Construction and Building Materials 421 (2024) 135566

Contents lists available at ScienceDirect

Construction and Building Materials

journal homepage: www.elsevier.com/locate/conbuildmat

Increased CO_2 fixation and reduced embodied energy of mycelium bio-composite materials grown on a mixed substrate over diurnal temperature cycles

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ARTICLE INFO

Keywords:
fungi
mycelium
sustainable building material
bio-composite
embodied energy
CO_2 sink

ABSTRACT

There is a pressing need for alternative construction materials that can facilitate the transition to a sustainable circular economy. Mycelium-based bio-composites are an example of such materials. They have a low embodied energy compared to concrete-based materials and commercial thermal insulators, and act as a net CO_2 sink. So far, mycelium materials have been produced using homogenous substrates and by growing at a fixed temperature. Growth at a fixed temperature accounts for 73% of the embodied energy and more than 40% of the CO_2 emissions. Here, mycelium bio-composites were grown using temperature cycles mimicking ambient temperature conditions during an Israeli transition season or a summer day in the Netherlands without impacting material qualities or time of production. These results verify a possible strategy to dramatically reduce energetic and CO_2 cost of mycelium materials fabrication, and the findings imply that monolithic structures can be grown *in situ* at outdoor construction sites. The use of mixed substrates allows a wide range of final properties that can be tuned by the composition. Also, thermal conductivity values as low as 0.026 W m^{-1} K^{-1} were obtained by growing the mycelium bio-composite on mixed or homogenous substrates. These results show that mycelium materials with superior thermal insulation properties can be grown at ambient temperature using mixed as well as homogenous waste streams.

1. Introduction

Linear processes, working by the paradigm of cradle to grave, are responsible for the majority of industrial greenhouse gas emissions [1–4]. The extraction and processing of raw materials from nature is enormously energy-intensive and contributes to rising levels of CO_2 in the atmosphere and consequent climate disruption [5–7]. Moreover, the disposal of products that are not biodegradable consumes landfill area and results in the deposition of toxic chemicals and micro-plastics in the ground and water bodies [8,9]. In order to transition to a circular economy, we must learn from nature, where there is no such thing as waste. Waste streams should be used to make new materials, that again can be recycled or that are biodegradable at their end of life. Mycelium based bio-composites meet these requirements. These materials are made from agricultural waste streams bonded together by the fungal mycelium that has colonized the plant-based substrate. Agricultural waste is abundantly available; for instance, straw represents about 60% of the plant weight of crops like wheat and corn [10,11] and is an excellent starting point to make mycelium bio-composites. Other substrates for fungal growth include by-products from fruit orchards, grapevines, and municipal pruning. In Israel, about 900,000 tons of agricultural residues are being produced annually and only 30% of it is being re-used in some way [12], while the rest is being burnt in the field or discarded. Thus, a great amount of waste is available as raw material for production of mycelium bio-composites, which could replace fossil- or cement- based materials.

Mycelium based bio-composites have properties that range from foams (e.g. polystyrene) to natural materials (e.g. wood) [13–15]. The

Abbreviations: EPS, extruded polystyrene; H, hemp shives; S, rapeseed straw; C, processed cellulose; RH, relative humidity, HCB, hollow concrete block; AAC, Aerated autoclaved concrete.

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https://doi.org/10.1016/j.conbuildmat.2024.135566
Received 14 August 2023; Received in revised form 11 February 2024; Accepted 21 February 2024
Available online 4 March 2024

foamy materials have excellent thermal and acoustic insulation properties, which is relevant for the construction industry. They can also absorb mechanical impact, which is important for its use as packaging [2,16,17]. Notably, mycelium bio-composite materials have a low embodied energy and act as a CO_2 sink [18]. Post-processing in the form of heat-pressing the material increases the mechanical strength dramatically, about 20–25 folds, and makes the material to behave as wood-like material [13]. So far, properties of mycelium bio-composites have been tuned by varying the fungal species or the type of organic waste stream [13,17,19,20]. Using hemp shives or wheat straw as a substrate results in a mycelium bio-composite with good thermal insulation (0.04 W m^{-1} K^{-1}) and low density (~100 kg/m^3) [16], while Alaska birch sawdust (particle size < 5 mm) results in higher density (~250 kg/m^3) and higher thermal conductivity (0.06 W m^{-1} K^{-1}) [21]. Water uptake by the material also depends on the substrate, with the weight increase at saturation varying from 43% for mycelium bio-composites grown on beech sawdust to 436% for those grown on rapeseed straw – and concurrent dimensional expansion ranging from 6.72% to 24.24% [13]. These differences are explained by the porosity of the mycelium bio-composite and its surface hydrophobicity [13].

So far, mycelium composite materials have been reported based on a single substrate and by growing at a constant defined temperature [13,16,22–24]. This has clear disadvantages. First, growth at a constant temperature in an incubation room is the most energy intensive step in the process, accounting for about 73% of the overall process energy, and more than 40% of the carbon emissions [18]. Second, the use of incubation rooms may limit the size of a mycelium bio-composite object. Growing at ambient conditions can save energy and allow for cast-in-place method to be applied in monolithic structures. Third, some substrates may have excellent fiber structures but limited nutritional value and vice versa. Thus, mixing different substrates can enable us to tune the properties to the desired use, controlling substrate strength and nutritional values. In addition, using more than one substrate to produce the material increases the volume of raw materials for the industry, which is particularly relevant when aiming for industries with infinite quantities of products as in the construction and packaging industries.

In this paper, the influence of alternating temperatures on the growth of mycelium bio-composites in both pure and mixed substrates was examined. Temperature cycles were set to mimic ambient temperature conditions during an Israeli transition season or the summer season in the Netherlands. Hemp shives, rapeseed straw and processed cellulose from sewage treatment plants (ReCell®) were used as pure or mixed substrates. The results show the synergistic effect of mixed substrates, with no degradation of the mechanical properties while retaining excellent thermal properties. The alternating temperature samples grew almost at the same rate as the samples at 25°C, while keeping the same mechanical and thermal properties, indicating the feasibility of growing the material in ambient conditions. Integrating the strategy of growing at ambient temperature to a former published LCA model [18], the results show that the embodied energy of mycelium bio-composite materials can be reduced to levels 11- to 15- fold lower than expanded polystyrene (EPS), while the CO_2 sink activity can be further increased by almost 4-fold, sequestering about 150 kg CO_2eq m^{-3}.

A brief overview of the research structure:

This paper consists of three experimental stages (see also Table 1):

1) Working with 3 temperature conditions (constant 25°C; alternating between 25°C to 15° C; constant 15°C), and 7 substrate types (hemp, straw, cellulose, hemp-straw, hemp-cellulose, straw-cellulose, hemp-straw-cellulose). The motivation for stage 1 was to check the possibility to grow mycelium materials at ambient temperature conditions, which would save the energy use of the incubator (about 73% of the process energy [18]), and to grow monolithic structures onsite (i.e. a whole wall). Using mixed substrates can allow to tune the final properties of the material, and increase the amount of raw materials when compared to homogenous substrate.

2) examination of the straw-cellulose composition in different ratios. The results from stage 1 led us to focus on the straw-cellulose combination, as a composition that combines mechanical strength with low thermal conductivity. Samples were grown at 7 different ratios in order to determine the influence of the ratio over the thermal and mechanical properties of the final material. The motivation was to check if there is an ideal ratio.

3) The results of stage 2 led us to choose the 1:1 ratio of straw and cellulose. In stage 3 we further investigated the influence of different temperatures on the growth of the samples, and their final properties. We worked with 7 different temperature conditions, five constant and two alternating over a daily cycle (5, 10, 30, 35, 40, 5–25 and 25–40°C). The motivation was to further investigate the possibility of growing at ambient temperatures, this time mimicking a hot summer day and a winter day in Israel. The constant temperatures were used as control experiments.

