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Article

The effectiveness of glass fines on the fire resistance of mycelium-based biocomposites

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SUMMARY

Mycelium-based biocomposites are emerging as a more sustainable alternative to plastic. Hence, they are widely applied in different industrial sectors such as construction, packaging, and manufacturing. Mycelium, the segment of the mushroom containing vegetative fungal threads, acts as a natural adhesive that binds substrate containing waste material, creating a mycelium biocomposite. The alteration of the material within the substrate can modify the properties of the mycelium biocomposite produced. In this experiment, the effects of different weight percentages (0-50wt%) of silica glass fines, small glass particles between 3mm and 8mm in size, on the fire resistance of *Pleurotus ostreatus* mycelium biocomposites were evaluated. The glass fines are rich in silica, enhancing the fire resistance of the composite through forming char and silica ash during heating. Fire reaction properties were tested using a Bunsen burner flame with surface temperature changes measured as a proxy for fire resistance. We hypothesized that when the wt% of glass fines in the substrate is increased, there will be a corresponding increase in fire resistance. The results indicated a significant reduction in surface temperature change at 30wt% and greater, which is attributed to improved thermal stability and silica ash formation. One-way ANOVA and Tukey-Kramer tests further confirmed that increasing the wt% of glass fines significantly enhanced fire resistance. These findings shed light on the potential of mycelium-based biocomposites as renewable materials for semi-structural applications in environments that require resistance to elevated temperatures.

INTRODUCTION

In 2022, 7.39 million tons of trash were generated in Singapore, with amounts estimated to rise exponentially each following year (1). Fortunately, mushrooms efficiently recycle waste as they can turn a sizable amount of dead organic matter into usable material without external energy input (2). As the substrate used alters the properties of the mycelium, mycelium biocomposites can be applicable in various areas of work such as construction, manufacturing and agriculture (2). Therefore, replacing non-renewable materials like plastic with mycelium-based composites would heavily support sustainability.

Oyster mushrooms belong to the Class *Basidiomycetes* of the Family *Agaricaceae* (3). Most *Agaricaceae* are saprotrophic and externally digest dead organic matter into nutrients to use for growth (3). Mycelium is the nutrient-rich vegetative network of root-like fungal threads that break down dead organic material (2). Mycelium forms from mushroom spores and grow underground in long filamentous branches, or hyphae, which, when grouped, create a fuzzy, cotton-like mass (3). Under suitable conditions, mycelium can develop into a fruiting body, which will eventually release fungal spores, repeating the cycle of mycelium growth (3).

Mycelium has the unique capability to convert waste materials such as sawdust and woodchips into usable nutrients (3). The mycelium acts as a natural adhesive that binds the substrate composed of dead organic waste together during growth, forming blocks known as mycelial biocomposites, which have been used as an engineering material (2). For example, mycelial biocomposites can be used as planar materials (e.g. sheets and plastic films), larger low-density material (e.g. plastics and foams), and semi-structural materials (e.g. furniture and flooring) (2). The properties and quality of these composites are dependent on the components of the substrate and the fungi used (4). Therefore, by altering the mycelium substrate, the properties of the biocomposite can be tailored towards its application.

Glass fines are a common additive supplemented to mycelium composites (2). Glass fines are small, round particles of glass between 3mm and 8mm that cannot be efficiently recycled due to the presence of micro-contaminants that are difficult to separate from the glass (5). They can be supplemented to mycelium substrates as a low-cost additive to increase fire resistance, which refers to the ability of a material to withstand burning and prevent the passage of heat when exposed to a flame (6,7). Improved fire-resistance may make the biocomposites more applicable for use in various environments with elevated temperatures, such as bricks for furnaces and chimneys (8). Glass fines contain high amounts of silica (SiO_2), a ubiquitous mineral found naturally in soils and decomposing matter (2). In composite substrates, silica contributes unique thermal properties such as a high melting point, low thermal conductivity, and promotes char formation under heat (2). Because the vegetative network of mycelium binds and transforms substrate material, a silica-rich substrate provides both structural mineral content and thermal resilience (5). Additionally, when exposed to high temperatures, the composites organic components decompose into carbonaceous char, while the silica content remains as silica ash (2). Together, these two residues accumulate on the surface of the composite to produce a thermal barrier that reduces heat transfer and improves thermal stability.

