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Research Article

Fungal Mycelium Bio-Composite Acts as a CO_2-Sink Building Material with Low Embodied Energy

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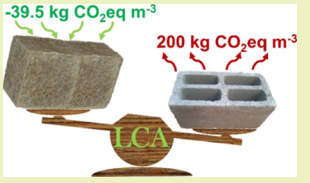
ABSTRACT: As part of the global transformation to a circular economy, modern society faces the challenge of developing sustainable building materials that do not deplete nonrenewable resources or generate environmentally destructive waste. Bio-composites based on fungal mycelium grown on agricultural waste streams have the potential to serve this purpose, reducing the ecological impact of the construction industry and the conventional materials on which it currently relies. In addition to the possible advantages in the production and postuse phases of their life cycle, mycelium bio-composites are lightweight and highly insulating, thus providing valuable thermal properties for reducing energy consumption and emissions over the operational lifespan of the building. In this study, a comprehensive life cycle assessment of mycelium bio-composites was conducted, focusing on the embodied energy (EE) and embodied carbon (EC). Part of the CO_2 that is emitted is the result of the fungal growth. Therefore, a novel calculation method was developed to assess the metabolic carbon emissions as a function of weight loss during the growth period. Using a cradle-to-gate model of the production process, the EE of the mycelium bio-composite was estimated to be 860 MJ m^{-3}, which represents a 1.5- to 6-fold reduction compared with that of the common construction materials. The EC was calculated to be −39.5 kg CO_2eq m^{-3}, its negative value indicating that the fungal bio-composite effectively functions as a CO_2 sink, in contrast to currently used construction materials that have a positive EC. The incubation stage of mycelium bio-composite production made up the largest portion (73%) of the overall energy, while metabolic CO_2 comprised a significant proportion (21%) of the overall emissions as well. Altogether, our results demonstrate that using bio-composite building materials based on fungal mycelium and local plant residues can provide a sustainable alternative to current practice.



KEYWORDS: *building materials, life cycle assessment, fungal mycelium bio-composite, embodied energy, embodied carbon, circular economy*

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INTRODUCTION

A central challenge for society is to transform our economy into a sustainable system by reducing CO_2 emissions and minimizing the production of waste through the use of nature-based circular processes.

Building operation and construction, including the production of building and insulating materials, are responsible for the largest share of global energy use (36%) and energy-related CO_2 emissions (39%).[1−4] More than 33 billion tons of cement-based concrete are produced every year worldwide, and the cement industry alone accounts for some 8% of global CO_2 emissions.[5,6] The energy demand and carbon footprint of the construction industry are expected to rise because of the growing world population and the fact that urbanization processes continue to accelerate in developing countries.[5] Hence, the realization of long-term sustainability goals is highly dependent on the development and use of building materials with a lower environmental impact.

Mycelium bio-composites are a new kind of material, which have gained increasing interest in the last decade.[7] These materials are based on a cellulose- or lignocellulose-rich substrate colonized by fungal mycelium and, as such, represent an upcycled agricultural residue. Their production is considered to be environmentally friendly since it relies entirely on circular biological processes, and the final product is biodegradable. In addition, these bio-composites tend to be extremely lightweight, with a typical density < 200 kg m^{-3}. Furthermore, they have a thermal conductivity that can be below 0.04 W/m·K,[8] which is lower than that of other biomass derived bio-composites for insulation (Table 1). Therefore, these materials can provide very effective thermal insulation, which is crucial in the operation of buildings where much of the energy is consumed for heating and cooling.[9,10] Moreover, fungal bio-composites provide very good acoustic insulation (70−75% absorbance at frequencies < 1500 Hz)[11] and have mechanical properties that are characteristic of natural materials (with a compressive strength ranging from 0.17 to 1.1 MPa and tensile strength ranging from 0.025 to 0.18 MPa),[12−14] being weaker than other bio-composites used for insulation (Table 1).

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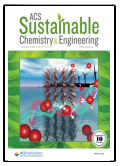

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Table 1. LCA Metrics and Physical Properties of the Fungal Mycelium Bio-Composite ("Mycoblock") as Compared with Those of Commercial Insulators, Other Bio-Composites, and Commercial Building Materials

Material	bulk density [kg m^{-3}]	thermal conductivity [W $m^{-1}K^{-1}$]	compressive strength [MPa]	EC [kg CO_2eq m^{-3}]	EE [MJ m^{-3}]
Mycoblock	163	0.04−0.08[8]	0.15−0.51[12−14]	−39.5	860.3
commercial insulation materials					
expanded polystyrene (EPS)	21[21]	0.036[21]	0.17−0.33[22]	224[23]	2710[24]-3565[23]
polyurethane (PUR)	30[23]−110[25]	0.03[26]	0.98[25]	525[23]	3030[24]-8790[23]
mineral wool	100[27]	0.035[27]	N/A	266[23]	1660[24]-3997[23]
class wool	25[23]	0.04[26]	N/A	100[23]	1438[23]
sheep wool	20[28]	0.036[28]	N/A	878[28]	930[28]
biomass-derived bio-composites for insulation					
Hemp-Lime	450[29]	0.11[29]	0.25−1.2[29]	−53.7−145[29]	1710[29]
Hay-Rosin	791[30]	0.0938[30]	5.82[30]	N/A	N/A
rise husk + PLA/PBAT	378[31]	0.08[31]	14.5[31]	N/A	N/A
flexible hemp batt	34[21]	0.04[21]	N/A	N/A	N/A
commercial building materials					
Concrete	2409[23]	2[32]	25[23]	361[23]	2581[23] −285224
concrete blocks	1400[23]	0.8[32]	15[23]	200[23]	1216[24]-2200[23]
autoclaved aerated concrete	550[23]	0.18[32]	2.3−3.8[33]	385[23]	1536[24]-4673[23]

