



WOOD2WOOD

A Wood-to-Wood Cascade Upcycling Valorisation Approach

» Deliverable 9.3

Glue separation from wood and bioremediation wood treatment

WP No.	9
WP Title	Chemical and Bioremediation Technologies for Wood Waste Upcycling (TRL3-5)
WP Leader	LERMAB
Due Date:	02/06/2025
Submission Date	16/06/2025
Authors	Arnaud Besserer; Nicolas Brosse; Melek Ayadi
Contributing Partners	Melek Ayadi; Coline Giustiniani
Status	Final



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Peer Reviewers	-
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GA Number	101138789
Project Full Title	Wood2Wood Project
Project Acronym	W2W
Funding Scheme	Horizon RIA
Start Date	01/01/2024
Duration	48 months
Coordinator	Dr. Angelos Amditis, Research and Development Director, ICCS
Project Website	In progress

PROJECT INFORMATION



REVISION AND HISTORY CHART

Version	Date	Author	Description of changes
0.1	02/06/2025	Arnaud Besserer; Nicolas Brosse; Melek Ayadi	Draft Version
1.0	30/06/2025	Melek Ayadi; Celine Giustiniani	Final submitted version
2.0			Revised submitted version (if required)

Dissemination Level		
PU	Public	X
RE	Restricted to a group specified by the consortium	
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GLOSSARY OF ACRONYMS

Acronym	Extended Definition
CLSM	Confocal Laser Scanning Microscopy
MWBP	Machining wood by-product
NIPU	Non-Isocyanate Polyurethane
NIRS	Near Infrared Spectrometry
PCA	Principal Component Analysis
SE	Steam explosion
SEM	Scanning electron microscopy
UF	Urea-formaldehyde
W2W	Wood2Wood

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EXECUTIVE SUMMARY

The Wood2Wood (W2W) project aims to revolutionize the recycling and upcycling of post-consumer wood (PCW) by developing sustainable, circular solutions for the construction and furniture industries. Deliverable 9.3 focuses on Use Case 2, which explores the application of steam explosion (SE) and fungal bioremediation to remove contaminants from BR1/BR2 wood waste and repurpose it into high-performance, environmentally friendly materials.

This report presents the results of experimental work conducted to evaluate the effectiveness of SE as a pretreatment method for enhancing fungal colonization and enabling the production of mycelium-based composites (myco-composites). The SE process was shown to significantly alter the physical and chemical structure of wood particles, improving their suitability as substrates for fungal growth. Optimal fungal development was observed at intermediate SE severity levels ($R_0 \approx 3.65$), which promoted dense hyphal networks, high extracellular polymeric substance (EPS) production, and improved material cohesion.

The deliverable also documents the initial production and validation of particleboards incorporating SE-treated wood and bio-based adhesives, including non-isocyanate polyurethane (NIPU). These panels demonstrated promising thermal insulation properties and structural performance, although further optimization is needed to meet industrial standards for internal bond strength.

Key findings include:

- i. SE effectively removes UF resins and enhances wood defibration, improving substrate bioavailability.
- ii. Myco-composites produced from SE-treated wood exhibit improved fungal colonization and mechanical properties.
- iii. NIPU adhesives derived from BR1/BR2 wood offer a viable alternative to conventional UF resins, supporting VOC-free panel production.
- iv. Thermal conductivity tests confirmed the potential of myco-composites as sustainable insulation materials.

Overall, Deliverable 9.3 confirms the feasibility of integrating SE and fungal bioprocessing into wood recycling workflows, supporting the W2W project's broader goal of establishing a circular, zero-waste bioeconomy for wood-based materials.

1. INTRODUCTION

1.1. PROJECT INTRO

Ambitious climate change reduction policies are increasing the demand for woody biomass in the EU while potentially restricting its supply. The amended Renewable Energy Directive ("RED III") aims to raise the share of renewable energy in the EU's overall energy consumption to 42.5% by 2030, up from the current share of 23%. The goal of integrating 30% wood in construction will also boost wood demand in Europe (Lerink et al., 2023). The updated regulation on land use, land-use change, and forestry (LULUCF) sets ambitious and binding national targets for increasing net greenhouse gas removals for the period 2026-2030, which will help achieve the EU's collective target of 310 Mt CO₂ equivalent net removals in the LULUCF sector by 2030 (EU, 2023). For member states that are already intensively using their forests, these carbon sink targets can only be met by reducing harvesting. Therefore, the only viable option will be to adapt forestry and wood utilization practices to both preserve forest resources and biodiversity while enhancing the benefits of CO₂ sequestration by trees (Bozzolan et al., 2024; Fulvio et al., 2025; Jonsson, 2024).

At the same time, approximately 40 million tons of post-consumer wood (PCW) are collected each year by collection systems in Europe, according to Eurostat's database. The European Wood Circus project estimated this volume at 70 Mt. Additionally, wood accounts for 20 to 30% of the total construction and demolition waste related to buildings. The increasing consumption of wood and derived products continues to rise. Recycling these streams can provide an abundant and cost-effective raw material source that has been well identified at the European level (European Commission, 2024) and is one of the strategic focuses of Horizon Europe and CBE project calls until 2031. Utilizing this post-consumer wood resource presents a challenge due to its heterogeneity (Altgen et al., 2025). Wood waste streams from industrial and urban waste contain significant amounts of fossil carbon from coatings, paints, plastics, and preservatives that could be recycled using innovative processes, making their valorization one of the priorities of Processes4Planet (P4P) for wood waste treatment. However, recycling processes are significantly complicated by the presence of additives (glues, varnishes, and paints), pollutants (wood treatment products and heavy metals), and contaminating materials (glass, plastics, metals, etc.), necessitating research and development efforts to improve them. Other challenges include the irregular and unpredictable availability of these waste streams and the complexity of sorting and cleaning processes.

In this context the W2W project aims to improve the use of post-consumer wood utilization for material recovery. This will be a major change regarding the current wood waste management strategies which prioritise landfill and energy recovery. To tackle these challenges, W2W proposes a comprehensive framework for multi-dimensional cascade (Besserer et al., 2021; Schmitz et al., 2023). In the project the valorisation of wood from C&D and furniture waste is consisting of four core components/pillars: 1) Advanced Separation and Sorting Technologies; 2) Upcycling Processes and Technologies; 3) Digital Tools for Improving Circular Flows of Secondary Materials; and 4) Supportive Framework in Policy, Market, and Skills. W2W will demonstrate efficient and sustainable value chains for the production of wood without pollutants; bio-composite building

materials; biopolymers; polyols; chemical detergents; and the recovery of nutrients, through the use of technologies and tools that allow the selection of optimal cascade paths for further uses of wood products and their materials. The W2W holistic approach will reduce the demand for virgin materials, reduce the amount of waste that ends up in landfills or incineration facilities, allow for the creation of new value-added products from waste materials, and support the transition towards a circular economy by promoting closed-loop systems where materials are continually repurposed and reused, extending their useful life.

1.2. PURPOSE OF THE DELIVERABLE

This deliverable will provide the results obtained in WP9 from the beginning of the project. It will focus on the use case 2 of the project that aims to remove contaminants from wood by using the steam explosion process.

Steam explosion pretreatment is a so-called thermomechanical process known for the treatment of lignocellulosic material. It involves hydrothermal treatment under pressure at high temperatures for relatively short periods of time. The steam explosion process aims to treat wood material to produce particleboard, increase access to cellulose in the wood CW (Ziegler-Devin et al., 2021). The steam explosion pre-treatment process is currently used mainly for the extraction and/or processing of wood components with the aim of adding value and using them in numerous fields such as food, cosmetics, etc.... It is also being developed on a pilot and/or industrial scale for various applications (Qian et al., 2022; Ziegler-Devin et al., 2021). The EV process is economically feasible and has a limited carbon footprint compared to alternatives (Kral et al., 2016; Kratky Lukas et al., 2018).

The first part of the Wood2Wood project was dedicated to the implementation of steam explosion technology on the recycling of post-consumer wood classified as BR1/BR2. This class of post-consumer wood mainly includes wood from furniture waste. It consists mainly of solid wood of various species, particleboard and fiberboard. However, OSB and plywood panels are also included. All these types of panels contain binders, mainly of the phenol-formaldehyde (PF) or urea-formaldehyde (UF) type, which need to be removed from the wood so that the particles, veneers or fibers can be reused in other materials.

Post-consumer wood was supplied by the French eco-organization Ecomaison (<https://ecomaison.com>). Currently, 70% of the BR1/BR2 post-consumer wood which is reused in the wood sector is grounded to be incorporated into particle board. Thus, we also considered to test the SE technology on the particle board mix used in the production chain of particle board manufacturer CF2P (<https://www.cf2p.eu/>).

The workflow of WP9 is represented on Figure 1.

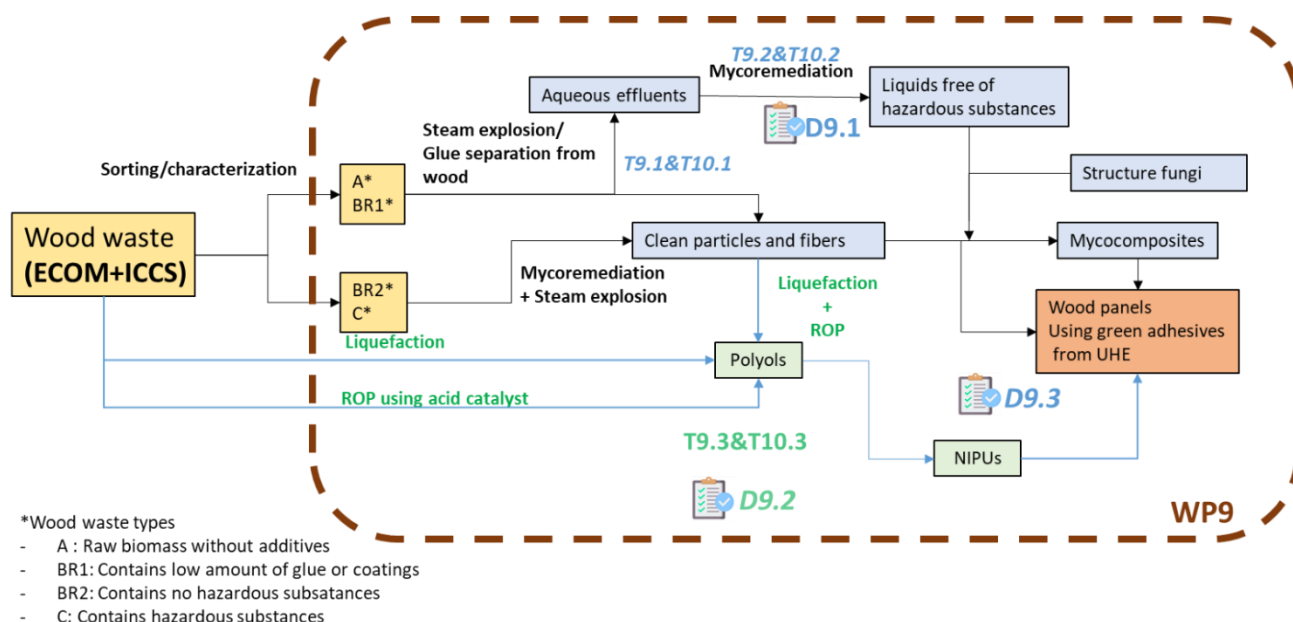


Figure 1: Workflow of the WP9 (use case 2).

