WOOD2WOOD

A Wood-to-Wood Cascade Upcycling Valorisation Approach

» Deliverable 9.1

Glue separation from wood and bioremediation wood treatment

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PU	Public	X
RE	Restricted to a group specified by the consortium	
CO	Confidential, only for members of the consortium (including the European Commission Services)	

Legal Disclaimer

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GLOSSARY OF ACRONYMS

Acronym	Extended Definition
BR1 / BR2	Wood classified as non-hazardous
CHON	Carbon, Hydrogen, Oxygen, Nitrogen
CH_2O	Chemical molecule of formaldehyde
CLEM	Correlative Light and Electron Microscopy
CLSM	Confocal Laser Scanning Microscopy
FTIR	Fourier Transform Infrared Spectroscopy
KER	Key Exploitable Result
MDF	Medium Density Fiberboard
MUF	Melamine-Urea-Formaldehyde
N	Nitrogen
NIR	Near-Infrared Spectroscopy
OSB	Oriented Strand Board
PCA	Principal Component Analysis
PDA	Potatoes Dextrose Agar
PDB	Potatoes Dextrose Broth
SE	Steam Explosion
SEM – EDS	Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy
SEM – WDS	Scanning Electron Microscopy with Wavelength Dispersive Spectroscopy
TRL	Technology Readiness Level
TRL 3	Experimental proof of concept
TRL 5	Technology validated in a relevant environment
UF	Urea-Formaldehyde
YE	Yeast Extract
W2W	Wood2Wood

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EXECUTIVE SUMMARY

In the context of the European Green Deal and the transition toward a circular economy, the Wood2Wood (W2W) project aims to revolutionize the recycling and upcycling of post-consumer wood. Deliverable 9.1 presents the progress and results of Work Package 9 (WP9), focusing on two key innovations: the separation of glues from wood using steam explosion (SE) technology and the bioremediation of wood effluents through fungal treatment.

i. Glue separation via steam explosion

The first part of the deliverable demonstrates the technical feasibility of removing urea-formaldehyde (UF) and melamine-urea-formaldehyde (MUF) resins from post-consumer wood using SE. Trials conducted at both laboratory (TRL 3) and pilot (TRL 5) scales confirmed that:

- Particle size significantly influences glue removal efficiency, with finer particles yielding better results.
- Optimal SE conditions achieved up to 86% glue removal.

A multi-scale analytical approach — combining elemental analysis, spectroscopy, microscopy, and image analysis — validated the effectiveness of SE and enabled detailed monitoring of glue removal.

ii. Fiber morphology and recycling potential

SE not only decontaminates wood but also facilitates fiber defibration, offering an alternative to mechanical grinding. Successive SE treatments led to:

- Progressive fiber separation and homogenization.
- Reduction in fine dust and increase in usable fiber clusters.
- Potential for reuse in composite materials and panel production.

iii. Bioremediation of SE effluents

The second part of the deliverable explores the use of a formaldehyde-resistant fungal strain to detoxify SE effluents. Key findings include:

- The fungus tolerates and metabolizes formaldehyde at concentrations of 1500 mg/L.
- A two-phase detoxification dynamic was observed: an initial acclimation period followed by active degradation.
- HPLC and biomass measurements confirmed significant pollutant reduction and fungal viability.

iv. Outlook and recommendations

The results of WP9 validate the W2W approach to cascade valorization of post-consumer wood. Future work will focus on:

- Optimizing SE parameters for different wood types.
- Standardize and scale up bioremediation processes.
- Developing rapid, non-destructive monitoring tools for SE process and detoxification of SE water effluent.

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 CO_2 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 CO_2 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 since 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 cell wall (Jacquet et al., 2015; 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 SE process is economically feasible and has a limited carbon footprint compared to alternatives (Kral et al., 2020, 2016; Kratky Lukas et al., 2018).

The first part of the Wood2Wood project was dedicated to the implementation of the 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 panel 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 ecoorganism 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 testing 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 in Figure 1.