In order to evaluate the overall impact of mycelium bio-composites on the environment, a comprehensive life cycle assessment (LCA) is needed. As can be seen in Table 1, not much LCA research has been done on bio-composites, and it is therefore hard to compare which of these materials is more sustainable. The first LCA model of fungal mycelium bio-composites was published recently by Stelzer et al.,[15] showing an improvement in the climate change, water scarcity, acidification, and smog criteria but worsening land use and eutrophication criteria when compared to commercial building materials. They have made an attempt to assess the environmental impact of a scaled-up process, comparing it to commercially produced bricks. While the LCA was done in great detail and mostly based on accurate lab results, two main contributors were left out of the calculations: the sequestered CO_2 in the substrate and the metabolic CO_2 emissions during the fungal growth. The results of the model were presented in six different categories of impact on the environment but without the embodied energy (EE), a key criterion to allow easy comparison to other building materials, especially with today's motivation to mitigate climate change by reducing exploitation of resources by the industry. While data about sequestered carbon in agricultural residues or the carbon content in plants are available,[16,17] no data have been reported in the literature about the metabolic carbon emissions from the fungal growth process on solid substrates.

In this paper, a comprehensive LCA for mycelium-based bio-composites is described that includes the metabolic fungal CO_2 emission. To this end, a method was developed to measure the metabolic CO_2 emissions during the production of a mycelium bio-composite. With a cradle-to-gate life cycle energy assessment (LCEA) and life cycle CO_2 assessment ($LCCO_2A$), we demonstrate that the EE and embodied carbon (EC) for the production of a unit volume of mycelium-based bio-composites are highly favorable when compared to commonly used construction and insulation materials and, in fact, that these mycelium bio-composites can act as a net CO_2 sink.

MATERIALS AND METHODS

CO_2 Measurements. The first step toward a comprehensive LCA of mycelium bio-composites is the quantification of CO_2 emissions resulting from fungal metabolism during the production of the mycelium material. To this end, the fungus *Trametes betulina* was grown on rapeseed straw and recycled cellulose (separately) in 280 mL microboxes for 14 days. The carbon content in the samples was quantified using an elemental microanalyzer at the start and during the growth process and related to the substrate dry weight. Mycelium bio-composites were produced using biological triplicates by growing the fungus *T. betulina* in 15 g of rapeseed straw (Gedizo, The Netherlands) and 30 g of recycled cellulose (ReCell, The Netherlands) with 35 or 70 g of water, respectively, in a cylindrical 280 mL microbox ($SacO_2$, Belgium). After sterilization, the substrate was inoculated with 3% spawn (*T. betulina* pregrown on millet grains; detailed below) based on the dry weight and incubated at 25 °C for up to 4 weeks, after which samples were dried at 60 °C and weighed. Control samples without fungal growth were made identically but were immediately dried without incubation at 25 °C. Samples were homogenized to a fine powder by grinding in a 450 W blender (Waring, USA) for 45 s followed by grinding in a TissueLyser II (QIAGEN, Germany) for 2 min. Samples were dried in a 60 °C oven, after which 3 μg was loaded in tin capsules for elemental microanalysis using 4−5 technical replicates. The capsules were kept in a desiccator with dried silica to avoid humidity absorption until element analysis using an EA1110 analyzer (Carbo Erba Instruments, Germany). 2,5-Bis(5-tert-butyl-benzoxazol-2-yl)thiophene (BBOT), acetanilide, and atropine were used for calibration. C-measurements had an error <1.47% based on a linear regression of the standards.

To calculate metabolic CO_2 emission, carbon loss in the samples was assumed to be caused by CO_2 emission to the atmosphere. The carbon loss was calculated as the difference between the carbon content at time 0 ($C_{t=0}$) and the final carbon content (C_f)

$$\Delta C = C_{t=0} - C_f \tag{1}$$

The carbon content was calculated as the weight of the sample multiplied by the carbon percentage as measured by elemental microanalysis. The CO_2 emission was then calculated from the lost carbon, adding the weight of oxygen in the molecule

$$m(CO_2) = n(C)\cdot MW(CO_2) = \left(\frac{m(C)}{MW(C)}\right)\cdot MW(CO_2)$$
$$= \frac{44}{12}\cdot m(C) = 3.667\cdot m(C) \tag{2}$$

where $m(CO_2)$ is the weight of CO_2, $n(C)$ is the amount of carbon lost in mol, $MW(CO_2)$ is the molar mass of CO_2, $m(C)$ is the weight of

