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# Production of mycelium-based biomaterial using various substrates

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This experiment was carried out to create a biomaterial with mycelium as its main component and binding part. The main object of this research was mycelium of *Pleurotus ostreatus*. Various substrates were used for mycelium growing: buckwheat husks, onion husks, garlic husks, coconut husk substrate, coffee grounds, birch sawdust, wheat grains, ground wheat, hemp husks, rock wool, and polystyrene. These different substrates and materials were mixed with the mycelium culture and grown for at least three months, with growth monitored weekly. The blocks of grown biomaterial were dried and tested for properties such as water absorption, moisture content, and biodegradation. The conducted studies showed that mycelium can successfully colonise and grow on different substrates, forming biomaterials with different properties. The choice of substrate influenced the physical characteristics of the obtained biomaterials. Mycelium can be used as a sustainable alternative for the production of various biomaterials. The results of this study add to the knowledge in the field of mycelium-based biomaterials and open the way for further research and development of this promising technology.

**Keywords:** mycelium, biomaterial, substrates, water absorption, biodegradation

## INTRODUCTION

Today, most industrially produced materials, including those used in construction and packaging, are non-recyclable and harmful to the environment. The use of these traditional materials requires energy, depletes natural resources, and pollutes the environment during manufacture and transportation (Italia et al., 2016). According to recent studies, the development of biocomposites from the mycelium substrate complex could potentially displace conventional materials. Instead of being produced, this biomaterial or composite is grown (Maximino et al., 2020). Mycelium is the vegetative portion of a fungus consisting of a mass of branching hyphae and a hollow, tubular structure that serves as the binding matrix and promotes rapid growth (Travaglini et al., 2014). It serves as a natural binder, binding to any nearby organic materials like sawdust, wheat bran, straw, and bagasse to form a super-tight web of thread (Elkhateeb, Daba, 2019). In comparison to traditional synthetic materials, mycelium-derived materials have several significant advantages,

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including low cost, low density, environmental protection, and low energy usage. The anticipatory technology soon to promote environmental sustainability is the application of microorganisms in biomaterial manufacturing, particularly in the construction and packaging sector (Peng et al., 2020). Eben Bayer and Gavin McIntyre, the creators of the Evocative company, first proposed using mycelium as a material in 2007 (Dougoud et al., 2018). Beyond bioremediation and medical use, mycelium is being used in the manufacture of biomaterials like biocement, bioblock, and bioenzymes. The major goal of this study is to explain how fungus mycelium may be combined with different substrates to produce various low-cost biomaterials and to assess the potential for the future.

## MATERIALS AND METHODS

To grow biomaterials such as plastic containers of about 20 × 15 cm (first set) and 13 × 10 cm (second set), a 250 ml measuring cylinder, mycelium- stock culture (organism: *Pleurotus Ostreatus*), a muslin cloth, and polyethylene fibre covering material were used.

Two sets of substrates were chosen for sample preparation. For the first set of samples, 100% was 500 ml, whereas 80% accounted for 400 ml and 20% for 100 ml (Table 1). The measuring of samples was made with a 250 ml-capacity measuring cylinder of . The substrates were pressed for no drop of water after sterilisation and then measured. For the second set of samples, 100% was 250 ml, whereas 80% accounted for 200 ml and 20% for 50 ml (Table 2). The measuring of samples was made with a 250 ml-capacity measuring cylinder. The substrates were pressed for no drop of water after sterilisation and then measured.

Inoculant (Mycelium seed culture) of about 20 grams is added to each measured substrate, then mixed, stabilised with hand pressure into respective containers, and finally covered with a muslin cloth followed by a polyethylene sheet (Fig. 1).

Table 1. **First set of chosen substrates and sample preparation, in percentage**

| Sample No. | Sample | Substrate | Percentage |
|---|---|---|---|
| 1. | Control | Sawdust | 100% |
| 2. | Sample 1 | Sawdust and coconut fibre | 80% + 20% |
| 3. | Sample 2 | Sawdust and buckwheat | 80% + 20% |
| 4. | Sample 3 | Sawdust and coffee grounds | 80% + 20% |

Table 2. **Second set of chosen substrates and sample preparation, in percentage**

| Sample No. | Sample | Substrates | Percentage |
|---|---|---|---|
| 1. | S-1 | Sawdust + buck wheat | 80 + 20% |
| 2. | S-2 | Sawdust + wheat bran | 80 + 20% |
| 3. | S-3 | Sawdust + wheat grains | 80 + 20% |
| 4. | S-4 | Sawdust + onion peels | 80 + 20% |
| 5. | S-5 | Sawdust + garlic peels | 80 + 20% |
| 6. | S-6 | Sawdust + polystyrene + sawdust + polystyrene + sawdust | 20 + 20 + 20 + 20 + 20% |
| 7. | S-6 | Sawdust + rock wool + sawdust + rock wool + sawdust | 20 + 20 + 20 + 20 + 20% |
| 8. | S-7 | Sawdust + hemp husk | 80 + 20% |


**Fig. 1.** Sample preparation to produce a biomaterial block. A – stock culture, B – sample mixed with substrate and fungal mycelium placed in the container, C – sample covered with muslin cloth and a polyethylene sheet

The samples were kept for incubation at about +22°C for the growth of mycelium all over the substrates. The samples were checked for proper moisture content during the time of growth. The first set of samples (Fig. 2) were grown for six months and the second set of samples (Fig. 3) for three months.