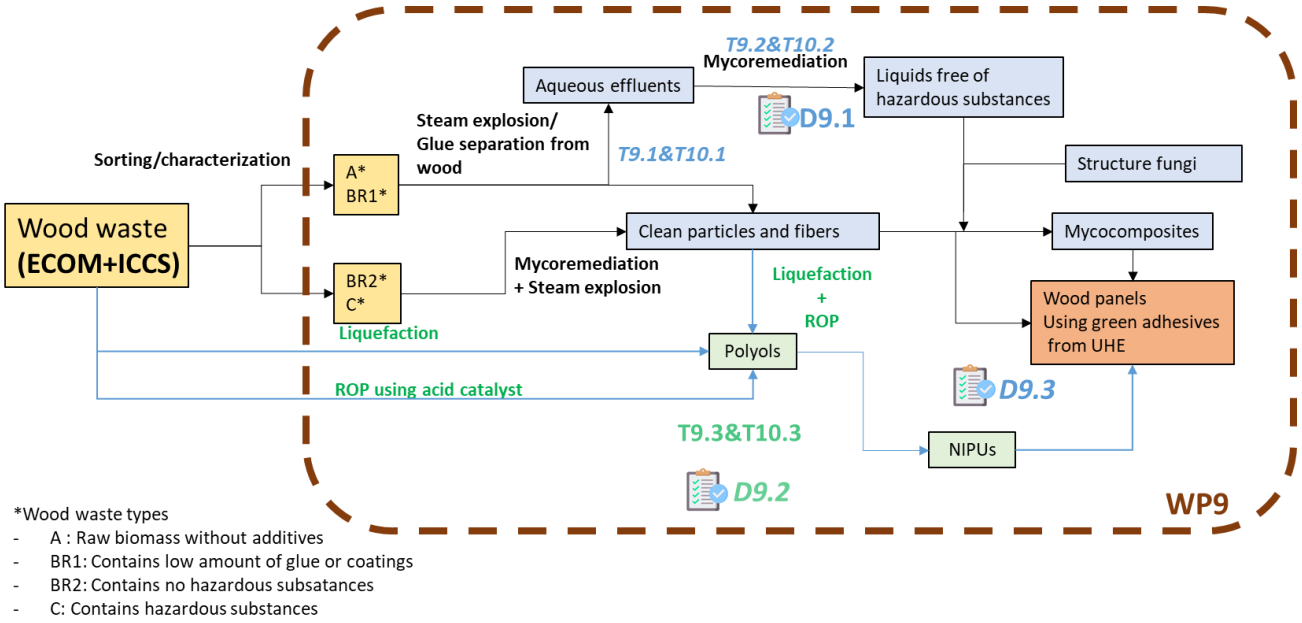


Figure 1. Workflow of the WP9 (use case 2). The tasks and deliverables are indicated. Blue, green and orange colors indicate parts that will be done by LERMAB, UHE and shared. Yellow indicates tasks attributed to ECOM and ICCS.

Deliverable 9.1 will mainly focus on the KER6: Wood glue separation and present the first results obtained for the KER7: Bioremediation wood treatment. The corresponding tasks are found in pillar 2 of the project. There are T9.1 Glue separation from wood and T9.2 Bioremediation wood treatment. The tasks T9.3 Liquefaction Process of mixed wood waste and green adhesives based on polyols and T9.4 Final products and initial validation will be treated in separate deliverables.

Also, the post-consumer falling into C class (containing hazardous substances) will be addressed in the next part of the project.

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.

