# Steam Explosion: A Booster for Fungal Growth in a Myco-composite

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**ABSTRACT:** A myco-composite is a sustainable material formed by combining mycelium with a lignocellulosic substrate; these components are binding together to create a light and eco-friendly alternative to traditional materials. In this paper, steam explosion (SE) was experimented as a pretreatment to promote the growth of *Trametes versicolor* on beech wood. An optimal fungal growth was observed for an SE treatment severity (log $R_0$) ≤ 3.96. Characterization and quantification of mycelium density and heterogeneity in the myco-composite were carried out using scanning electron microscopy (SEM), nitrogen measurement, and near-infrared spectroscopy (NIRS) combined with multivariate analysis. The combination of these techniques was shown to be relevant for quantifying the fungal density in the material in relation with the growing matrix used. These results pave the way for a multicriterion optimization of the fungal/matrix choices to obtain myco-composites with specific properties.

**KEYWORDS:** *steam explosion, myco-composite, solid state fermentation, mycelium-based material*
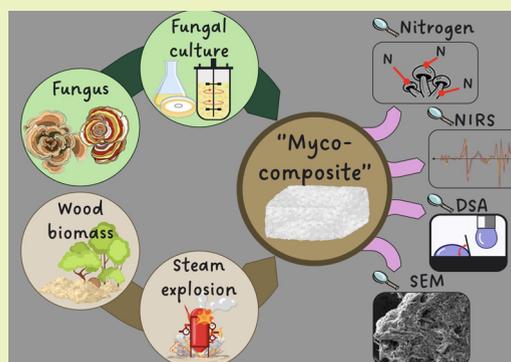
## INTRODUCTION

A myco-composite refers to a composite material made by combining fungal mycelium with various organic substrates, with the mycelium acting as a natural adhesive, binding the substrate particles together to create a strong and durable material. In recent years, myco-composites have gained more attention for their sustainability and eco-friendly properties. They can be used in various applications, including packaging materials, building insulation, and even as a substitute for leather.[1] The versatility and environmentally friendly nature of myco-composites make them a promising option for industries seeking more sustainable alternatives. The fungal strains used in the manufacture of myco-composites are mainly white-rot basidiomycetes.[2] Among the strains investigated in the literature, three fungi are predominant for the production of a myco-composite:[3] *Trametes versicolor*, *Ganoderma lucidum*, and *Pleurotus ostreatus*.

The nature of the substrate is an important element in the myco-composite manufacturing process, and the challenge is to find the best substrate/fungus combination allowing the maximum fungal growth with little or no nutrient supplementation. Various substrate/fungus combinations have been studied in research works.[3−7] Myco-composites are mainly developed from biomass or agricultural biomass residues, such as wheat straw, hemp, etc. Some of the most recent studies use wood as the fungus growth medium.[8] However, given the natural recalcitrance of wood, due in particular to the low accessibility of its components and the high lignin content, the number of examples of wood-based myco-composites is limited.

The steam explosion process (SE) is a pretreatment method broadly used to break down lignocellulosic biomass before fermentation. In SE, the biomass is saturated with steam under high pressure (typically 8−20 bar) during few minutes and then the reactor is rapidly depressurized. This sudden drop in pressure causes water inside the biomass to vaporize quickly. The rapid expansion of steam within the biomass generates mechanical forces that break down the lignocellulosic structure.[9] The combination of a high-temperature cooking step and rapid depressurization causes the biomass to undergo significant physical and chemical changes such as partial hydrolysis of hemicelluloses and relocation of part of the lignin to the fiber surface. This treatment makes the lignocellulosic biomass and cellulose more accessible to enzymes in subsequent processes. This approach has been extensively studied over the past two decades as a pretreatment for the production of cellulosic ethanol and biogas by microorganism fermentation of wood components.[10] Various studies have been carried out to assess the environmental impact and cost of pretreatment. It has been shown that SE is economically feasible[11] and has a limited carbon footprint when compared with conventional alternatives.[12,13]

However, to the best of our knowledge, SE prior to fungal steam fermentation has never been studied for myco-composite production. Myco-composites generally exhibit a complex hierarchical structure, with interwoven networks of mycelium linking the substrate materials.[14] Characterizing this structure is challenging, requiring techniques capable of probing different length scales, from macroscopic to microscopic, and differentiating mycelium from its support. In the work published to date, the myco-composite has been studied primarily by microscopic observation and by assessing the properties of the material produced. For example, Appels et al. investigated nine myco-composites derived from various biomass sources and *T. versicolor*.[15] They employed an approach combining thermogravimetric analysis, tensile and bending tests, and visual assessment to quantify the fungal content within these materials. Sun et al. investigated the production of yellow birch myco-composites through controlled incubation of *T. versicolor* at various time intervals.[2] To compare the mycelium development within the composite material, they combined visual assessments of cross sections with rigorous mechanical testing and comprehensive thermal and acoustic characterizations. Thus, a review of the literature shows that myco-composites have mainly been characterized as a material for their phonic, mechanical, thermal, and other properties. However, it appears that there is a lack for specifically quantifying the amount of mycelium in the myco-composite.

In the present work. a steam explosion pretreatment was experimented on beech wood prior to fungal fermentation using *T. versicolor*. Different temperature and time conditions were studied. Characterization and quantification of the mycelium fraction of the composite were carried out using SEM, nitrogen measurement, and NIRS combined with multivariable calibration. This latter technique offered a powerful analytical tool with minimal sample preparation for fungal growth characterization, proving a rapid analysis.

## ■ MATERIALS AND METHODS

**Biological Materials.** European beech (*Fagus sylvatica*) wood harvested in the Vosges (France) was used as a biomass. Wood was previously cut into small elements before grounding it into particles with a cross-beater mill equipped with 8 mm sieves (Cross beater mill SK100, Retsch, Germany).