To evaluate the potential of silica-enriched substrates in improving the thermal performance of mycelial materials, we examined to what extent increasing the weight percentage

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(wt%) of silica glass fines (0, 10, 20, 30, 40, 50 %) in mycelium substrate affected the fire resistance of the oyster mushroom *Pleurotus Ostreatus* (*P. ostreatus*) mycelial biocomposites. We quantified heat resistance by percentage change in temperature (%) when exposed to a Bunsen burner flame. *P. ostreatus* greatly enhances the compressive strength of its biocomposites compared to other mushrooms and are the most used fungal species in mycelial biocomposites (2).

Silica-rich additives like glass fines and rice hulls have been shown to enhance thermal stability through the promotion of carbonaceous char and silica ash (9). We therefore hypothesised that when the wt% of glass fines in the substrate is increased, there will be a corresponding increase in fire resistance, with decreased temperature change as a proxy.

Our results demonstrated a significant improvement in fire resistance at 30wt% glass fines and above, supporting the hypothesis that silica enrichment enhances the thermal stability of *P. ostreatus* mycelial biocomposites. These findings suggest potential for silica-enriched mycelial biocomposites to be used as fire-resistant, sustainable alternatives in future construction and packaging applications.

RESULTS

The investigation involved two parts: growth and heating. Mushroom spores obtained from an oyster mushroom growing kit were inoculated into a nutrient-rich, sterilized substrate and grown for an extended period. Materials within the substrate include hardwood sawdust, hardwood chips, millet, glass fines and agricultural lime (**Table 1**). After incubation, the substrate, now bonded together by mycelium and grown as a 500g substrate block, was cut into pieces of equal size around 2 - 3 cm. They were heated in the oven to denature the mycelium before being tested for fire resistance, quantified by percentage change in surface temperature following flame exposure. Samples were suspended 10 cm above the flame, heated on the underside for 20 seconds with surface temperature measured on the exposed face using a laser thermometer. The experiment was conducted in an air-conditioned room, which explains the low initial temperatures, ranging from 18.5°C to 21.8°C across all groups and displaying no significant temperature differences prior to heating (**Table 2**). Upon heating, the 0wt% glass fines group increased from an average initial temperature of 19.3°C to a final temperature ranging from 71.2°C to 81.0°C. By contrast, for the 50wt% group, the final temperatures were significantly lower, ranging from 49.7°C to 63.8°C, with one outlier value of 90.2°C.

When heated with the Bunsen burner flame, the biocomposite's surface began forming a thin layer of black substances. This was the formation of char and silica ash, which formed due to the mycelium block being exposed to high heat. As initial temperatures for each trial were different, the percentage change in temperature was calculated for each temperature (**Table 3**). For measuring variability, standard deviation was used as it consistently yielded higher uncertainty estimates than the laser thermometer's stated accuracy.

The control group (0wt% glass fines) had the largest average temperature change of 285% while the average change in temperature of the 50wt% glass fines biocomposite was significantly less at 186% **(Figure 1)**. The most substantial drop occurred between the 20wt% and 30wt% glass fine groups, with a decrease in average temperature change from 240% to 204%. These results demonstrate that as the weight percentage of glass fines increases, the average temperature change decreases. However, the relatively large size of the standard deviation indicates that there was large error present within the experiment, particularly for the 20wt% and 40wt% results. The most significant average temperature change was between 20wt% and 30wt% temperatures, indicating a threshold effect whereby fire-resistance improves after glass fines reach 30wt%. The processed temperature changes for each wt% replicate and their corresponding standard deviations demonstrate a consistent reduction in mean temperature change as glass fine concentration increases beyond 20wt% (**Table 3**).

For further analysis, we ran ANOVA and Tukey-Kramer tests to identify both whether the temperature difference is significant, and which specific groups significantly differ from one another. The ANOVA test indicated a statistically significant difference (p = .002). Following this, a Tukey-Kramer post hoc test identified which specific group differences were significant. It showed that significant improvements in fire resistance only emerged at or above 30wt% glass fines when compared to the control (0wt% glass fines) mycelium. As the P-value was less than the q-value, the ANOVA test declared there was a significant difference in the fire resistance of *P. ostreatus* biocomposites across different wt% of glass fines. Additionally, the Tukey-Kramer test showed that the wt% pairs 0% – 30%, 0% – 40%, 0% – 50%, 10% –40% were all significantly different (**Figure 1**). Therefore, it can be concluded that by increasing the weight percentage of glass fines by at least 30wt% will there be a significant change in fire resistance.