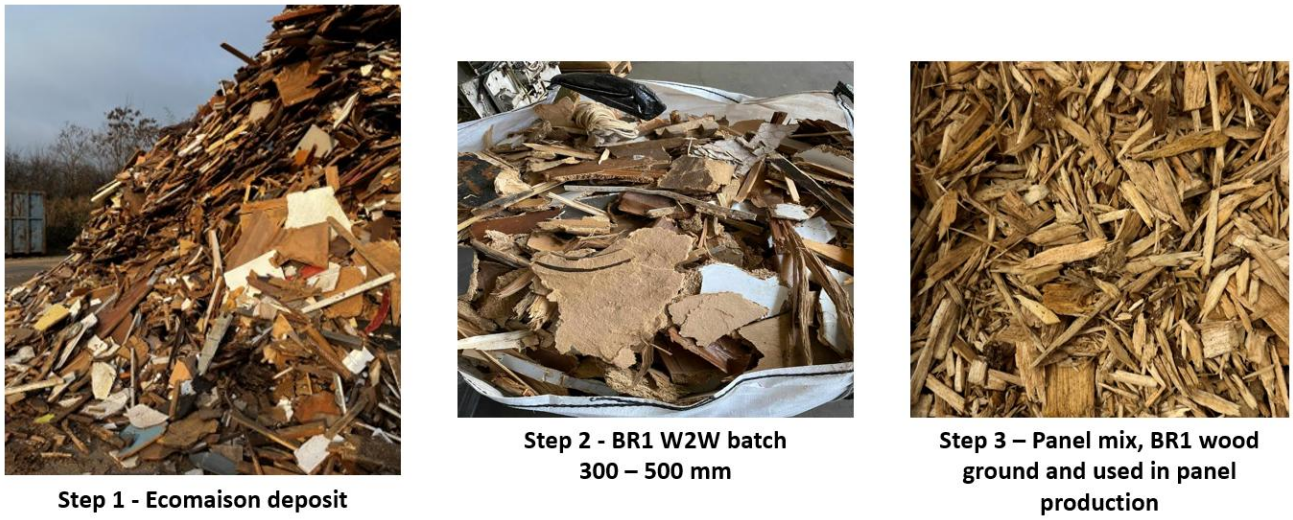
1.4. Structure of the Deliverable

This deliverable is structured in 2 main chapters. The first chapter presents the results of the contaminant removal from the BR1 wood. In the second chapter, the first results concerning the myco-remediation of the aqueous phase will be presented.

2. CHAPTER 1 DECONTAMINATION BY STEAM EXPLOSION

Presentation of the post-consumer wood biomass used in the study

A representative sampling of the BR1/BR2 post-consumer wood deposit was carried out by Ecomaison at sorting centers throughout France. The wood in question is primarily sourced from municipal waste collection centers and is subsequently shredded into various fractions. To ensure the highest degree of representativeness of the samples, they were collected from the 300–400 mm fraction. These samples are intended to serve as a source of raw material for WP 5, 7, and 9. Given the high variability of post-consumer wood, the initial objective was to ascertain its relative composition across the various types of wood. Figure 3 shows some examples of biomass categories that are commonly found in waste wood deposits. The BR1 wood is generally used for panel production. Figure 2 describes the transformation from the initial deposit, where the harvested material is stored, to the shredded particles that will be used in panel factory. At stage 3, manufacturers add wood from roundwood shredding to their panel mix.. Biomass is composed of 60% Step 1 wood and 40% raw wood.



Step 1 - Ecomaison deposit

Step 2 - BR1 W2W batch 300 – 500 mm

Step 3 – Panel mix, BR1 wood ground and used in panel production

Figure 2. BR1 wood processing stage, from its storage area to grinding for use in particleboard

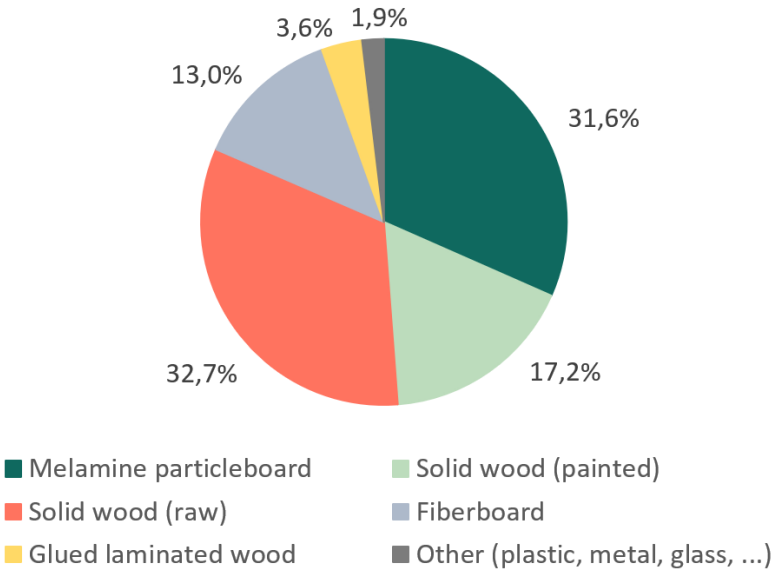


Figure 3. Percentage of different types of wood in BR1 deposit

Given the heterogeneity of BR1 wood, we classified it into different categories (Figure 4) and studied the behavior of each material during glue removal. In the next part, we are going to study the elimination of adhesive on BR1 wood, panel mix, fiberboards (MDF) and particleboards.