2. Material and methods

2.1. Culture conditions

Trametes hirsuta [25] was grown as biological triplicates on pure hemp shives (H) (Kanabat, La Chanvrière, France), rapeseed straw (S) (Gedizo trading int., Netherlands), processed cellulose (C) (ReCell®, Netherlands) or 1:1 or 1:1:1 w/w mixtures thereof (HS, HC, SC, and

Table 1
Outline of the experimental stages carried out in the research.

Stage	Temperature	Substrate	Time	Measurements	Rational	Motivation
#1	25°C 15–25°C 15°C*	H, S, C, HS, HC, SC, HSC	14 days *25 days	Thermal properties; Mechanical properties; Water absorption; Expansion in high humidity	Is it possible to grow mycelium materials at ambient temperature of an Israeli transition season/ Dutch summer. Learn about growing mycelium materials on heterogenous substrates.	Saving 73% of the fabrication energy; Growing cast-in-place structures; Tuning of final properties by the substrate; increasing the amounts of raw materials.
#2	25°C	S:C ratios: 100:0, 85:15, 70:30, 50:50, 30:70, 15:85, 0:100	14 days	Thermal properties; Mechanical properties;	Assess how the S:C ratio influences the thermal and mechanical properties.	Showing the range of properties possible from the combination of two substrates. Finding "favorite" composition for certain purposes.
#3	5–25°C (+) 25–40°C (-) 5°C (-) 10°C (-) 30°C (+) 35°C (-) 40°C (-)	S:C (1:1)	14 days	Thermal properties; Mechanical properties; Water absorption; Expansion in high humidity	Expanding the temperature range, mimicking an Israeli summer and winter day. Adding control points of constant temperature conditions along the temperature cycles.	Extending the possible growth at ambient conditions to a longer period, or more climate regions, and learn the temperature limits of it.

* In stage 3, the temperature conditions that marked with (+) have grown well within 14 days, while the ones marked with (-) did not grow.

HSC). The hemp shives and the rapeseed straw were ~1 cm long, while the cellulose consisted of 0.03–0.05 mm fibers in 1–2 mm bundles. The substrate was mixed with water in a 3:7 ratio (w/w), transferred to a 280 mL microbox (SacO2, Belgium), sterilized for 30 min at 121 °C and inoculated with sorghum-based spawn (3% spawn wet weight per substrate dry weight). The microbox was then placed in an incubator at 5*, 10*, 15, 25, 30*, 35*, or 40* °C or using temperature cycles. An Israeli transition season or a summer season in the Netherlands were mimicked with the following cycle: from 15 °C to 25 °C in 8 h; keeping 25 °C for 4 h; from 25 °C to 15 °C in 8 h; keeping 15 °C for 4 h, while an Israeli summer and winter season were mimicked by similar cycles between 25 and 40 °C* and 5 and 25 °C*, respectively (Fig. 1) (asterisks indicate temperature that were used for SC substrate only). Samples were taken out of the microbox after growing for 14 days, were air-dried for 3 days, and treated at 60 °C for 2 h to inactivate *T. hirsuta*. Resulting materials can be seen in Fig. 2A. Samples that were constantly incubated at 15 °C did not sufficiently grow within 14 days and therefore growth was extended to 25 days. Similar experiments were done at 25 °C by mixing 15, 30, 50, 70 and 85% straw with 85, 70, 50, 30, 15% cellulose, respectively.

2.2. Thermal insulation and mechanical properties

Thermal insulation properties of the mycelium bio-composites were determined using TEMPOS (METER, USA) according to ASTM D5334 [26]. Heat capacity was determined with the SH-3 sensor, using a two min measurement, and probing samples at three different places (cylinder top, side, and bottom). This was followed by determining the thermal conductivity by inserting the KS-3 sensor, using a 1 min measurement, at three different spots in the cylinder side wall. The measurement deviates from the standard by using lower current to induce slower heat accumulation, as recommended for insulating materials. The results presented are the average of nine measurements, three measurements from each sample of biological triplicates. Mechanical properties of the samples were measured after heat pressing the mycelium bio-composites (Fig. 2B). Heat-pressing is a post processing method that was used before, but compression properties were not measured [13]. To this end, samples were pressed at 28.25 MPa and 150 °C for 5 min (custom made steel mold, 3889CE.4PR0000 hot press, Carver Inc., USA). Compression of the pressed mycelium composite was measured using an Instron 3380 testing system following ASTM D3501–94 [27], with a rate of 0.5 mm min^{-1}. The elastic modulus was calculated from the initial linear part using linear regression. Strength of the materials was measured at 20% strain, to allow comparison to published compression results of mycelium materials [24].

2.3. Water absorption and expansion at high relative humidity

Water absorption and expansion of the materials were measured according to ASTM C1585 [28] (deviation from the standard is detailed below) using biological triplicates of the heat pressed samples (see above). Samples were placed in containers filled with distilled water maintained at 25 °C assessing the weight after 0.5, 1, 2, 5, 11 and 24 h. The specimens were immersed in water to half of their height. For each measurement, samples were removed from the water surface, manually removing the superficial water with paper towel, and weighed immediately. Some of the specimens had disintegrated during the experiment, and only specimens that kept the structure to the end were included in the results. For expansion measurements, samples were dried at 60 °C, after which they were incubated at 22 °C and 85% relative humidity (RH). Thickness (at 3 positions per sample) and weight were measured after 0, 0.5, 1, 2, 5, 11 and 24 h.

2.4. Statistical analysis

Statistical analysis was done using One-way ANOVA ($p \leq 0.05$) (Analysis ToolPak in Excel) to examine whether the substrate or temperature influenced the fungal material's property. For example, for the thermal conductivity of materials grown on seven different substrates and at three different temperatures, 10 ANOVAs were done. Three ANOVAs were done for each temperature condition to determine if the different substrates result in different thermal conductivity. Another 7 ANOVAs were done on each substrate separately to see if the temperature has an effect on the thermal conductivity. The ANOVAS were followed by a t-test (two-tailed distribution, heteroscedastic, $p \leq 0.05$) to distinguish statistical groups.

3. Results and discussion

3.1. Thermal insulation and mechanical properties of mycelium composites grown at different temperature and using different substrates or mixtures thereof

T. hirsuta was grown for 14 days at 25 °C, a 15–25 °C cycle (Fig. 1), or for 25 days at 15 °C on hemp shives (H), rapeseed straw (S), processed cellulose (C) or 1:1 or 1:1:1 w/w mixtures thereof (HS, HC, SC, and HSC, respectively). The thermal conductivity of the resulting composites was between 0.026 and 0.042 W m^{-1} K^{-1} except for C that showed significantly higher values irrespective of growth temperature (0.058–0.068 W m^{-1} K^{-1}) (Fig. 3A; Table 2). Differences in thermal conductivity were also found between the other substrates. For instance, conductivity was significantly lower when the mycelium had been grown at 25 °C on S (0.026 W m^{-1} K^{-1}) when compared to growth at the same temperature on H, SC, or HSC (0.031–0.032 W m^{-1} K^{-1}). Growth

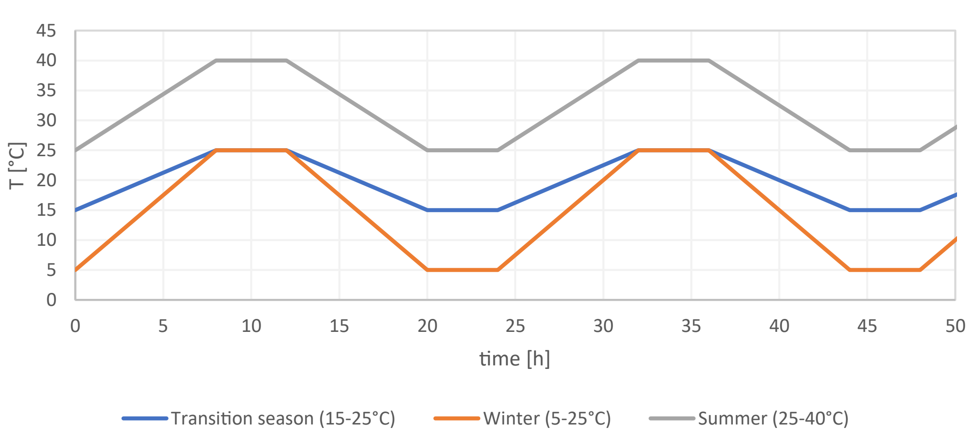

Fig. 1. Temperature cycles used in this study mimicking seasons in Israel.