**Steam Explosion Pretreatment.** The steam explosion system (ADF, France) for material pretreatment involves a hydrothermal treatment (Figure S1). The wood particles were initially vacuum-impregnated with water for 20 min. Afterward, the liquid was removed and 100 g of the wood particles was placed inside the reactor. High-temperature steam ranging from 180 to 210 °C was injected into the reactor for 5 or 10 min. The condition used and corresponding sample nomenclature are given in Table 1. After the designated time, a pneumatic valve was opened, resulting in the bursting of biomass within the splitter due to the pressure difference. A mixture of the liquid and solid components was recovered. The separation of effluents and exploded particles occurred in several steps: initially, a sieve was used to

**Table 1. Summary of Steam Explosion Conditions for Biomass Production**

| temperature (°C) | residence time (min) | pressure (MPa) | severity factor ($R_0$) | notation |
|---|---|---|---|---|
| 180 | 10 | 1.6 | 3.36 | $R_{0\ 3.36}$ |
| 190 | 10 | 1.9 | 3.65 | $R_{0\ 3.65}$ |
| 210 | 5 | 2.8 | 3.94 | $R_{0\ 3.94}$ |
| 210 | 10 | 2.8 | 4.24 | $R_{0\ 4.24}$ |

separate most of the particles. Afterward, centrifugation (Multifuge X4R Pro, Thermo Scientific, USA) and Buchner vacuum filtration with 25 μm filter were combined. The exploded particles were then oven-dried at 103 °C for 24 h and stored under ambient conditions.

**Medium Preparation and Fungal Culture.** All media were sterilized at 121 °C for 20 min before inoculation (vapor line 135-M, VWR, Germany). To obtain a fungus mat, an initial culture of *T. versicolor* CTB 863 A was grown on malt-agar medium containing 20 g·L$^{-1}$ malt extract for 7 days. Five agar plugs (i.e., diameter of 8 mm) from *T. versicolor* Petri dishes were inoculated in a 250 mL baffled Erlenmeyer flasks with 50 mL of malt medium (20 g·L$^{-1}$). The inoculated Erlenmeyer flasks were placed in an incubation shaker (Innova 44, New Brunswick Scientific, Germany) for 2 days at 28 °C and 100 rpm. After 2 days, the culture was used to inoculate a sterile 7 L bioreactor filled with a 20 g·L$^{-1}$ malt medium at 5 L final volume (Global Process Concept, PRO-LAB, France). The culture conditions were maintained at 28 °C, 80% O$_2$, 250 rpm, and pH 5. Probes were used to monitor growth parameters (pH, temperature, redox potential, oxygen, and optical density of fungal pellets) and to select optimal growth conditions. Once the optimal conditions were achieved, the content of the bioreactor was harvested under sterile condition. The mixture of medium and fungus was separated using centrifugation at 4200 rpm for 10 min at room temperature (Multifuge X4R Pro, Thermo Scientific, USA). The supernatant was removed and reserved for further analysis, while the fungus was used to produce the myco-composite.

**Myco-composite Production.** The substrate consists of either steam-exploded beech particles or native beech particles (used as a control). Both types of particles were sterilized with water (i.e., 60 g of wood with 120 mL of water) at 121 °C for 20 min. A ratio weight/volume of 1:2 of fungus was mixed with the substrate in a microbox (Greiner bio-one, France) (i.e., 30 mL of fungus for 60 g of wood). The microbox was placed in an incubator (climatic chamber KBF LQC 240, Binder, Germany) at 28 °C and 60% RH for 14 days. To encourage fungal growth on the underside of the mold, the molds were turned upside down during the incubation period. After 14 day incubation, the myco-composites were removed from the mold and dried in an oven overnight at 65 °C. The whole production process is illustrated in Figure 1.

**Scanning Electron Microscopy (SEM).** The scanning electron microscopy (SEM) Jeol IT200 was used to analyze samples. Samples were coated with a gold/palladium 4.8 Å metallizer (ACE600 metallizer, LEICA, Germany). Samples were analyzed in high vacuum mode with an SE detector. The working distance was 10 mm. The acceleration voltage and probe current were set to 10 kV and 60%, respectively. The images were processed with ImageJ. To measure hyphal width, 10 hyphae were analyzed from 10 different images for each condition (n = 100).

**Infrared Spectroscopy.** Samples (native or steam explosion-pretreated beech particles and ground myco-composite) were analyzed in mid-infrared (MIR) and near-infrared (NIR) using an infrared spectrometer (Spectrum 100 IR/NIR, PerkinElmer, USA) equipped with an IngaAs detector and a CaF$_2$ beamsplitter. The spectra were obtained from 10000 to 4000 cm$^{-1}$ with 2 cm$^{-1}$ resolution, and an average of 20 spectra was recorded for each sample. Spectral data were computed by using a script on Rstudio (Rstudio.com, version 2023.12.1+402) and Chemospec plugin.[16] The second derivative was calculated from raw NIRS spectra.[17] Principal component analysis (PCA) was performed on the entire spectra to cluster samples according their physicochemical properties. Finally, the PCA scores and loadings were analyzed to identify the most contributing variable on each principal component.[18]

