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Research Article

Production of Mycoblock from the Mycelium of the Fungus *Pleurotus ostreatus* for Use as Sustainable Construction Materials

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As the global population rises, resource depletion and environmental pollution also aggravate. To meet the needs of the population, different products have been manufactured. However, most industrially manufactured products are not eco-friendly, costly, and locally unavailable. To solve these problems, using and enhancing locally available biomaterials are the key option. Three substrates sawdust, bagasse, and coffee husk and the fungus *Pleurotus ostreatus* were used. Mycelium was fully colonized by 9, 14, and 27 days on potato dextrose agar (PDA), sorghum grain, and substrate, respectively. The mycelium growth on coffee husk showed the fastest growth rate whereas that of the sawdust was slowest. The fully colonized substrates were molded for 7, 14, and 21 days by plastic mold to maintain their regular 3D structure. The result shows that the block made with sawdust at 21 molding period has higher compressive strength and density of 750 kPa and 343.44 Kg/m^3, respectively, followed by bagasse and coffee husk. These variations were due to the mycelium density difference between the substrates. Physicochemical and mechanical characteristics such as mycelium morphology, bimolecular and elemental analysis of substrates, density, water absorption, and compressive strength of the block were analyzed. This technology has the potential to replace conventional construction and packaging materials used for indoor applications such as insulation, partition walls, and other design and architectural applications. It also benefits in terms of its low cost, green synthesis approach, nontoxicity, low environmental emission, recyclability, and local availability.

1. Introduction

The rapid rate of global population growth leads to environmental pollution and natural resource depletion. The human population increase could aggravate both resource depletion and environmental pollution. Similarly, the rise of the human population is also the main cause of urbanization. The United Nations study projection showed that about 66% of the world population will live in urban areas by 2050 [1]. The increasing urban population will lead to a significant increase in urban energy consumption and urban emissions [2]. Modernization of the construction sector has a significant role to play in reducing urban emissions [2]. Most construction materials nowadays are made of cement, gypsum, metals, wood products, and polymer products. These materials need high cost, consume energy, are environmentally unfriendly, and are nonrecyclable. A recent study shows that about 8.7 gigatons, which is about 10% of the world emission, of carbon dioxide, is from the construction sector [3], either from demolition or construction [4]. To minimize the environmental effect caused by the construction sector, applications of innovative materials (low/zero carbon buildings) are the key options [2].

As compared to others, cement is one of the most widely used construction materials worldwide [5–7]. Cement-based materials are hydrophobic, high strength, and durable [7].

However, they are not subject to decomposition, create environmental pollution, are susceptible to cracking, and require high-cost. Eight to ten percent of the global total carbon dioxide emissions were released from the manufacturing of cement only [8]. To keep the construction sector clean and sustainable, technological improvement should be needed.

The practice of business as usual in the construction sector will not create a sustainable environment and circular economy. The advancement of technology in construction materials has become one of the most important recent issues in the field of Biotechnology and Civil Engineering research studies. There is a possibility of making sustainable construction material from a complex of fungal mycelium and organic substrates [9, 10]. Mycelium is a vegetative part of fungi that has a long, branching, and filamentous structure called hyphae and acts as a natural adhesive and is used to create a network of extremely dense fibers, attached to the organic substrate (sawdust, straw, coffee grounds, wheat bran, and bagasse) [11, 12]. The organic matter bounds with this hyphal structure and forms fungal skin. When this process is ceased through drying or heating, the incomplete process results in a mycelium-based block (Mycoblock). Mycoblock is a block made of organic substrates and uses mycelium as a natural adhesive. In addition to being applied in biocomposite production, the fungal mycelium can also be applied in a variety of other environmental technologies [13, 14].

Existing research shows that Mycoblock is used in a variety of applications such as packaging materials, insulations, partition walls, utensils, furniture, and different design and architectural [15]. It can significantly reduce the reliance on fossil fuels and the embodied energy required for construction and lower waste left at the end of buildings' life cycles [16]. Because it is entirely biodegradable, does not produce waste when appropriately discarded, and produces a lower carbon footprint *s* compared to conventionally manufactured building materials [16], the quality of the product can be enhanced through methodological diversifications such as types of substrate, types of strain, length of cultivation time, molding type, and molding temperature [17].

The genera belonging to Pleurotus are widely used and studied by different scholars for the application of Mycoblock followed by *Trametes* and *Ganoderma genera* due to their contamination resistance and faster growth than other fungal genera [16, 17]. Hot-pressing shifts the property of blocks from foam-like to wood-like by enhancing their stiffness and homogeneity [16]. The current study is mainly focused on the production of noncement-based biomaterials from organic wastes for alternative and low-cost construction materials by using fungal mycelium as a natural adhesive. The study also identifies the comparative strength of different substrates (bagasse, sawdust, and coffee husk) for better mycelium-based blocks.

2. Materials and Methods

2.1. Strain Cultivation.
The fungal strain *Pleurotus ostreatus* (P. *Ostreatus*) was obtained from Shitaki international mushroom plc. In Addis Ababa., PDA (39 g/L) was used for

the growth of the strain after autoclaving at 121°C for 15 minutes (min). The warm liquid media (50–60°C) was poured carefully into a sterile Petri plate until 2/3 of the plates were filled [18]. A piece of mass of mycelium was picked using an inoculation loop and placed at the center of a cooled PDA agar plate under an aseptic condition to refresh the strain. The fully colonized refreshed strain was triplicated by taking a disk of (6 x 6) mm2 mycelium grown on agar. Finally, the plates were incubated at 28°C until grown mycelia fully cover the Petri plate. Mycelia growth was visually observed and measured using a ruler in terms of diameter on the culture plate every three days intervals, and growth rate (GR) was calculated using equation (equation (1)) [19]. The pure culture was stored for further study according to the preservation method used by [20].