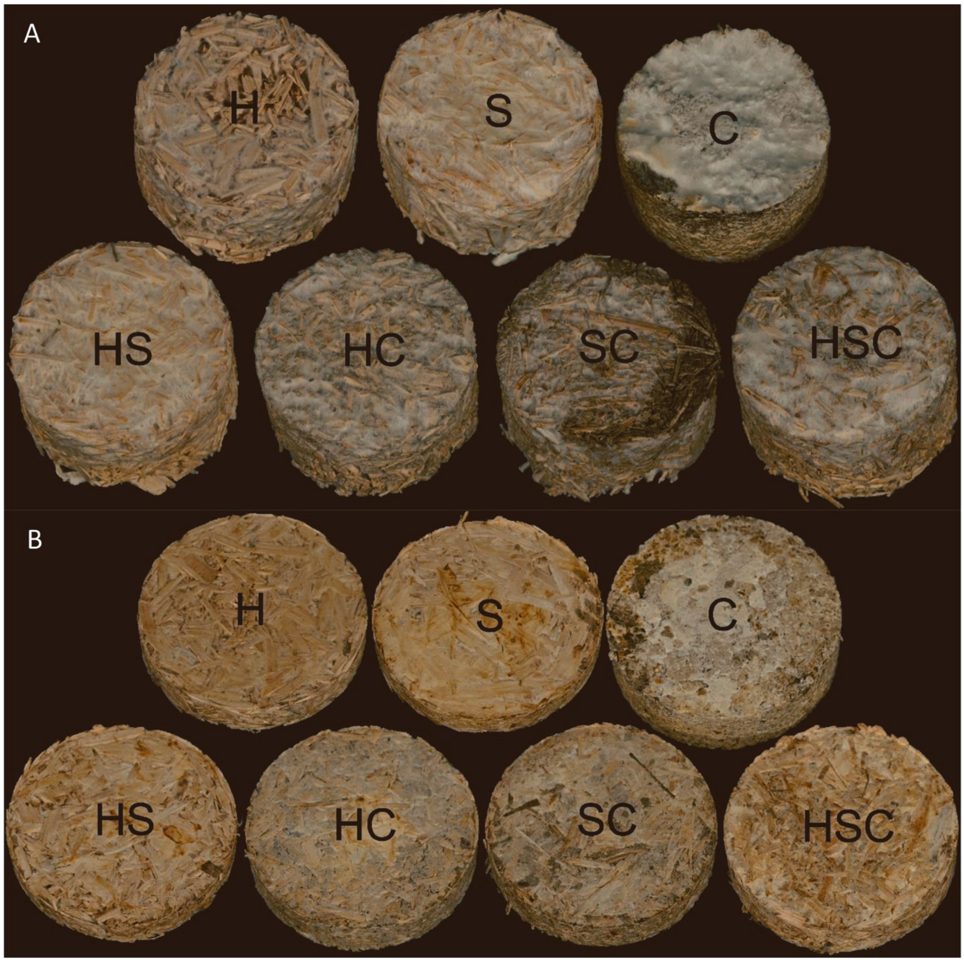

Fig. 2. Mycelium bio-composites grown on hemp (H), rapeseed straw (S) and cellulose (C) and combinations thereof before (A) and after (B) heat-pressing.

temperature had no effect on thermal conductivity when H or S were used as a substrate with values of 0.030–0.031 and 0.026–0.030 W m^{-1} K^{-1}, respectively (Fig. 3A). Growth temperature did have an effect in the case of the other substrates. For instance, thermal conductivity of C-based materials was lower when grown at 25 °C compared to 15 °C (0.058 and 0.068 W m^{-1} K^{-1}, respectively). Mixing H or S with C yielded conductivities much closer to H or S than to C. Heat capacity reflected the same trends as the thermal conductivity (Fig. 3B; Table 2). C based samples grown at 25 °C, 15–25 °C and 15 °C had a higher heat capacity with values of 752, 718 and 812 kJ m^{-3} K^{-1}, respectively, than the other substrates that ranged between 489 and 616 kJ m^{-3} K^{-1}. Growth temperature had no significant effect on heat capacity (Fig. 3B). Together, it is concluded that the thermal properties are not affected by the temperature cycle when compared to growing at 25 °C. Thermal properties are mainly influenced by the air cavities in the material, and growth temperature is expected to mainly influence the growth rate of the mycelium. Thus, there does not seem to be a connection between the growth rate and the amount of air cavities inside the material. The substrate, on the other hand, does influence the thermal properties. A finer substrate (C) will have a higher packaging factor, and, as a consequence will leave less air cavities to insulate. On the other hand, more gross substrates (H and S), which also have air pockets inside them, have a much lower packaging factor, and hence many more air cavities. The results from the mixed substrate show behavior more similar to the gross substrate, which imply that many of the air cavities are being kept emty, and do not fill with cellulose. Moreover, the results show that we can tune the thermal properties by mixing substrates, and thus achieve a certain amount of cavities that will translate to a desired thermal conductivity.

Samples were heat pressed and their compression strength was determined. Failure was absent for all samples even at a strain as high as 50% (see Fig. 4 for typical stress strain curves). Compression strength was between 2.5 (H-based material grown at 25 °C) and 5.7 MPa (C-based material grown at 15 °C). Statistical analysis showed that stress at 20% strain was influenced by the substrate used (Fig. 3C; Table 2). For instance, H-based materials grown at 25 °C were weaker than all other materials grown at the same temperature condition. Temperature impacted the compression strength in the case of H, HS, SC, and HSC materials. For instance, H-based materials grown at 25 °C (2.5 MPa) were weaker than those grown at 15–25°C (3.1 MPa). Similar results were obtained when the elastic moduli were compared. They ranged between 24 and 53 MPa (Fig. 3D; Table 2). The influence of the process temperature can influence the strength of the materials by the growth of the fungus in the given time. Since mycelium is the binder of the organic aggregates, it is understandable that more developed growth will lead to improved mechanical properties. The Maillard Reaction may be involved in forming a stronger material, with increasing cross-linking with higher mycelium content. What we see in the results is no significant difference between the constant and the alternating temperature conditions. Thus, the growth of mycelium is expected to be similar. The results indicate that the substrate does have a role in determining the mechanical properties. We see that growing the mycelium materials on a mixed substrate can strengthen it. For instance, straw-cellulose (SC) mixtures had a compressive strength of 4.5 MPa and elastic modulus of elasticity of 40 MPa, as compared with 4.2–4.3 MPa and 36–37 MPa respectively for materials produced with S or C only. These can be