The tasks and deliverables are indicated. Blue, green and orange colors indicate parts that will be done by LERMAB, UHE and shared. Yellow indicates tasks attributed to ECOM and ICCS.

Deliverable 9.3 corresponds to task 9.4 “Final products and initial validation” of the WP9 found in pillar 2 of the project. The objective of this task is to initiate the testing of the production of particleboard incorporating BR1/BR2 wood treated by steam explosion, either as a substitute for native BR1/BR2 particles or in the form of incorporated myco-composites included in the inner layer of the particleboard. In both cases, the objective is to examine the potential for utilizing SE-treated wood in a panel production process that has a reduced environmental impact and adverse health effects for humans. To reduce VOC emissions as much as possible, and as part of a logic of circularity and maximum optimization of the use of BR1/BR2 wood, the first tests will be carried out on the manufacture of panels bonded with the biosourced glue produced in task 9.3. The biobased binder under consideration is produced from polyols that are obtained by subjecting BR1/BR2 wood to a chemical transformation process, thereby producing an isocyanate-free polyurethane binder (NIPU). The utilization of this binder is intended for the domain of panel production, thereby superseding the conventional Urea-Formaldehyde (UF) glue that has historically been employed. The subsequent phase of the project will entail the multicriteria characterization of the resulting materials, namely myco-composites and hybrid particleboard. The findings documented in Deliverable 9.3 are contingent upon technological advancements and the outcomes reported in Deliverables 9.1 and 9.2. An independent preliminary study was conducted to assess the impact of structural and chemical modifications to the wood wall caused by the steam explosion process on particle growth and cohesion by the fungus. Preliminary experiments were conducted with the objective of producing myco-composites from BR1/BR2 wood.

1.3. INTENDED AUDIENCE

The audience for this deliverable is scientific experts and R&D managers in companies. Public decision makers would also be concerned.

1.4. STRUCTURE OF THE DELIVERABLE

This deliverable is structured in 2 main chapters. The first chapter presents the results of the studies that were performed to evaluate the effect of SE on wood biomass to reuse it in myco-composite application. The effect of SE on fungal growth and the first characterization of the fungal growth pattern with the myco-composite properties are shown. In the second chapter, the initial panel production at TRL3 is presented. Four types of panels were produced: myco-composite boards, standard particle board made of industrial panel mix and glued with UF resin, SE treated BR1/BR2 wood glued with UF resin and particle boards made of industrial panel mix and glued with NIPU resin produced from BR1/BR2 wood.

On the first part of the project the goal was to implement and test the steam explosion technology on BR1/BR2 post-consumer stream harvested from the industry at TRL5 and to start the particleboard and myco-composite production.

2. Chapter 1: Steam Explosion treatment and myco-composite production

2.1. PRESENTATION OF THE TECHNOLOGY USED: THE STEAM EXPLOSION

Post-consumer BR1/BR2 wood, beech (*Fagus sylvatica*) wood particles and machining wood by-product (MWBP) were used as substrate biomass. The wood underwent an initial size reduction into small pieces, followed by grinding with a cross-beater mill equipped with 8 mm sieving grids (Cross beater mill SK100, Retsch). A steam explosion pilot unit (Figure 2) operating at Technology Readiness Level 5 (TRL 5) developed and commissioned in LERMAB at Épinal (France), with its official inauguration which took place in September 2023, was used as pretreatment for MWBP and post-consumer wood. This process, classified as thermomechanical, is well-known for processing lignocellulosic biomass. It involves hydrothermal treatment under pressure at high temperature for relatively short durations. Two stages can be identified in the pre-treatment of lignocellulosic material: the chemical treatment of the material by steam at high temperature and the mechanical treatment with the defibration of the material linked to the sudden opening of the reactor leading to the restructuration of the material by depressurization. The initial stage is the steam cracking phase. This stage corresponds to the time it takes for the biomass in the reactor to be heated by steam at the set temperature and pressure. This stage is delineated as a succession of four sub-

steps (Kessler et al., 1998): steam injection, steam treatment, fibrillation of the material, and, finally, degradation of the wood components.

The severity factor is a parameter frequently utilized to assess the pretreatment process. The present study combines treatment temperature (T) and residence time (rt) into a single value, defined by the following equation

$$S = rt \times e^{\frac{T-100}{14.75}}$$

Equation 1: Severity factor calculation

(R.P. Overend and E. Chornet, 1987; Faucher, Marzetti, and Crescenzi, 1991).

The well described effect of steam explosion on wood is fragmentation of the biomass partial hydrolysis and relocation of the cell wall macro-polymers.

To optimize treatment efficiency, MWBP particles were first soaked in water for 20 min and then the biomass is fully loaded into the reactor from the top. Saturated steam is injected into the chamber. The pretreatment process is initiated once the reactor reaches a uniform target temperature, ensuring consistent thermal exposure throughout the biomass. The condensation of steam heats the biomass, enabling its “capillary-like” microporous structure to become saturated with hot liquid water. (Chornet et al., 1991). After maintaining the material under these conditions for the predetermined residence time, a control mechanism activates the rapid release of pressure by opening a pneumatic discharge valve. At the pilot output, a mixture of fibers and effluents is collected. This mixture must then be separated to allow each component to be processed individually. The exploded particles were then oven-dried at 103 °C for 24 hours and stored under ambient conditions.



Figure 2: Steam explosion pilot at LERMAB (CH4NGE, France)

2.2. THE MYCO-COMPOSITES

2.2.1. Definition of a myco-composite material

Mycelium-based composites or myco-composites are a promising category of bio-based materials, created by cultivating mycelium—the vegetative structure of filamentous fungi—on solid organic substrates. Fungi are eukaryotic microorganisms found in virtually all environments, exhibit remarkable diversity in both form and function and acclimation potential. Many species play a vital ecological role by breaking down ligno-cellulosic biomass and recycling carbon in terrestrial ecosystems or are responsible for timber decay in buildings. Due to their unique metabolic and physiological traits, fungi hold significant biotechnological potential and offer sustainable, efficient, and cost-effective solutions to modern environmental issues.

When mycelium grows on lignocellulosic substrates, it forms a lightweight composite material. This material consists of a three-dimensional network where natural reinforcement fibers from the substrate (such as wood) are interwoven with the filamentous cells of the mycelium. The fungal organism is a crucial component of the composite—not only because of its cellular structure, which imparts unique properties, but also due to its growth behavior. During development, the mycelium acts as a “natural glue,” enabling self-assembly and eliminating the need for synthetic, fossil-based polymer binders (Figure 3).



Figure 3: Multiscale visualization of a myco-composite material. From left to right : macroscopic view of a wood particles based myco-composite material glued with *Trametes versicolor*; correlative microscopy (CLSM-SEM) showing the fungal hyphae in green and the wood in red; SEM detail showing hyphae connecting two wood particles.

Once the substrate is fully colonized, the material is heat-treated to deactivate the organism and remove moisture (Elsacker et al., 2020).

Here, as we were using waste streams, the entire production process can be considered environmentally sustainable. Importantly, the resulting mycelium composites are made entirely from biocompatible materials and are fully biodegradable, supporting a circular production model.

2.2.2. Proof of concept of using SE on wood recycling: effect of the process on wood particle material and its subsequent use for myco-composite production

In the deliverable 9.1, the potential of SE in UF-resin removal from BR1/BR2 wood is described. It is shown that SE is an efficient process to remove glue from post-consumer wood. Because wood recycling includes not only the bioremediation step but also the reuse of the treated particles, we plan to reuse these particles into panels showing improved environmental characteristics but with constant or better technical properties than current particle boards. Thus, it was necessary to investigate the compatibility of SE on untreated wood (A class) with the production of myco-composite because it involves the interaction of a living micro-organism with a modified lignocellulosic matrix. First experiments on the effect of SE in the repurposing of particles to particle boards with standard and innovative binders will be presented in a second time.

2.2.3. Effect of steam explosion treatment on mycelium growth and myco-composite production

In the first study steam explosion (SE) was experimented as a pretreatment to promote the growth of *Trametes versicolor* on Beech wood (Aguilar et al. 2024). A set of SE experiments using severity coefficients of S_0 3.36, S_0 3.65, S_0 3.96 and 4.24 were tested. We develop a multi-modal set of characterization methods using scanning electron microscopy (SEM), nitrogen measurement, and near-infrared spectroscopy (NIRS) combined with multivariate analysis. Macroscopic pictures and SEM observations of obtained myco-composites are given in Figure 4. At the macroscopic level, fungal colonization within the particle mat appears uneven. The surface of the materials displays a mix of white and brown regions, corresponding to areas of high and low fungal growth, respectively. This visual contrast was used to conduct a semi-quantitative assessment of fungal colonization through grayscale segmentation of the composite surface.

The proportion of surface covered by mycelium increased with the severity of the substrate pretreatment, rising from 54% for untreated beech wood to 69% for a severity factor of $S_0 = 3.96$. This indicates that steam explosion (SE) pretreatment enhances the substrate's suitability for fungal growth. However, at a higher severity level ($S_0 = 4.24$), colonization declined, with mycelium coverage returning to levels similar to those observed in untreated wood.

SEM analysis of samples taken from within the composite volume confirmed the heterogeneous colonization by *Trametes versicolor*. In untreated wood, fungal colonization was minimal, with only a sparse presence of hyphae, which corresponded to a brittle and fragile material structure. In contrast, SE-pretreated wood yielded composites with a denser, more cohesive structure and a visibly higher hyphal density under SEM (Figure 4).

Previous studies have also shown that the composition of fungal cell walls and the organization of the hyphal network can be influenced by the substrate, leading to significant variations in the mechanical properties of the resulting composites.