**Elementary Analysis CHONS.** Nitrogen content was measured on 3 randomly harvested samples of the myco-composite (1 cm$^3$), fungal pellets washed with UHQ water, or native beech particles without fungi. The samples were frozen in liquid nitrogen and ground into a homogeneous powder using a ball mill Cryomill (Cryomill grounder, Retsch, Germany). An elemental combustion analysis was performed using a Flash EA 1112 (Thermo Scientific, USA) to quantify nitrogen. Combustion was performed at 950 °C in the presence of copper oxide
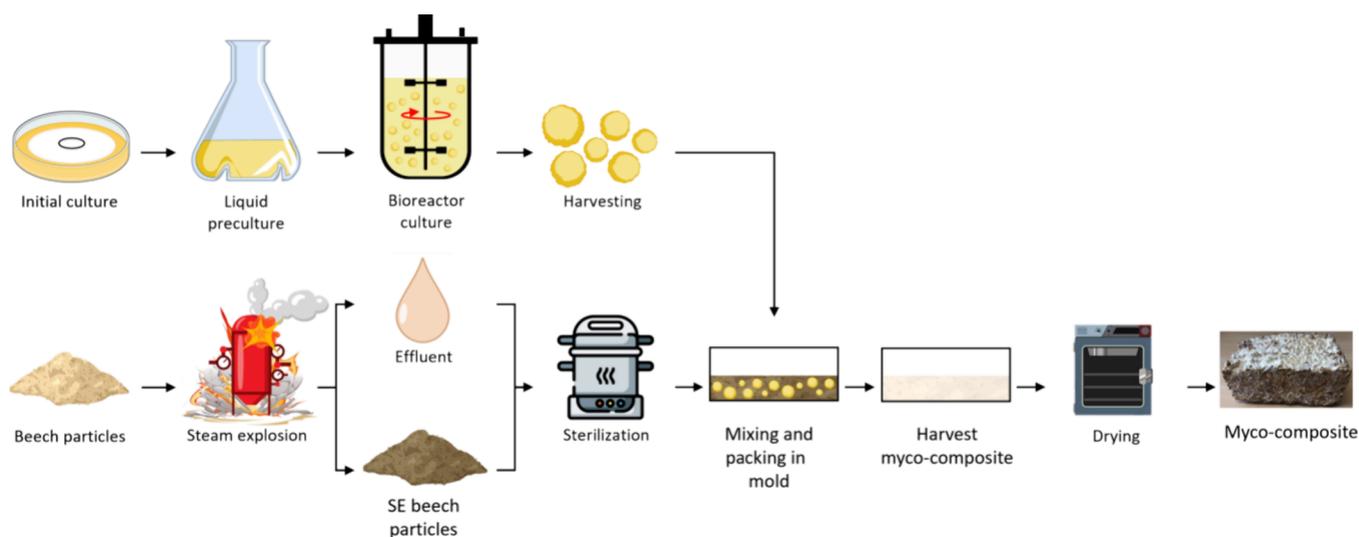
**Figure 1.** Myco-composite manufacturing process from pretreatment and fungus cultivation to the final product, the myco-composite.
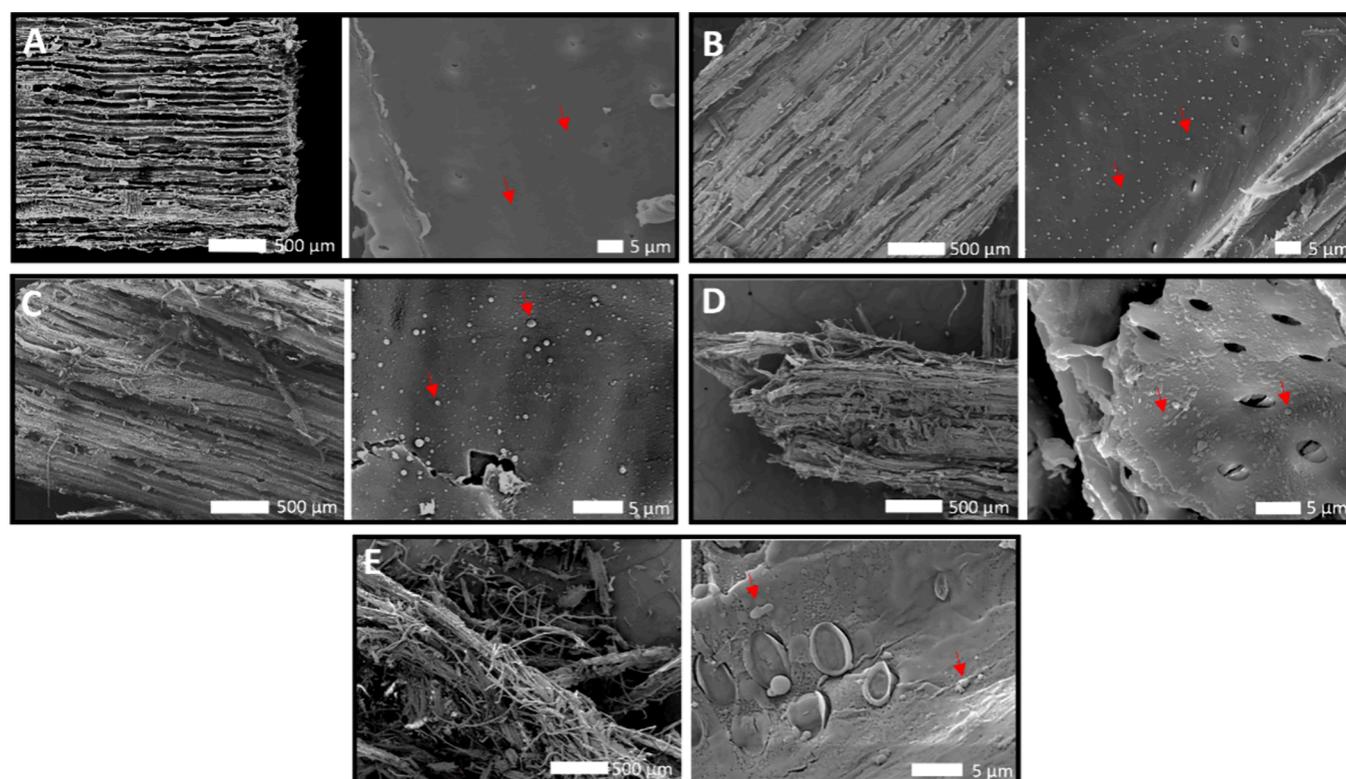


**Figure 2.** Scanning electron microscopy (SEM) observation of wood particles before and after steam explosion. Low-magnification SEM image (left) and high-magnification SEM image (right). Red arrow: lignin deposit. (A) Beech, (B) $R_{0\ 3.36}$, (C) $R_{0\ 3.65}$, (D) $R_{0\ 3.96}$, and (E) $R_{0\ 4.24}$.

under an oxygen flow for 15 s. This combustion produces $CO_2$, $H_2O$, $SO_2$, and $NO_x$, which are reduced to $N_2$ by copper. All of the system was swept by a helium stream. The combustion products were measured by gas chromatography (Chromosorb-filled column, catharometer detector) under the conditions determined by the manufacturer. The results were finally recorded and analyzed by Eager Smart 300 software with an absolute accuracy of $\pm0.3\%$.