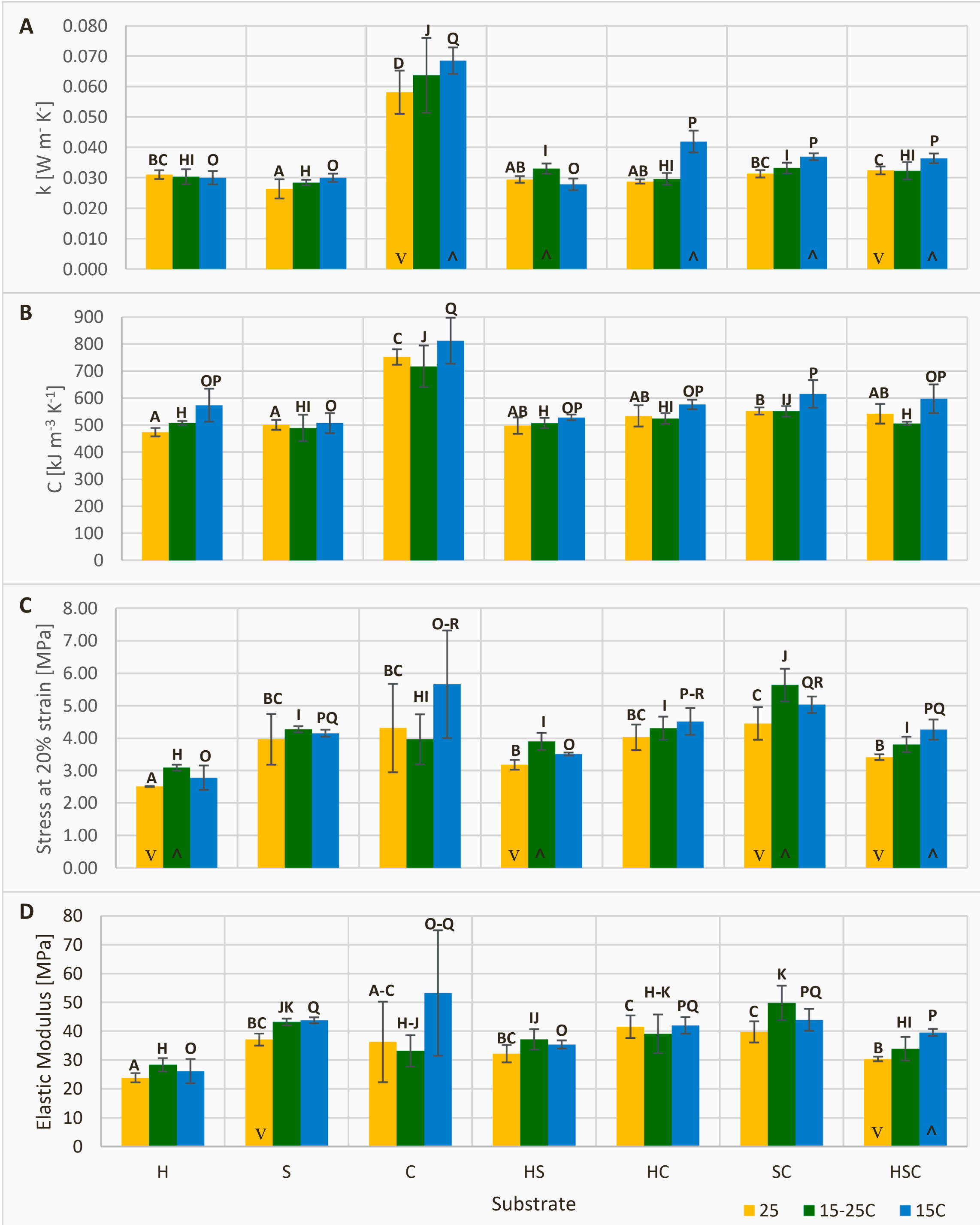

Fig. 3. Thermal conductivity [W m· K·] (A), heat capacity [kJ m^{-3} K·] (B), Strength at 20% strain [MPa] (C), and elastic modulus [MPa] (D) of the mycelium composites made with different substrates and at different growth temperatures. Samples at 15 °C did not grow sufficiently after 14 days and were therefore grown for 25 days. Bars represents standard deviation. Letters (A-D, H-K, and O-R) represent statistical groups for each of the growth temperatures. ^ or v mark a sample within a substrate that has a higher or lower value, respectively, than the other two samples of the same substrate. If two samples were significantly different from each other but not from the third one, both ^ and v were used to indicate which showed a higher and lower value, respectively.

attributed to two main reasons- 1) the nutrition level of the substrate, which support the mycelium growth in the substrate, and result in better binding of the particles. The final result is higher strength. 2) the hierarchical structure of the material and the density of the substrate- straw and hemp are stiff and gross, with particles about 1 cm in length, which yield many air pockets in the structure. The cellulose, on the other hand, is very fine with particles one or two orders of magnitude smaller, and leaves very little voids when soaked with water. The bigger particles act as macro-particles that resist compression loads but leave large cavities in the material that can be filled with cellulose fibers. Therefore, cellulose has higher values in the mechanical properties than the straw and hemp. Here again we see the potential on tuning the properties to the desired values by combining different substrates, as can be seen in the mixed substrates results.

Table 2
Properties of mycelium bio-composites that were grown on different substrates for 14 days at 25 °C or using a 15–25°C temperature cycle or for 25 days at 15 °C. ± represents standard deviation. Statistical analysis was performed for each growth temperature using the.