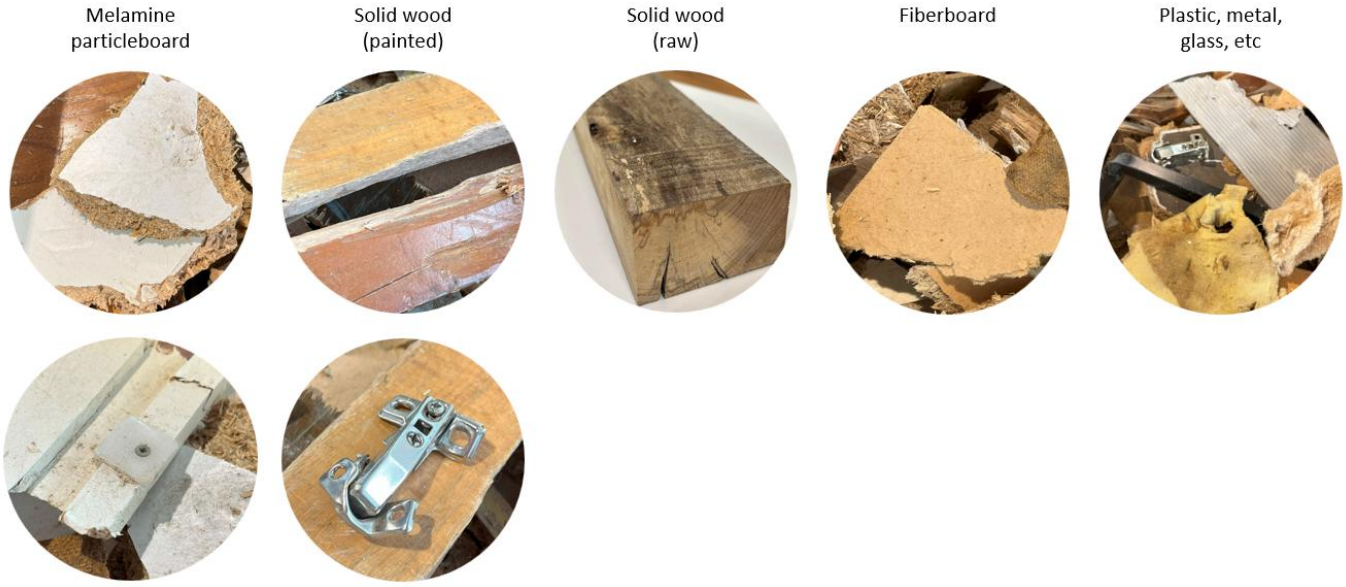


Figure 4. Example of biomass in the big bag supplied by Ecomaison

Presentation of the technology used: the steam explosion

In the lab, we have two pilots to perform steam explosion experiments. They are mostly differing by their size (100g for the TRL 3 and up to 25L/ 3kg for TRL 5). The devices are shown in Figure 5.

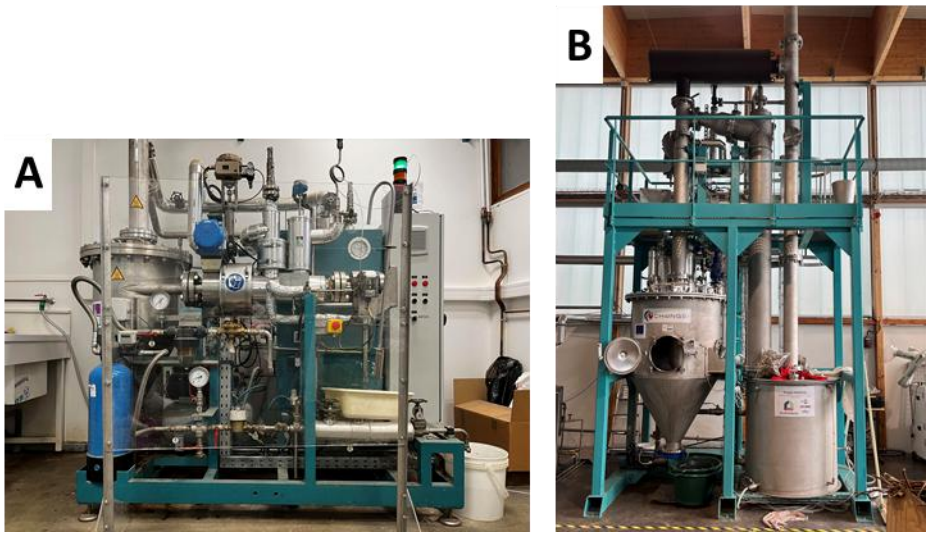


Figure 5. Steam explosion pilots available at LERMAB and used in this study. A/Small scale (100g) TRL 3 pilot and B/ Large scale (up to 3kg) TRL 5 devices.

