then placed horizontally on the UTM. The bending test refers to JIS A 5908-2003 (Ningrum et al., 2022). Three replications of the analysis were performed, with Styrofoam as a positive control. The morphology of biofoams was analyzed using Kayence digital microscopy (Rodhibilah et al., 2022), and Field-Emission Scanning Electron Microscopy (FESEM, Thermo Scientific Quattro S) (Sutiawan et.al., 2022). The thermal properties of biofoam were performed using Thermogravimetric Analyzer (TGA, Perkin Elmer Inc, USA) (Bruscato et al., 2019), and the functional group characterization of biofoam was analyzed using Fourier Transmission Infra-Red (FTIR, Perkin Elmer) (Ningrum et.al., 2023).

## RESULTS AND DISCUSSION

### Determination of *Rhizopus oligosporus* from tempeh inoculum

In the previous research, we studied the effect of temperature and substrate composition on the fungus *Rhizopus* sp. obtained from tempeh inoculum. Hence, in this study, identification of the type of *Rhizopus* contained in the tempeh inoculum was carried out both macroscopically and microscopically.

Fig. 1 depicts a macroscopic and microscopic examination of the Raprima tempeh inoculum. Macroscopically (Fig. 1A and 1B), the isolate looks grayish white; the structure was like cotton and uniform, indicating that the isolate consists of one colony. Meanwhile, the brownish-black spores and colorless chains can be seen microscopically (Fig. 1C and 1D). Sine & Soetarto (2018), explained that the characteristics of *Rhizopus oligosporus* colonies are gray- white with a height of ≥1 mm, smooth or rather rough cell walls, a diameter for one colony ranging from 10-18 µm and a length >1000 µm. In addition, *Rhizopus oligosporus* also has sporangia globosa, which are brownish-black in color, with a diameter of 100-180 µm, many chlamydospores, colorless chains, and granules that form hyphae, sporangiospores, and sporangia. *Chlamidospora globosa* is elliptical or cylindrical in shape, with a size of 7-30 µm. This finding is related to the work of Duniaji et al. (2019), and Sine & Soetarto (2018). Therefore it can be concluded that the Raprima tempeh inoculum is made up of *Rhizopus oligosporus* fungi.

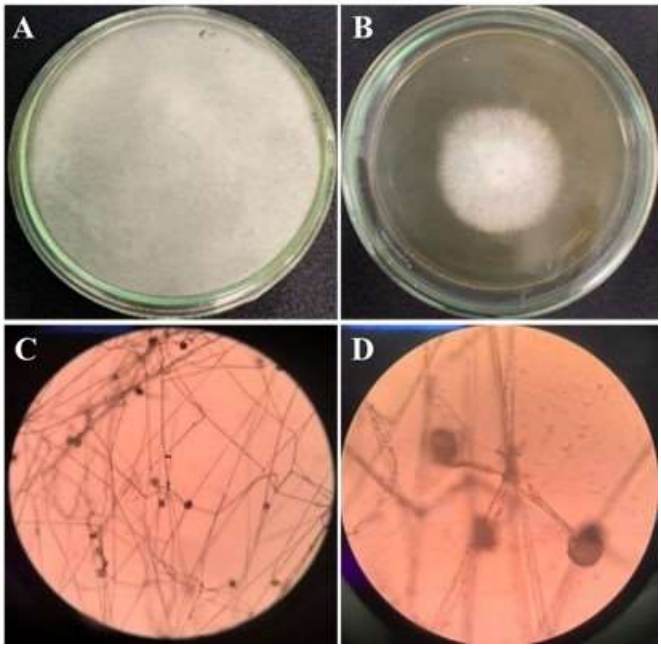


**Figure 1: Determination of *Rhizopus oligosporus* from Raprima tempeh inoculum. A) isolate in the PDA media before purification, B) isolate in the PDA media after purification, C) microscopic observation at 100x magnification, D) microscopic observation at 400x magnification.**

**Chemical content analysis of lignocellulosic source and soybean starch**

In the fabrication of mycelium-based biofoam, PL and SCT are the main matrixes in the growth substrate for Rhizopus oligosporus. The lignocellulose content in the PL and SCT greatly influences the growth of mycelia due to it acts as a carbon source for mushroom nutrition. According to Table 2, PL has more lignin than SCT; however, the cellulose and extractive content were lower than SCT.