## RESULTS AND DISCUSSION

**Effect of Steam Explosion (SE) Pretreatment on Beech Wood Particles.** Steam explosion is a versatile process that was previously shown to alter wood properties on both wood

blocks[19] and also wood particles.[20] It has been described as being particularly effective in overcoming lignocellulosic biomass recalcitrance and making polysaccharides easily digestible by biocatalysts for cellulosic ethanol production. The increase in digestibility is due to both chemical (hydrolysis of hemicelluloses) and mechanical effects (defibration due to the explosive decompression stage). In this work, we focused on European beech (*F. sylvatica*) wood particles of similar size and shape to those found in particleboard, namely, particles that are 8 mm long and 2−3 mm wide. Wood particles were treated by steam explosion (SE) according to 4 combinations of residence time and temperature (Table 1). The calculated severity factor
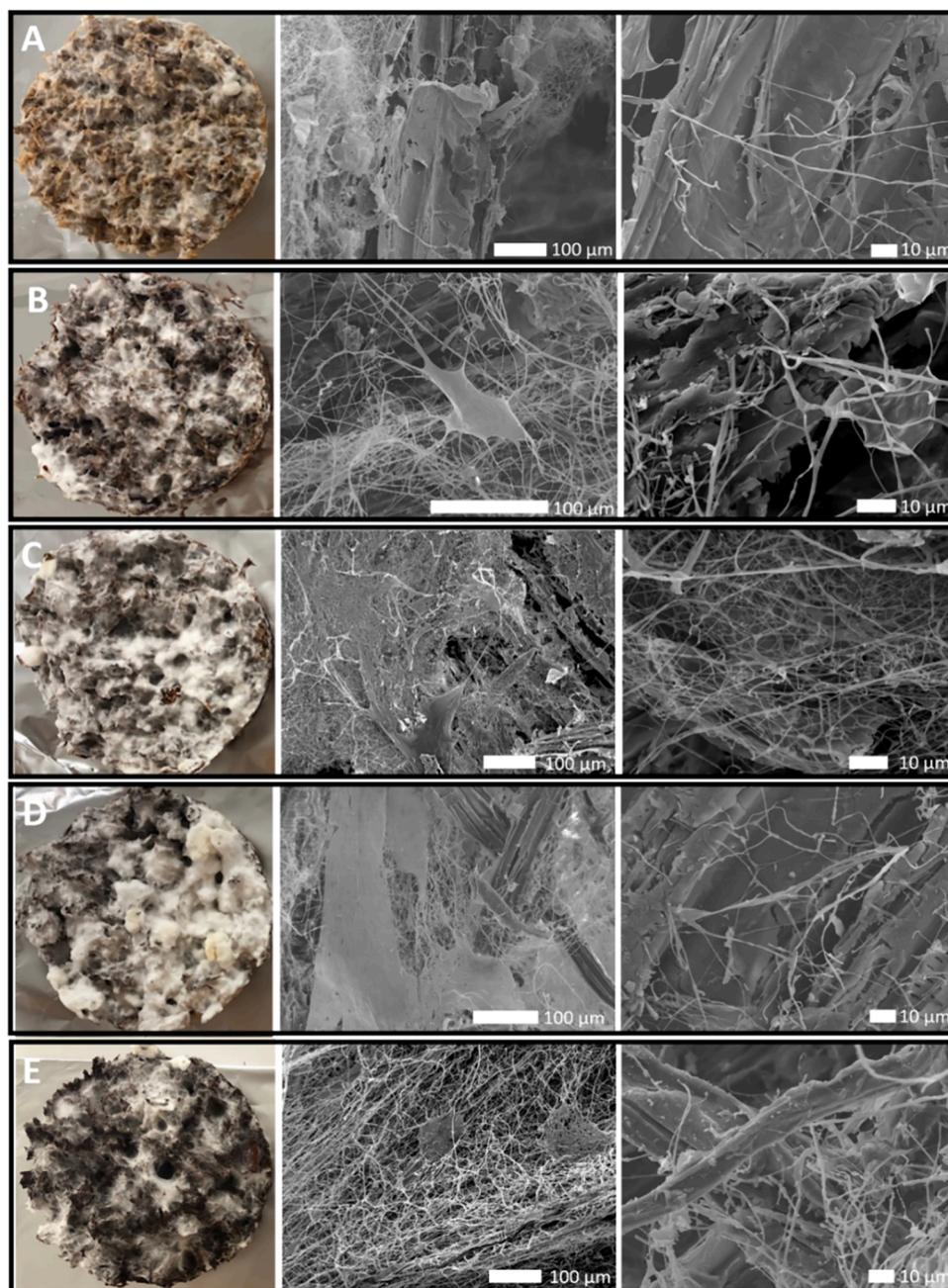
**Figure 3.** Macroscopic observations after 14 days of growth (white: fungus; dark brown: wood particles) and scanning electron microscopy (SEM) observations of the myco-composite: (A) beech, (B) $R_{0\ 3.36}$, (C) $R_{0\ 3.65}$, (D) $R_{0\ 3.96}$, and (E) $R_{0\ 4.24}$.

according to the equation defined by Overend and Chornet is also given and will be used thereafter as a sample name.[21] This severity coefficient was described to provide an estimation of the impact of SE on the hemicellulose hydrolysis.[9]

To obtain a detailed view of the SE conditions, beech wood particles were investigated by scanning electron microscopy (SEM). Representative pictures are shown in Figure 2. Low-magnification observations showed a correlation between the increase in the severity factor and the intensity of wood defibration. On native beech wood particles, the wood cell structure was observed with vessels and fibers tightly packed together. The sample treated under mild conditions, $R_{0\ 3.36}$ and $R_{0\ 3.65}$, showed a change in the beech particles with a partially defibrated surface. The droplets observed on the surface of the fibers are due to lignin redeposition (red arrows, Figure 2).

Indeed, steam explosion was widely reported in the literature to trigger lignin droplet deposits on the cell wall surface, the abundance and size of which seem to depend on the species studied.[22−25] At higher severity ($R_{0\ 3.96}$ and $R_{0\ 4.24}$), the surface became blistered and burred. Greater defibration with higher fiber exposure is clearly observed. The entire cell wall was modified as the temperature of the SE treatment increased. All these observations confirm that SE pretreatment increases the exposure and accessibility of the cell wall to microorganisms and that the effect depends on the severity of the treatment.