The grown samples were then dried in a convection oven after measuring the length, breadth, and weight of the samples. The samples were dried at +70°C for 10 h.

**Determination of the moisture content.** The change in the weight and the moisture content of each sample were calculated in percent-

**Fig. 2.** Observation of biomaterial before and after the growth from the first set of samples

**Fig. 3.** Observation of biomaterial before and after the growth from second set of samples

age. The moisture content was calculated by subtracting the dry weight of the sample from the wet weight and divided by the dry weight.

$$\text{Moisture Content (M) in Percentage} = \frac{\text{Wet weight } (W_w) - \text{Dry weight } (D_w) \times 100\%}{\text{Dry weight of the sample } (D_w)}.$$

The calculation was done in reference to ISO 16979:2003.

**Determination of water absorption property.** The mycelium composites were tested using the partially immersed sample method described in reference to ASTM C 1585 for measuring the rate of water absorption by hydraulic-cement concretes. The experiments were carried out at ambient temperature (+20°C).

The weight change in respect to the initial weight was used to calculate the water absorption by the following formula:

$$m = M_t / a \times d$$

where: $m$ is absorption of water (in mm), $M_t$ = weight change of the sample (in grams), $a$ = the exposed area of the sample (in mm$^2$), and $d$ is the density of the water (in gram\mm$^3$).

To test the property in each grown biomaterial, the samples were shaped into cubes of 3.5 × 3.5 cm (Fig. 4), and testing was carried out in both cold (+12°C) and hot (+100°C) water for 1 h (Fig. 5).

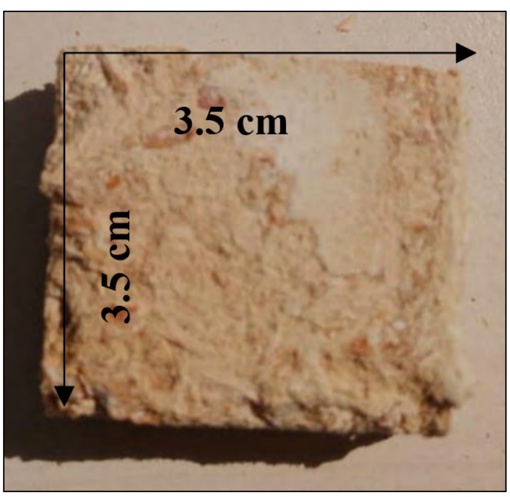


**Fig. 4.** The grown material from each sample shaped into cubes of 3.5 × 3.5 cm

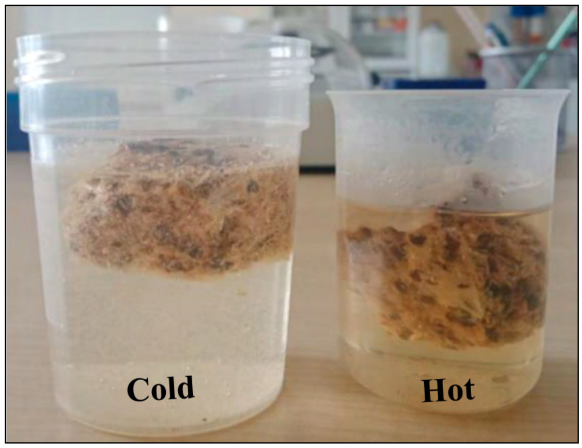


**Fig. 5.** Sample tested for water absorption property in both cold and hot water

**Determination of biodegradation of the biomaterial.** Pieces of each grown sample were buried in the compost soil for a period of 20–30 days and the degradability of the samples was determined by observing the changes in the structure of the biomaterial (deformation) by comparing before and after degradation of the samples.

## RESULTS

**Moisture content of the samples.** The moisture content in each grown biomaterial of different substrates was calculated using the formula mentioned above. The results are presented in percentages in Table 3.

**Water absorption property.** The property of water absorption for each grown biomaterial of different substrates was calculated using the formula mentioned above, and the results (in mm) are presented in Table 4.