**Table 2: The chemical component of sugarcane trash and pineapple leaf**

| Chemical content | Sugarcane trash (SCT)[14] | Pineapple leaf (PL) |
|---|---|---|
| Extractive (%) | 13.44 ± 1.70 | 11.96 ± 0.89 |
| Total lignin (%) | 19.03 ± 2.01 | 37.09 ± 3.62 |
| Holocellulose (%) | 67.40 ± 1.05 | 46.55 ± 0.19 |
| Alpha cellulose (%) | 32.76 ± 2.13 | 21.54 ± 1.17 |
| Hemicellulose (%) | 34.64 ± 3.18 | 25.01 ± 0.98 |

Soybean starch is one of the ingredients in the fabrication of biofoam that function as a nitrogen source. According to the analysis using CHN analyzer, the soybean starch was composed of 79% carbon, 12% hydrogen, and 9% nitrogen. In addition, soybean starch also contains other micronutrients that are important for the growth of *Rhizopus oligosporus*, such as phosphor, bromium, sulphur, calcium, and magnesium (Rodhibilah et al., 2022).

**Fabrication and morphological analysis of Biofoam**

The biofoam fabrication was referred to our previous research (Rodhibilah et al., 2022), wherein the used particle size of PL and SCT (lignocellulosic) was 20 mesh, the mass ratio of soybean starch, $CaCO_3$, and distilled water was 5:2:12 (% w/w) to the amount of lignocellulosic used, and the incubation temperature was 29 $^oC$. That is the best condition for the growth of the fungus *Rhizopus* sp. While the variation of inoculum concentrations was carried out to obtain the right inoculum percentage, the mycelia were able to grow optimally. According to the observation on the $7^{th}$ day after incubation as shown in Table 3, the growth of mycelia on a substrate consisting of 25% and 30% *Rhizopus oligosporus* inoculum was more evenly distributed and covered the surface of the substrate compared to the addition of 20% inoculum. There were very few mycelia that grew on a substrate consisting of 20% inoculum because *Rhizopus* lacks nutrition due to the incomparable ratio between inoculum and soybean starch. As we know, *Rhizopus oligosporus* is a proteolytic microorganism, making it easier to grow in substrates containing high protein concentration (Endrawati & Kusumaningtyas, 2018).

Table 3 also demonstrates that mycelia grow better on an SCT substrate than PL or a combination of SCT and PL due to PL having a higher lignin content compared to SCT. *Rhizopus oligosporus* has a cellulose enzyme which able to degrade cellulose (dos Santos et al., 2016), while it does not have a ligninase enzyme to degrade lignin. Moreover, *Rhizopus oligosporus* can produce the α-amylase (Han et al., 2003), (Kanti, 2016), protease, lipase (Han et al., 2003), (Nugraha et al., 2022), glutaminase, α-galactosidase (Han et al., 2003), endoglucanase (dos Santos et al., 2016), and glucoamylase enzymes (Nahar et al., 2008).

Fig. 2 shows the microscopic image of the growth of *Rhizopus* mycelia where the translucent white filaments are mycelia and the substrate was a yellowish or brownish color. According to Manan et al. (2021), the fungi colonize their substrate via elongated filamenous cells called hyphae, which grow and form a three-dimensional (3D) interwoven flamentous network, known as mycelium. The mycelium secretes enzymes broke down the substrate (such as starch, cellulose, or lignin) into simpler components that can be used as nutrients. Fungi utilize these nutrients and increase their biomass by growing on the surface of the substrate as well as penetrating it, while some grow out of the substrate and form a compact or fluffy layer called “fungal skin”

**Table 3: *Rhizopus oligosporus* mycelia-based biofoam**

| Concentration of inoculum | Sample Code | | |
|---|---|---|---|
| 20% | S1  | P1  | SP1  |
| 25% | S2  | P2  | SP2  |
| 30% | S3  | P3  | SP3  |