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 destructuring 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 by Kessler et al. as a succession of four sub-steps, schematized in Figure 30 (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 (Chum et al., 1990; Overend and Chornet, 1987).

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

2.1. GLUE REMOVAL FROM WOOD

2.1.1. Glue removal from MDF fiber (TRL 3)

At the beginning of the Wood2Wood project data available in the literature concerning the use of hydrothermal or steam explosion process in post-consumer wood recycling process was rather scarce and mostly focused on MDF (Gibier et al., 2022; Hagel and Saake, 2020).

Previous works performed at LERMAB aimed to use SE to investigate the glue removal from MDF fiber in MDF boards (Troilo et al., 2023). It allowed the implementation in the lab of the technologies

required to characterize the efficiency of the SE on glue removal from wood fibers. By using a multi-modal and multi-scale approach, we used Near Infrared spectroscopy (NIRS) coupled with elemental analysis to monitor the UF resin removal from wood. Indeed, nitrogen content in wood is very low but high in UF resin. Thus, it can be used as a proxy as UF-resin content determination in the fiber. One example of the results is given in Figure 6.

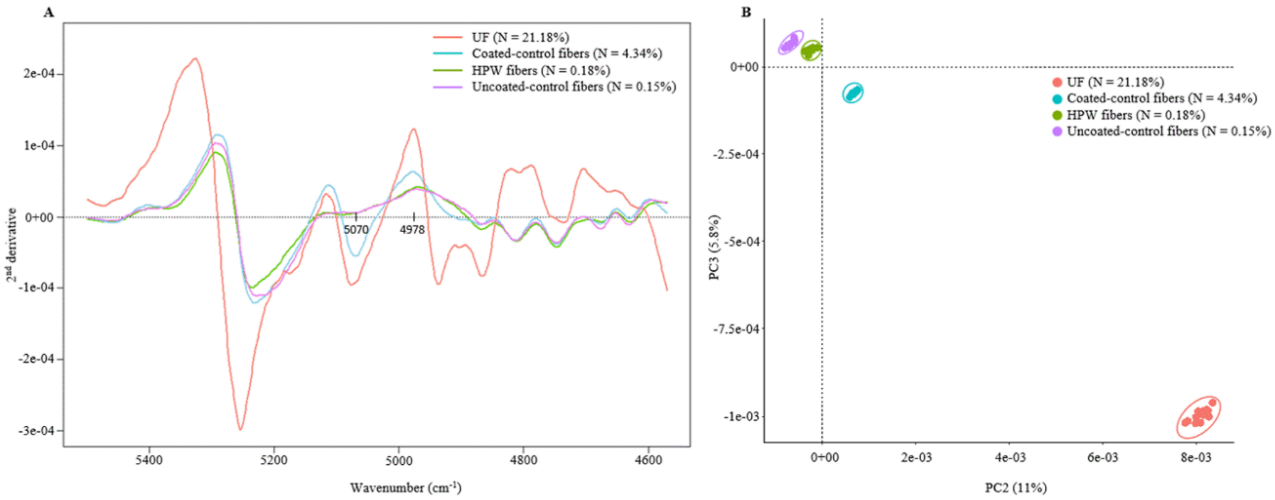


Figure 6. NIRS detection of UF resin in MDF samples. (A) Mean second derivative of cured UF (red), coated control fibers (blue), HPW (green), and uncoated control fibers (violet). The mean second derivative was calculated between 5500 and 4570 cm−1; n = 15. (B) PCA of cured UF, coated control fibers, HPW, and uncoated control fibers. The plot shows PC2-PC3 scores. (From Troilo et al., 2023)

Two bands 4978 cm^{-1} and 5070 cm^{-1} were identified on the NIRS second derivative spectra as characteristics from UF resin. After principal component analysis (PCA), the different groups of samples were clustering according to their nitrogen content. In accordance with literature data, hydrothermal treated fibers showed a nitrogen content close to control fibers. However, to validate this nondestructive measurement and to investigate the effect of SE on spatial distribution of UF resin on fibers, correlative microscopy experiments were performed. Results illustrated on Figure 7 showed that mapping of the resin on the fibers was possible by fluorescence microscopy.