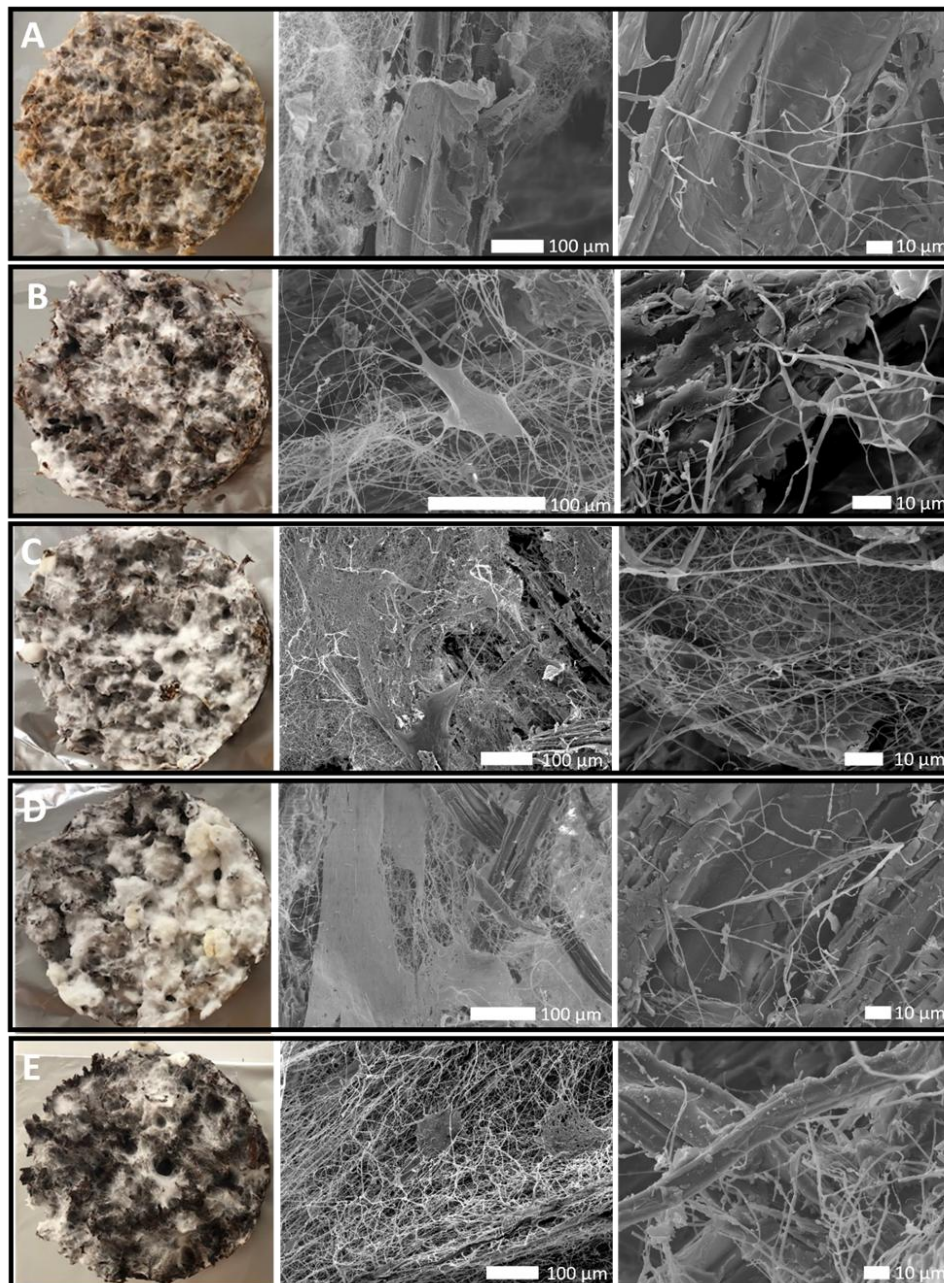


Figure 4: Macro and microscopic observation of myco-composites after 14 days of growth. Macroscopic observations after 14 days of growth (white: fungus; dark brown: wood particles) and scanning electron microscopy (SEM) observations of the myco-composite: (A) beech, (B) S0 3.36, (C) S0 3.65, (D) S0 3.96, and (E) S0 4.24.

These observations were only qualitative. To quantify fungi in the composite, we took advantage of the chemical difference in cell wall composition of fungi and wood. Wood primarily contains carbohydrates (cellulose and hemicelluloses) and lignin, and its nitrogen content is very low. On the contrary, the fungal cell wall contains chitin and proteins. An AT-FTIR analysis of fungal mycelium, virgin Beech wood particles and myco-composite clearly showed that nitrogen (amine groups) are markers for the fungus (Figure 5).

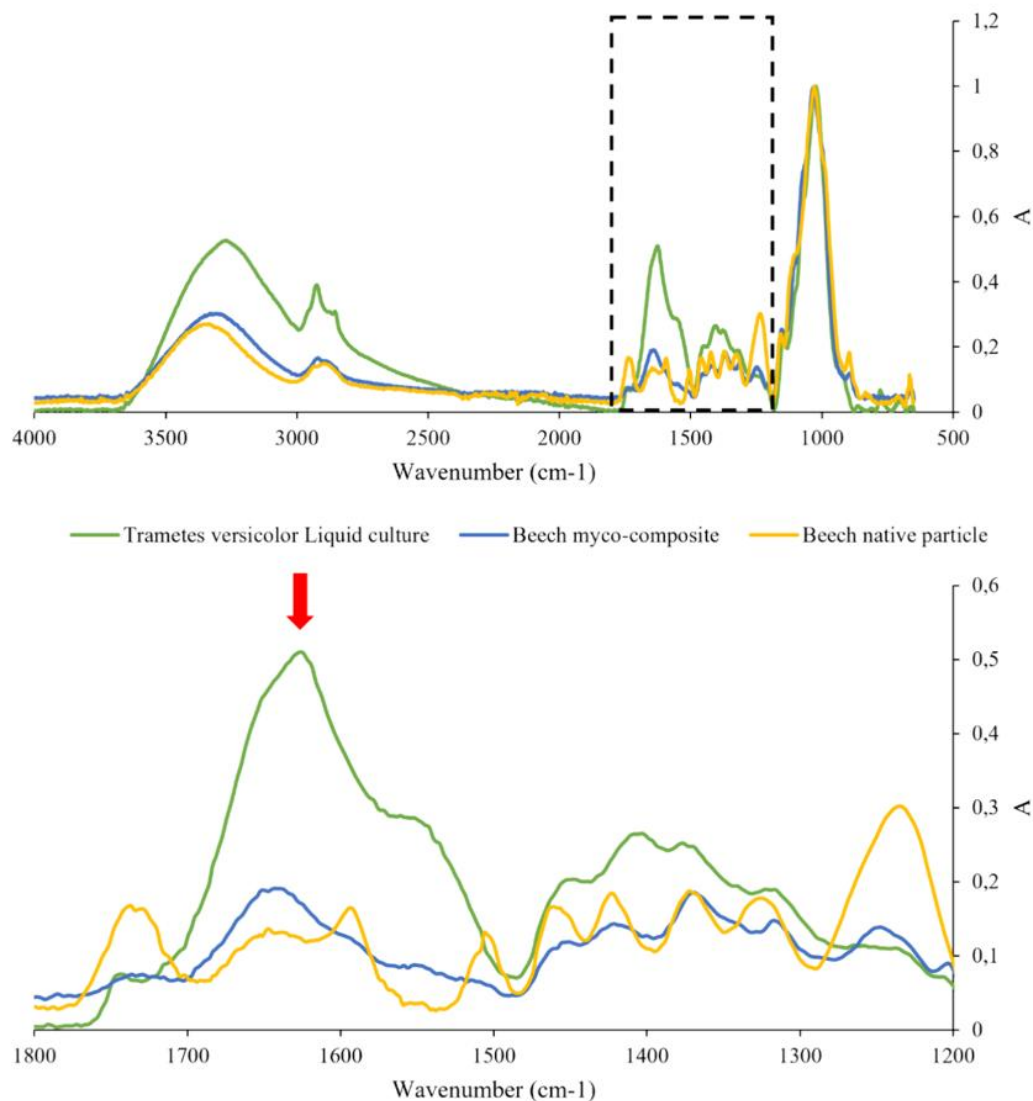


Figure 5: AT-FTIR detection of amine groups in Beech wood particles, beech myco-composite, and *T. versicolor* pellets. (A) MIR spectra from 4000 to 500 cm⁻¹ and (B) MIR spectral focus on the zone from 1800 to 1200 cm⁻¹

Finally, NIRS analysis was combined with elemental quantification. After mathematical computation of the NIRS data we showed that NIRS data and nitrogen levels 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 S_0 3.36, S_0 3.65, and S_0 3.94. For more severe conditions (S_0 4.24), the fungus did not develop optimally.

The combination of these techniques was found to be relevant for quantifying the fungal density in the material in relation to the growing matrix used. An optimal fungal growth was observed for an SE treatment severity of $S_0 \leq 3.96$.

2.2.4. Linking SE pretreatment, fungal colonization and myco-composite production

In the framework of the W2W project, we investigated further the relation between SE treatment, fungal colonization and the resulting myco-composite properties. It was recently published (Figel et al., 2025). This study highlights the potential of steam explosion (SE) as an effective pretreatment to enhance the bioavailability of beech wood components for myco-composite production. Wood samples were subjected to steam explosion under four different severities (S_0 3.36; S_0 3.65; S_0 3.94; S_0 4.24), and their chemical composition and micro-structure were analyzed by dynamic image analysis, scanning electron microscopy, drop shape analyzer, near infra-red spectroscopy and anionic chromatography. Beech wood particles were treated by SE according to 4 combinations of residence time and temperature.

The calculated severity factors are also given and will be used thereafter as a sample name. As expected, the more severe the treatment became, the more the particles defibrated and showed a fluffy appearance (Figure 6). As the severity factor increased, the particles changed color from creamy white to dark brown. The visual inspection of the biomass suggested that mild SE conditions (S_0 3.36 and S_0 3.65) were not strongly altering the particle morphology. The differences between native beech particles and those with an S_0 of 3.36 were barely noticeable. On the contrary, the S_0 4.24 particle sample mostly consisted of a fluffy mixture of single fibers or small fiber bundles. As the severity of wood pretreatment increases, the degree of defibration enhancement becomes more pronounced.

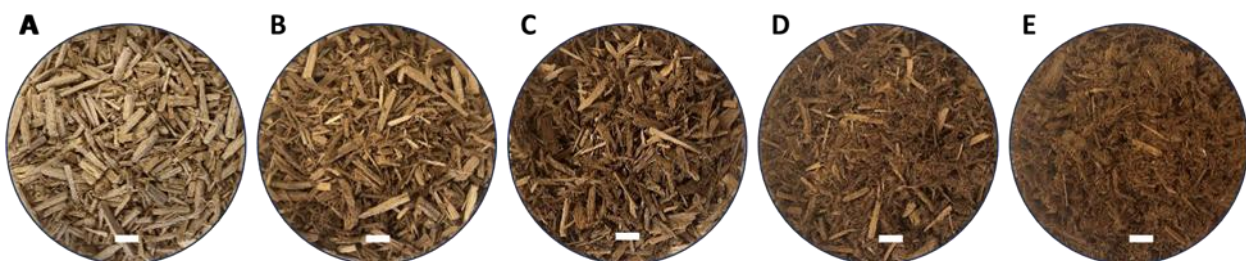


Figure 6: Macroscopic modification of wood particles caused by steam explosion.

Severity coefficient as follow: A = native, B = S_0 3.36, C = S_0 3.65, D = S_0 3.94 and E = S_0 4.24. The scale bar represents 1 cm

Automated imaging techniques were employed to quantitatively evaluate how steam explosion (SE) treatment affects the macrostructure of wood by analyzing particle morphology. The CAMSIZER analysis results (Figure 7) revealed that although all samples shared a peak particle size of 3.15 mm, they could be grouped into two distinct clusters.