**Fungal Growth in Steam-Exploded Wood Myco-composites at Varying SE Severity Levels.** To evaluate the effect of the wood modification caused by steam explosion on the production of myco-composites, hyphal pellets of *T. versicolor* were incubated for 14 days with beech wood particles

pretreated at varying SE severity levels. The total amount of water was equal in all the tested conditions. The mycelium-based material obtained after drying was in the form of blocks with a density of 0.148 ($\pm$0.017) g·cm$^{-3}$. This density range aligns with what has been reported in the literature, i.e., between 0.06 and 0.30 g·cm$^{-3}$.[26]

Macroscopic pictures and SEM observations of obtained myco-composites are given in Figure 3. At the macroscopic scale, the fungal growth in the particle mat appears to be heterogeneous. Indeed, the surface of the materials exhibited both white and brown areas that indicate intense and low colonization by the fungus, respectively. We used this property to perform a semiquantitative evaluation of the fungal colonization by gray level segmentation of the myco-composite surface. Representative samples used for this evaluation are presented on Figure 3. The mycelium surface area increased with the severity of pretreatment, rising from 54% for raw beech to 69% for $R_0$ = 3.96. Detailed surface quantification data are plotted in Figure S2. This suggested that SE treatment created a growth substrate that enhances the fungal development. However, a drop in colonization at high severity ($R_{0\ 4.24}$) was observed with a mycelium surface similar to that of native wood. The SEM observations performed on sample harvest inside the myco-composite volume clearly reveal heterogeneous colonization by *T. versicolor*. For untreated wood, there is weak colonization by the fungus with a limited number of hyphae. This observation is confirmed by the brittle and fragile appearance of the material. On the other hand, SE-pretreated wood produced myco-composites with a more compact appearance, better cohesion, and higher hyphal density observed by SEM (Figure 3). Several other works reported that the parietal composition of hyphae and the hyphal network organization could be affected by the substrate.[6,27−29] This leads to marked changes in mechanical properties.[11,28,30]

The hyphal diameters were measured from the SEM images, and it was observed that hyphal size significantly varied with treatment severity (Figure 4). Similar diameter values ($\sim$0.7

$\mu$m) were measured for native beech and for SE-treated beech $R_{0\ 3.96}$ and $R_{0\ 4.24}$. In contrast, myco-composites produced from $R_{0\ 3.36}$ and $R_{0\ 3.65}$ conditions displayed $\sim$1 $\mu$m hyphae, which correspond to skeletal hyphae (thread-like) in a trimitic hyphal composition.[31,32] These two conditions are therefore potentially conducive to the production of larger and, therefore, presumably stronger mycelia.[32,33]

To quantify the fungal colonization in the material, the myco-composite samples were harvested in the volume of the material and ground using a ball mill to ensure homogeneity and an elementary analysis was conducted (Table 2). Measuring the

**Table 2. Elementary Analysis of Myco-composites**

| sample | N (%) | C:N (%) | H:N (%) |
|---|---|---|---|
| native beech wood particles | <0.05 | n.d. | n.d. |
| pure fungal pellets | 3.27 $\pm$ 0.05 | 13.53 $\pm$ 0.6 | 2.04 |
| myco-composite beech | 0.32 $\pm$ 0.13 | 143.9 $\pm$ 7.3 | 18.6 $\pm$ 0.8 |
| myco-composite $R_{0\ 3.36}$ | 0.64 $\pm$ 0 | 73.1 $\pm$ 0 | 9.6 $\pm$ 0 |
| myco-composite $R_{0\ 3.65}$ | 0.61 $\pm$ 0.18 | 76.6 $\pm$ 3.6 | 10.1 $\pm$ 0.5 |
| myco-composite $R_{0\ 3.94}$ | 0.54 $\pm$ 0.13 | 87.5 $\pm$ 3.7 | 11.6 $\pm$ 0.5 |
| myco-composite $R_{0\ 4.24}$ | 0.50 $\pm$ 0.04 | 92.5 $\pm$ 3.0 | 11.5 $\pm$ 0.9 |

concentrations of nitrogen can be used to indirectly quantify the fungal content in myco-composites made in the same culture conditions. Indeed, the fungi contain nitrogen-rich molecules such as chitin and protein in their cell wall that are required for fungal adhesion to the substrate such as hydrophobins.[28,34] On the contrary, wood particles contained less than 0.05% of nitrogen in mass. To our knowledge, *T. versicolor* is not able to fix $N_2$ from air. Thus, the global nitrogen pool in the myco-composite material would originate from the fungus introduced. Attenuated total reflectance−Fourier transform infrared spectroscopy (AT-FTIR) measurements were carried out on washed mycelium from liquid culture (as fungus pellet). It showed characteristic amide bands, especially the one at 1620 cm$^{-1}$.[35] This band is not present in wood alone but could be detected in the myco-composite, supporting the possibility to use amine and nitrogen as proxy for mycelium quantification in the myco-composite volume (Figure 5).[36]