	Thermal conductivity [W m-1 K^{-1}]	Heat capacity [kJ m^{-3} K^{-1}]	Density [kg m^{-3}]	Compression strength at 20% strain [MPa]	Elastic modulus [MPa]	Water absorption after 24 h [%]	Expansion at 85% RH after 24 h [%]	Weight added at 85% RH after 24 h [%]
	25°C (Not pressed)		25°C (Heat pressed)					
H	0.031 ± 0.001^{BC}	474 ± 15^{A}	322 ± 2^{A}	2.5 ± 0.01^{A}	24 ± 2^{A}	404 ± 20^{B}	19 ± 3^{BC}	12 ± 0.4^{G}
S	0.026 ± 0.003^{A}	501 ± 18^{A}	430 ± 46^{C}	4.0 ± 0.8^{BC}	37 ± 2^{BC}	358 ± 29^{AB}	$25\pm8^{A\text{-}C}$	$12\pm1.5^{D\text{-}H}$
C	0.058 ± 0.006^{D}	752 ± 29^{C}	634 ± 24^{E}	4.3 ± 1.4^{BC}	$36\pm14^{A\text{-}C}$	281*	9 ± 1^{A}	9 ± 0.4^{D}
HS	0.029 ± 0.001^{AB}	498 ± 30^{AB}	370 ± 15^{B}	3.2 ± 0.2^{B}	32 ± 3^{BC}	385 ± 21^{AB}	21 ± 3^{C}	13 ± 0.3^{H}
HC	0.029 ± 0.001^{AB}	534 ± 39^{AB}	464 ± 14^{C}	4.0 ± 0.4^{BC}	42 ± 4^{C}	-	17 ± 2^{BC}	10 ± 0.3^{E}
SC	0.031 ± 0.001^{BC}	552 ± 13^{B}	516 ± 28^{D}	4.5 ± 0.5^{C}	40 ± 4^{C}	361 ± 11^{A}	16 ± 2^{BC}	$11\pm0.7^{E\text{-}G}$
HSC	0.032 ± 0.001^{C}	542 ± 36^{AB}	415 ± 4^{C}	3.4 ± 0.1^{B}	30 ± 1^{B}	349 ± 2^{A}	14 ± 2^{B}	11 ± 0.4^{F}
	15–25°C (Not pressed)		15–25°C (Heat pressed)					
H	0.030 ± 0.002^{HI}	508 ± 8^{H}	353 ± 5^{H}	3.1 ± 0.1^{H}	28 ± 2^{H}	441 ± 9^{G}	20 ± 2^{K}	12 ± 0.4^{N}
S	0.028 ± 0.001^{H}	489 ± 49^{HI}	450 ± 7^{J}	4.3 ± 0.1^{I}	43 ± 1^{JK}	376 ± 9^{E}	23 ± 2^{K}	13 ± 0.7^{NO}
C	0.064 ± 0.012^{J}	718 ± 77^{J}	614 ± 24^{L}	4 ± 0.8^{HI}	$33\pm5^{H\text{-}J}$	273 ± 1^{C}	7 ± 1^{I}	9 ± 0.4^{L}
HS	0.033 ± 0.002^{I}	507 ± 19^{H}	409 ± 15^{I}	3.9 ± 0.3^{I}	37 ± 4^{IJ}	398 ± 8^{F}	21 ± 2^{K}	13 ± 0.4^{O}
HC	0.030 ± 0.002^{HI}	525 ± 20^{HI}	464 ± 15^{J}	4.3 ± 0.4^{I}	$39\pm7^{H\text{-}K}$	-	13 ± 2^{J}	11 ± 0.4^{M}
SC	0.033 ± 0.002^{I}	551 ± 19^{IJ}	527 ± 12^{K}	5.6 ± 0.5^{J}	50 ± 6^{K}	337 ± 7^{D}	15 ± 0.4^{J}	11 ± 0.7^{MN}
HSC	0.032 ± 0.003^{HI}	506 ± 6^{H}	420 ± 11^{I}	3.8 ± 0.2^{I}	34 ± 4^{HI}	$324\pm51^{C\text{-}G}$	14 ± 2^{J}	11 ± 0.5^{M}
	15°C (Not pressed)		15°C (Heat pressed)					
H	0.030 ± 0.002^{O}	574 ± 61^{OP}	371 ± 20^{O}	2.8 ± 0.4^{O}	26 ± 4^{O}	-	-	-
S	0.030 ± 0.001^{O}	507 ± 37^{O}	445 ± 6^{P}	4.2 ± 0.1^{PQ}	44 ± 1^{Q}	-	-	-
C	0.068 ± 0.004^{Q}	812 ± 85^{Q}	643 ± 41^{R}	$5.7\pm1.7^{O\text{-}R}$	$53\pm22^{O\text{-}Q}$	-	-	-
HS	0.028 ± 0.002^{O}	529 ± 10^{OP}	406 ± 3^{O}	3.5 ± 0.05^{O}	35 ± 1^{O}	-	-	-
HC	0.042 ± 0.004^{P}	576 ± 18^{OP}	495 ± 20^{PQ}	$4.5\pm0.4^{P\text{-}R}$	42 ± 3^{PQ}	-	-	-
SC	0.037 ± 0.001^{P}	616 ± 51^{P}	525 ± 4^{Q}	5.0 ± 0.3^{QR}	44 ± 4^{PQ}	-	-	-
HSC	0.036 ± 0.002^{P}	598 ± 53^{OP}	460 ± 16^{PQ}	4.3 ± 0.3^{PQ}	40 ± 1^{P}	-	-	-

data within each column. Samples in the same statistical group are indicated by letters.
*Result from only 1 sample; - C substrate grown at 25 °C, in the water absorption measurement.

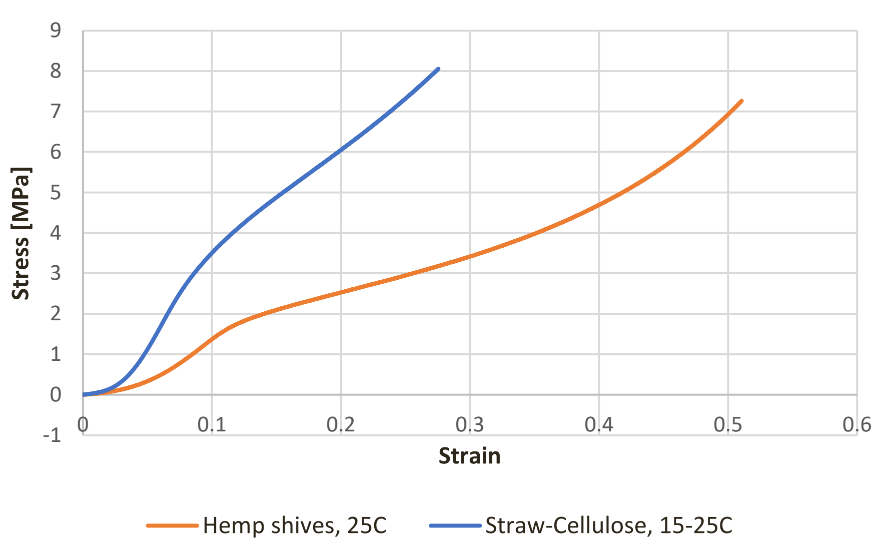

Fig. 4. Typical stress-strain curves of heat pressed H and SC mycelium-based composites (the end point of the curves does not indicate the point of failure but the end of measurement).

A follow up experiment was done to see how different ratios of two substrates influence the final material's properties. The thermal conductivity and mechanical properties were determined for mycelium materials that resulted from growing the fungus in 100, 85, 70, 50, 30, 15, 0% rapeseed straw mixed with 0, 15, 30, 50, 70, 85, and 100% cellulose, respectively. The thermal conductivity of the non-pressed materials depended on the percentage C in the substrate. Thermal conductivity of 0–30% C materials was the lowest (0.026–0.028 W m^{-1} K^{-1}), while those of 100% C were the highest (0.058 W m^{-1} K^{-1}) (Fig. 5A; Table 3). Heat capacity increased with increasing C content. Samples with 0–50% C gave values ranging between 501 and 552 kJ m^{-3} K^{-1}, and 100% C being the highest with 752 kJ m^{-3} K^{-1} (Fig. 5A; Table 3). The results suggest that at lower C% many of the air cavities between the straw pieces remain empty, and thus the conductivity remains the same. At higher C%, the conductivity is rising, probably as a result of reduction in the air cavities, and the formation is a cellulose matrix filled with straw pieces.

The strength at 20% strain and the elastic moduli of the different materials were not different and ranged from 4.22 to 4.79 MPa (Fig. 5B; Table 3) and 35–43 MPa (Fig. 5C; Table 3), respectively. In search for statistical difference, stress from 10% strain was examined as well. The results showed differences between the samples, with 100% C materials being the strongest (3.2 MPa) and 15 and 30% being the weakest with 1.7 MPa. This can be explained by the higher density of pure cellulose materials.

The SC (1:1) substrate was used in the next set of experiments due to the low thermal conductivity (0.031 W m^{-1} K^{-1}) and good compression results (strength of 4.5 MPa at 20% strain and elastic modulus of 40 MPa). SC-based materials were grown at temperature cycles of 25–40 °C and 5–25 °C (Fig. 1), mimicking a hot summer day or desert winter day in Israel, respectively, as well as at a constant temperature of 5, 10, 15, 25, 30, 35 and 40 °C. Samples did not fully colonize the substrate, if at all, when incubated for 14 days at 5, 10, 35, 40 and 25–40 °C as evidenced by no visible mycelium growth, and no substrate binding in the resulting materials in all of the technical replicates. These samples were not further analyzed. The highest thermal conductivity of the materials that had been fully colonized was obtained with material grown at 15 °C (0.037 W m^{-1} K^{-1}), while the other temperatures or temperature cycles resulted in a similar conductivity (0.029–0.033 W m^{-1} K^{-1}) (Table 4). Heat capacity values of the alternating temperature conditions (540–560 kJ m^{-3} K^{-1}) were within the range of the constant temperature conditions (508–625 kJ m^{-3} K^{-1}). As an overall overview of how the fabrication temperature influence the thermal properties, we see that the results are in the same range for both