DISCUSSION

The investigation results are consistent with previous literature. A study examining the fire resistance of mycelial biocomposites with substrates containing glass fines has demonstrated that adding 50wt% glass fines would substantially increase fire resistance (9). These findings correspond with our results, displaying that the fire resistance

Material Name	Amount of material for each weight percentage (%)						Total Mass Required (± 0.1g)
	0 (± 0.1g)	10 (± 0.1g)	20 (± 0.1g)	30 (± 0.1g)	40 (± 0.1g)	50 (± 0.1g)	
Sawdust	285.0	256.5	228.0	199.5	171.0	142.5	1282.5
Wood Chips	140.0	126.0	112.0	98.0	84.0	70.0	630.0
Millet	75.0	67.5	60.0	52.5	45.0	37.5	337.5
Glass fines	0.0	50.0	100.0	150.0	200.0	250.0	750.0
Total Mass	500.0	500.0	500.0	500.0	500.0	500.0	3000.0

Table 1: Weight requirements of substrate materials at different weight percentages of glass fines. The table shows the mass (± 0.1 g) of each component (sawdust, wood chips, millet, and glass fines) used to prepare mycelial biocomposites at 0–50wt% glass fines. "Total Mass" for each substrate was fixed at 500 g per replicate, with the cumulative requirement of each material provided in the final column.

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Weight %	Temperature of mycelial blacks before and after flame exposure									
	Trial 1 (± 0.1°C)		Trial 2 (± 0.1°C)		Trial 3 (± 0.1°C)		Trial 4 (± 0.1°C)		Trial 5 (± 0.1°C)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
0%	19.5	73.7	19.8	81.0	19.2	72.3	18.5	71.2	19.4	72.8
10%	19.6	73.6	19.8	75.3	20.3	81.1	19.6	62.8	19.8	70.3
20%	20.0	62.3	19.5	83.8	21.8	60.0	19.6	63.7	19.9	70.8
30%	19.8	61.8	20.2	64.6	19.6	53.3	19.2	50.5	19.5	67.7
40%	**19.7***	**89.9***	21.3	67.6	19.6	65.4	19.4	44.8	19.4	51.2
50%	19.1	56.5	20.8	63.8	19.4	49.7	**21.2***	**90.2***	20.2	57.6

Table 2: Raw data of initial and final surface temperatures of mycelial biocomposites for each wt% of glass fines. "Initial" represents the surface temperature of the biocomposite recorded prior to flame exposure, and "Final" refers to the temperature taken immediately after 20 seconds of exposure to a Bunsen burner flame. Wt% refers to the percentage by mass of glass fines relative to the total dry mass of the composite. Measurements were taken using a laser thermometer with values reported to three significant figures. Values with an asterisk indicate statistical outliers determined using the IQR rule (14).

properties of mycelial biocomposites with glass fines were significantly greater at 30wt% and above. Despite changing the substrate component instead of the weight percentage, this literature similarly reported that silica incorporation improves the fire reaction properties of mycelial biocomposites, aligning with the trend observed in this study (9). Furthermore, other studies reasoned that adding glass fines contributed to the high silica concentration in the substrate, thereby increasing the fire-retardant and fire-resistant properties of the composite (2). Therefore, as the investigation and similar studies have obtained consistent results despite the varying equipment and methodology, this knowledge could support developments of fire-resistant building materials, providing promising alternatives to traditional materials that may improve sustainability while avoiding the use of polystyrenes or foams that can pose environmental concerns when burnt.

Considering the investigation studied the effects of fire resistance rather than heat resistance, manufacturers must be able to distinguish between the two. As heat encompasses various forms besides just fire, other manifestations of heat may not yield similar results as those found in the investigation (7). Consequently, understanding this disparity is pivotal for accurately assessing product performance and ensuring safety standards are met across different heat-related scenarios.

The data depicts a significant change in fire resistance as the wt% of glass fines increased. The statistical tests accepted the alternative hypothesis and measured a significant difference from substrate levels 30wt%, 40wt% and 50wt%, which signifies that higher wt% of glass fines display greater increases in fire resistance. The sharp decline in average temperature change between 20wt% and 30wt% glass fines supports the results of the statistical results showing that significant improvement occurs from 30wt% and greater (**Figure 1**). Hence, the amount of glass fines could be tailored depending on the application. For example, 30wt% glass fines may be applicable in situations with less intense fires, while 50wt% glass fines may be used in situations with close-proximity flames. Higher amounts of glass fines would correlate with higher production of silica and char when under intense flames (2), which act as functional fire retardants that improve the fire resistance of the composite material during heating (8).