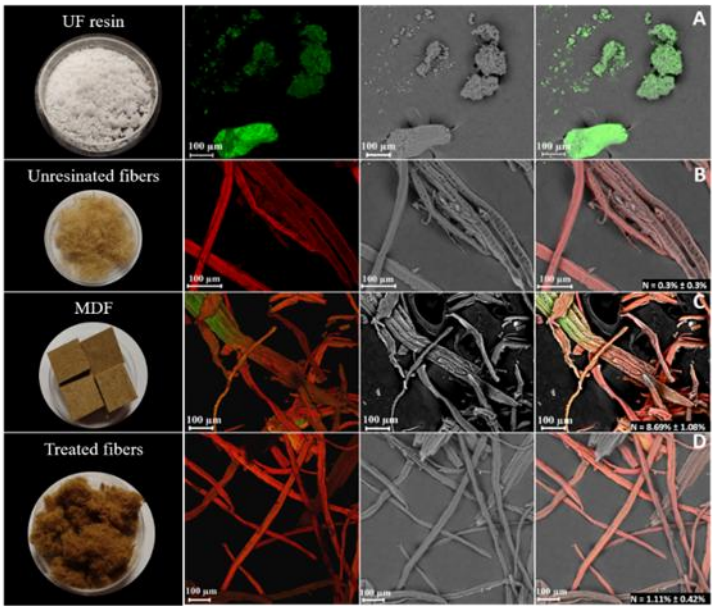


Figure 7. From left to right: CLSM (maximal intensity projection), SEM, and CLEM images of (A) UF resin, (B) uncoated control fibers, (C) coated control fibers, and (D) SE treated MDF fibers stained by safranin. Relative nitrogen percentage determined by WDS on fiber pellets; mean of 15 acquisitions are given on CLEM images. Error values are 95% CI.

Furthermore, by using SEM-EDS on the same observation field it was possible to map nitrogen in the green fluorescent areas and SEM-WDS quantification was in accordance with bulk elemental analysis. This technology was used to monitor the UF-resin removal subsequently to MDF treatment by SE at TRL 3. An efficient (up to 86%) cleaning of the resin was obtained on post-production MDF boards at TRL 3.

2.1.2. Glue removal from post-consumer BR1/ BR2 (AIII) wood (TRL 3)

In the framework of the Wood 2 wood project, the first batch of experiments was to test the UF removal from BR1/BR2 post-consumer wood at TRL 3 (small pilot). Based on the results obtained on MDF, SE experiments were performed using optimal conditions. NIRS and elemental analysis were performed subsequently to evaluate the resin removal by using nitrogen quantification as proxy. Two types of biomasses were used: i) BR1/BR2 wood made of fragments of particleboards panel around 2 cm size and ii) industrially grounded particles (8 mm size) mix used for particle board production (60% BR1/BR2 mixed with 40% fresh wood; less than 1.5% of MDF fibers). Results on Figure 8 showed that UF resin removal by SE was obtained on both biomass types. The high dispersion of the value illustrated the variability of the biomass. Finally, the shift observed between native and steam explosion treated biomass was higher for panel mix than for raw BR1/BR2 fragment. This would suggest an effect of the particle size in the biomass.