2.1.1. Spawn Preparation.
Sorghum grain locally called (ZENGADA) purchased from the local market was cleaned and soaked. Then, the cleaned and soaked grain was spread on a water permeable cloth to remove the excess water until 50% moisture (Equation (2)). To maintain the pH, 2% lime on a dry weight basis of grain was added and mixed thoroughly [21–23]. Glass bottles filled with 100 g (on a wet weight basis) grain lime mixture were autoclaved at 121°C for 60 min and allowed to cool overnight in aseptic condition. After cooling a quarter (1/4) of 9-days-old culture from the Petri dish was inoculated and incubated at 28°C until the substrate was fully colonized. The mycelia invasion rates were inspected every three days' intervals.

2.1.2. Substrate Collection, Preparation, and Inoculation.
Three substrates were collected from the following places: coffee husk (CH) and sawdust (SD) from Addis Ababa around Haile garment and Bagasse (Bg) from the Metehara sugar factory. These substrates were selected due to their abundance and local availability. The average size of the substrate was obtained by homogenizing it manually with a scissor below 2 cm [24, 25], and other unwanted materials such as plastics, metals, and stones were cleaned out from the substrate manually.

About 10% Teff bran (on a dry weight basis) was added to each prepared substrate as a supplement and to provide adequate void space between substrate substances [9, 21, 25]. The substrate-supplement mixture was soaked separately in excess tap water overnight to soften the substrate. Then, the soaked mixture was drained off the excess water until moisture content become 60% to 70% [26, 27]. For moisture measurement, about 40 g samples from each type were taken randomly [9], and the result was analyzed according to Equation (2) [21].

For the purpose of buffering, preventing substrate adhesion and facilitating air circulation between substrates, 3% of calcium sulfate (on a dry weight basis) was added to each mixture and mixed thoroughly [22, 27, 28]. The adequate amount of substrate mixture was sterilized in an autoclave at 121°C for 60 min and allowed to cool overnight under aseptic conditions [22, 29]. Each substrate (1000 g) with 60 to 70% moisture was inoculated with 10% spawn (100 g) [25, 30].

Then, the sample bags were kept in a dark room at $(22 + 1°C)$ until mycelium fully colonized the substrate. The mycelium growth rate was inspected every five days to determine mycelium quality and density.

2.2. Production Phase.

Blocks were made after passing the following three phases: molding, incubation, and denaturation. About 200 g of fully colonized mycelium of each substrate was added to 11 cm × 8 cm x 4 cm size plastic mold for three incubation periods (7, 14, 21 days) [26, 31], under aseptic conditions. The incubation temperature was adjusted to 22 + 1°C. At the end of each growth period, the sample was taken out of the mold and ready for weight measurement and denaturation. To terminate the mycelium overgrowth, for dehydration, and decrease the toxicity level of the strain, heat of about 50°C for 48 h was applied [32].

2.3. Physico-Chemical and Mechanical Characterization Techniques

2.3.1. pH Level. pH was measured after taking 1 : 10 w/w of the sample from the fully colonized substrate and control and soaked for one hour (hr) [33, 34].

2.3.2. Water Content. To ensure mycelium development, the moisture content of both inoculated substrate and control were measured by taking a 40 g sample from each bag, and the result was analyzed by (equation (2)) [35].

2.3.3. Elemental Analysis. The substrate elemental content was evaluated by a device EA 1112 Flash CHNS/O- analyzer by taking 0.2 g of samples' powder grinded with the size of below 150 μm.

2.3.4. Scanning Electron Microscopy (SEM). The surface morphology of the mycelium fibers grown on PDA and different substrates were analyzed using SEM (INSPECT F50, Japan).

2.3.5. Fourier-Transform Infrared Spectroscopy (FT-IR). The chemical composition of fungi mycelium fiber grown on different existed substrates was analyzed by FT-IR spectroscopy (Perkin Elmer, USA) in the range of 4000 to 500 cm 1. Then, 0.5 g of sample grinded below ≤150 μm in size was taken for analysis.

2.3.6. Water Absorption. After dry weight was obtained, each block was submerged in excess water for 32 hrs. Weight was recorded every 8, 24, and 32 hours until stable weights were obtained [18, 36]. Then, the data were analyzed with the (equation (3)) [35].

2.3.7. Density. Densities were calculated by measuring the weight and volume of each block after heating 50°C for 48 h as per equation (4) [37].

2.3.8. Compressive Strength. A compressive strength test was carried out by a compressive testing machine (3000 kN) with a pace rate of 2.4 kN/s. The samples were gently placed on the lower beam and compressed till the specimens fractured completely.

2.4. Statistical Analysis.

The experimental design was completely randomized in a 3 x 3 factorial method with three substrates and three cultivation periods. Each test was triplicated and the result was taken from the mean:

$$Growth rate = \frac{Df - Di}{di}, \qquad (1)$$

$$moisture content (\%) = \frac{(Mw - Dw)}{Mw} \times 100, \qquad (2)$$

$$Water absorption (\%) = \left(\frac{Wf - Wi}{Wf} \times 100\right), \qquad (3)$$

$$Density = \frac{m}{y}. \qquad (4)$$

where Df is diameter at the last evaluation day, Di is diameter at the initial evaluation day, di is the evaluation day interval, Dw is dry weight of the substrate, M is mass, Mw is moistened weight of the substrate, V is volume, Wf is final weight of the Mycoblock (after 24 hr submerged in water), and Wi is initial weight of the Mycoblock (dry weight before submerged to water).