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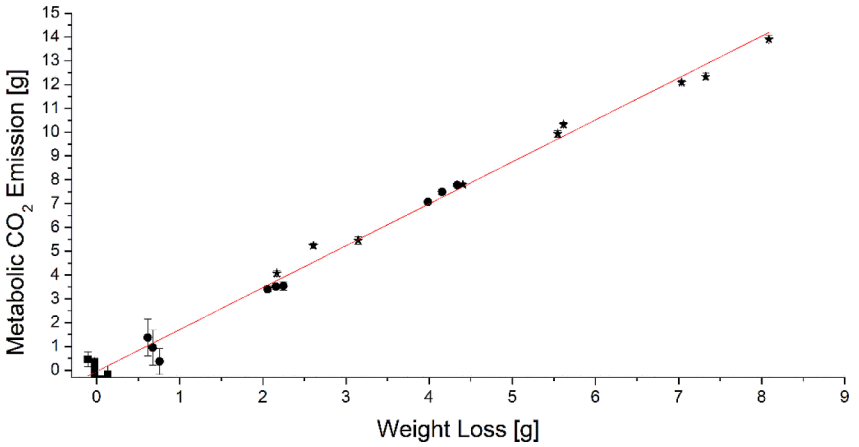

Figure 1. Calibration curve between the weight loss of *T. betulina* and CO_2 emission when grown on rapeseed straw (circles) and recycled cellulose (stars). Squares represent control samples.

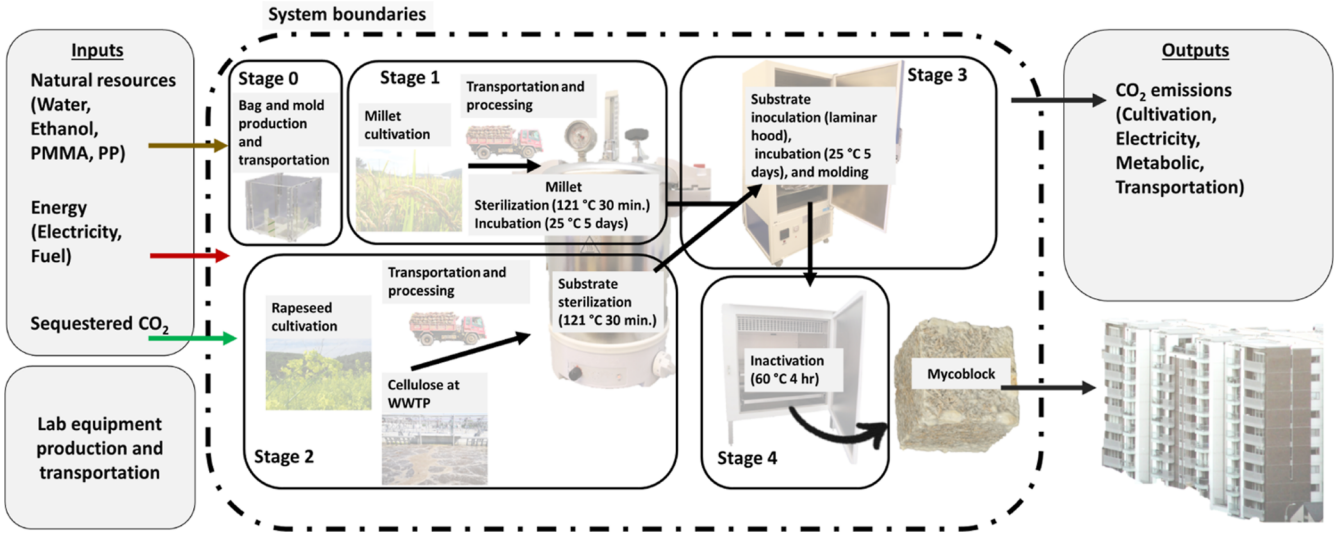

Figure 2. Flowchart of the production process of a fungal mycelium bio-composite ("mycoblock"). The system boundaries include the production and processing of the raw materials, the CO_2 fixation of the substrate, the production of bags and molds, transportation, and the growth process of the fungus. Manufacturing and transport of the lab equipment (autoclave, incubator, and laminar flow hood) and machinery for the processing of the raw materials (e.g., grinding instruments) were not included in the analysis.

carbon lost, and *MW*(C) is the molar mass of carbon. A calibration graph was plotted to assess the correlation between the weight loss and CO_2 emissions using a confidence interval of 0.95 (Figure 1).

Life Cycle Assessment. *Goal and Scope Definition.* The goal of the cradle-to-gate LCA was to evaluate the environmental impact of fungal mycelium material production focusing on the EE and EC. The functional unit was defined as 1 m^3 of the material. System boundaries included the production of bags, molds, and spawns; the processing of raw materials; and the manufacturing process (Figure 2). Transportation was taken into account in the former two stages. Manufacturing and transport of the lab equipment (autoclave, incubator, and laminar flow hood) and machinery for the processing of the raw materials (e.g., grinding instruments) were not included in the analysis. The manufacturing process is calculated for a lab scale using lab equipment.