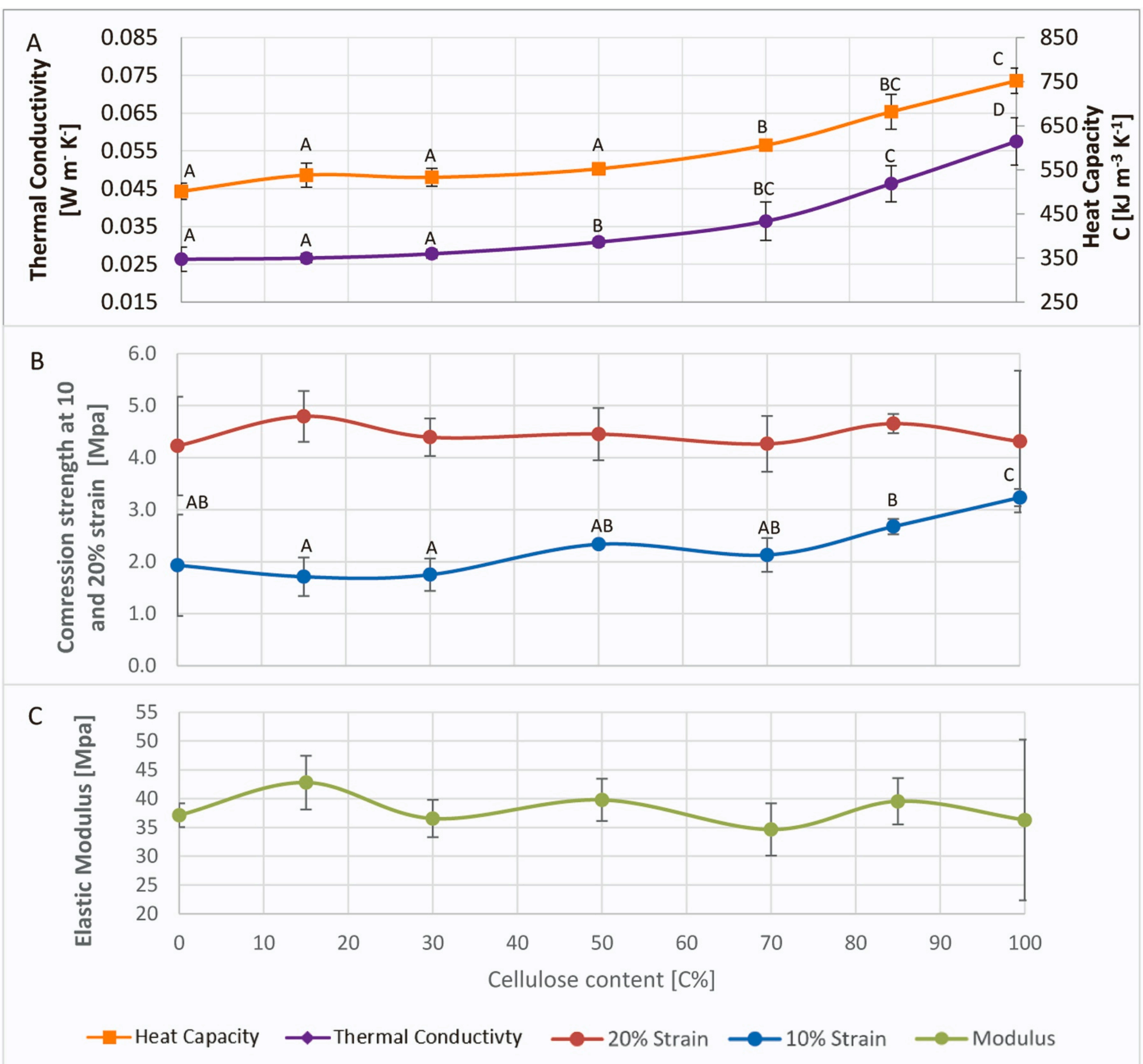

Fig. 5. Thermal conductivity and heat capacity (A), compression strength at 10% and 20% strain (B) and elastic modulus (C) of mycelium composites grown at 25 °C using different ratios of rapeseed straw and processed cellulose. Bars represent standard deviation. Samples in the same statistical group are indicated by letters. No letters are used when all data are within a single statistical group.

Table 3
Properties of mycelium bio-composites that were grown on rapeseed straw mixed with processed cellulose at different ratios, incubated for 14 days at 25 °C. Statistical analysis was performed using the data within each column. ± represents standard deviation. Samples in the same statistical group are indicated by letters. No letters are used when all data are within a single statistical group.

%C	Thermal conductivity [W m-1 K^{-1}]	Heat capacity [kJ m^{-3} K^{-1}]	Density [kg m^{-3}]	Compression strength at 10% strain [MPa]]	Compression strength at 20% strain [MPa]	Elastic modulus [MPa]
	Not pressed		Heat pressed			
0	0.026±0.003 ^{A}	501±18 ^{A}	430±46 ^{A}	1.9±1.0^{AB}	4.2±0.9	37±2
15	0.027±0.001 ^{A}	538±27 ^{A}	486±35^{AB}	1.7^{a}±0.4 ^{A}	4.8±0.5	43±5
30	0.028±0.001 ^{A}	533±20 ^{A}	460±10 ^{A}	1.7^{a}±0.3 ^{A}	4.4±0.4	37±3
50	0.031±0.001^{B}	552±13 ^{A}	516±14^{B}	2.3^{c}±0.1^{AB}	4.5±0.5	40±4
70	0.036±0.005^{BC}	606±11^{B}	511±42^{ABC}	2.1±0.3^{AB}	4.3±0.5	35±5
85	0.046±0.005 ^{C}	682±40^{BC}	570±15 ^{C}	2.7±0.1^{B}	4.7±0.2	40±4
100	0.058±0.006^{D}	752±29 ^{C}	634±24^{D}	3.2±0.2 ^{C}	4.3±1.4	36±14

Table 4
Properties of mycelium bio-composites that were grown on rapeseed straw: cellulose substrate (1:1) for 14 days at different temperatures. ± represents standard deviation. Statistical analysis was performed using the data within each column. Samples in the same statistical group are indicated by letters. No letters are used when all data are within a single statistical group.

Growth Temp. [°C]	Thermal conductivity [W m-1 K^{-1}]	Heat capacity [kJ m^{-3} K^{-1}]	Density [kg m^{-3}]	Compression strength at 20% strain [MPa]	Elastic modulus [MPa]	Water absorption after 24 h [%]	Expansion at 85% RH after 24 h [%]	Adsorption at 85% RH after 24 h [%]
	Not pressed		Heat pressed					
15	0.037±0.001^{B}	625±51^{AB}	533±7 ^{A}	5.0±0.3^{AB}	44±4^{AB}	-	-	-
25	0.031±0.001 ^{A}	548±13^{B}	489±10 ^{A}	4.5±0.5 ^{A}	40±4 ^{A}	361±11^{B}	16±2	11±0.7 ^{A}
30	0.029±0.003 ^{A}	508±17 ^{A}	557±28^{AB}	5.9±1.3^{AB}	51±14^{AB}	306±27^{AB}	20±3	13±1.4^{AB}
5–25	0.030±0.005 ^{A}	540±31^{AB}	575±21^{B}	4.8±0.5^{AB}	48±4^{B}	332±2^{AB}	17±5	13±0.3^{B}
15–25	0.033±0.004 ^{A}	560±19^{AB}	527±12 ^{A}	5.6±0.5^{B}	50±6^{AB}	337±7 ^{A}	15±0.4	11±0.7^{AB}