3. Results and Discussion

3.1. Growth Conditions and Morphological Analysis of Mycelium Fibers.

The growth condition of *P. ostreatus* mycelium fibers on PDA, grains, and three substrates (CH, SD, Bg) were illustrated in Table 1 and Figures 1(a)–1(c). The mean growth rate of mycelium grown on PDA was higher than grain and substrates as indicated in (Table 2). The color of the cotton-like structure of mycelium fibers grown on PDA covered the entire Petri dish (90 mm in diameter) within 9 days (Figure 2(a)), whereas that of the spawn takes 14 days for entire growth (Figure 2(b)), which showed similar results with the study of [21]. The mycelium growth rate for culture and spawn decreased as incubation time increased (Figures 1(a) and 1(b)); whereas the mycelium growth rate between different substrates was varied (Figure 1(c)). This is might be due to nutrient limitations.

The highest running rate was observed in coffee husk followed by bagasse and sawdust numerically 25, 26, and 27 days, respectively. These growth differences might be due to variation of aeration between substrate particles and nutritional content [21]. The mycelium growth rate of the current study is comparable with the study of [22] which took 25 and 27 days for full colonization of *P. ostreatus* mycelium on the coffee bulb and wood chips supplemented with Teff straw, respectively. Figure 3 shows the mycelium growth condition on different substrates. The figure illustrates that the growth rate was inversely proportional to the

TABLE 1: Growth features of mycelium grown on PDA and different substrates.

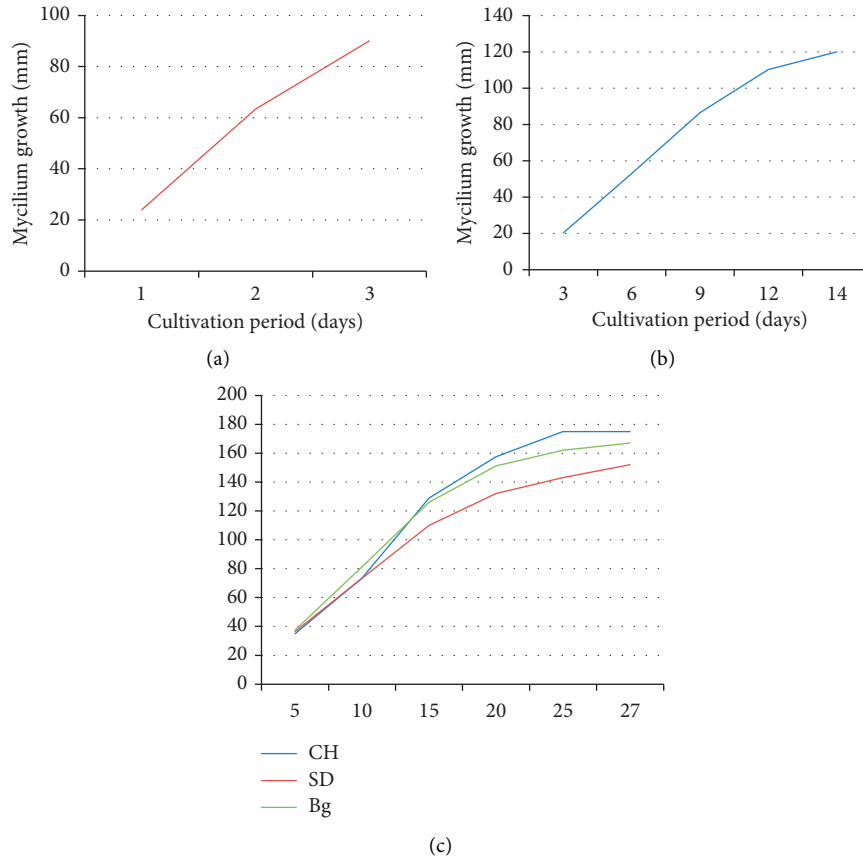|	Growth length (mm)	Growth period (days)	Growth rate (mm/day)
Plate culture	90	9	10.00
Spawn	120	14	8.19
Coffee husk	175	25	7.00
Bagasse	167	26	5.82
Sawdust	152	27	5.14

FIGURE 1: Mycelium growth rate grown on (a) PDA, (b) grain (spawn), and (c) different substrates: CH (coffee husk), SD (sawdust), and Bg (bagasse).

TABLE 2: Mean and standard deviation of mycelium growth rate at different growth media.

Statistical measurements	PDA	Spawn	CH	SD	Bg
Mean growth rate (mm/day)	10.00	8.19	7.00	5.14	5.82
Std. deviation	2.19	2.78	2.79	2.02	3.04

length of the incubation period and height of growing materials and directly proportional to the density of mycelium, which might be due to aeration difference across a height [37] and difficulty in nutrient extraction out of compacted substrates [18]. No mycelium growth was observed in control samples.

Unlike the growth rate, the density of the mycelium at each substrate was inversely proportional to the incubation period which is in agreement with [38], which might have happened due to substrate elemental content (Table 3). The

elemental composition of the substrate has a high effect on fungal mycelium development[39].