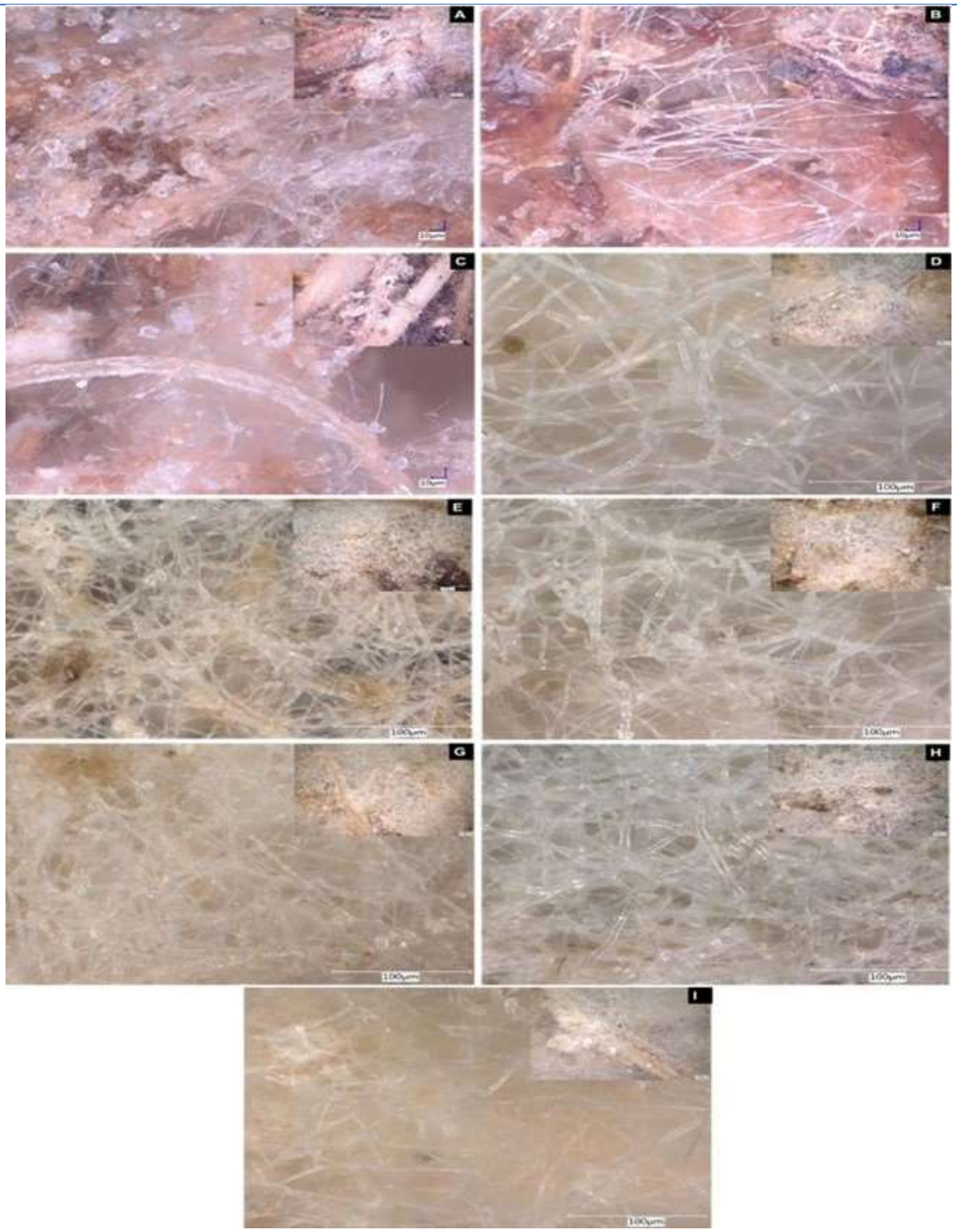


**Figure 2: Morphology biofoams using Kayence digital microscopy A) S1 B) P1 C) SP1 D) S2 E) P2 F) SP2 G) S3 H) P3 I) SP3.**

**Density of biofoam**

The results of biofoam density can be seen in Fig. 3. The biofoams have a higher density than Styrofoam as a control, the densities were 0.196 – 0.298 g/cm$^3$ and 0.015 g/cm$^3$.respectively. On the SCT and PL substrates, the greatest density was found in samples S2 and P2 (containing 25% inoculum), while on the mix of SCT and PL substrates, the greatest density was obtained from sample SP3 (containing 30% inoculum). Moreover, the smallest density was found in the sample containing 20% inoculum, whether using SCT, PL, or a mixture of both as a substrate. This is consistent with Table 3, where a sample with a higher density contains more mycelia that are more evenly distributed.