**Biodegradation of the biomaterial.** Biomaterials, especially made from substrates such as sawdust, coconut fibre, buckwheat, and coffee grounds, were kept for biodegradation for a period of 30 days. The samples were observed twice, at 20 days and 30 days. As expected, the samples started to degrade (Figs 6–9).

It was observed that mycelium started to grow over the biomaterial thus contributing to the degradation and growth of the fungus. From the results analysed in biodegradation, it

Table 3. **Determination of the moisture content in samples grown, including first and second sets of samples, in percentages**

| Sample No. | Sample | Length and breadth | Weight before drying | Weight after drying | Moisture content |
|---|---|---|---|---|---|
| 1. | Sawdust | 14 × 10 cm | 127.6g | 119.3g | 6.9% |
| 2. | Sawdust + coconut fiber | 14 × 10 cm | 119.9g | 111.7g | 7.3% |
| 3. | Sawdust + buck wheat | 14 × 10 cm | 119.4g | 111.9g | 6.7% |
| 4. | Sawdust + coffee grounds | 14 × 10 cm | 121.6g | 113.3g | 7.3% |
| 5. | Sawdust + buckwheat | 10 × 6 cm | 43.5g | 35.9g | 21.1% |
| 6. | Sawdust + wheat grains | 10 × 6 cm | 67.7g | 44.4g | 52.4% |
| 7. | Sawdust + ground wheat | 10 ×6 cm | 41.2g | 37.3g | 10.4% |
| 8. | Sawdust + onion peels | 10 × 6 cm | 39.1g | 35.1g | 11.3% |
| 9. | Sawdust + garlic peels | 10 × 6 cm | 48.9g | 42.4g | 5.3% |
| 10. | Sawdust + polystyrene | 10 × 6 cm | 82.8g | 53.4g | 55% |
| 11. | Sawdust + rock wool | 10 × 6 cm | 65.5g | 52.4g | 25% |
| 12 | Sawdust + hemp husk | 10 × 6 cm | 54.3g | 43.9g | 23.6% |

Table 4. **Determination of water absorption rate in samples grown, including first and second sets of samples, in mm**

| Sample | Water absorption rate (cold water – 1 h) | Water absorption rate (hot water – 1 h) |
|---|---|---|
| Second set of samples – water absorption in 100 ml for each sample | | |
| Wheat grains | 0.00009 | 0.000165 |
| Ground wheat | 0.000105 | 0.000146 |
| Garlic peels | 0.000110 | 0.000251 |
| Buckwheat | 0.000116 | 0.000154 |
| Hemp husk | 0.000131 | 0.000157 |
| Polystyrene | 0.000137 | 0.000192 |
| First set of samples – water absorption in 200 ml for each sample | | |
| Sawdust | 0.000106 | 0.000108 |
| Coconut fibre | 0.000135 | 0.000156 |
| Coffee grounds | 0.000066 | 0.000100 |


**Fig. 6.** Biomaterial kept for degradation – before 20 days. A – sawdust + coffee grounds; B – sawdust + buckwheat; C – sawdust + coconut fibre; D – sawdust

**Fig. 7.** Biomaterial observed after 20 days of biodegradation

**Fig. 8.** Biomaterial observed after 30 days of biodegradation

is clearly seen that mycelium can still be alive after drying.

The drying process can only kill the growth of mold, if any, but the mycelium goes into the state of hybernation, and whenever it finds suitable environment, it starts to grow. It can be physically observed in Fig. 9 that half of the placed biomaterial has undergone biodegradation within three to four weeks and the remaining biomaterial will also degrade in the following few weeks. Also, it is noted from the experiment conducted and described by Lazaro Vasquez et al. (2020) that the bio-based material can take up to 90 days to degrade under natural conditions in the soil.

**Shaping of the biomaterial.** This work was done to show that mycelium-based material can be created in any form of own choice to be used as packaging material. The sawdust + buckwheat combination was used to create these shapes using moulds (Fig. 10). The formed structures were firm and lighter in weight.