Mycelium-based blocks were formed at 7, 14, and 21 days of incubation with different physico-chemical characteristics. The skin on the surface of the block was formed through growing radial direction and stimulated the generation of the outer skin when the expanding biomass of mycelium came in contact with the molds and formed a fairly strong protective layer on the surface of the sample [7] (Figures 4(a)–4(c)). In contrast, the block made with control had no mycelium skin and had an indefinite structure. Mycoblock with no heat applied was spongy in texture, was white in color, and increased in size. Whereas Mycoblock exposed to heat was strong, was brown in color, and showed reduction in size. Block made with coffee husk was fractured when exposed to heat, which might be due to low mycelium density. A similar result was reported in [40].

(a)

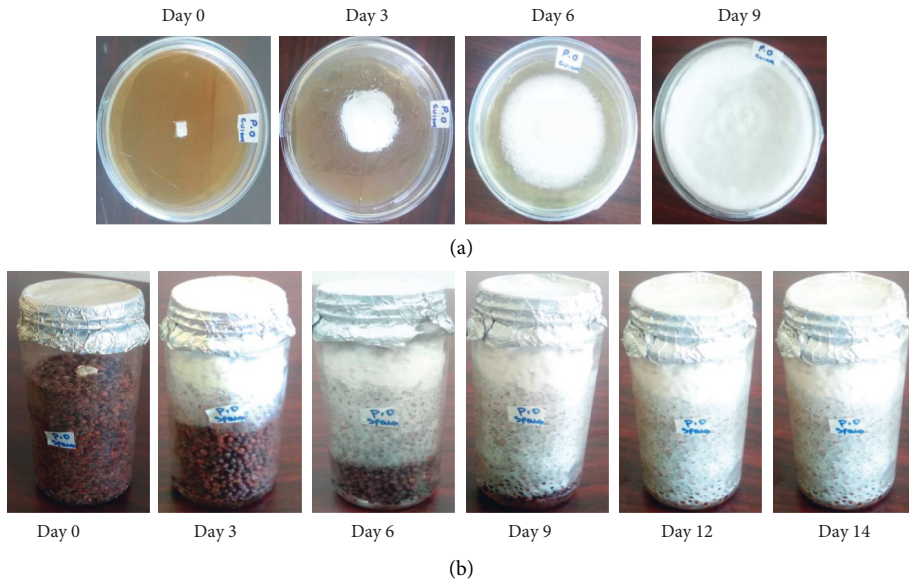Day 0   Day 3   Day 6   Day 9   Day 12   Day 14

(b)

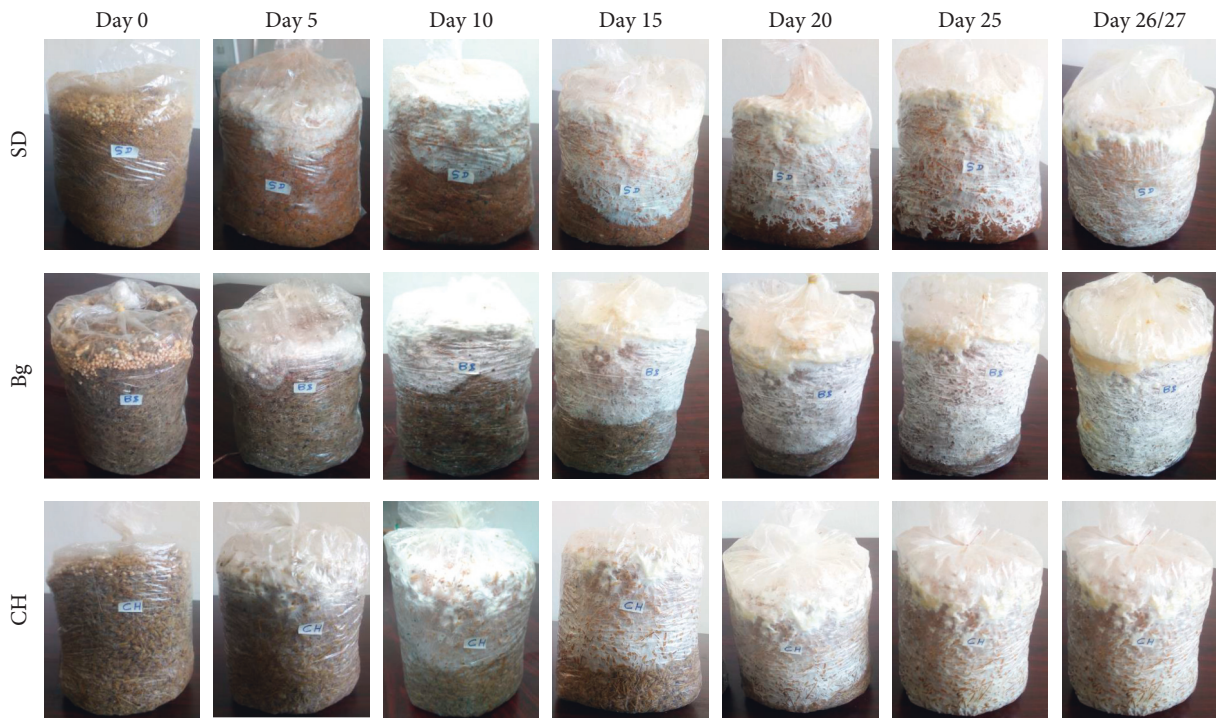FIGURE 2: Mycelium growth on (a) PDA and (b) spawn.