Moreover, the biofoam obtained from this study has a lower density than the biofoam based on the mycelia of the fungi *Pleurotus sanguineus*, P. *albidus*, and L. *velutinus*, whose densities were 0.32, 0.30, and 0.35 g/cm$^3$, respectively (Bruscato et al., 2019).

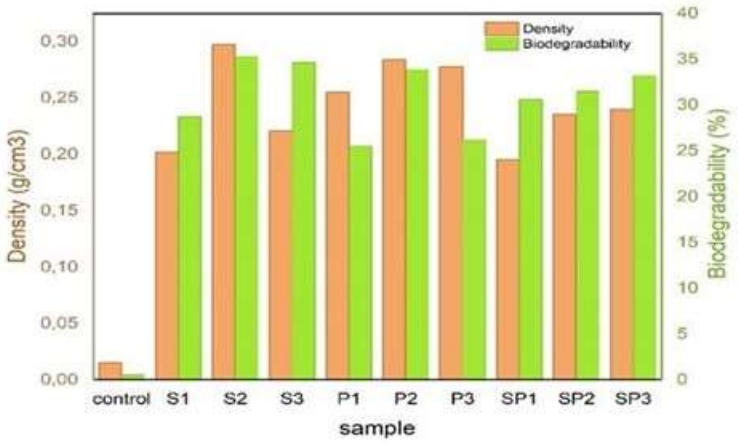


**Figure 3: Density and biodegradability of biofoams**

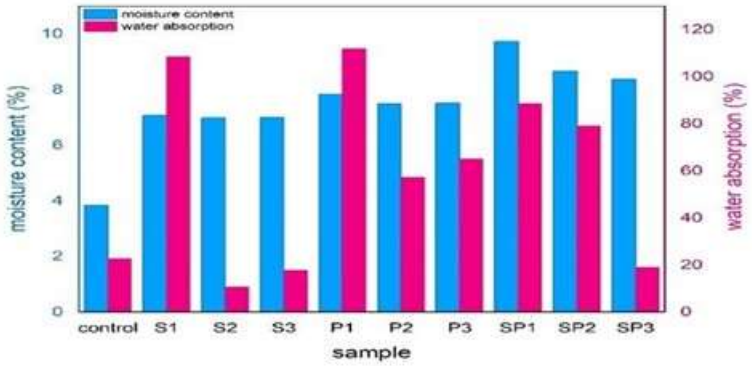


**Figure 4: Moisture content and water absorption of biofoams**

**Biodegradability**

The biodegradability of biofoam was around 25.54 – 35.29%, while the biodegradability of control was lower than biofoam, which is 0.523% for 14 days (Fig. 3). The biodegradability analysis also revealed that the better the mycelia growth on the substrate, the faster the biofoam degrades in the soil. All of the biofoams produced in this research have met the standard as biodegradable material according to the international standard (ASTM 5336), which stated that the biodegradable packaging takes 60 days to be completely degraded (Hendrawati et al., 2017). Biofoam can be degraded in the soil due to it is formed from organic materials that can be broken down by microorganisms. Microorganisms are capable of producing enzymes that can break the polymer chains of organic matter into simpler compounds (Obradovic et al., 2017). According to Dailin et al (2022), some microorganism such as *Salmonella, Pseudomonas putida, Pseudomonas aeruginosa, Paenibacillusurinalis, Bacillus* sp., *Xanthomonas* sp., and *Sphingobacterium* sp., were able to breakdown both styrene and polystyrene.

**Moisture content and water absorption of biofoam**

Moisture content analysis aims to determine the moisture absorption ability of the sample at room temperature. The moisture content of biofoam is around 6.985 – 9.737%, while the moisture content of the control is 3.843% (Fig. 4).

Moreover, Fig. 4 also shows the water absorption of biofoam is around 10.6-111.9% and 22.643% for the control. Three biofoams had lower water absorption than the control, namely S1 (10.6%), S2 (17.66%), and SP3 (19.13%). These three biofoams also meet Indonesia's National Standard (SNI), and the maximum water absorption for biofoams is 26.12% (Hevira et al., 2021).