Production of the Mycelium Bio-Composite. Growing mycelium-based bio-composites is a multistep process that can be done with many variations. The process that is covered in this LCA is detailed here. It is based on the standard growth protocol used in the lab with a 20 × 20 × 40 cm fungal mycelium bio-composite, a "mycoblock". All material quantities are normalized with respect to the functional unit (1 m^3) of the analysis.

Stage 1: The spawn was prepared by sterilizing a mixture of 2.98 kg of millet grain and 2.98 L of water in an aerated bag ($SacO_2$, 47 × 57 PP) in a UTKBS-200LV autoclave (MRC, Israel) at 121 °C for 30 min (cooled down to room temperature without a temperature input), followed by inoculating the mixture with 184 g of the spawn from a former batch and incubating the millet at 25 °C for 5 days (MRC BOD-550 incubator, Israel).

Stage 2: The substrate was prepared by mixing 96.5 kg of recycled cellulose and 96.5 kg of rapeseed straw by hand in batches of 2.1 kg, followed by mixing 450.33 L of water (4.9 L per batch). The substrate was sterilized (as in stage 1) in 92 aerated bags ($SacO_2$, 47 × 57 PP) each with 7 kg of the substrate.

Stage 3: The spawn was added to the substrate (3 wt % of the dry substrate, 63 g per bag) in a laminar flow hood (MRC BBS-DDC 940, Israel). The inoculated substrate was incubated in four batches (each with 23 bags) in an incubator (MRC BOD-550 incubator, Israel) at 25 °C for 8 days, after which the colonized substrate was cast into poly(methyl methacrylate) (PMMA) molds (20 cm × 20 cm × 40 cm; about 9.44 kg of the substrate into each mold). Growth was prolonged for 4 days at 25 °C in the incubator, after which the mycelium bio-composite was transferred to a ripening box (polypropylene crates with small slits, where four blocks can be placed without touching each other) for another 2 days at 25 °C in the incubator.

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Stage 4: After a total growth period of 14 days, the mycelium bio-composite materials were dried at ambient temperature, followed by a heat treatment for 4 h at 60 °C to inactivate the fungus.

Life Cycle Inventory. The life cycle inventory (LCI) reported here is based on EE and EC coefficients taken from the work of this study, data from the scientific literature, the Ecoinvent 3 database, and reasoned engineering assessment when no other option was available. The basic unit is a "mycoblock" with dimensions of 20 × 20 × 40 cm to match the size of a standard hollow concrete block. The calculations in the inventory are based on this unit and multiplied by 62.5 to achieve the functional unit of 1 m^3. Inputs in the process can be divided into two categories: material inventory and process inventory. The material inventory was composed of disposable materials (e.g., alcohol and aerated bags) and wear-subjected materials (e.g., molds). Supporting Information, Text S1, describes the different inputs in the process, which are summed up in Supporting Information, Table S1, normalized per cubic meter. It contains assumptions for the amount of the material used and the number of equipment use cycles (e.g., a PMMA mold can be used on average 100 times before replacement). The packaging materials of raw materials are excluded due to lack of information in the Ecoinvent 3 database. Transportation processes are calculated using a 16-ton lorry with an Euro6 engine as all the transportation distances range from 15 to 200 km. The process inventory (Supporting Information, Table S2) comprised the different processes in the method, describing the required inputs of the material and energy and the outputs. Energy usage was measured using smart switch plugs for electricity, measuring the real consumption of the different stages in the process. The different processes are divided into batches depending on the equipment size. The description of each individual batch and the number of batches in each process are specified in Supporting Information, Text S1. The electrical generation fuel mix is based on standard values for the power grid in the Netherlands.

The raw materials for the production process are treated as waste streams composed of carbohydrates and therefore have a negative carbon starting value. Soil organic carbon was not included in this model as it is assumed that the mycelium materials will be reintroduced back to the field at the end of life. The calculations for rapeseed straw include transportation from fields (a 100 km range) to a processing plant, grinding, and another transportation of 100 km to the production factory. The energy input for the straw processing is 21.67 kW h per ton,[18] from the stage of field collection to final processing (crushed 5−10 mm straw fibers, which represents a higher level of processing than is required for our bio-composites). Since specific data on rapeseed straw were not available in Ecoinvent 3, the option of "wheat straw" was used as a proxy, adding to it the energy for processing and the sequestered carbon. Recycled cellulose is a waste stream from wastewater treatment plants (WWTPs) that requires processing from sludge to a workable substrate. Since this processing is part of the WWTP, in this case, a single 100 km transportation step was included in the LCA. Based on an LCA done as part of project SMART-plant under Horizon 2020,[19] the overall energy demand for the cellulose treatment, in contrast to that for a standard WWTP, is negative (i.e., the filtration and drying of the cellulose consume less energy than what is saved downstream in the wastewater treatment). Drying of cellulose is accomplished mainly with excess heat from the treatment process to a level of 90% dry matter. Final drying and pelleting require 50 kW h of energy per ton of cellulose. As an estimation, drying without compression would only require about 50% of the energy. Since this process is not relevant to the wastewater treatment, we included 25 kW h ton^{-1} as energy needed for producing the recycled cellulose.