FIGURE 3: Mycelium growth conditions on Bg, SD, and CH at different growth periods.

TABLE 3: Elemental content of the substrates.

Substrates	N (%)	C (%)	C/N ratio
SD	1.67	48.21	28.87
Bg	1.86	35.73	19.21
CH	1.87	35.21	18.83

3.1.1. Morphological Analysis. The morphology of *P. ostreatus* hyphae grown on PDA and different substrates were identified by light microscope and SEM (Figures 5(a)

and 5(b)). Figure 5(a) illustrated that the light microscope image of hyphae grown on PDA had septa, anastomosis, and clamp connections in their filaments which is similar to [41] study report. Clamp connections are more common in most of Basidiomycota which is formed during cell division of secondary hyphae [42], whereas anastomosis helps the hyphae to attach to one another [37]. SEM images of pure mycelium clearly show the tubular hyphae and the interwoven network (Figure 5(b)). The SEM image for the control sample has more air voids in between the substrates

(a)

(b)

(c)

(d)

(e)

FIGURE 4: Image showing Mycoblock made from different substrates: (a) heat exposed, (b) nonheat exposed, (c) control, (d) block side view, and (e) rectangular sheets.

(Figure 5(c)). In contrast, SEM images for mycelium colonized substrates have interwoven hyphae networks between substrate particles with less air voids (Figures 5(d)–5(f)). The difference in voids spaces might be due to the network of hyphae.

3.2. Physical and Chemical Characterization of Mycelium Fibers.
Mycelium development on each substrate was evaluated through selected properties such as pH and water content, as shown in Figures 6(a)and 6(b). There were changes in pH and water content values in mycelium-developed substrates and the control [43, 44]. The pH of the control samples was higher than mycelium-developed samples (Figure 6(a)), which is probably due to enzymatic digestion [33]. Similarly, the water content of each substrate inoculated with *P. ostreatus* had higher water content than

substrate without fungi (Figure 6(b)). This variation might be due to mycelium density variation between substrates [44].

Mycelium chemical composition and the chemical nature of different substrates were analyzed by FT-IR spectra. Mycelium-based materials (MBm) made from selected substrates are expected to inherit the microstructure and properties of the feeding material [45]. All expected essential biomolecules such as polysaccharides, proteins, lipids, and Chitin were observed (Figure 7), which is in line with the result reported in [18]. The author's report shows that all the essential characteristic biomolecules such as proteins (1644 to 1546 cm^{-1}), lipids (3000–2800 cm^{-1}, 1740 cm^{-1}), nucleic acids (1255–1245 cm^{-1}), chitin (1318–1415), and polysaccharides (1200–900 cm^{-1}) were observed from Mycoblock made of agricultural wastes. The presence of chitin in fungal mycelia even at minor fractions is crucial for the material's
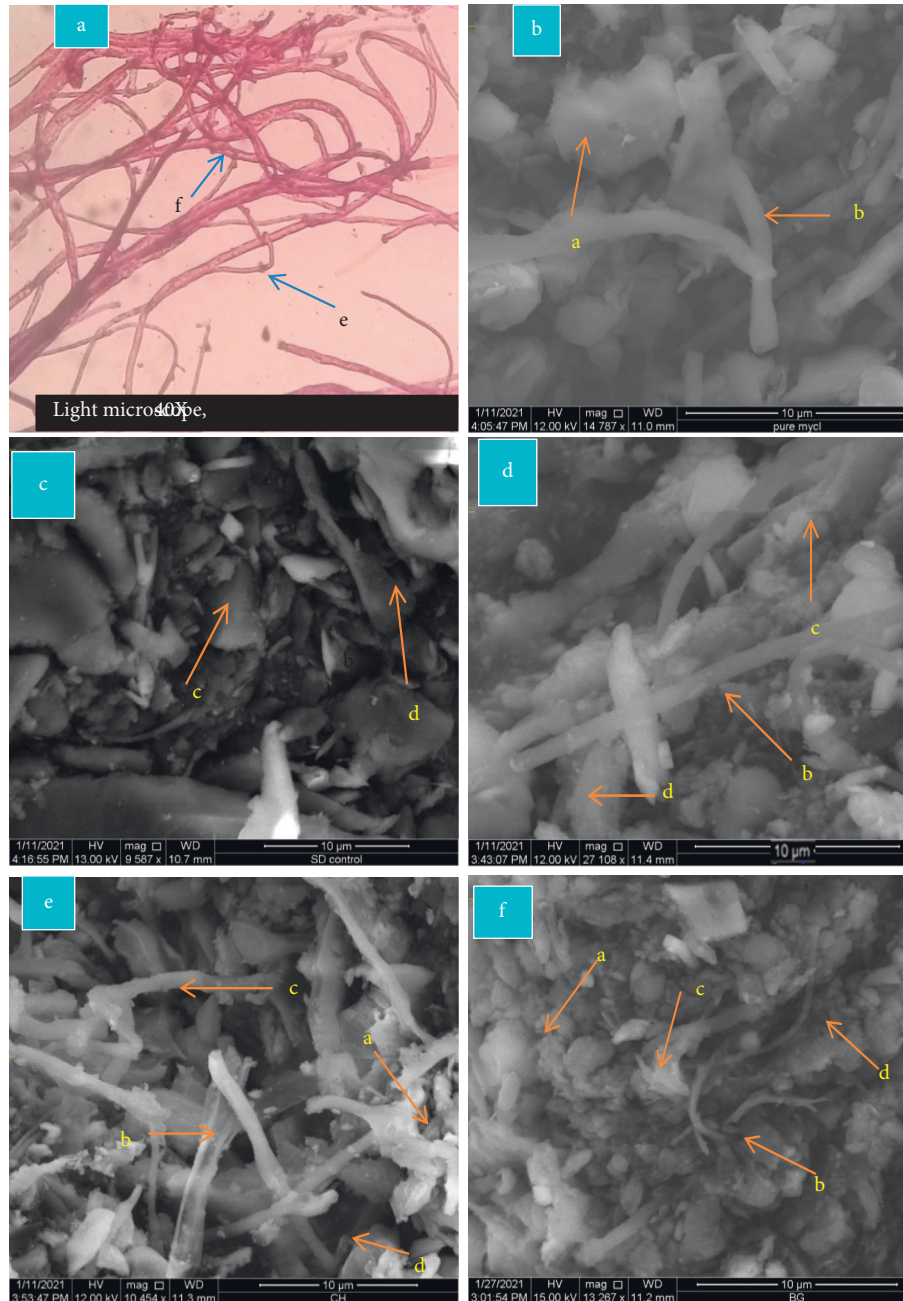
FIGURE 5: Microscopic images of mycelium grown on different substrates. (a) Electro microscopic image of pure mycelium. (b) SEM image of pure mycelium. (c) SEM image of pure SD. (d) SEM image of inoculated SD. (e) SEM image of the inoculated coffee husk. (f) SEM image of inoculated Bg, where (A) fused hyphae, (B) mycelium, (C) substrate, (D) air voids, (E) hyphae septa, and (F) hyphae anastomosis under a light microscope.