Furthermore, biofoams with 20% inoculum had higher moisture content and water absorption than other biofoams with more inoculum. The mycelia formed was not evenly distributed on the surface or inside the substrate, allowing water or moisture to be easily absorbed by the substrate. Biofoam with a 25% and 30% inoculum concentration had the lowest moisture content and water absorption on SCT or PL and mixture substrates, respectively. This is following the results in Table 4, which show that the most optimal mycelial growth is in samples S2, P2, and SP3. The high value of water absorption in biofoam is also caused by the structure of the biofoam material, which based on its morphology, mycelia based-bio foam is a porous material therefore when analyzing water absorption, the water particles will enter into the pores of the biofoam and cause a large

value of water absorption.

Fig. 5 depicts the difference in the growth of *Rhizopus oligosporus* on S2 (using SCT as the substrate) and P2 (using PL as the substrate). *Rhizopus oligosprosus* spores and mycelia were distributed similarly on the surface (Fig. 5A) and in the cross-section (Fig. 5B) of the S2. Meanwhile, in the P2, the surface distribution of *Rhizopus oligosprosus* spores (Fig. 5C) was greater than the cross section of the substrate (Fig. 5D).

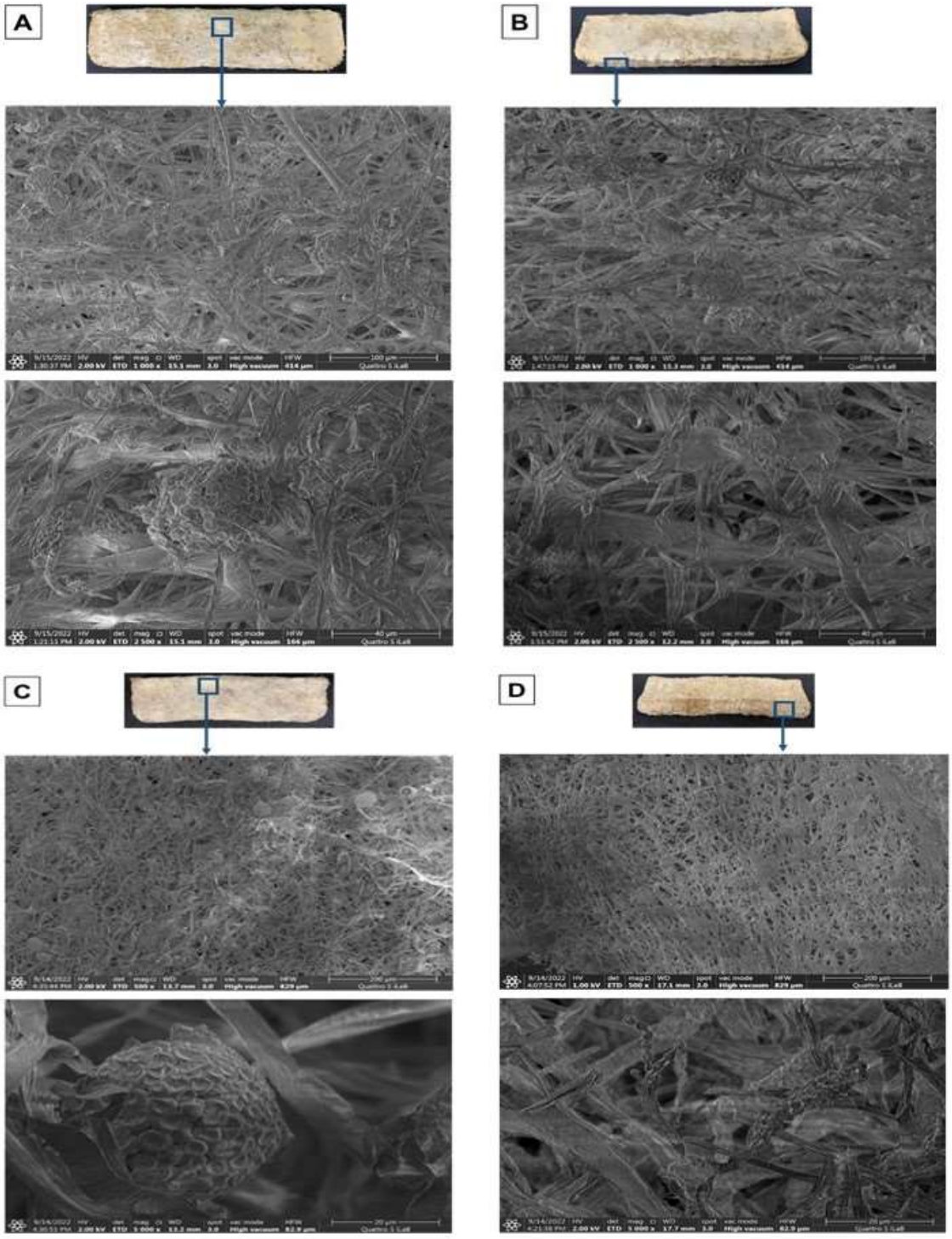


**Figure 5: Morphology of biofoam using FESEM: A) surface of S2, B) cross section of S2, C) surface of P2, and D) cross section of P2.**

One of the factors influencing biofoam's high or low water absorption capacity is the distribution of spores and mycelia. Previous research found hydrophobins, amphoteric proteins that cause mycelia to be hydrophobic, in the mycelia of several molds (Linder et al., 2005), including *Aspergillus niger* (Valsecchi et al., 2018), *Aspergillus fumigatus* (Tanaka et al., 2022), and *Trichoderma longibrachiatum* (Moscatiello et al., 2018). However, more research into the amphoteric protein in *Rhizopus oligosporus* is required.