However, as the mycelial blocks were not heated for prolonged periods and not ignited in flames due to safety concerns, it cannot be assured that the mycelial blocks will withstand high heat for extended periods. These may have been sources of systematic error (**Figure 1**). Moreover, the insubstantial amounts of silica ash and char produced during this short heating time may not have been enough to display the extent of their fire-retardant effects, resulting in potentially unreliable results and suggesting a promising direction for further research. In addition, the mycelium block had an uneven surface which may have caused errors and outliers in heat measurement. This was due to difficulties in positioning the laser thermometer and the variations in heating distance, potentially reflected by anomalous results (**Table 2**). Despite these errors, the processed data displayed the efficacy and significance of glass fines in enhancing fire resistance.

In conclusion, there was a clear change in the fire resistance as the amount of glass fines increased. The statistical tests found that only by adding at least 30wt% of glass fines to the substrate would there be a significant difference in fire resistance. To further investigate this topic, a longer exposure time to flames could be explored to determine the extent to which silica ash and char act as a fire retardant. Based on qualitative observations, a small amount of silica ash and char was formed from the heating, which plays a crucial role in flame resistance (2). An exploration which involves controlling the weight percentage of glass fines but changing the exposure time to flame could provide greater insight into the effectiveness of silica ash and char as fire retardants, expanding the scope of the investigation through improving our understanding of how and if the fire-retardant properties of these mycelial composites are enhanced over time. With this information, future manufacturers may replace non-renewable thermoplastics with flame-resistant mycelium biocomposites, allowing them to emerge in structural applications across various industries.

MATERIALS AND METHODS

Substrate requirements

Polypropylene bags (TonGass, Amazon ASIN:

Weight wt%	Percent Change 1 (%)	Percent Change 2 (%)	Percent Change 3 (%)	Percent Change 4 (%)	Percent Change 5 (%)	Average (%)	Standard Deviation
0%	278	309	277	285	275	285	14.0
10%	276	280	300	220	255	266	30.4
20%	212	330	175	225	256	240	58.3
30%	212	224	172	163	247	204	35.4
40%	**356***	217	234	131	164	187	47.5
50%	196	207	156	**325***	185	186	21.9

Table 3: Processed data displaying the percentage change in initial and final surface temperatures of mycelial biocomposites for each wt% of glass fines. Averaged values report the mean percentage change for each wt% of glass fines, excluding values with an asterisk identified as statistical outliers determined by the IQR rule. Standard deviation was calculated for all wt% values to indicate the variability across the values.

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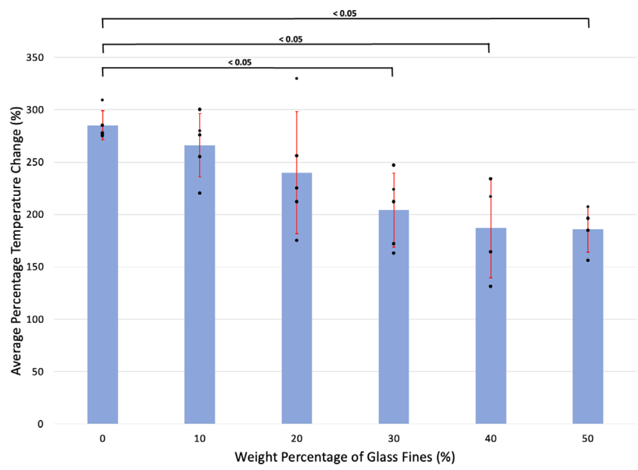

Figure 1. Glass fines reduce temperature change in mycelial biocomposites under flame exposure. Bar graph showing the average percentage temperature change (± standard deviation) of *P. ostreatus* mycelial biocomposites with 0–50wt% glass fines. Each bar includes individual datapoints (n = 5 biological replicates per wt%). Composites were exposed to a Bunsen burner flame for 20 seconds, and surface temperature change was measured using a laser thermometer. A one-way ANOVA indicated a statistically significant difference between groups (p = 0.00155). Tukey-Kramer post hoc analysis showed significant reductions in temperature change at ≥ 30wt% glass fines compared to 0wt%.

B08P2RWZXF) that were sufficient for containing 500 g of substrate for mushroom spore growth were used. Oyster mushroom substrate was composed of sawdust (LoranTrad, Etsy Listing ID: 1320911826), wood chips (Camerons Products, Amazon ASIN: B000RU01VG) and millet (Simply Natural, Amazon ASIN: B07B9VHKLH) in a ratio of 57:28:15 respectively for efficient mycelium growth (10). Glass fines (Lechloris, Amazon ASIN: B0CXDTYBCS) were incorporated at 0%, 10%, 20%, 30%, 40% and 50% by weight of the total 500g substrate mass. As the mass of glass fines takes priority, this ratio will dictate the proportions for remaining components of the substrate after incorporating the glass fines while maintaining a constant total mass of 500g (**Table 1**).