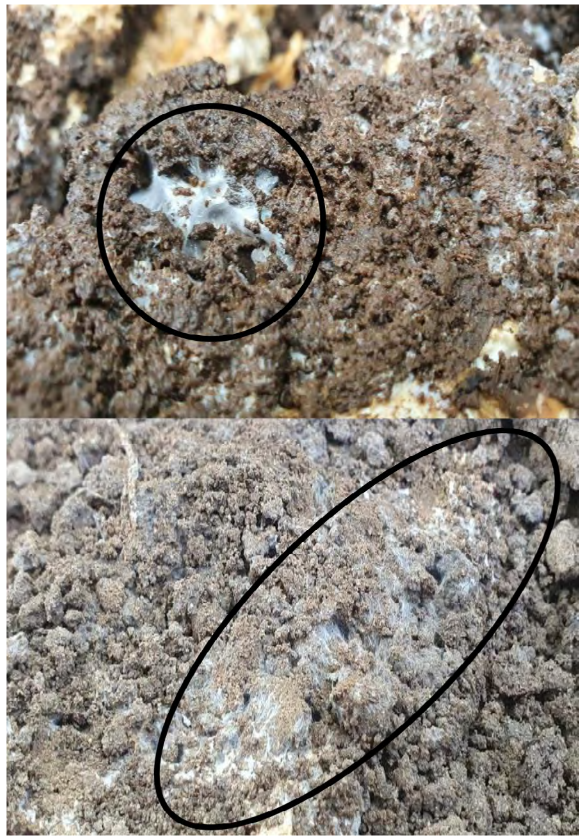

**Fig. 9.** Growth of mycelium over the biomaterial kept for biodegradation

**Fig. 10.** Shaped biomaterials made from sawdust and buckwheat substrate combination

## DISCUSSION

The outcomes of this study contribute significantly to the field of biological materials since they give a thorough picture of the manufacturing procedures and physical characteristics of the composites made from mycelium. A number of studies (Attias et al., 2020; Dougoud et al., 2018; Joshi et al., 2020; Maximino et al., 2020) have been conducted on mycelium-based biomaterial production. In this work, different substrates were considered, which had not been used for biomaterial production. The substrates involved are primarily waste materials contributing to waste reduction. The biomaterials produced have a major advantage of being biodegradable and causing no harm to the environment. Previous research studies observed that when straw was used as a substrate, it exhibited a faster mycelium growth rate when compared to sawdust, and, in a similar manner, bagasse exhibits faster mycelium development than sawdust and its mixture (Joshi et al., 2020). This is because sawdust contains glucan, which is complex and varied in terms of nutrients. Straw and bagasse also have softer particle characteristics than sawdust, which makes it easier for fungi to use nutrients from soft substrates than from hard substrates (Attias et al., 2020). A different combination and concentration of substrates, addressed by other researchers (Gao and Zou., 2020; Irbe et al., 2022; Elsacker et al., 2020; Alemu et al., 2022), was not considered in this work. Compared to previous studies, the findings of this work provide new insights into the varied strengths and properties of mycelium composites.

## CONCLUSIONS

All the samples exhibited good growth of mycelium except for the onion peel composite, due to its antifungal property against *Pleurotus ostreatus* mycelium. The best growth was observed in substrates such as wheat grains, grinded wheat, and garlic peels.

The moisture content of different substrates varies from 5.3% to 55%.

The water absorption property in the biomaterial made from substrate which includes wheat grains (0.00009 mm) and ground wheat (0.000105 mm) was found to be exceptional. Mostly, all other samples had high water absorption property.

Biodegradation of the material made from sawdust and coffee grounds have a higher level of degradation when compared to that of buckwheat and coconut fibre.

This research expands the knowledge in the field of mycelium-based biomaterials and paves the way for further exploration and development of this promising technology.

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## GRYBIENA PAREMTA BIOMEDŽIAGŲ GAMYBA NAUDOJANT ĮVAIRIUS SUBSTRATUS

*Santrauka*

Šiuo eksperimentu siekta sukurti biomedžiagą, kurios pagrindinė ir rišamoji dalis būtų grybiena. Tyrimo objektas – *Pleurotus ostreatus* (gluosninė kreivabudė) grybiena. Grybienos auginimui buvo naudojami įvairūs substratai: grikių, svogūnų, česnako, kanapių lukštai, kokoso lukštų substratas, kavos nuosėdos, beržinės pjuvenos, kviečių grūdai, malti kviečiai, akmens vata bei polistirenas. Su grybienos kultūra sumaišyti minėti substratai buvo auginami mažiausiai 3 mėnesius, augimas stebimas kiekvieną savaitę. Išaugintų ir išdžiovintų biomedžiagos blokų buvo tikrinamos tokios savybės kaip vandens absorbcija, drėgmės kiekis ir biologinis skaidymas. Atlikti tyrimai rodo, kad grybiena gali sėkmingai kolonizuotis ir augti ant įvairių substratų, sudarydama skirtingomis savybėmis pasižyminčias biomedžiagas. Nuo pasirinkto substrato priklausė gautų biomedžiagų fizikinės charakteristikos. Grybiena gali būti tvari alternatyva įvairių biomedžiagų gamybai. Šio tyrimo rezultatai papildo žinias apie grybienos pagrindu pagamintas biomedžiagas ir atveria kelią tolesniam šios perspektyvios technologijos tyrimui ir plėtrai.

**Raktažodžiai:** grybiena, biomedžiagos, substratai, vandens absorbcija, biologinis skaidymas