The nitrogen contents from the fungus may vary from 0.23 to 15% depending on the type of mycelium analyzed and the substrate colonized.[37] In the present study, the nitrogen content was 3.27% ($\pm$0.05) in pure mycelium. The same quantities of fungi in the same physiological state were introduced in the wood substrates. Thus, we considered that a constant nitrogen pool was added in the different samples. After 14 days of growth, when the entire myco-composite was ground and subsamples were used for elemental analysis, the nitrogen content was below the detection threshold, suggesting that the global fungal biomass was low when compared to the wood biomass. Thus, the average total nitrogen content of the myco-composite was too low to be quantified. Because visual inspection showed heterogeneous colonization of the wood particle mat, it makes sense to check whether the nitrogen content could be correlated with different mycelium abundances at the local scale. In a first attempt, elemental analysis was performed on the surface exhibiting a high colonization level and in the volume of the myco-composite with a much lower visible mycelium density. The results (Figure 6) showed clearly that local mycelium density was correlated to nitrogen content. Thus, the elemental analysis was performed on subsamples of the core layer of the diverse myco-composite sample to investigate if the variation of
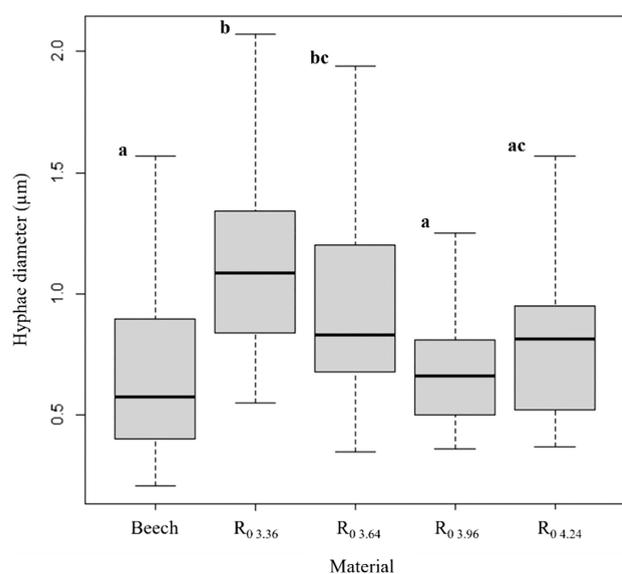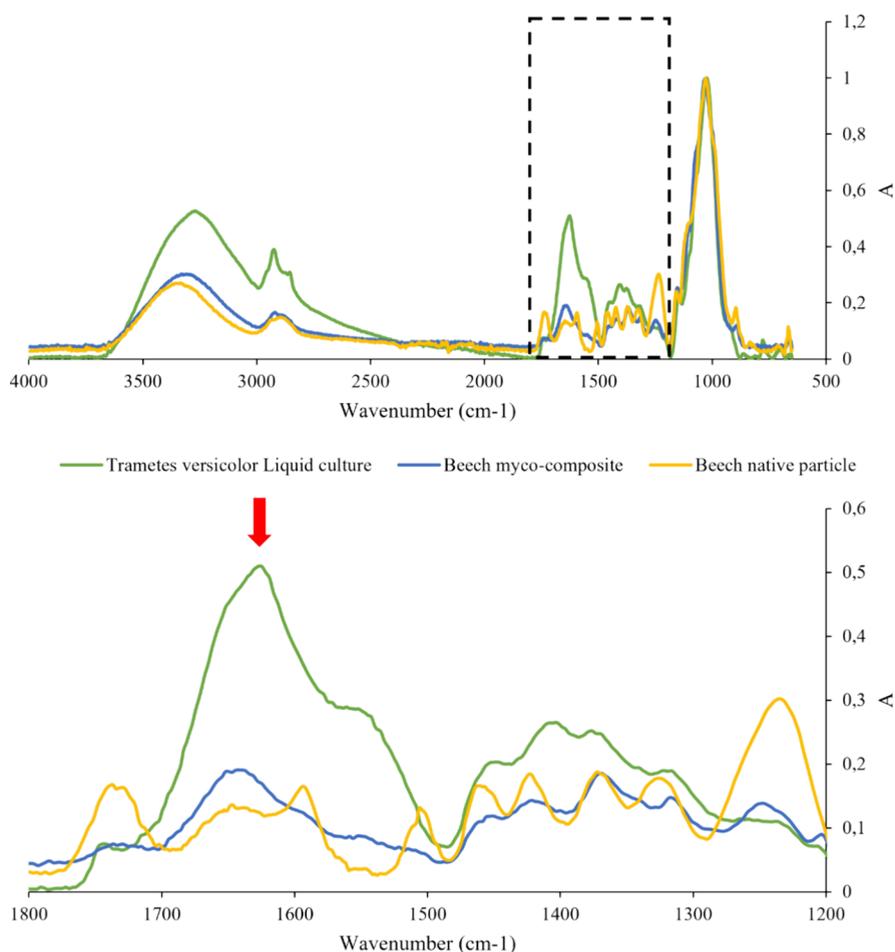


**Figure 4.** Average diameter of hyphae from *T. versicolor* in myco-composites produced under various steam explosion conditions measured from SEM images. Letters indicate groups that are significantly different (ANOVA, $p < 0.05$).

**Figure 5.** AT-FTIR detection of amine groups in beech wood particles, beech myco-composite, and *T. versicolor* pellets. (A) MIR spectra from 4000 to 500 cm$^{-1}$ and (B) MIR spectral focus on the zone from 1800 to 1200 cm$^{-1}$.

hyphal network density in the material could be correlated with the physicochemical changes of the wood substrate.

The results in Table 2 illustrate that despite the heterogeneous growth observed in the material, the local hyphal density in the volume was higher in the wood particles treated in mild severity conditions. This quantitative analysis is directly linked to fungal physiology. Fungi are known to recycle their cellular material (especially nitrogen) by autophagy.[38] It has been suggested on the basis of ecophysiological models (which could be applied to SE wood) that the more aversive the environment, the more important the recycling strategy for fungal development and environment exploration by running hyphae.[39] In our case, we hypothesized that the stricter SE conditions would strongly hamper the fungal development due to strong reduction in hemicellulose content and an increase in cell wall surface hydrophobicity. We observed that the harsher the conditions, the more the cellular recycling is stimulated and hyphal network density is decreased and, ultimately, more nitrogen is diluted in the hyphal network.

To develop a nondestructive technology to quantify hyphal colonization and distinguish the effect of SE on wood particles from fungal abundance, NIRS was performed on the myco-composites and beech particles. After spectral data computation, the PCA plots allowed us to successfully cluster samples according to the SE conditions used (Figure 7A and Figure S3). The loading analysis allowed us to attribute the band related to hemicellulose and lignin to the first component (PC1). Thus,

PC1 discriminates samples based on the impact of SE treatment on the wood. It captures variations related to the severity of the treatment. Bands related to protein and nitrogen-containing compounds were identified as the main contributing bands on the second component (PC2). Thus, PC2 discriminates samples based on the presence of fungi in the particles. Statistical analysis (one-way ANOVA) on PC2 scores from the PCA (Figure 7B) showed that this component allows specific discrimination between myco-composite samples. Physicochemical changes of the wood substrate caused by SE did not affect the analysis since native and SE beech wood particles did not differ. Finally, a significant difference found between myco-composites grown on native and SE particles suggested that fungal abundance, i.e., hyphal network density, could be evaluated by NIRS.