Substrate preparation

Substrate materials for the 0wt% condition were measured using a digital scale and added into a glass beaker. The materials were then transferred into a container and distilled water was incrementally poured into the container and mixed with the substrate until a moist clump formed that did not drip when squeezed (11). The mixed substrate was then poured into a polypropylene bag. This process was repeated for the remaining five weight percentages of glass fines. 10g of agricultural lime (Hi-Yield, Amazon ASIN: B0CYJR6BJT) was added to each bag to adjust the pH for optimal mycelial growth (12). The pH was tested using a pH meter with the optimal pH level for the substrate being around ~pH 7.8 (12). Bags were temporarily sealed and sterilized in an autoclave at 121°C for 120 minutes at 15 psi (11).

In a biosafety cabinet, 10g of mushroom spawn from an oyster mushroom growing kit (Forest Origins, Amazon ASIN: B08W9XH89M), was extracted using a spoon and digital scale. The bags were unsealed one at a time, and the spawn was thoroughly mixed into the substrate using a spoon before resealing. This was repeated for all six bags. Each bag was then labelled with its respective weight percentage using a permanent marker.

Incubation

After substrate preparation, all bags were placed in the same dark cupboard and left to incubate at standard atmospheric conditions (25°C and ≥98% humidity) to promote hyphal growth. Each bag received 59 mL of distilled water every two days to maintain consistent moisture levels. Growth was monitored over a 17-day period until full colonization of the substrate by mycelium was observed **(Figure 2)** (13). Although the mycelium itself does not possess flame-retardant properties and serves only as a binding agent, inconsistent colonization could affect thermal stability, as sparse substrate may degrade more rapidly under heat.

Testing the fire resistance

Substrate blocks were carefully removed from the bags. Each block was cut into five equal pieces and placed in an oven at 180°C for two hours to denature the fungi and halt further growth (8). This step also ensured that absorbed moisture was removed to prevent inaccurate temperature change measurements during testing. Equipment for the combustion process was assembled. Each substrate sample was suspended 10 cm above the Bunsen burner using a tripod stand and a clay-pipe triangle. The clay-pipe triangle supported the sample securely while allowing heat to rise evenly from the flame without obstruction. The burner was positioned so the tip of the flame nearly reached, but did not directly touch, the underside of the sample, ensuring consistent heating by convection rather than combustion. The surface temperature was measured after 20 seconds. This duration allowed for the formation of silica ash and char, while preventing the composite from catching fire. Tongs were used to remove each piece, and the process was repeated for the remaining four pieces of the block. The entire procedure was

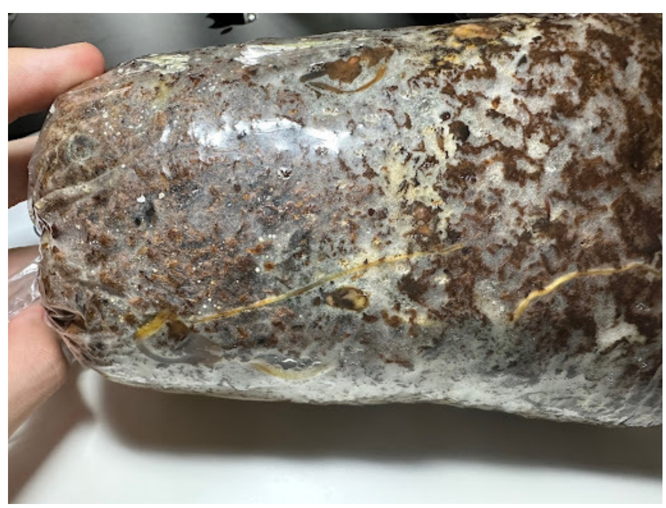

Figure 2: Close-up image of substrate colonization by mycelium and mushroom fruiting bodies. The polypropylene bag contained brown substrate, white thread-like mycelium and the yellow mushroom fruiting bodies.

then repeated for the other five substrate bags.

Statistical tests

We ran an ANOVA and Tukey-Kramer test to determine whether temperature differences were significant as well as which specific groups significantly differ from one another. Due to the limited sample size and discrete weight percentage intervals, correlation tests such as Spearman's or Pearson's were not suitable. Instead, a one-way ANOVA test was conducted to evaluate whether the fire resistance (measured by temperature change) differed significantly across the six groups with varying glass fines concentrations.

The one-way ANOVA was conducted at a 95% confidence level ($\alpha = 0.05$) to determine whether differences between the groups were statistically significant.

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