Elemental microanalysis showed a carbon content in the straw and cellulose of 43.11 and 43.44%, respectively. Since the source of this carbon is atmospheric CO_2 absorbed by plants, the total CO_2 values can be calculated as follows.

$$m(CO_2) = n(C)\cdot MW(CO_2) = \frac{m(C)}{MW(C)}\cdot MW(CO_2) = \frac{MW(CO_2)}{MW(C)}\cdot \%C\cdot m = \frac{44}{12}\cdot \%C\cdot m \quad (3)$$

where $m(CO_2)$ is the mass of CO_2, $n(C)$ is the amount of carbon in mol, MW is the molecular weight, and %C is the measured carbon percentage from the elemental microanalysis—determined to be 1.581 and 1.593 g of sequestered CO_2 per gram of straw and recycled cellulose, respectively.

The final density of the "mycoblock" material was 161 kg m^{-3}, while the average weight loss was 16.58 ± 1.8% (see Supporting Information, Table S3). According to these results, 193 kg of the substrate (combining straw and cellulose at a 1:1 ratio) and 450.3 L of water are required to grow 1 m^3 of the mycelium bio-composite material.

Life Cycle Energy and CO_2 Assessment. The life cycle assessment was conducted using SimaPro v.8, and EC was calculated using the IPCC 2013 GWP 100a V1.01 method (modified to account for metabolic CO_2, as described above). Data for commercial building materials, which may be replaced by mycelium materials, were taken from the literature.

Uncertainty and Sensitivity Analysis. Since the process for commercial production of mycelium materials is still under development, there are uncertainties that can influence the results of this LCA. We have performed sensitivity analyses to scrutinize the variations in the overall EE and EC that result from different scenarios, including (1) the incubation time, (2) the transportation distance of the raw materials, and (3) the EE and EC of raw materials.

(1) *Incubation time.* The incubation time is a crucial parameter in the growth process and influences the properties of the final material. A range of 1−3 weeks were used in the sensitivity analysis. One week of incubation results in EE = 316.6 MJ m^{-3} and EC = 85.2 kg CO_2eq m^{-3} in the SimaPro calculation. Increasing or reducing the incubation time by 1 week will increase or reduce the EE of the material by 37%. The fixed carbon will range between −124.7 and +45.7 kg CO_2eq m^{-3} when varying between 1 and 3 weeks of incubation.

(2) *Transportation distance of the raw materials.* A more optimistic scenario is described by positioning the manufacturing plant close to the grinding and wastewater treatment plants, avoiding the second transportation stage of the straw and reducing the total cellulose transport distance to 15 km. In such a scenario, the transportation EE and EC are, respectively, reduced by 48.4 MJ m^{-3} and 2.99 kg CO_2eq m^{-3} when compared to the two 100 km steps in transportation for straw and 100 km for cellulose. The other extreme is to have the manufacturing plant further away from the raw materials, increasing the distance to 200 km for both ground straw and cellulose. In this scenario, the transportation EE and EC increase by 52.4 MJ m^{-3} and 3.23 kg CO_2eq m^{-3}, respectively. The resulting impact is small compared to the overall CO_2 emission (−0.98% for the short distance scenario and 1.05% for the long distance scenario) but is more significant in the EE of the process (−5.6 and 6.1%, respectively).

(3) *EE and EC of raw materials.* The highest level of uncertainty in the data pertains to the energy needed to produce the raw materials. The influence of doubling or halving the energy needed to produce straw and cellulose was assessed in the sensitivity analysis. Doubling the processing energy will add 16 MJ m^{-3} EE and 3 kg CO_2eq m^{-3} EC to the process, while reducing it by half will result in respective decreases of 9 MJ m^{-3} and 1.5 kg CO_2eq m^{-3}.

Taking the extreme scenarios for all three parameters, we obtain an EE ranging from 486.3 to 1245 MJ m^{-3} and an EC ranging from −129 to 52 kg CO_2eq per m^3 for the fungal mycelium materials.

RESULTS

Metabolic CO_2 Measurements. A linear relationship (M_{CO_2} = 1.761*Δm − 0.0521) was obtained between the amount of emitted CO_2 and the dry weight loss Δm irrespective of the substrate that was used. During 14 days of fungal colonization, 15.07% ± 0.67% and 18.08% ± 2.37% of the dry weight mass of the rapeseed straw and the recycled cellulose