To evaluate the sensitivity and robustness of NIRS fungal quantification, the correlation between the nitrogen content and NIRS data was evaluated. The regression plot (Figure 8) shows that the nitrogen content could be correlated ($R^2 = 0.99$) to the PC2 score from the PCA. By connecting nitrogen levels with fungal quantity, we observed that this result can be directly related to fungal abundance, consequently, fungal growth depending on the substrate. These results showed that the modification of beech particles through SE promoted the growth of fungi in the myco-composite under the conditions $R_{0\ 3.36}$, $R_{0\ 3.65}$, and $R_{0\ 3.94}$. For more severe conditions ($R_{0\ 4.24}$), the fungus did not develop optimally. The combination of NIRS data and nitrogen content seems to be correlated to the hyphal
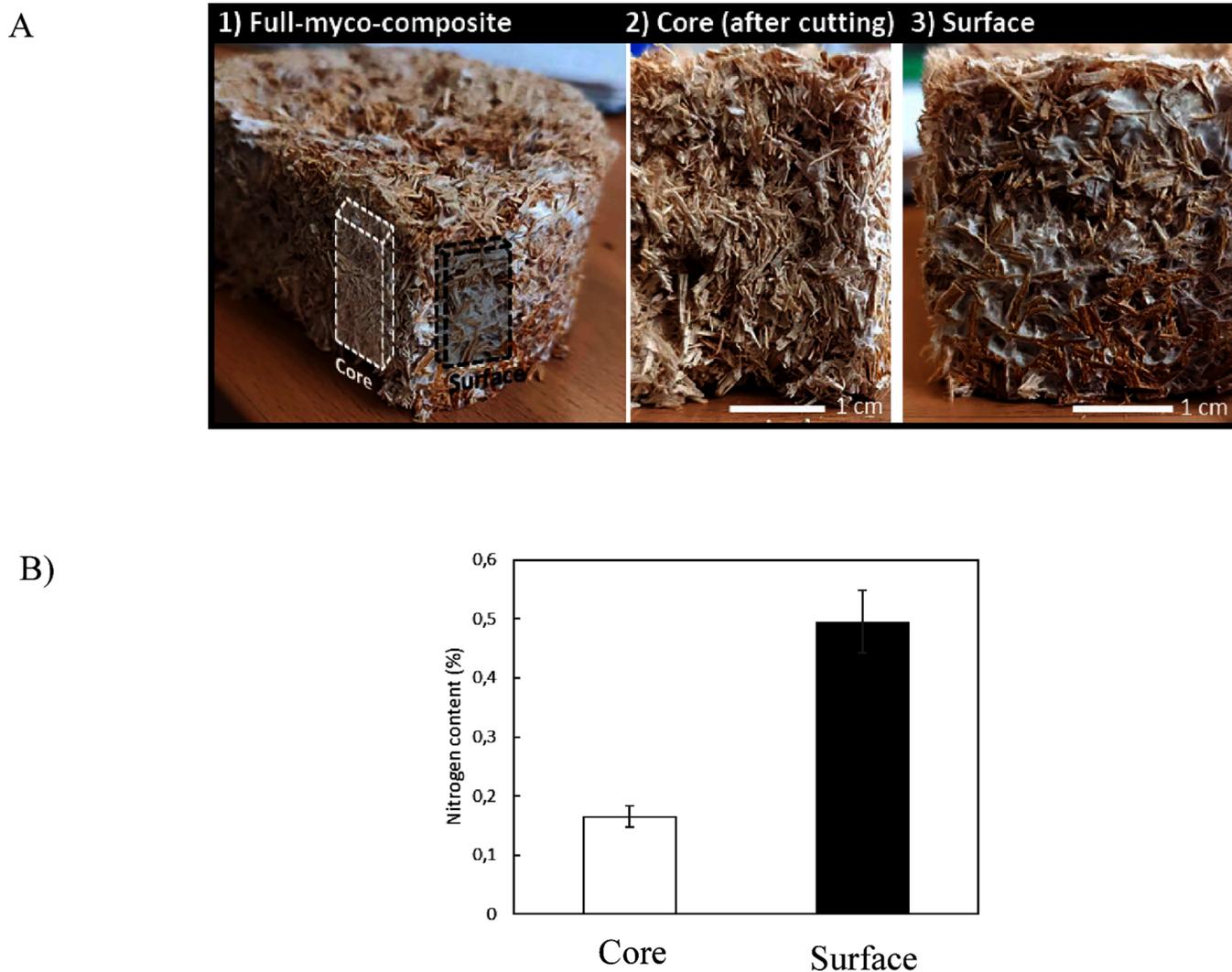
**Figure 6.** Correlation between nitrogen quantification and mycelium abundance. (A) Observation of (1) the full, (2) core, and (3) surface parts of the beech wood myco-composite. (B) Nitrogen content in different samples harvested in the core (white bar) or on the surface (black bar) of the myco-composite. Error bars are 95% CI ($n = 5$).

network structure because samples exhibiting the trimitic hyphal morphology ($R_{0\ 3.36}$ and $R_{0\ 3.65}$) are clustering together.

Taken together with microscopy data, the results suggest that the strong alteration of cell wall chemistry results in the potential generation of fungal growth inhibitors on the cell wall surface. For a similar mycelium abundance found in $R_{0\ 3.65}$ and $R_{0\ 3.94}$ myco-composites, the morphology of hyphae was significantly different, suggesting that the fungus develops a gradual adaptation to its substrate upon colonization. On malt extract, the hyphal morphology of *T. versicolor* is trimitic.[40] The monomitic morphology observed in the $R_{0\ 3.94}$ myco-composite strongly suggests a stress reaction of the fungus. Skeletal and ligative hyphae found in the trimetic hyphal system were shown to be brought up to 86% of the mechanical resistance and be 5.5-fold more resistant than generative hyphae.[32] Combination of fast hyphal density quantification by NIRS and nitrogen quantification was in agreement with the microscopic data. It brings valuable morphological characteristics of the mycelium that could be used to predict overall fungal development in the material and infer the myco-composite properties.