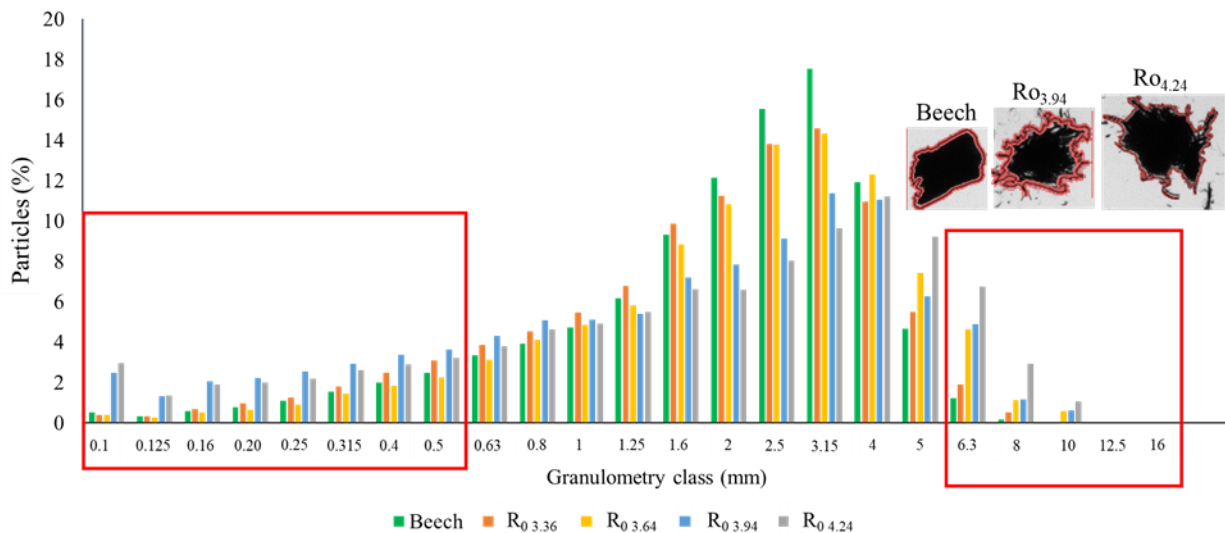


Figure 7: Narrowest particles width distribution (xMa_{min}).

Samples treated at higher severity levels ($S_0 = 3.94$ and 4.24) contained significantly more fine particles ($< 500 \mu m$) — 4.6% and 11.6% , respectively — compared to lower severity treatments ($S_0 = 3.36$ and 3.65) and untreated beech (around 0.7%).

Additionally, $S_0 = 4.24$ samples had the highest frequency of particles larger than $5 mm$. The total particle count was similar for untreated and lower severity samples ($\sim 535,000$), but dramatically higher for $S_0 = 3.94$ (~ 5.9 million) and $S_0 = 4.24$ (~ 14.3 million). The sample analysis showed that fine particles were mostly individual fibers, while larger ones were fiber aggregates, likely formed due to electrostatic interactions during measurement.

Further morphological analysis with SEM (Figure 8) confirmed that higher SE severity led to extensive wood defibration, while lower severity primarily altered the wood's ultrastructure.

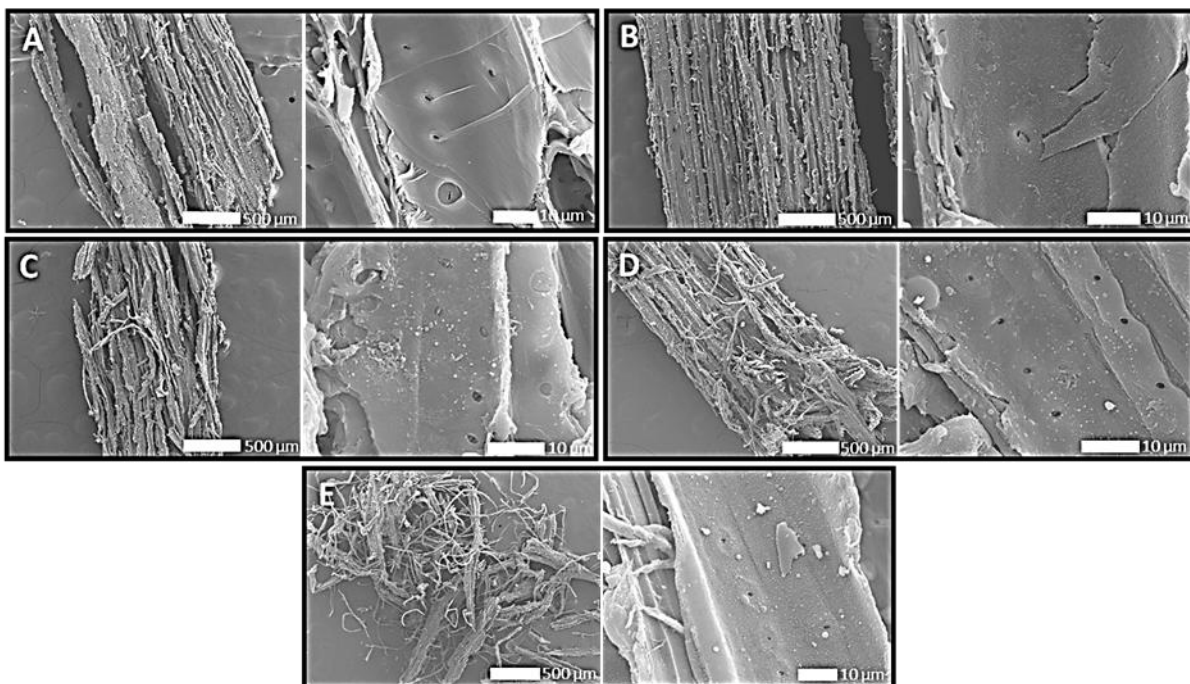


Figure 8: Scanning electron microscopy (SEM) observation of wood particles before and after steam explosion.

Low magnification SEM image (left) and high magnification SEM image (right). A) Beech, B) S_0 3.36, C) S_0 3.65, D) S_0 3.94, and E) S_0 4.24. Yellow arrows: lignin deposit.

Low magnification observations confirmed a correlation between the increase in severity factor and the intensity of wood defibration (Figure 8). On native beech wood particles, the wood cell structure was observed with vessels and fibers tightly packed together. On samples treated with the mild treatment conditions, S_0 3.36 and S_0 3.65 showed a noticeable change on the surface of the beech particles. At high severities, the surface became blistered, and burrs were clearly visible, confirming the wood particle defibration observed above. The entire cell wall was modified as the severity of the SE treatment increased. This indicates that the degree of SE treatment directly influences both the extent and nature of structural changes in the wood

The surface modifications of beech particles were also examined by near-infrared spectroscopy (NIRS). This non-destructive method was successfully used to monitor the effect of the steam explosion on the wood biomass (Troilo et al., 2023; Aguilar et al., 2024). Thus, we tested the possibility to separate the SE treated wood sample by using this technology. After spectra acquisition and the calculation of the secondary derivative, three zones with high variability between the samples were identified on the spectra. The first region was located between 6000 and 5750 cm^{-1} , the second between 5450 and 5100 cm^{-1} and the third located from 4955 to 4600 cm^{-1} . Principal Component Analysis (PCA) was used to develop a clustering analysis of the sample subsequent to NIRS. PCA is a statistical technique used to reduce the dimensionality of data and explore relationships between variables. This analysis was performed on each of the three regions of variability on the secondary derivative, and the associated loading analysis was performed. The loading analysis allowed the identification of the variables that bring the highest contribution to the inter-sample variability on each of the principal components. Independently of the spectral region considered for the PCA analysis, the samples were successfully clustered according to the severity of the SE treatment. It was possible to delineate three groups: the group containing only native beech, a second group with R_0 3.36 and R_0 3.65, and a third group with S_0 3.94 and S_0 4.24. Based on band assignment and loading analysis, the PCA of the range from 6000 to 5750 cm^{-1} was the most relevant to characterize the SE effect on the wood cell wall (Figure 9).

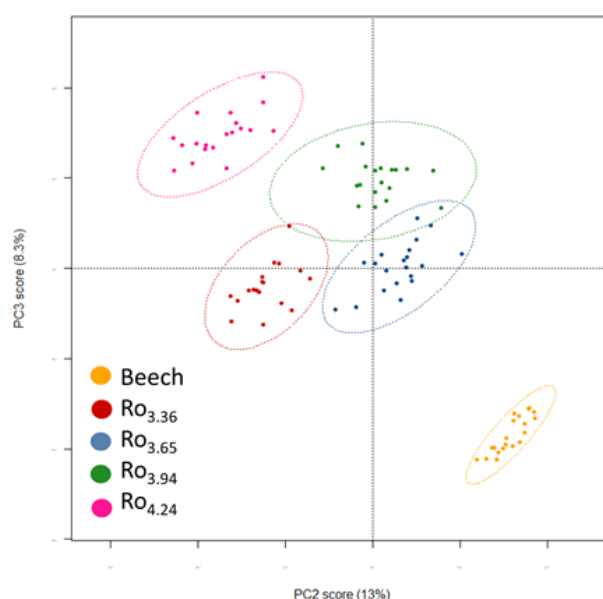


Figure 9: PCA analysis on the sub region of interest.

PCA from 6000 to 5750 cm^{-1} with PC2 and PC3. Clear discrimination between beech native particle, low severity factor (S_0 3.36 and S_0 3.65) and high severity factor (S_0 3.94 and S_0 4.24).

Indeed, variability on the PC2 axis was attributed to hemicellulose content and lignin to PC3. The sample clustering clearly exhibited a linear relation between the severity condition and the lignin to hemicellulose ratio. NIRS analysis on the wood particles suggested that the more severe the treatment, the more the lignin was relocated to the cell wall surface, which is confirmed by MIR analysis. Additionally, the increase in the steam explosion treatment severity triggered strong hemicellulose removal and possibly *in situ* chemical modification. Taken together, NIRS and MIR analysis brought chemical complement data to the SEM observations.

In this study we also analyzed the aqueous effluents. Mono and poly-saccharide content were analyzed by ionic chromatography. Inhibitory compounds (furfural and 5-HMF) known to be generated by SE were analyzed as well. Based on these preliminary works, we can conclude that the inhibitors contained in the SE effluent are not in sufficient concentration to explain the fungal growth inhibition. It can therefore be concluded from this study that the aqueous effluents produced by SE, rich in complex sugars and containing low concentrations of potentially inhibitory substances, can constitute a nutrient medium for the growth of *T. versicolor*.

Impact of the substrate modification on fungal growth was evaluated by NIRS, macroscopic and SEM techniques used in combination as previously described. Macroscopic observations of samples A, B, and C confirm that colonization with S_0 3.36, S_0 3.65 and S_0 3.94 is more effective than with beech particles and S_0 4.24 particles, reaching an optimum at S_0 3.65 which validates our previous study (Figure 10).