structural and mechanical properties [46, 47]. Chitin is a long-chain polymer of N-acetyl glucosamine. This long-chain polymer forms into antiparallel chains and reinforces by being crossed-linked to β (1, 3) glucan with covalent bonds [35].

3.3. *Physical Characterizations of Mycoblock.* The physical properties of the current Mycoblock were affected by two factors, incubation time and substrate type. As incubation

time increased, the density of the block had increased and decreased water absorption up to 11.95% and 1.9% respectively (Figure 8). This result agrees with the previous study made in [7] that the density of Myco block prepared using sawdust mycelium composite increased from 195 kg/m3 to 280 kg/m3 as the incubation period increased because the voids between the fibers are filled as the mycelium continues to grow and the substrate is bonded more strongly together which in turn increases the density [46]. Similarly, longer inoculation time increased mycelium composition
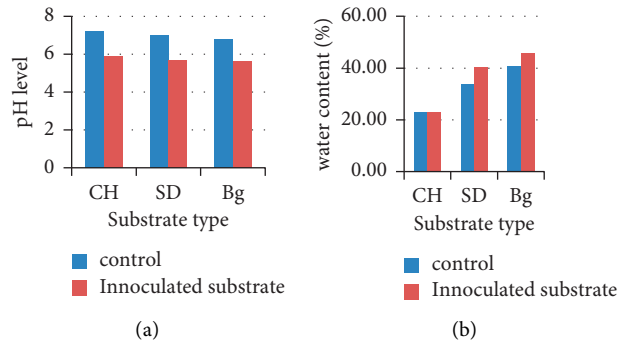
(a)

(b)

Figure 6: Effect of mycelium development on pH and water content.
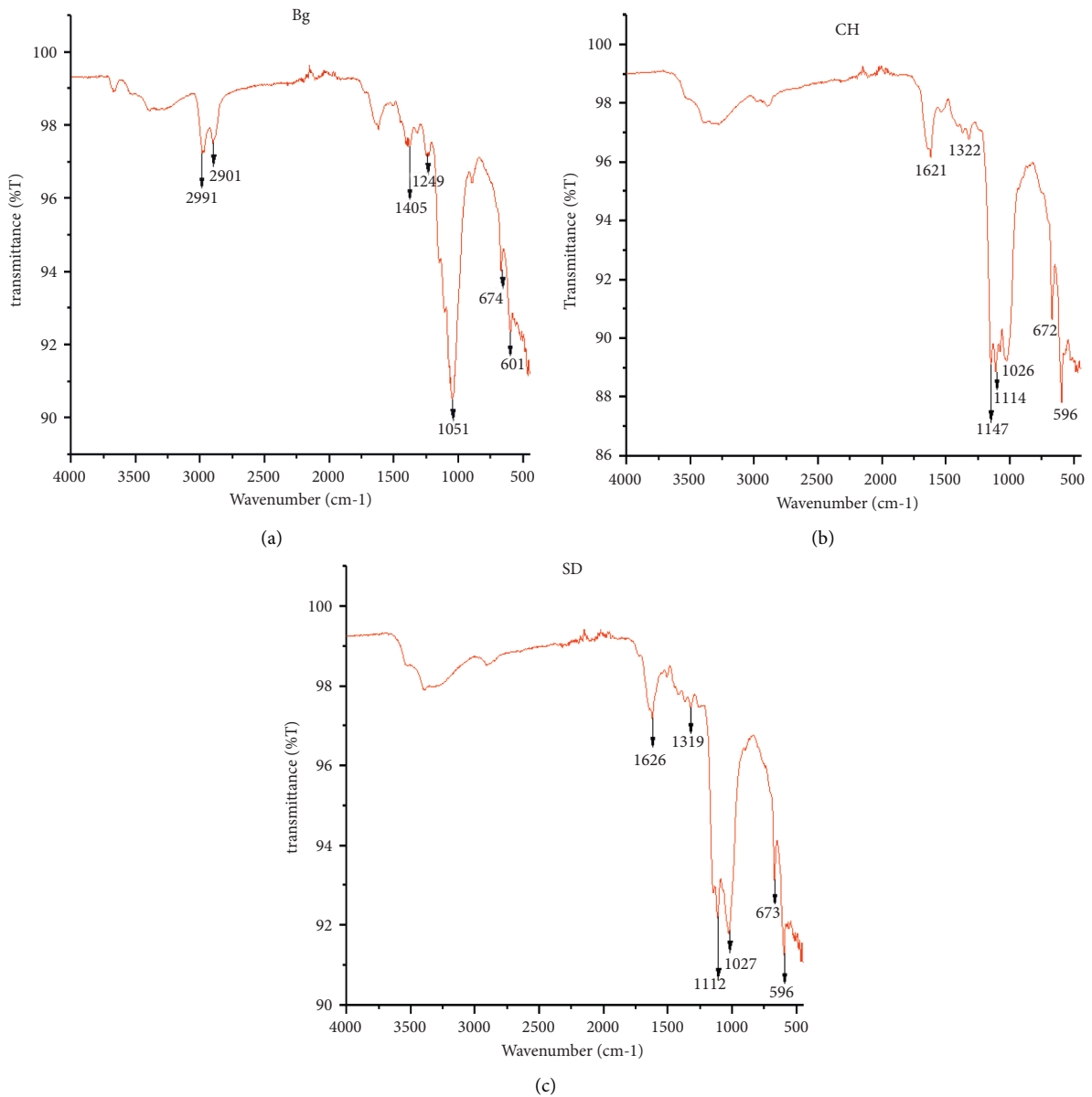