From a technical-economic point of view, SE is considered to be energy-efficient, even though a significant amount of energy is required for heat generation. Sui and Chen have modeled the heat transfer of the steam explosion process.[41] They have shown that energy efficiency can be improved by controlling biomass humidity (<40%), reducing particles size, and adopting low-temperature and long-duration operating parameters. The pretreatment conditions optimized in this study for fungal growth (190 °C, 10 min, and particles size of 8 mm) therefore correspond to relatively limited energy consumption.

## ■ CONCLUSIONS

In this study, it was shown that steam explosion, which combines a hydrothermal cooking step with explosive decompression, is an effective pretreatment to enhance fungal colonization of beech wood by *T. versicolor* for the preparation of myco-composites in mild condition. SE has been used as a valuable tool to selectively modify the properties of wood cell walls, resulting in increased overall fungal growth, leading to a denser mycelium. The association of elementary analysis and near-infrared spectroscopy coupled with multivariate analysis was a relevant technique for quantifying fungal development. SEM allowed the fine analysis of the hyphal structure. Altogether, these techniques allowed inferring the myco-composite material quality. It has
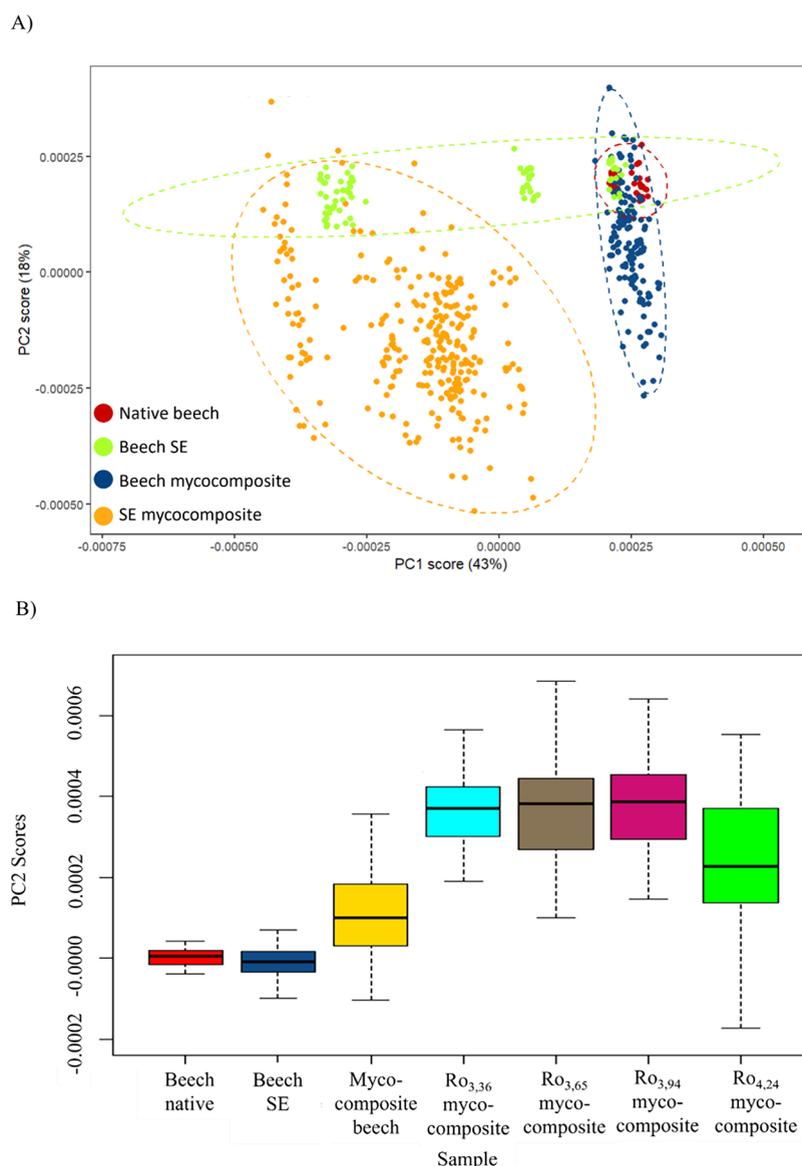
**Figure 7.** Multivariate analysis of NIRS. (A) PCA of native and steam explosion-treated beech particles and myco-composites grown on native and steam explosion-treated beech particles. The mean second derivative was calculated between 10000 and 4000 cm$^{-1}$. The plots show PC1 and PC2 scores. (B) Boxplot of the second component's scores for each sample.
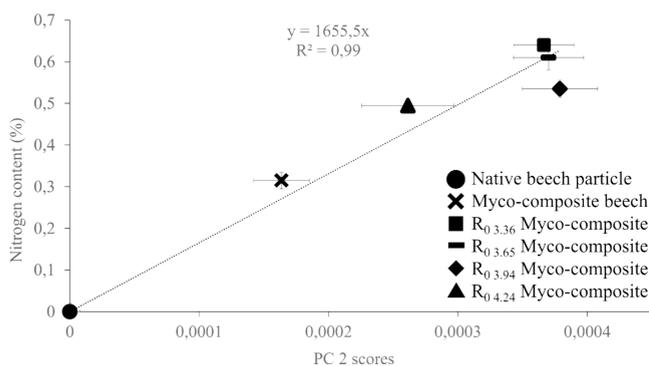


**Figure 8.** Nitrogen content as a function of the medium scores of component no. 2 of myco-composites and native wood particles.

been concluded from this study that optimal fungal growth is observed for an SE treatment severity <3.96. We hope that this study, which provides insights into the interaction between pretreatment, fungal growth, and material properties, will contribute to the development of myco-composites.

## ■ ASSOCIATED CONTENT

### ⓢ Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssuschemeng.4c03099.

> SE system schematic, quantification of the mycelium area on the myco-composite surface in the different samples, and PCA with details of the sample (PDF)

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## Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

## Notes

The authors declare no competing financial interest.

## ■ ABBREVIATIONS

SE; steam explosion; SEM; scanning electron macroscopy; NIRS; near-infrared spectroscopy; NIR; near-infrared; MIR; mid-infrared; MIRS; mid-infrared spectroscopy; PCA; principal component analysis; ANOVA; analysis of variance; ATR-FTIR; Attenuated total reflectance—Fourier transform infrared spectroscopy

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