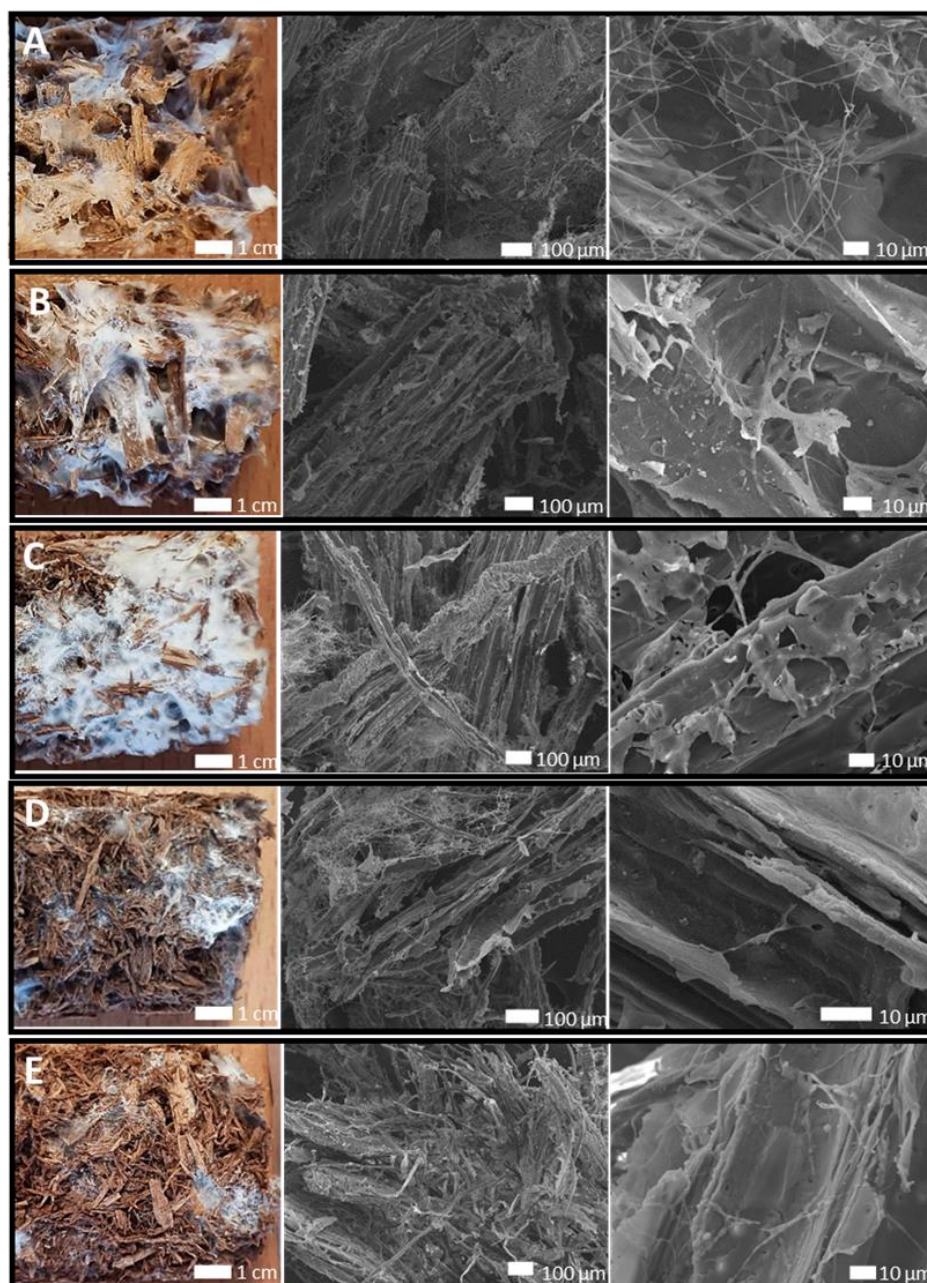


Figure 10: Multi-scale investigation of Se effects on fungal growth and cohesion

Macroscopic pictures after 14 days of growth (white: fungus and dark brown: wood particles) and scanning electron microscopy (SEM) observations of the myco-composite, A) Beech, B) S_0 3.36, C) S_0 3.65, D) S_0 3.96, and E) S_0 4.24.

Scanning Electron Microscopy (SEM) revealed a denser presence of extracellular polymeric substances (EPS) surrounding hyphae grown on substrates treated at severity levels $S_0 = 3.36$ and 3.65 . EPS plays a crucial role in fungal adhesion by forming a temporary interface between the hyphae and the wood, anchoring the fungus at the degradation site. This matrix also contains extracellular enzymes and structural proteins, and its production is triggered when the fungus detects a suitable substrate. We found a correlation between fungal colonization, hyphal structure, and a possible link with the mechanical properties of the resulting mycelium composites.

Specifically, the composite made with $S_0 = 3.65$ showed increased fungal growth, thicker hyphae, greater EPS secretion, and a trimitic hyphal structure. These findings suggest that the extent of fungal colonization and the cohesion of hyphae may significantly influence the mechanical performance of mycelium-based materials.

Compression tests and thickness recovery were successfully used to evaluate the link between hyphal network density, hyphal bounding to wood, and mechanical properties. (Aiduang et al., 2022; Elsacker et al., 2019; Zhang et al., 2023) Interestingly, myco-composites material produced from poorly colonized substrates (S_0 4.24 and Beech) showed similar Young's modulus ($9.10 \cdot 10^{-4}$ MPa) to the ones measured from S_0 3.94 myco-composites (Figure 11 a).

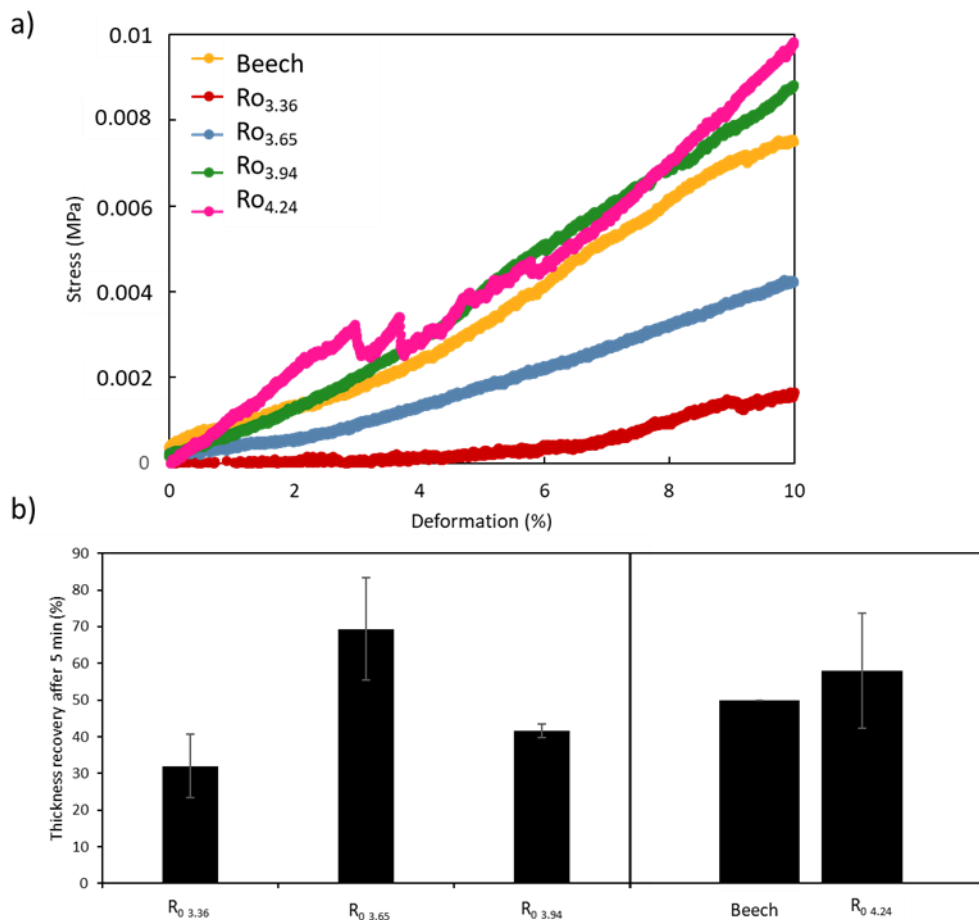


Figure 11: Axial compression tests on myco-composites

a) Stress-strain curve of the myco-composite materials and b) Thickness recovery of the myco-composites following a 5-minutes compression period. (ANOVA, $p < 0.05$, post hoc Tukey HSD analysis).

It suggests that these composites have an internal structure and microporosity close to native wood. The compression test results obtained for fungal S_0 3.94 myco-composites did not differ from the S_0 4.24 and Beech ones. Thus, despite fungal colonization being similar in S_0 4.24, beech, and S_0 3.94 myco-composites, we conclude that the network observed in S_0 3.94 myco-composites was only poorly cohesive. By comparison, myco-composites obtained with S_0 3.36 and S_0 3.65 substrates exhibited higher flexibility and a significantly lower Young's modulus ($2.10 \cdot 10^{-4}$ MPa and $4.10 \cdot 10^{-4}$ MPa, respectively). Thus, a very similar colonization level but with different amounts of EPS

and hyphal morphology leads to a contrasting stiffness behavior. To further investigate the possible influence of the mycelium properties on myco-composite material, thickness recovery tests were performed. The results showed significant variation in the structural integrity of the myco-composites despite comparable fungal growth (Figure 11 b). Myco-composites produced from S_0 3.65 achieved the highest thickness recovery (69 ± 14 %) compared to myco-composites produced from S_0 3.36 and S_0 3.94 (respectively 32 ± 8 % and 41 ± 2 %), suggesting optimal substrate accessibility for fungal colonization and material cohesion. The complex and dense mycelial network within myco-composites produced from S_0 3.65 might restrict the movement of wood chip particles and result in better mechanical and cushioning properties.

In conclusion, steam explosion (SE) pretreatment significantly modifies the physical and chemical properties of beech wood particles, enhancing their suitability as substrates for myco-composite production. Increasing the severity factor (S_0) improved defibration, water retention, and surface hydrophobicity, while releasing sugars with minimal accumulation of inhibitory compounds such as furfural and HMF. These modifications improve substrate bioavailability and support fungal colonization. Importantly, an intermediate SE severity yielded optimal results. Excessive severity ($S_0 > 3.7$) impaired fungal development, due to adverse changes in wood-water interactions. This finding highlights the critical role of pretreatment severity in balancing substrate accessibility and nutrient preservation. These findings support SE as a tunable and sustainable strategy for converting lignocellulosic biomass into high-performance myco-composite materials.

3. Chapter 2 Initial product validation- Panels production

3.1. PROOF OF CONCEPT OF MYCO-COMPOSITES BOARDS FROM BR1/BR2

All the data presented in this section is preliminary and will be studied and confirmed in phase 2 of the Wood-to-Wood project.

3.1.1. Myco-composite development

In a first attempt, we investigated the possibility of using BR1/BR2 wood to produce myco-composites boards. In the first step the substrates used was a mix of (BR1/BR2 wood and beech machining wood by-products (MWBP). The production process is illustrated in Figure 12.