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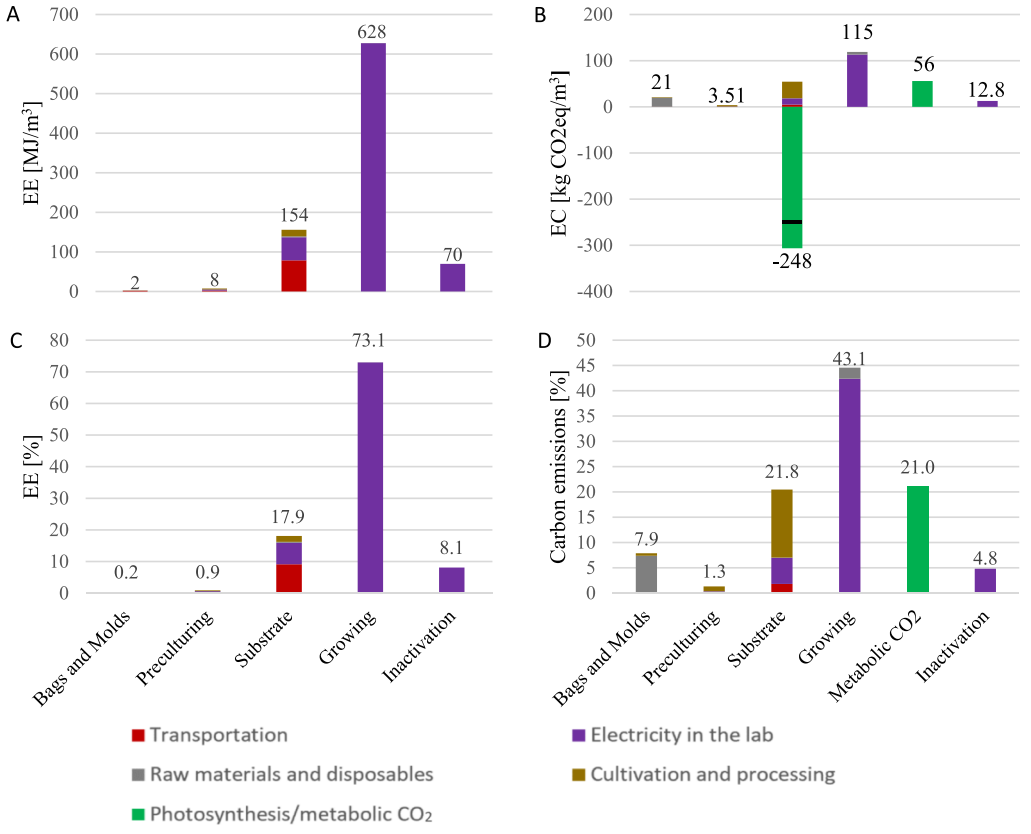

Figure 3. Energy consumption (A), overall CO_2eq emission (B), relative energy consumption (C), and relative CO_2eq emissions (D) of the different stages in the fungal mycelium bio-composite production process. The relative CO_2eq (D) is calculated as a percentage of the "positive" emissions only, neglecting the sequestered CO_2 in order to highlight the emission "hotspots" in the process. It is possible to see the different contributions to the energy or emissions by the colors described in the legend below the columns.

were lost, respectively (Supporting Information, Table S3). Similar results were obtained when using a 1:1 ratio of rapeseed straw and recycled cellulose as the substrate (Supporting Information, Table S4).

Life Cycle Assessment. A comprehensive LCA was performed for the production of 1 m^3 mycelium bio-composite blocks (40 cm × 20 cm × 20 cm each) resulting from the growth of *T. betulina* in rapeseed straw mixed with recycled cellulose (1:1 w/w) for 14 days (see the Materials and Methods). The cradle-to-gate LCA model was built in SimaPro v.8[20] with the overall energy consumption and CO_2 emissions per cubic meter of the blocks as outputs of the process. Production and processing of the raw materials were included as well as the CO_2 fixation of the substrate, the production of bags and molds, transportation, and the growth process of the fungus (Figure 2 and Supporting Information, Tables S1 and S2). Stages of the process of mycelium bio-composite production were defined as follows: (0) production of molds and bags; (1) preculturing the fungus on millet including the cultivation of the grain, transportation, sterilization, inoculation, and culturing; (2) production of the substrate including cultivation of rapeseed (modeled as wheat) and separation of cellulose at the WWTP, transportation and processing of the straw and the cellulose, and sterilizing in bags; (3) growing the materials, including inoculation in a laminar flow hood, incubation, and molding; and (4) inactivation of the blocks at 60 °C (see Figure 2). The model resulted in an EE value of 860.3 MJ and an EC of −39.5 kg CO_2eq per cubic meter for the "mycoblock" (Table 1). The latter means that the material is fixing CO_2 and thereby acting as a net carbon sink, which is explained by the high rate of CO_2 fixation during the growth of rapeseed and the source of cellulose in the field.

Hotspots in the Manufacturing Process. The growing stage of the fungus is by far the most energy-demanding stage of the bio-composite production, consuming 73% of the overall energy used throughout the five-stage process (Figure 3A,C). Nearly all (99.5%) of the growing-stage energy is consumed for maintaining the incubator temperature, while the remaining part is used by the laminar flow hood during the inoculation. Notably, the energy for preculturing and for the production of molds and bags used in the process is negligible. The growing stage of the fungus is also highest in CO_2 emissions, accounting for 64.1% of the total (Figure 3B,D). Fungal metabolic CO_2 (as detailed in the Materials and Methods) represents 21% of the emitted CO_2 in the whole process and about 33% of the emissions during the growing stage. Another significant CO_2 source (7.9%) is the production of the bags and molds, which are made from fossil fuel-based polymers.