**Mechanical properties of biofoam**

Bending analysis can be used to determine the modulus of rupture (MOR) and modulus of elasticity (MOE) of biofoam. In this analysis, the mechanical properties of samples S1, P1, and SP1 were not characterized because there was less mycelium growing on the substrate. Fig. 6 showed that biofoams have a MOR value ranging from 0.027 – 0.053 MPa, with SP3 having the lowest MOR and P2 having the highest. The substrate consisting of lesser inoculum (25%) had higher MOR than the substrate consisting of 30% inoculum. In the case of MOE value, higher inoculum concentration increased the MOE value. Biofoams had a lower MOR and a higher MOE than the control, which had a MOR and MOE of 0.223 MPa and 5.221 MPa, respectively. The findings indicate that *Rhizopus oligosporus* mycelia affect the strength and elasticity of biofoam made from SCT or PL substrates. The optimum strength was obtained at a concentration of 25% inoculum, while the optimum elasticity was obtained at a concentration of 30% inoculum.

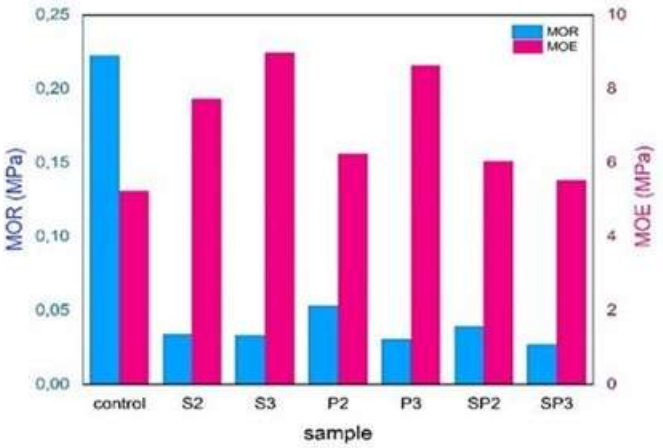


**Figure 6: Mechanical properties of biofoams**

**FTIR Analysis**

Fig. 7 shows that the FTIR spectra of the three samples (S2, P2, and SP3) were similar. However, the intensities were fluctuated. It implies that the functional groups in their samples are similar as well. Fig. 7 also reveals that the intensity of SP3 was higher than S2 and P2, owing to the fact that SP3 was a combination of the two substrates (SCT and PL). This is the first time an FTIR analysis of *Rhizopus oligosporus* mycelia-based biofoam has been released. The functional groups and their wave number can be seen in Table 3.

For styrofoam as the control, the peak at 3025 $cm^{-1}$ is for aromatic C-H stretching vibration, 2921 $cm^{-1}$ for C-H stretching, and 2850 $cm^{-1}$ for C-H stretching alkenes. The following three peaks, at 1601, 1492, and 1452 $cm^{-1}$ indicate aromatic C-H bond stretching vibration. The aromatic C-H deformation vibration is represented by the peaks at 1181, 1028, 841, 755, and 696 $cm^{-1}$.