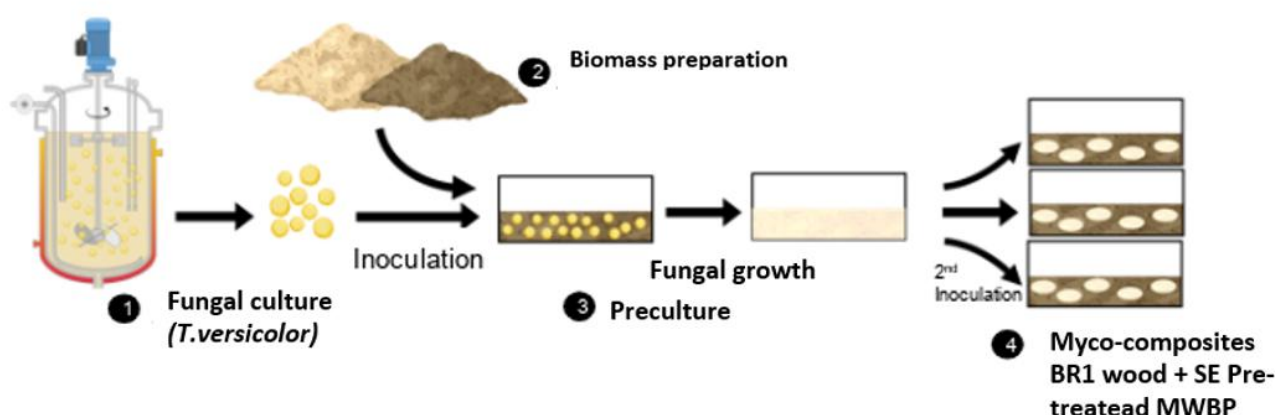
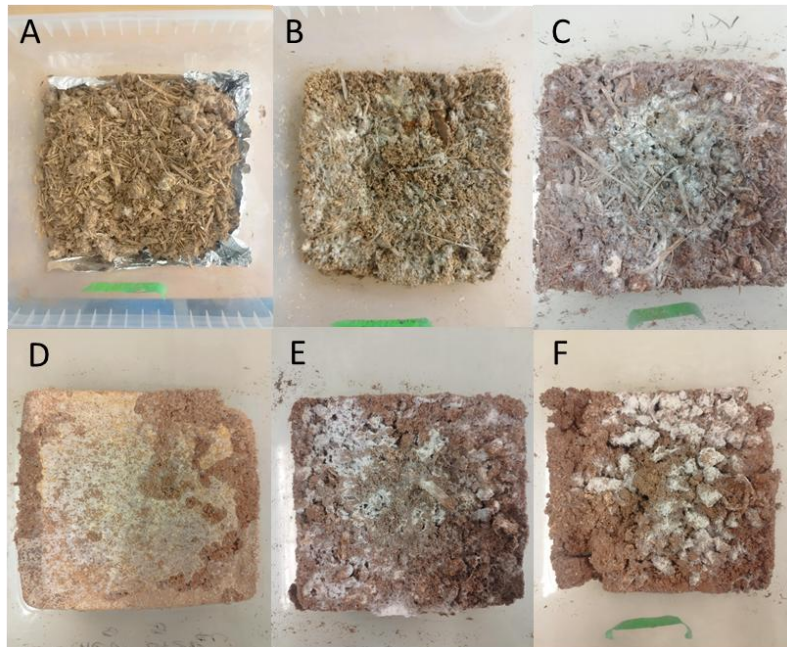


Figure 12: Workflow of the myco-composite production method

After 30 days of incubation and drying period, we observed enhanced fungal colonization and improved structural cohesion in mycelium-based composites when the machining wood byproduct (MWBP) was pretreated using steam explosion. This pretreatment appeared to significantly improve the substrate's suitability for fungal growth, resulting in a more uniform and denser mycelial network (Figure 13). In contrast, untreated (BR1+malt) showed much poorer fungal development and led to composites with weaker mechanical integrity (no cohesion). The growth on mixed BR1/MWBP was promoted when using either SE MWBP particles alone or in combination with MWBP SE effluents.

This improved performance could be attributed to several factors induced by the steam explosion. The results presented above (Aguilar et al., 2024; Figel et al., 2025) provide explanations about the effect of SE on fungal growth and agree on the observations. Additionally, steam explosion might help eliminate inhibitory compounds such as extractives (Serrano-Martínez et al., 2024) or resins

that would otherwise hinder fungal growth and, at the contrary, provide stimulatory compounds that boost the fungal growth.



*Figure 13: Illustration of the myco-composites produced with wood waste substrates
 (A) BR1 + malt, (B) BR1 + MWBP, (C) BR1 + SE MWBP + SE effluents, (D) MWBP, (E) SE MWBP and (F) SE MWBP + SE effluents*

The forthcoming experiment will combine growth on SE BR1/BR2 wood in combination with WMBP. This provides an integrated production solution of panelboards in a circular zero-waste bioeconomy paradigm. It would be interesting to study the thermal performance of steam-exploded BR1 wood as well as to explore the use of mixed fungal strains (co-cultivation) to further improve the performance of myco-composites. Different fungi may exhibit complementary enzymatic activities and growth behaviors, potentially leading to faster colonization, more robust networks, or even novel material properties.

3.1.2. Thermal conductivity

The steady-state thermal conductivity of the various myco-composites developed in this study was assessed using square specimens measuring $150 \times 150 \text{ mm}^2$. Measurements were carried out with a guarded hot/cold plate apparatus, in accordance with the NF EN 12667 standard, which is widely used for evaluating the thermal performance of building insulation materials. This technique employs the heat flow meter method, grounded in Fourier's law of heat conduction, where a unidirectional heat flux is established through the thickness of the specimen. By maintaining stable temperature conditions on both sides of the sample and recording the resulting heat flow and temperature gradient, the thermal conductivity (λ) can be accurately quantified under steady-state conditions.

Initial measurements were conducted with the cold and hot plates set to 0 °C and 20 °C, respectively, ensuring a mean test temperature of 10 °C (displayed commercial value for thermal insulation materials). To examine the influence of moisture content on thermal performance, two sets of conditions were applied to the samples. In the first, specimens were oven-dried at 70 °C to attain near-zero relative humidity, effectively removing bound and free water. In the second, samples were equilibrated under controlled climatic conditions of 23 °C and 50% relative humidity until reaching a quasi-constant mass, simulating real-world indoor environmental conditions.

The thermal conductivity (λ) in this unidirectional configuration can be described using the simplified expression of Fourier's law, which relates the heat flux to the material's thermal conductivity, the surface area, and the temperature gradient across the specimen according to the equation:

$$q = -\lambda A \left(\frac{dT}{dx} \right)$$

Equation 2: thermal conductivity calculation

Where:

q is the steady-state heat flux in the x-direction (W/m^2),

A is the cross-sectional area of the sample (m^2),

and $-dT/dx$ is the temperature gradient (K/m).

This method enables a reliable comparison of the hygrothermal behavior of the myco-composites, providing insight into their potential as sustainable insulation materials. Each specimen, with a cross-sectional area of $150 \times 150 \text{ mm}^2$, was positioned between the two plates of the heat flow meter apparatus. The upper plate, referred to as the hot plate, served a dual function, acting simultaneously as the heat source and the temperature sensor. A unidirectional heat flux was generated through the sample's thickness, from the hot side to the cold side, establishing steady-state thermal conditions. This setup ensures precise determination of the material's ability to conduct heat under standardized and controlled conditions.

Figure 14 presents measured thermal conductivity at 0-20 °C of the different developed myco-composites preconditioned at 70°C, 0% RH (dry conditions) and 23°C, 50% RH (humid conditions) as a function of their density. The comparison of dry and wet thermal conductivity revealed the influence of moisture content on thermal performance of the myco-composites. The results showed that the thermal conductivity of dried materials (70 °C, 0% RH) ranged between 0.04881 and 0.05494 W/m.K and between 0.0503 and 0.05775 W/m.K for wet materials (23 °C, 50% RH). The variations between dry and wet state ranged between 3% (SE MWBP + BR1 + SE effluent) and 10% (MWBP + BR1). While increasing the moisture content, heat conduction increases due to the presence of water particles in the sample, which engenders an increase in the thermophysical characteristics of the material. It should be noted, however, that the increase in thermal conductivity under 50% relative humidity has allowed the materials to remain within the standards of thermal insulation with a thermal conductivity of $<0.25 \text{ W/m.K}$. These Preliminary tests have

shown promising results, particularly regarding fungal growth and insulation properties, even in the absence of adhesive removal. In some cases, materials composed of MWBP and BR1 wood (non-steam-exploded) still supported satisfactory fungal colonization and exhibited good insulating performance compared to commercialized thermal insulation materials (sheep wool, hemp mycelium composite, etc.).

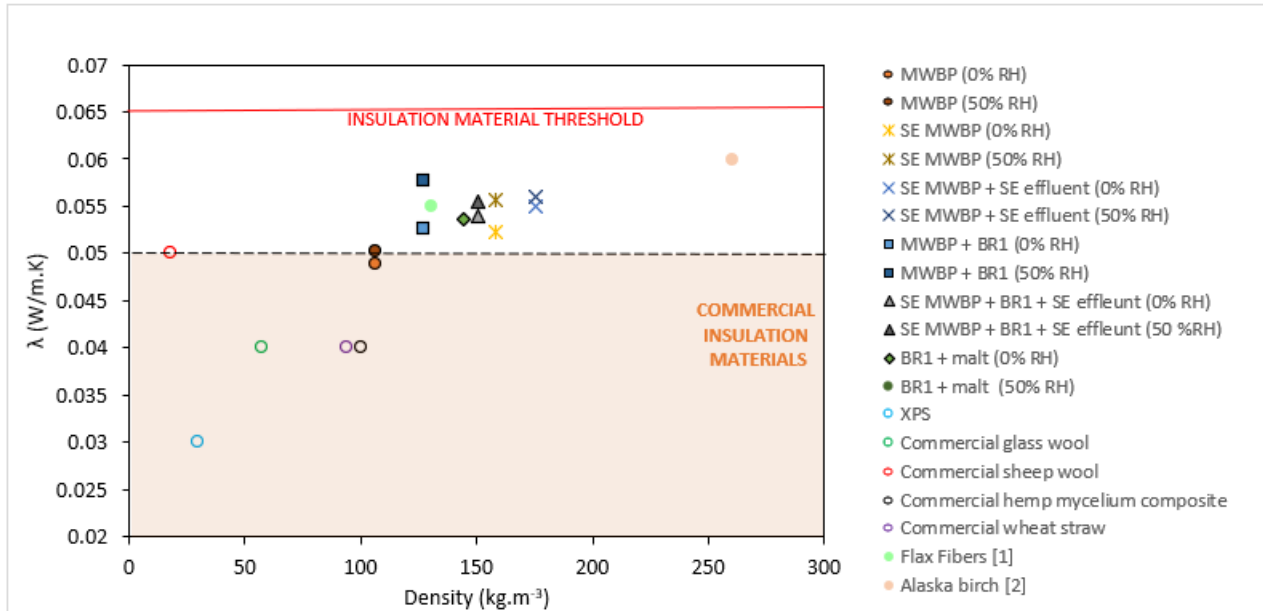


Figure 14: Thermal conductivity (W/m.K) of produced myco-composites measured on conditioned materials at 0% RH and 50% RH and of commercialized thermal insulation materials.

Data from Elsacker et al., 2019 [*], Papadopoulos [*], Xing et al., 2018 [*], Yang et al., 2017 [*].

Taken together, our data demonstrated the feasibility of myco-composite boards using a mix of wood waste (BR1/BR2 + SE-MWBP) that exhibited interesting thermal behavior. Further improvements such as the use of BR1/BR2 wood treated by SE as substrate for myco-composite production and incorporation of myco-composite in particle boards to decrease weight and improve the use of recycled components will be the next step of the project.

3.2. PRODUCTION OF A STANDARD 3-LAYERS PARTICLEBOARD PANEL

3.2.1. Production of particleboard using UF binder

3.2.1.1. ADHESIVE CHARACTERIZATION

The dry content (D) of the UF adhesive was determined by measuring the initial mass (M_1) and the constant mass (M_2) after heat treatment at 103 ± 2 °C for 24 hours, using the following equation:

$$D(\%) = \left(\frac{m_1}{m_2} \right) \times 100$$

The dry content of UF adhesive was determined to be 67%.

3.2.1.2. PRODUCTION

Experimental particleboards were produced at the laboratory scale using a three-layer structure composed of bonded and compressed wood particles (Mixed wood particles) in mixture with commercial UF adhesive and ammonium sulfate hardener. The mixed wood particles and commercial UF adhesive were kindly provided by the CF2P company (<https://www.cf2p.eu/>). The central layer, known as the core layer (CL), was formed using coarse wood chips, which play a critical role in ensuring the mechanical strength and structural integrity of the panel. In contrast, the outer layers, referred to as surface layers (SL), were made from finer wood particles (Figure 15).