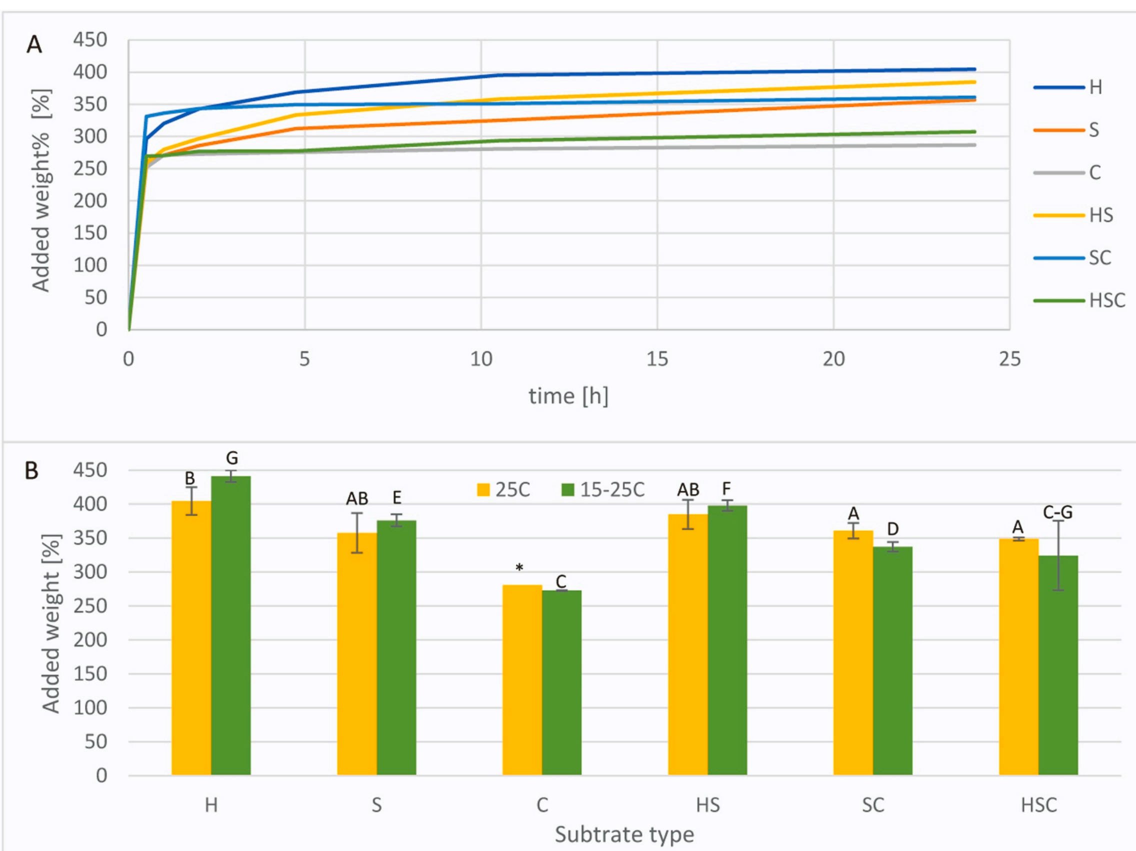

Fig. 6. Water absorption during 24 h of mycelium bio-composites made of different substrates at 25 °C (A) and water absorption after 24 h of mycelium bio-composites made of different substrates at 25 °C or 15–25°C (B). Bars represent standard deviation. Samples in the same statistical group are indicated by letters (A, B for 25 °C and C-G for 15–25 °C).

constant growth temperature and alternating temperature. That is expected, as have been stated before, as it make sense that the temperature does not influence the amount of air cavities in the samples.

As in the thermal properties, the compression strength and the elastic modulus of samples grown at alternating temperature (4.8–5.6 MPa and 48–50 MPa, respectively) were within the same range of materials grown at constant temperature (4.5–5.9 MPa and 40–51 MPa, respectively). This is another indication for the similar growth of the material at the alternating temperature conditions. Together, these data show that growing at alternating temperatures does not impact the materials mechanical properties and the heat insulation properties. On the other hand, the substrate does affect heat insulation and mechanical properties of mycelium materials, and we can use the combination of different substrates to tune the properties of the final material.

3.2. Water absorption and expansion at high relative humidity

Water absorption and expansion at high RH were measured on the heat pressed samples resulting from the different substrates and temperature conditions (i.e., 15–25 and 25 °C). The heat pressed samples grown at 25 °C absorbed 281–404% water (Fig. 6A). Maximum absorption was obtained after 5–10 h. The absorption after 24 h (Fig. 6B; Table 2) shows that the substrate type affected the material's absorption. For instance, pure C-based materials absorbed much less water (281% or 273% when grown at 25 or 15–25 °C, respectively) than all other materials (except for HSC grown at 15–25 °C with very big variance). In contrast, H-based materials showed highest absorption, with 441% when grown at 15–25 °C. 6 out of 6 HC samples did not survive the water absorption test, and 3 out of 6 C samples deteriorated as well. As for the influence of the temperature, the results show clearly that it does not influence the absorption, as seen in the mixed substrates experiment (Table 2) and the different growing temperature for SC-based materials (Table 4).

The expansion in height of dry heat pressed mycelium materials at 22 °C and 85% RH ranged between 7% and 25% (Fig. 7A). Adsorption of water and height expansion were most rapid in the beginning of the exposure to humidity but saturation was still not attained after 24 h. Expansion of C-based material grown at 25 °C was lower (i.e. 9%) than all other materials grown at the same temperature except for S, while it was even the lowest (i.e. 7%) of all materials when grown at 15–25 °C (Fig. 7B; Table 2). Similar results were obtained when weight increase was assessed (Fig. 7B; Table 2). Growth temperature had no effect on height expansion and only had an effect over weight increase in the case of the HC substrate (Fig. 7B).

A relation was noted between the porosity of the materials, which is substrate dependent, and the water absorption. Higher porosity (H and S substrate) leads to higher uptake of water, while the denser substrate (C substrate) shows lower uptake. Interestingly, samples that contain C were more sensitive to deterioration in the water uptake experiment than samples without this substrate, in spite of the lower absorption of the C-based materials. The low adsorption of C-based samples implies that the surface area of the substrate, which is much bigger than in the S- or H-based samples, is not the main parameter for adsorption. Previously, a water absorption of 246% was found in heat-pressed mycelium