Sensitivity Analysis. The sensitivity of the LCA model to variations in particular parameters was analyzed with respect to the time of fungal growth in the substrate, the distances for transportation, and the processing energy of raw materials (detailed in the Materials and Methods). The sensitivity analysis shows that transportation distances and processing energy of the raw materials have a relatively minor impact on the LCA, with the EE ranging from 802.9 to 928.7 MJ m^{-3} and the EC between −44 and −33.27 kg CO_2eq m^{-3} when assuming 115 km of transportation for straw and cellulose with half the processing

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energy or 500 km of transportation and double the processing energy. As mentioned above, the incubation time is a critical value in the process, which is confirmed in the sensitivity analysis. When growing the fungus for 3 weeks instead of 2 weeks, the material is no longer a CO_2 sink—with an EC value of +45.7 kg CO_2eq m^{-3} and an EE of 1176.9 MJ m^{-3}. In contrast, incubating for 1 week results in an EC of −125 kg CO_2eq m^{-3} and an EE of 543.7 MJ m^{-3}.

DISCUSSION AND CONCLUSIONS

Mycelium materials can play a key role in the transition to a circular economy because the production of these biodegradable materials involves upcycling of organic waste streams.[34] Here, we show that these materials can act as a net CO_2 sink, helping achieve net-zero or even negative emission buildings.

Metabolic CO_2 Emissions. A comprehensive cradle-to-gate energy and carbon LCA of a mycelium bio-composite was performed. To this end, a method was developed to include the metabolic CO_2 that results from the growth process of the fungus. Because metabolic CO_2 is of a biogenic source, it is often excluded from the LCA calculation (as is the case for the commonly used IPCC 2013 GWP_{100} method).[35] However, this biogenic source can represent a considerable part of the total emissions, illustrated by the fact that the metabolic CO_2 of the mycelium bio-composite analyzed here represented 21% of the total emitted CO_2. As a result, the magnitude of the "negative" net EC was reduced by about 60%—from −95.5 to −39.5 kg CO_2eq m^{-3}. Still, the bio-composite is a net CO_2 sink, in sharp contrast with common construction materials such as cast concrete products and EPS (Table 1).

The amount of metabolic CO_2, as calculated using an elemental analyzer (where samples were grinded, see the Materials and Methods), was related to the loss in dry weight of the substrate. Theoretically, the amount of metabolic CO_2 could have been calculated from the chemical equation of burning cellulose based on the assumption that all the weight loss is due to CO_2 emissions. This would yield a conversion ratio of 1.63 g CO_2 emissions for each 1 g substrate reduction. As described in the results, the experimental conversion ratio is 1.761 g CO_2 per 1 g of the reduced substrate (the slope of the calibration curve), a difference of 8%. The explanation for this gap can be found in the different carbon contents of the substrate and the fungus—a lower carbon content in the fungus allows higher mass with less carbon. We measured the total weight loss and not the weight loss of the substrate itself, and therefore, losing 2 g of the substrate and gaining 1 g of the fungus will result in 1 g weight loss, with higher carbon loss than if we would burn 1 g of the substrate. Therefore, the latter simple and nondestructive method can be used for future LCAs with *T. betulina* and similar or identical substrates. Determining the relation between the metabolic CO_2 and dry weight loss for other fungi and substrates will be beneficial to assess LCAs for other mycelium materials and also for industrial production of edible and medicinal mushrooms and the use of waste streams that are produced by this industry.[34] Notably, this method will also enable us to identify low-CO_2-emitting fungi that would be of preferred use for the production of fungal materials.

Comparison of the EC and EE of Common Building Materials. The EE of the mycelium bio-composite was found to be 860.3 MJ m^{-3}, which is 1.5−6-fold lower than that of current construction and insulation materials such as concrete, concrete blocks, autoclaved aerated concrete, EPS, and PUR (Table 1). However, mycelium bio-composites are expected to have a shorter life than the current construction materials and might need to be replaced a few times during the 50 year operation of a building. If we assume that the life time of the material is one-third of the EPS or PUR life time, the EE of mycelium bio-composite insulation will be 2580 MJ m^{-3}, lower than that of EPS (2710[24]−3565[23] MJ m^{-3}) or PUR (3030[24]−8790[23] MJ m^{-3}). It should be noted that in many cases, the EE of the construction materials only represents 10−20% of the life-cycle building energy costs,[36] with 80−90% of the overall energy consumed during the use-phase of the building, mainly for climate control.[9,37] The latter can be reduced by improving the insulation of the building and in some new buildings get below 50%.[29] Notably, the thermal conductivity of mycelium materials is very low with values similar to those of commercial insulators such as EPS. Thus, mycelium bio-composites can also contribute to the reduction of the energy consumption during the use-phase of the building.[38]

The advantage of using the fungus as the binder in the bio-composite is the low energy needed for its growth and the encapsulation of air bubbles which improve the thermal insulation of the grown material, which will help in reducing the operational energy (OE). When comparing the matrix of mycelium to other possible matrixes as lime or cement, as in "hempcrete", the energy and carbon footprint of the latter are higher. In hemp-lime composites, the density is 450 kg m^{-3}, and the amount of lime is 63% in weight.[29] The EC of lime is 1.43 kg CO_2 kg^{-1}, and the EE is 5.3 MJ kg^{-1}. For a cubic meter of hemp lime, there is a need for 283.5 kg of lime, with a cost of 405 kg CO_2 and 1502 MJ, without transportation costs, and without the expected calcification of the product at the end. Replacing lime with cement[23] (assuming the same weight) will result in a higher EE (11.8 MJ kg^{-1} or 3345 MJ m^{-3}) and a bit lower EC (1.3 kg CO_2eq kg^{-1} or 368.55 kg CO_2eq m^{-3}), without the potential to sequester CO_2 in the calcification process over time. Both alternatives end up with a higher EE and higher EC compared to those of the fungal bio-composite (the EC of growing the mycelium sum up to 208 kg CO_2eq m^{-3}).