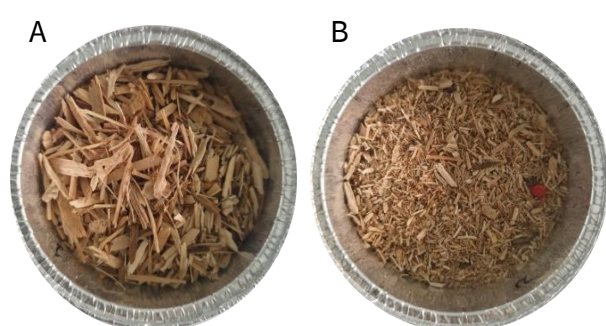


Figure 15: Industrial panel mix particles used for particles boards production at lab scale. (A) Particules of the core layer(CL) and (B) chips from the surface layer (SL) from industrial panel mix

These layers contribute primarily to the surface hardness and smoothness of the panel, enhancing both its durability and aesthetic finish. This layered configuration allows for an optimized balance between strength and surface quality in the final product. The production process steps of wood particle boards are presented in Figure 16.

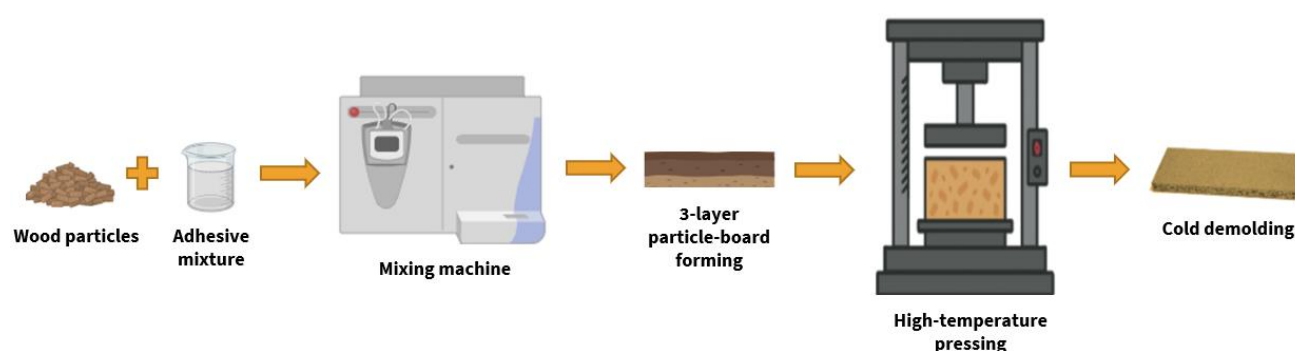


Figure 16: Illustration of particleboard production process

As a first step, the urea-formaldehyde (UF) adhesive and the ammonium sulfate hardener were sourced and used to produce the 3-layer particle boards. UF adhesive is a thermosetting aminoplast resin, commonly used in wood-based panel production due to its high reactivity and

cost-effectiveness. In this case, the resin had undergone partial polymerization, with the curing process deliberately halted to maintain its applicability during the manufacturing stage. This controlled pre-polymerization ensures optimal viscosity and reactivity for effective bonding during the pressing phase. The panels were produced at the laboratory scale. The intended final properties of the panel are summarized in Table 1 (Troilo et al., 2023).

Table 1: Intended parameters of Mixed wood particles/UF adhesive particle boards

Parameters	Requirements
Thickness (mm)	15
Density (kg/m ³)	650
Length (mm)	350
Width (mm)	350

The characteristics of each layer are presented in Table 2.

Table 2: Characteristics of core layer and surface layers

Characteristic	Core Layer (CL)	Surface Layers (SL)
Layer proportion (%)	69	31
Glue application rate (GAR %)	7.5	9.5
Solid content of adhesive (ES _{adhesive} %)	67	67
Hardener rate (HR %)	5.5	2.5
Solid content of hardener (ES _{hardner} %)	50	50
Solid urea per dry glue content (%)	3.5	0

Based on the calculated formulation, the precise quantities of each component were measured to manufacture the laboratory-scale particleboards. This approach ensured the production of a triplicate for repeatability. The process began by introducing the dry wood particles into the gluing machine (Figure 17). Subsequently, the adhesive formulation comprising the UF resin and hardener was added. The mixture was then thoroughly blended within the gluing system for a duration of three minutes to ensure consistent coating of the particles with the adhesive. This step is crucial for achieving proper bonding during the hot-pressing stage.



Figure 17: The gluing system used for particle boards production at lab scale

Following the mixing process, the adhesive-coated particles were manually distributed in a uniform manner onto a metal plate using a 350 x 350 mm² forming frame (Figure 18). This manual layering allowed for control of thickness during mat formation. Each layer was then subjected to a cold pre-pressing step using a panel of matching dimensions to lightly compact the mat and enhance layer cohesion prior to hot pressing. To define the target panel thickness, two spacers were placed on either side of the mat, serving as physical guides during compaction. Finally, a second metal plate was positioned on top of the layered mat to complete the press stack configuration and prepare it for subsequent thermal pressing. The panels were then hot-pressed at 200 °C under a sequential pressure regime: initially at 32 bar, followed by 20 bar, and finally reduced to 3 bar. This stepwise pressure reduction was employed to minimize the risk of panel rupture during pressing and to facilitate the controlled release of steam generated during the process. After pressing, the panels were placed horizontally and allowed to stabilize at ambient temperature to relieve internal stresses and prevent warping.



*Figure 18: Panel shaping in a 350*350 mm² wood mold*

After structuring and shaping, the particleboards were subjected to hot pressing at a temperature of 200 °C, following a controlled sequence of progressively decreasing pressures: an initial high pressure of 31 bars was applied to ensure thorough compaction and resin activation. This was gradually reduced to 20 bars and then to 3 bars to carefully manage internal stresses and prevent mechanical failure such as panel cracking or delamination. The stepwise pressure reduction also facilitates the controlled release of steam generated by moisture evaporation within the panel, which is critical to avoid void formation or internal delamination.

After the pressing cycle, the panels were allowed to cool while resting flat at ambient temperature, a necessary step to stabilize the material dimensions and minimize warping caused by thermal gradients (Figure 19). Finally, to achieve dimensional uniformity and prepare the panels for further processing or testing, the surfaces were sanded to a consistent thickness and smooth finish. This post-pressing surface treatment also helps improve adhesive bonding in subsequent lamination processes or coating applications.



Figure 19: Pressed mixed wood/UF adhesive panel

3.3. PRODUCTION OF PARTICLEBOARD PROTOTYPE USING BIO-BASED NIPU BINDER

To reduce VOC emission and recycling issues, we started to test the possibility to substitute UF – resin by Non isocyanate polyurethane (NIPU) binder produced at UHE in the task 9.2 of the WP9. This NIPU binder was produced at the lab scale from BR1/BR2 wood. It is available in limited amounts.

3.3.1. Adhesive characterization

The dry content (D) of the NIPU adhesive was determined by measuring the initial mass (M_1) and the constant mass (M_2) after heat treatment at 103 ± 2 °C for 24 hours, using the following equation:

$$D(\%) = \left(\frac{m_1}{m_2} \right) \times 100$$

The dry content of NIPU adhesive was determined to be 68.43%.

3.3.2. Production

At this stage of the study, the limited amount of non-isocyanate polyurethane NIPU adhesive (Figure 20) made available (only 40 g) did not allow to produce fully UF-free particleboards. This constraint, however, guided our experimental strategy and gave rise to a complementary and structured approach. On one hand, we focused on evaluating the performance of the provided bio-based NIPU adhesive by utilizing it with mixed-wood to produce a 3-layer-panel, to gain a first understanding of the NIPU behavior and bonding performance. On the other hand, we carried out the production of 3-layer panel using UF mixed with SE BR1 wood to investigate its properties. By isolating and analyzing each of these two key components, adhesive NIPU and steam-exploded BR1 wood, we were able to explore their individual contributions and limitations under controlled conditions. This dual approach lays the foundation for future developments: the ultimate goal is to define the optimal parameters required to produce particleboards that are not only high-performing but also entirely bio-based and VOC-free using SE BR1 material with NIPU adhesive. Accordingly, these constraints justify why VOC-free panels have not yet been produced at this stage of the project.

The identical formulation presented above was applied in the production of a three-layer particleboard with mixed wood particles, utilizing a NIPU adhesive as the binding agent with mixed wood particles as well as UF adhesive with SE BR1 wood particles. In the last case, SE BR1 particles (Figure 21) were used in CL while fine mixed wood particles were used in the SL. In the case of NIPU adhesive, no hardener was added to the formulation during panel preparation since it was incorporated during the synthesis of the adhesive. The required final properties of the intended panels are summarized in Table 3. Given the limited supply of NIPU adhesive, it was necessary to reduce the panel dimensions to 150×150 mm², deviating from the standard size of 350×350 mm². The developed panels are presented in Figure 22.



Figure 20: NIPU adhesive received from UHE (quantity 40 g).



Figure 21: Macroscopic view of SE BR1 wood.

Table 3: Intended parameters of Mixed wood/NIPU particle boards (A) and SE BR1/UF (B)

Parameters	A - Mixed wood particles	B - SE BR1 wood particles
Adhesive	NIPU	UF
Thickness (mm)	15	15
Density (kg/m ³)	650	650
Length (mm)	150	350
Width (mm)	150	350

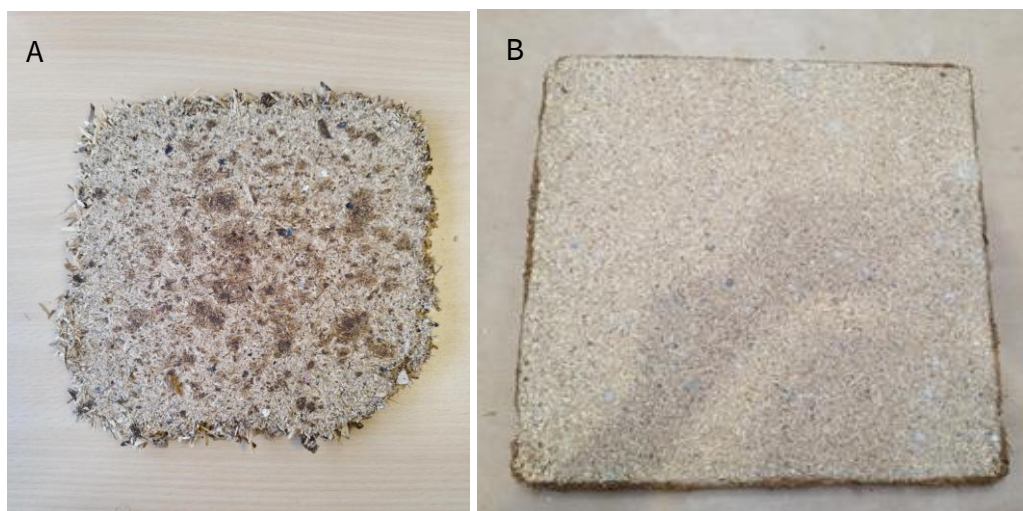


Figure 22: Panel mix wood particles/NIPU (A) n and (B) SE BR1/UF (B) developed panels

3.3.3. Panel characterization

3.3.3.1. DENSITY PROFILE

The density profiles of the developed panels cut into samples of $50 \times 50 \text{ mm}^2$ were assessed using a laboratory measurement system based on X-ray technology. The device employed, the DAX 5000 (GreCon, Germany), enables fast and accurate evaluation of the raw density of wood-based panels (Figure 23). The resulting data including both graphical density profiles and numerical values are recorded separately for each panel and can be accessed through the system's dedicated software.