(a)

(b)

(c)

Figure 7: FT-IR spectra band grown on different substrates (a) Bg, (b) CH, and (c) SD.

such as chitin [48], which positively affects the compressive strength of the materials [49]. On the contrary, an extensive incubation period leads to complete degradation of the feeding substrate, which causes a decrease in compressive
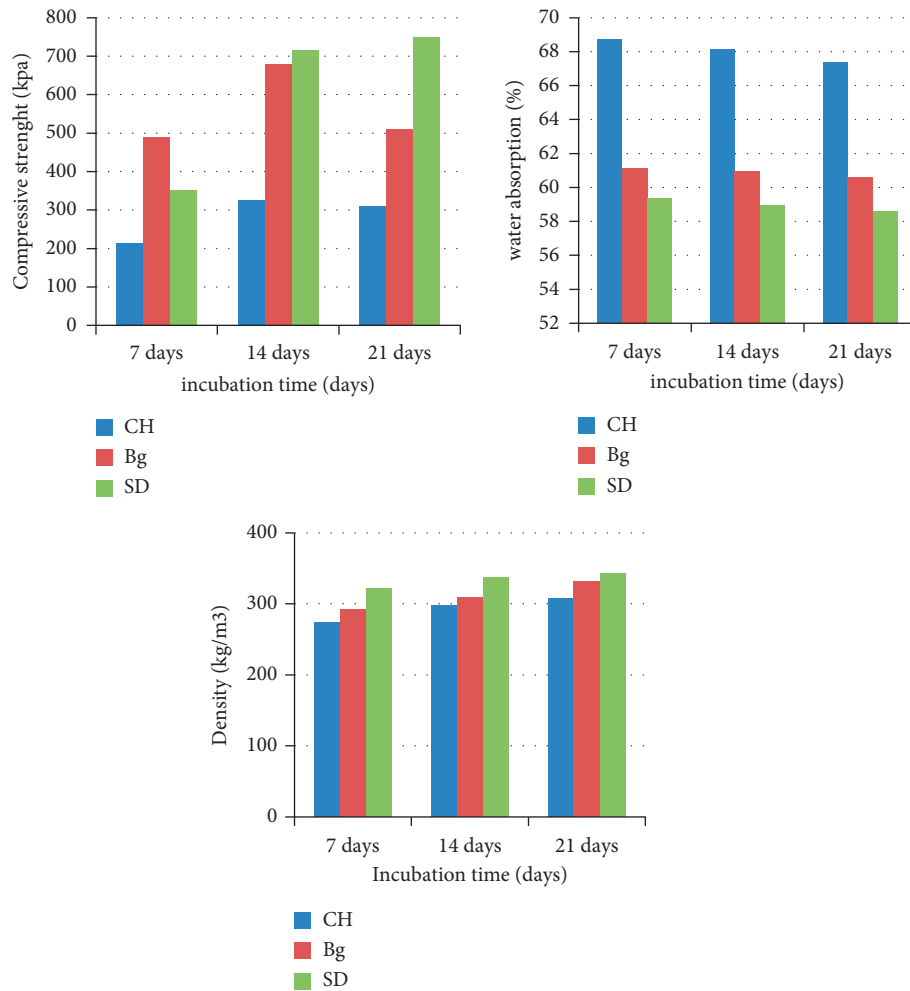
FIGURE 8: Mycoblock physical and mechanical characteristics with varying substrates and incubation period.

strength [19, 50]. A study report done in [51] also strongly agree with the current study that, as the incubation period increases further, it causes more organic substrate degradation, and results in less substrate and more hyphal structures. Since most of the compressive strength of Mycoblock is from the substrates, longer growth times result in less compressive strength.