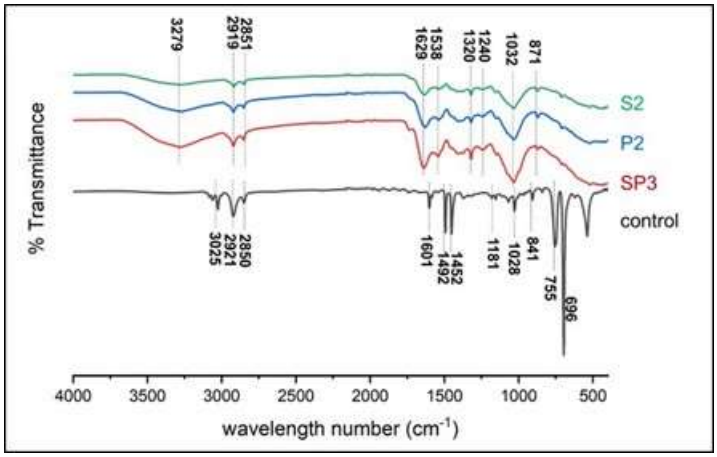


**Figure 7: FTIR spectra of biofoams and styrofoam as control**

**Thermal analysis**

Thermal analysis data using thermogravimetry (TGA) for *Rhizopus oligosporus* mycelia-based biofoam and Styrofoam as the control are shown in Fig. 8. Water evaporation, degradation of lignocellulose (cellulose, hemicellulose, and lignin), and residue degradation are the three stages of thermal degradation that emerge in biofoam. The first stage occurs at $T_{max}$ of 55.85 $^{o}$C for S2, 59.59 $^{o}$C for P2, and 55.49 oC for SP3, with mass losses of 6.153%, 7.355%, and 7.494%, respectively. Because SP3 has a higher moisture content than P2 and S2, it has the greatest mass loss while S2 has the smallest. The second stage occurs at $T_{max}$ of 326.02 $^{o}$C for S2, 335.05 $^{o}$C for P2, and 328.44 oC for SP3, with mass losses of 50.123%, 53.442%, and 57.752%, respectively. At this stage, cellulose and hemicellulose are degraded, while lignin is degraded but at a slower rate. The third stage appeared at $T_{max}$ 731.87 $^{o}$C for S2 and 730.64 $^{o}$C for SP3, with mass losses of 5.808% and 5.49%, respectively.

**Table 4: Functional group of biofoam**

| Assignment | Wave number ($cm^{-1}$) | | | |
|---|---|---|---|---|
| | S2 | P2 | SP3 | Reference (Elsacker et al., 2019) |
| O-H stretching hydrogen bonds | 3279 | 3278 | 3280 | 3600-3000 |
| $CH_2$, $CH_2OH$ in cellulose | 2917 | 2919 | 2920 | 2980-2835 |
| $CH_2$ symmetric stretching | 2849 | 2851 | 2851 | 2940-2840 |
| Absorbed O-H associated with lignin or cellulose | 1629 | 1626 | 1634 | 1633 |
| C=C stretching of the aromatic ring (syringyl) in lignin | 1538 | 1537 | 1540 | 1560-1520 |
| $CH_2$ wagging in cellulose | 1320 | 1320 | 1320 | 1317 |
| Sycingyl ring, C-O stretching in lignin and xylan, nucleic acids | 1241 | 1240 | 1239 | 1240 |
| C-O stretching in cellulose | 1032 | 1031 | 1031 | 1047-1004 |
| Anomere C-group, glucan β-anomerC-H bending, C-H deformation in cellulose | 872 | 871 | 871 | 896 |

Degradation of the residual compounds in the biofoam occurs at this stage. Since PL, the main substrate for P2, has a high lignin content, the $T_{max}$ in the second stage is higher than those of S2 and SP3. Furthermore, $T_{max}$ data was not available at P2 in the third stage because it was possible that the lignin degradation process had not been completed, possibly requiring testing at a higher temperature to determine the $T_{max}$. According to Hammoui et al. (2015), at a temperature of 810 $^oC$, at least the final degradation products of lignocellulosic fibers consisting of non-degraded wastes and impurities happened.