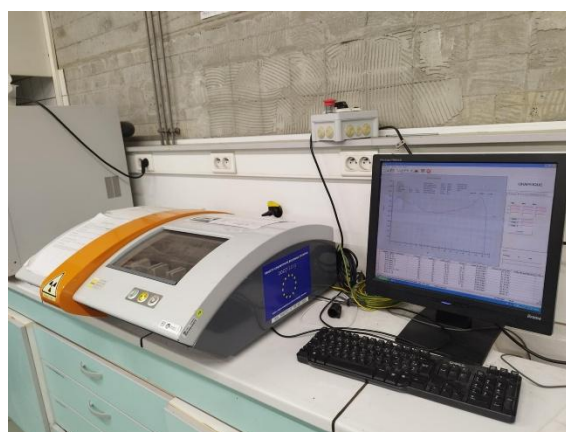


Figure 23: Photography of DAX 5000 machine (GreCon, Germany)

The density profile graph (Figure 24) shows how density varies across the thickness of particleboards produced with different adhesives and wood types. All profiles display a typical double-peak shape, with higher densities at the surfaces and lower density at the core, due to heat transfer and compression during hot pressing. Panels made with UF adhesive exhibit higher core densities ($700\text{-}750 \text{ kg/m}^3$), indicating stronger compaction. In contrast, the profile of the panel

bonded with NIPU adhesive is flatter and overall, less dense, suggesting lower reactivity or stiffness during pressing.

The type of wood particles also plays a crucial role. The panel made of SE BR1 wood particles shows a more uniform and symmetric profile with a denser core. The steam explosion process increases porosity and enhances adhesive penetration by disrupting the cell structure and modifying surface chemistry. UF adhesive and SE-treated wood result in a balanced density distribution. These results highlight how both adhesive formulation and biomass pretreatment strategies directly influence panel structure and properties.

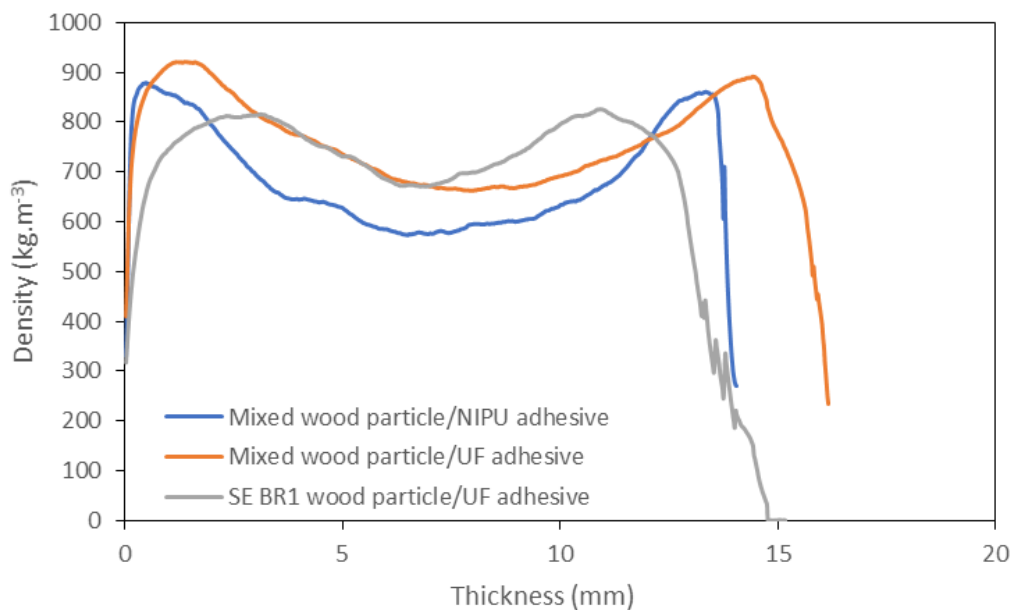


Figure 24: Density profile of the panels produced at lab scale

3.3.4. Internal bond strength

The internal bond strength of the particle boards was determined in accordance with the EN 319 standard. To carry out the measurement, panel specimens measuring $50 \times 50 \text{ mm}^2$ were used. Metal loading blocks were bonded to the surface of each specimen using hot-melt adhesive. The internal bond test was then conducted using a universal testing machine configured for tensile and bending tests (INSTRON) (Figure 25). For this experiment, seven samples were tested for each material. Prior to the test, the specimens with glue on blocks were preconditioned at 23°C and 50% RH for 24 h and then put under a load. The test was performed at a tensile speed of 2 mm/min, where the maximum load sustained by the test piece with a precision of 1% was recorded.

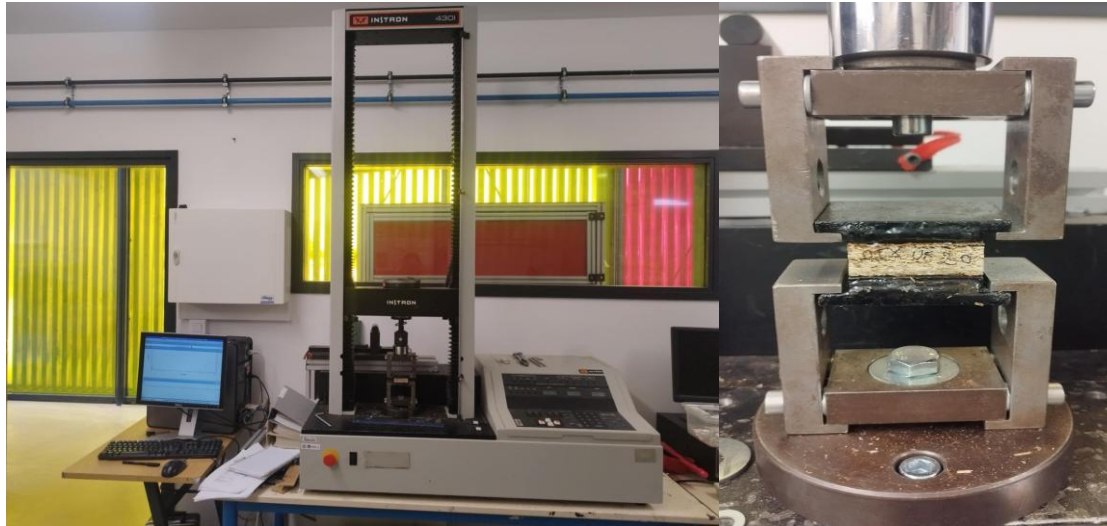


Figure 25: Mechanical testing for internal cohesion of panels
(A) INSTRON tensile and bending testing machine, (B) Mixed wood particle/UF sample during internal bond strength test

During the mechanical specimen preparation stage, it was observed that the samples bonded with NIPU adhesive detached in the middle when mounted onto the heated sample holders. This detachment can be directly linked to the internal density profile of the NIPU-bonded panels, which revealed a lower density at the core compared to panels bonded with UF adhesive. A lower core density could indicate poorer particle consolidation and weaker internal bonding. Insufficient compaction in this region reduces internal cohesion. Additionally, an eventual lower reactivity or stiffness of the NIPU adhesive may have contributed to less effective bonding in the panel's interior. The combination of reduced adhesive penetration and limited mechanical interlocking in the low-density core likely may have caused the observed failure at the mid-thickness during handling and heating while bonding on heated test blocks. These results indicate that the current formulation and processing conditions used with NIPU may require optimization, either through adjusting pressing parameters, adhesive content, or particle preparation, in order to improve core compaction and ensure sufficient internal cohesion for structural applications.

The panel produced with mixed wood particles demonstrated an average strength of 0.30093 N/mm², whereas the SE BR1 panel, manufactured using steam-exploded wood, recorded a much lower value of 0.05012 N/mm². Despite the better performance of the mixed wood panel, its strength still falls below the minimum threshold specified by EN 312 intended for type P2, which is 0.35 N/mm² (Table 4).

The poor performance of the BR1 panel likely stems from a combination of factors. The steam explosion process significantly changes the wood's morphology, increasing its porosity which may have interfered with potentially reducing its compatibility with UF adhesive using the described formulation. Additionally, the UF adhesive may have deteriorated due to age (opened 3 weeks before testing), impairing its curing behavior and weakening bond formation. As the next step, additional panels will be produced to generate a larger data set. This will also provide opportunities

to optimize adhesive formulations, pressing parameters, and raw material preparation to enhance core compaction and overall panel performance.

Table 4: Internal bond strength of the developed panels

Panel	Mixed panel wood particle/UF	BR1 wood particle/UF adhesive
Thickness (mm)	16.34 ± 0.40	14.47 ± 0.52
Internal bond strength (N/mm ²)	0.30 ± 0.20	0.05 ± 0.01

4. Conclusion

The work presented in Deliverable 9.3 of the Wood2Wood project demonstrates a significant advancement in the valorization of post-consumer wood through the integration of steam explosion (SE) pretreatment and myco-composite production. This deliverable focused on Use Case 2, which aims to remove contaminants from BR1/BR2 wood waste and repurpose it into environmentally sustainable and high-performance materials.

The implementation of SE technology at TRL5 enabled the effective defibration and partial decontamination of lignocellulosic biomass, particularly targeting the removal of urea-formaldehyde (UF) resins. The process was shown to alter the physical and chemical structure of wood particles, enhancing their porosity, surface chemistry, and water retention capacity. These modifications were critical in improving the bioavailability of the substrate for fungal colonization.

A comprehensive set of analytical techniques—including SEM, NIRS, FTIR, and CAMSIZER imaging—was employed to characterize the effects of SE on wood morphology and its subsequent influence on fungal growth. The results revealed that intermediate SE severity levels (notably $S_0 = 3.65$) provided the most favorable conditions for the growth of *Trametes versicolor*, leading to dense hyphal networks, high EPS production, and trimitic hyphal morphology. These biological features were directly correlated with improved mechanical properties and structural cohesion in the resulting myco-composites.

The study also highlighted the importance of balancing SE severity. While mild treatments did not sufficiently enhance substrate accessibility, overly severe conditions ($S_0 = 4.24$) negatively impacted fungal development, likely due to excessive lignin relocation and altered wood-water interactions. This underscores the need for precise control of pretreatment parameters to optimize both biological compatibility and material performance.

Initial panel production trials further validated the potential of SE-treated wood in particleboard manufacturing. Panels incorporating SE-treated BR1/BR2 wood and bio-based NIPU adhesives demonstrated promising thermal insulation properties and structural integrity, although further optimization is needed to meet industrial standards for internal bond strength. The integration of myco-composites into particleboard structures also opens new avenues for lightweight, VOC-free, and fully circular building materials.

In summary, this deliverable confirms that steam explosion is a viable and tunable pretreatment method for enhancing the recyclability of post-consumer wood. When combined with fungal bioprocessing, it enables the creation of novel, sustainable materials that align with the goals of a circular bioeconomy. The findings lay a strong foundation for the next phases of the project, which will focus on scaling up production, refining formulations, and exploring co-cultivation strategies to further improve material properties and environmental performance.

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#These publications (Aguilar et al.2024 & Figet el al., 2025) were produced within the framework of the Wood to Wood project and include acknowledgements to the European Commission for its financial support.



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