The current study shows that the compressive strength of Mycoblock increased up to 53.07% as the incubation period increased depending on substrate type. The strength of Mycoblock prepared using both coffee husk (CH) and bagasse (Bg) decreased from 309 kPa to 352 kPa, and 679 kPa to 511 kPa, respectively, as the incubation period increased from 14 days to 21 days, while saw dust (SD) Mycoblock remained increased from 352 kPa to 750 kPa. The mean strengths of CH, Bg, and SD were 283 kPa, 559.67 kPa, and 605.33 kPa, respectively.

This result agrees with the work in [7]; the compressive strength of Mycoblock increased from 350 kPa to 570 kPa as incubation time increased from one week to three weeks. Result reported in [19] also supports the current study report; the authors conclude that an extensive growth period of sawdust above 4 weeks resulted in decreased material strength. The main reason might be due to the physical

nature of the substrate [50] and its chemical contents [9]. Glucan-forming substrate (sawdust) is stronger than non-glucans-forming (softwoods) substrates [9] due to a thick layer of lignin that holds together laminates of cellulose fibrils in cross-orientation [50].

The maximum values in density and compressive strength of Mycoblock for sawdust and bagasse were 343.44 kg/m^3 and 750 kPa and 331.65 kg/m^3 and 511 kPa, respectively. The current study finding is a better result in compressive strength and density than the recent studies report, which has the maximum compressive strength and density of 498 kPa and 249 kg/m^3 Mycoblock made from mycelium substrate complex [51, 52]. In contrast, coffee husk had lower density and compressive strength which was about 292.35 kg/m^3 and 283.00 kPa, respectively. The vice versa was true for water absorption. SD had minimum water absorption capacity followed by Bg and CH, numerically 58.96%, 60.87%, and 68.07%, respectively. The same study also supports the current study that Mycoblock made from mycelium and saw dust has higher compressive strength and density than bagasse [18]. The same author reported that the lower strength and density of bagasse as compared to sawdust was because it has maximum substrate size and low mycelium penetration. The overall mean and standard

TABLE 4: The mean and standard deviation of Mycoblock at different properties and substrates.

Properties	Statistics	CH	SD	Bg
Water absorption (%)	Mean	68.07	58.96	60.87
	Std. deviation	0.67	0.27	0.39
Compressive strength (kPa)	Mean	283.00	605.33	559.67
	Std. deviation	60.36	220.13	103.93
Density (kg/m3)	Mean	292.35	334.11	310.92
	Std. deviation	17.47	10.99	19.89

deviation of physical properties of Mycoblock for three substrates are illustrated in Table 4.

The compressive strength of SD was 9.47% and 56.53% higher than Bg and CH, respectively. Similarly, the density of SD was 10.49% and 9.72% higher than Bg and CH, respectively. Based on this, it can be concluded that the compressive strength of Mycoblock is highly affected by substrate type rather than incubation time differences. The variations between substrate types were due to particle size, particle density, particle water holding capacity, and particle nutritional content. Improving molding type and heat application during the fabrication method could increase the density and compressive strength of Mycoblock by 2 to 3 folds than cold press [50, 53].

Mycoblock obtained in the current study fulfills the mechanical standards for applications in partition, architectural design, and insulation. It could replace polymer-based materials such as expanded polystyrene; the most widely used material for construction, which has the density in the range of 16–48 kg/m3 [54] and compressive strength in the range of 69–400 kPa [7]. The current finding which is about above two folds higher in compressive strength than polystyrene could help in substituting conventional noneco-friendly materials. The mycelium-based block is 49 times cheaper than cement and gypsum-based blocks [55]. It was pointed out that only 18.92 USD is needed per m3 of Mycoblock, whereas 936.87 USD per m3 was needed for cement-based block [18, 55]. Even though it is below the standard of cement-based materials in strength, density, and water absorption, it has also additional, and most significant benefits are the green synthesis approach, local availability, and nontoxicity [13]. The main challenge with Mycoblock technology is the sensitivity issue. Because it is growing rather than manufacturing, the life cycle of the selected strain for the technology is affected by different environmental factors. Know a day's mycelium-based composite is applied in a variety of applications such as packaging, insulation, partition wall, design, and architecture [13].

4. Conclusion

In this study, three substrates such as sawdust, bagasse, and coffee husk were used to produce Mycoblock using a fungal strain *Pleurotus ostreatus*. Aseptic conditions were strictly considered and pH and water contents were managed for the growth of the mycelium and compared with the control. Important parameters including substrate elemental analysis, SEM, FT-IR, density, compressive strength, and water absorption were analyzed to confirm the standard of the Mycoblock. The values obtained from physico-chemical and mechanical analysis varied with different substrates and incubation time periods. Maximum compressive strength and density were 750 kPa and 343.44 kg/m^3, respectively, with a 21 incubation period. Mycelium-based block most significantly benefits due to its low cost, green synthesis approach, nontoxicity, and low environmental emission. Apart from this, mycelium-based blocks have the potential to replace synthetic polymers used for construction materials. Furthermore, the development and expansion of the current study could be used for several renewable and sustainable applications such as wall insulating panels, packaging material, and production of furniture materials, as biodegradable and zero waste alternatives.

Data Availability

All data presented or analyzed during this study are included within the article.

Conflicts of Interest

The authors declare they have no conflicts of interest.

Authors' Contributions

DA contributed to the writing of the article. MT and YG contributed as advisors.

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