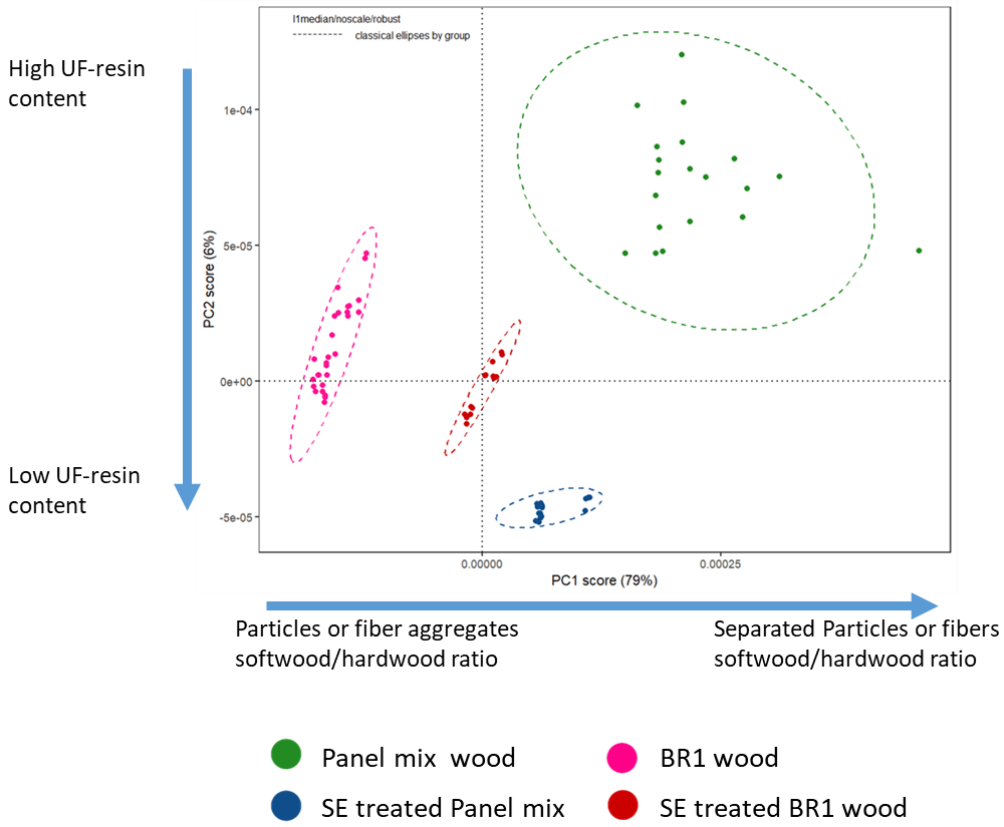


Figure 8. Monitoring the SE process efficiency at TRL 3. PCA were performed on NIRS spectra obtained on BR1/BR2 wood and panel mix. Both effect of particle size and glue removal can be observed.

To validate nondestructive measurement by NIRS, an elemental CHON analysis was carried out on the same sample. After SE treatment, water washing of the biomass and drying, a CHON elemental analysis was performed. The nitrogen content was used as a proxy for UF-glue quantification. The results are in Table 1.

Table 1. Nitrogen quantification by elemental analysis without and with SE .

Biomass	BR1/BR2	SE BR1/BR2	Panel Mix	SE Panel Mix
Nitrogen (mass %)	3.36 ± 0,29	1.86 ± 0,02	1.64 ± 0,15	0.40 ± 0,06

The results of the elemental analysis confirmed nondestructive measurements. The initial Nitrogen (UF-resin) content was 48% lower in panel mix than in BR1/BR2 wood. This can be explained because in the panel mix between 50 and 60% of the mass is particles partly containing UF-resin. Fiber represents less than 1.5%. On the contrary, the BR1/BR2 samples are 100% of the particles from the particleboard. Interestingly, the size of the processed biomass seems to influence the efficiency of UF resin removal. In the same conditions, the UF removal was more efficient (76%)

with the small size particle (*i.e.,* panel mix) than with the BR1/BR2 fragments (45%). Taken together, these results validated the NIRS methodology to monitor the UF-removal by SE process. The efficiency of the SE process seems to rely on the size of the post-consumer wood fragment to process.

2.1.3. Post-consumer wood from furniture industry (BR1/AIII) wood Upscaling at TRL5

Optimization of the SE conditions from TRL3 to TRL5 pilot

To enable the scaling up of the steam explosion (SE) process for extractives (UF) removal, we conducted a comparative study between two pilot systems at TRL3 and TRL5. Since the TRL5 pilot is relatively new in the lab and a straightforward correlation based solely on the severity factor was not possible, we focused our analysis on cell wall modifications observed in the wood biomass treated by the SE process. This approach allows for a more accurate optimization of the parameters required for scale-up. As observed previously on Beech wood, the density and morphology of the pseudo lignin deposit are good criteria for evaluating the intensity of the SE on wood (Aguilar et al., 2024). Thus, to find equivalent parameters to process wood in both pilots, we performed visual and semi-quantitative assessments of the pseudo-lignin deposit on the cell wall surface by SEM. Representative pictures are given in Figure 9.