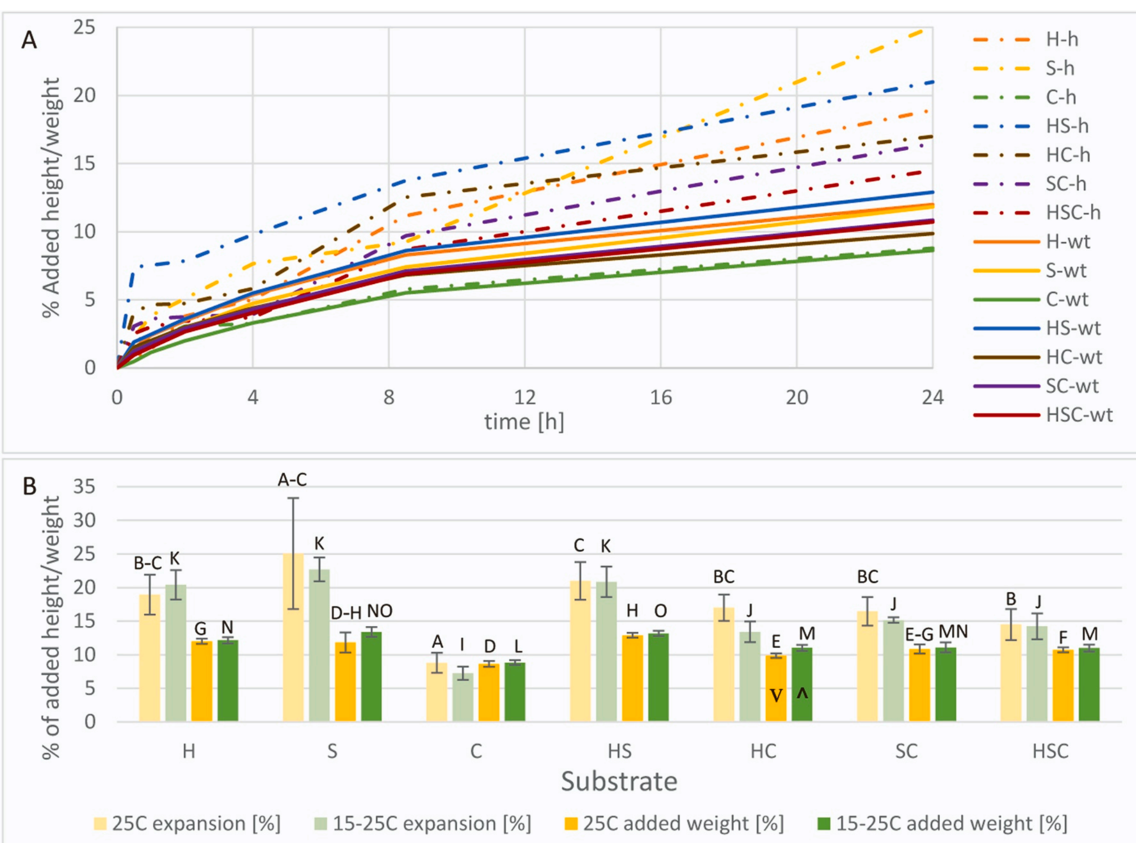

Fig. 7. Expansion in height (dashed lines) and weight gain (solid lines) resulting from adsorption of water of mycelium bio-composites made from different substrates and grown at 25°C during 85% RH exposure for 24 h (A) and expansion in height (light tones) and weight gain (dark tones) resulting from adsorption of water after a 24-h exposure of mycelium bio-composites (grown at 25 °C or 15–25 °C) to 85% RH (B). Bars represent standard deviation. Letters in B represent statistical groups within each condition (A-C and I-K, expansion in height of material grown at 25 °C and 15–25 °C, respectively and D-H and L-O, added weight of material grown at 25 °C and 15–25 °C, respectively). If two samples within a substrate group were significantly different from each other ^ and ᵛ were used to indicate which was significantly higher and lower, respectively.

materials when *Trametes multicolor* was grown on rapeseed straw, about two thirds of the result in our study [13]. Other heat pressed materials (*Pleurotus ostreatus* grown on rapeseed straw or cotton) showed lower values as well. As for expansion and weight gain due to adsorption, previous values were as low as −0.14 to 2.5% expansion (i.e., 4–10 folds lower than in our study) and 8.12–10.96% added weight (same range as in our study). The difference for both water uptake and expansion in thickness can be explained by the difference in sample preparation. In the former study samples were grown for 24 days (in this study 14 days), heat pressed at 150 °C for 20 minutes (in this study 5 min), and under <30KN (in this study 115KN). The difference can lead to more cross-linking of the fungal polysaccharides which result in a structure less capable for water uptake (less porous), and less sensitive to expansion.

4. Conclusions

The reported results show that mycelium bio composites can grow at ambient temperature conditions without harming the thermal or mechanical properties of the materials. Growth at ambient temperature thus provides a way to save the energy needed for the incubator as well as related carbon emissions in the fabrication process. Mycelium materials were produced in this study with a thermal conductivity as low as 0.026 W m^{-1} K^{-1}, which is lower than commercial insulating materials but also lower than values previously reported for mycelium materials (Table 5). The explanation for the excellent thermal insulation properties is the high porosity of the mycelium materials, with many air pockets trapped within and between the substrate particles. Such a material has the potential to act as a superior thermal insulator in the construction industry, reducing energy consumption during the use-phase of buildings [29,30], while using sustainable materials.

The heat-pressed mycelium materials reveal a wood-like behavior of the material, with strength ranges from 2.5 to 5.7 MPa. Hollow concrete blocks (HCB) and aerated autoclaved concrete (AAC) are characterized by a strength of 3.5–15 MPa [31] and 3–4.5 MPa [32], respectively. The results show potential for using mycelium bio composites in the building industry replacing concrete based materials such as HCB or AAC. Surely, mycelium bio composites cannot be used as a load bearing material. For this, concrete or preferably wood should be used.

Using mixed substrates allows us to modulate the material's properties. Moreover, using mixed substrates has the advantage of an increased local availability of waste to be used as substrates for the production of mycelium materials.

Mycelium materials grown at alternating temperatures (15–25 or 5–25 °C) have a similar quality as materials grown at a constant temperature of 25 °C, which is a commonly reported practice in literature [13,16,22,33]. The fact that the temperature cycle hardly affects the properties of the materials is an unexpected result, since colonization at 15 °C has a substantial impact when compared to growth at 25 °C. The reason for this is not yet known, but the importance of this finding is significant because growth at ambient temperature (mimicked by the 15–25 °C cycle) could save over 70% of the energy and > 40% of the CO_2 emission when compared to growth in an incubator at a constant temperature of 25 °C [18]. It should be noted that optimal growth is restricted to a certain temperature window. We showed that a temperature cycle with a maximum temperature of 40 °C did not work well. Growth at a constant temperature of 5, 10, 15, or 35 °C also did not result in full colonization of the substrate. For growing the material at 15 °C, for example, extra 11 days were needed to achieve full colonization, which might turn it already to a CO_2 emitter rather than fixator. In many regions in the world, temperature cycles in the range of 15–30 °C occur, at least for part of the year – and under such conditions, energy consumption and CO_2 emissions could be drastically reduced by growing at ambient temperatures. In addition, large structures (e.g., building walls) could be grown outdoor, on the actual construction site. Despite the promising results, some issues are yet to be addressed. Growing the material at large scale and as a cast-in-place method requires big quantities of material, and development of the right technology that will allow mixture, sterilization, and inoculation in an automized way is needed. Infections are another issue to address- in this study we had very little to no infections, but those are lab conditions. Working outside can lead to many infections. Pre-colonizing the substrate under controlled conditions reduces the infection incidence because the established mycelium can compete with infecting microbes. Yet, actually growing at scale will identify the main issues to be solved. Another issue to address is the time for growing the material- in this study we show a 14 days process from sterilization to full inoculation. That may be slow in terms of big industry but could be tackled by pre-colonization of the substrate under controlled conditions similar to horticulture. The results for water absorption of the heat-pressed material indicate that the mycelium materials should be coated when used as an outdoor product or when used in humid indoor conditions.

To conclude, the results presented in this paper strongly support the claims that mycelium materials can be valuable additions to a circular economy and more sustainable construction industry.

Table 5
Thermal conductivity of mycelium bio-composites (this and previous studies) and commercial insulating materials.

Material	Thermal conductivity [W $m^{-1}K^{-1}$]
Mycelium bio-composites – original data	0.026–0.068
Mycelium bio-composite – literature	0.04–0.08[16]
Expanded polystyrene (EPS)	0.036[34]
Polyurethane (PUR)	0.03[35]
Mineral wool	0.035[36]
Glass wool	0.04[35]
Sheep wool	0.036[29]

Funding

This work was supported by the Israeli Ministry of Energy, by KKL-JNF, and by The Goldman Sonnenfeldt School of Sustainability and Climate Change at BGU.

CRediT authorship contribution statement

Achiya Livne: Writing – original draft, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Han Wösten:** Writing – review & editing, Supervision, Resources. **David Pearlmutter:** Writing – review & editing. **Erez Gal:** Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Acknowledgements

We thank Prof. Alva Peled for the use of the Instron laboratory equipment.

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