In contrast to mycelia-based biofoam, Styrofoam undergoes only one stage of thermal degradation, which occurs at $T_{max}$ 408.59 $^oC$ with a mass loss of 98.48%. This is reliable with Bruscato et al. (2019), findings that EPS degrades at $T_{max}$ 440 $^oC$ with almost negligible residue percentage.

## CONCLUSION

Sugarcane trash (SCT) and pineapple leaves (PL) can be used as substrates for *Rhizopus oligosporus* mycelium-based biofoam, with the addition of soybean starch, $CaCO_3$, and water. The best *Rhizopus oligosporus* growth media combination is 25% inoculum for SCT or PL and 30% for the mixed substrate (both SCT and PL). Three biofoams (S2, P2, and SP3) outperforms others in terms of characterization, with low density, moisture content, and water absorption, but high biodegradability, and mechanical strength.

## CONFLICT OF INTEREST

The authors declare that no competing interest.

## ACKNOWLEDGEMENT

The authors would like to thank to the Padjadjaran University for the funding support from the academic leadership grant supervised by Ratu safitri, and the Integrated Laboratory of Bioproduct (iLab), Research Center for Biomass and Bioproducts, National Research and Innovation Agency (BRIN) for the facilities, scientific, and technical support.

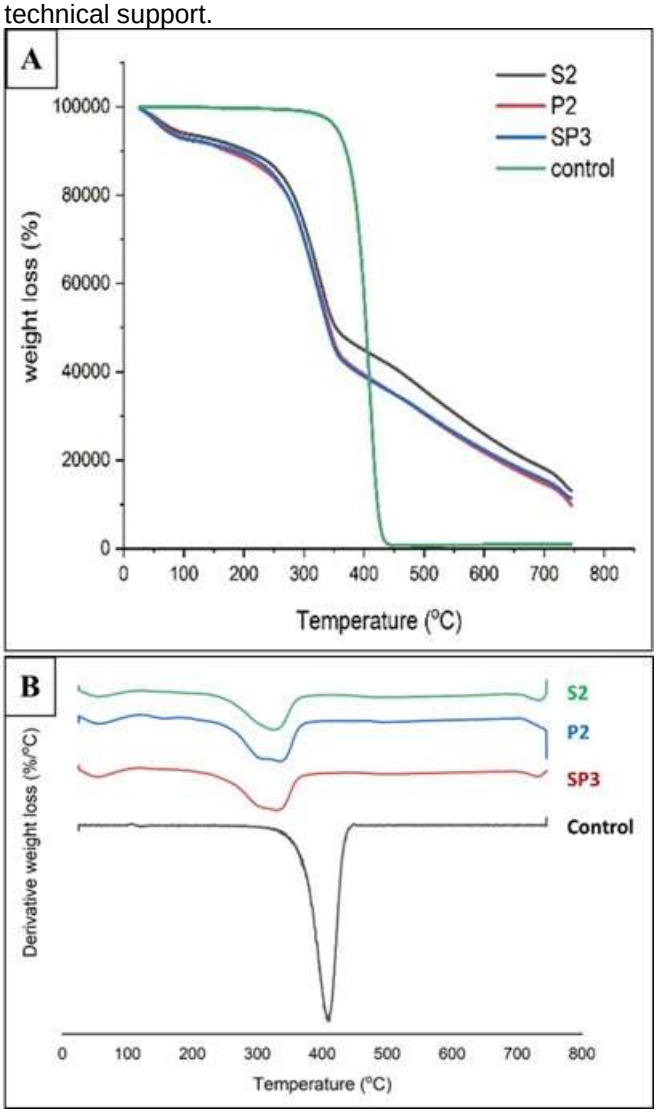


**Figure 8: TGA analysis of biofoam. A) weight loss, B) derivatives weight loss.**

## AUTHOR CONTRIBUTIONS

**Fadia Idzni Rodhibilah:** Investigation, Data curation, Writing-original draft. **Reny Rosalina:** Validation, Writing-review & editing **Fazhar Akbar:** Formal Analysis, Resources. **Tri Yulian:** Formal Analysis, Visualization. **Ratu Safitri:** Validation, Writing-review & editing. **Riska Surya Ningrum:** Conceptualization, Methodology, Formal Analysis, Investigation, Writing-original draft, Project administration.



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