Carbon Sequestration. The negative carbon footprint, or EC, of the material comes from the sequestered CO_2 during the plant growth, which later acts as the substrate for the fungal growth. The substrate (rapeseed straw and recycled cellulose) sequesters 306 kg of CO_2 m^{-3} via photosynthesis and emits 58.3 kg CO_2eq m^{-3} during the cultivation process, with most of the emissions in the substrate stage (58%) resulting from the straw cultivation itself. Since we modeled the straw using the existing "wheat straw" option in Ecoinvent 3, which is a byproduct of wheat cultivation, the straw does not count as a waste with zero energy but as an agricultural byproduct. It is important to note that taking residues from the field could reduce the soil organic carbon and minerals over time and would therefore not be sustainable.[39] However, mycelium materials can be reintroduced on the fields at their end of life and as such will not have an impact on the soil quality. In addition, using pure waste streams such as cellulose from WWTP or municipal pruning waste will reduce the EC and, in the long run, will support the reintroduction of the carbon lost in today's waste streams back to the soil.

Process Efficiency Improvements and Sensitivity Analysis. In the present study, the fungal growth process (stage 3, Figure 1) was found to be the major contributor to the EE and EC of the mycelium bio-composite because of the energy input required to control the climatic conditions during this process. The energy efficiency of this stage can be improved

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when scaling up the production process. Scaling up the LCA to a factory level is important in order to compare to commercial products, where the production process has been improved for decades. In addition, scaling up will demand more energy input for processes such as mixing the substrate and moving from place to place, which are now done by hand. There is a big uncertainty in scaling up and therefore should be taken as limited. Scaling up the sterilization process from a 200 to 10,000 L autoclave, using a method as suggested in a paper by Piccinno et al.[40] for scaling up LCA processes, would reduce the needed energy for sterilization by 7.6% per m^3 of the material. Since the absolute numbers for the 200 L autoclave are higher than the values we have measured, we stress here the difference in percentage only. In the same way, calculating the difference for the inactivation at 60 °C in a 10,000 L oven instead of the 550 L oven we have used in the described process yielded a reduction of 8.2%. Applying these results into our model will reduce the sterilization energy from 58.59 to 54.20 MJ and the inactivation energy from 69.12 to 63.45 MJ, overall saving 10.06 MJ, or 1.2%, of the overall process. In the scale-up model suggested by Stelzer et al.,[15] which is based on opinions of experts in the field, sterilization was done with steam, reducing the energetic cost to 28.99 MJ m^{-3}, reducing the sterilization energy in our model by 29.6 MJ or 49.5%. Since it is based on experts' knowledge with practical experience with sterilization and not generalized values, we believe it is more accurate than the method mentioned above. It is harder to assess the incubation energy of a scaled-up factory using the method mentioned above since the desired temperature is equal to the room temperature. Since this LCA model is based on our specific growth method, it is impossible to quantify its uncertainty to mycelium bio-composites in general. However, the general trend is that scaling up will reduce the needed incubation energy for a cubic meter of the material. Moreover, the EE and EC can be improved by reducing the incubation time. A reduction in incubation time may be achieved by using a higher amount of the spawn in the inoculation stage. For instance, a reduction of the incubation time by 25% using triple the amount of millet would reduce the EE to 717 MJ m^{-3} and the EC to −75 kg CO_2eq m^{-3}. Alternatively, one could opt to use ambient temperature rather than artificial temperature control. Assuming this would increase the production process time by 1 week to achieve the same fungal growth, the EE and EC would then be 232.6 MJ m^{-3} and −154.7 kg CO_2eq m^{-3}, respectively.

Together, production of mycelium bio-composites has low energy costs and CO_2 emissions, and there is still room for improvement of these values. Moreover, mycelium bio-composites are biodegradable or even compostable, and therefore, these materials do not need to be disposed of by landfilling and will not accumulate in the environment. Construction and demolition waste account for about half of the solid waste generated every year worldwide[41] amounting to about 4 billion tons per annum in the EU, US, China, and India alone.[42] Thus, even if a modest proportion of conventional construction materials would be replaced by mycelium bio-composites, a significant impact could be achieved by reducing energy costs, CO_2 emissions, solid waste, and environmental pollution.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssuschemeng.2c01314.

Material inventory and process inventory for production of a 1 m^3 fungal biocomposite, results from the metabolic carbon experiment, data about cubic samples grown using the method mentioned in the Materials and Methods, and detailed LCI description for the different materials and processes in the production process with description of the assumptions for every step (PDF)

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Notes

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