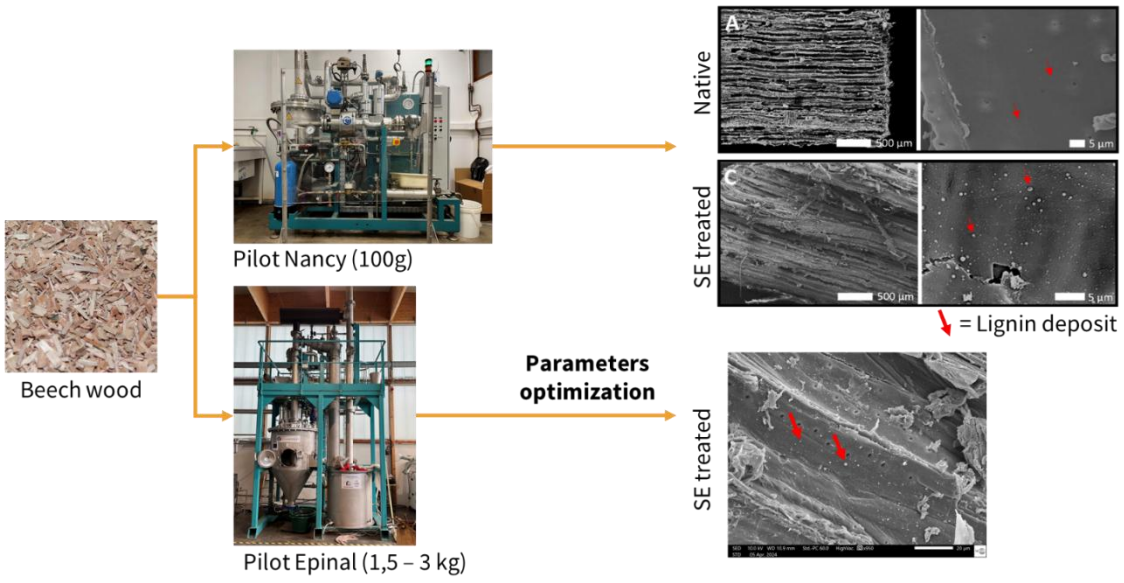


Figure 9. Experimental workflow to optimize SE setting at TRL3 and TRL 5.

Similar wood defibration morphological alterations were observed between the two pilots for high severity conditions at TRL3 and TRL5. These observations were confirmed at the micron scale by characterizing the lignin deposits on the cell wall surface. The density and morphology of the deposit were found to be similar between the two pilots. In conclusion, this preliminary set of experiments allowed us to find equivalent conditions of SE treatment between 2 experimental devices. This allowed the setup of possible optimal conditions to process UF-resin removal from

BR1/BR2 wood. The next step will be to investigate the UF-resin removal from different biomass processed with TRL5 SE pilot.

UF-resin removal from post-consumer wood by Steam explosion at TR5

The steam-explosion process has been scaled up to a larger pilot unit to generate sufficient biomass and effluents for analysis. It is being evaluated on the various biomass types supplied by Ecomaison (Figure 4). We are investigating the effect of steam explosion pretreatment on glue removal for each biomass type. After the steam explosion process, the biomass is named SE *Biomass*. The samples tested are as follows:

- BR1 wood from Ecomaison
- Panel mix from CF2P
- Particleboard, majority present in the deposit
- MDF, high glue concentration

The glue removal process can be done with quantitative methods such as the Kjeldahl analysis and SEM-EDX. And qualitative methods such as the FT-IR NIR analysis and fluorescence microscope.

Kjeldahl analysis was used to monitor the decontamination process. This method measures the nitrogen content in solid or liquid samples. Urea-formaldehyde (UF) and melamine-urea-formaldehyde (MUF) are the most used adhesives in particleboard and MDF, respectively. The urea molecule, $CO(NH_2)_2$, contains nitrogen, which can be tracked during analysis. In this study, the Kjeldahl analysis was performed on solid samples using only a few milligrams of material. The conditions of steam explosion are described in Table 2.

Table 2. Condition of steam explosion for each biomass

Biomass	Steam explosion condition
Panel mix	High severity
MDF	High severity
Particleboard	High severity
SE1, SE2, SE3, SE4, SE5	Mild severity

Figure 10 and Figure 11 show the nitrogen content of the panel mix and MDF before and after steam explosion, as well as that of BR1 wood. The glue content in BR1 wood and panel mix is quite similar, around 3.6%, due to the panel mix being derived from BR1 wood. MDF, which requires a higher amount of adhesive in its production process, shows a significantly higher nitrogen content, reaching 8.3%. After the steam explosion, a reduction in nitrogen content is observed, indicating partial decontamination. The decontamination threshold is around 1.56%, regardless of the initial biomass composition. Despite the amount of glue in the beginning, the high severity conditions of steam explosion seemed to remove adhesive from the wood.

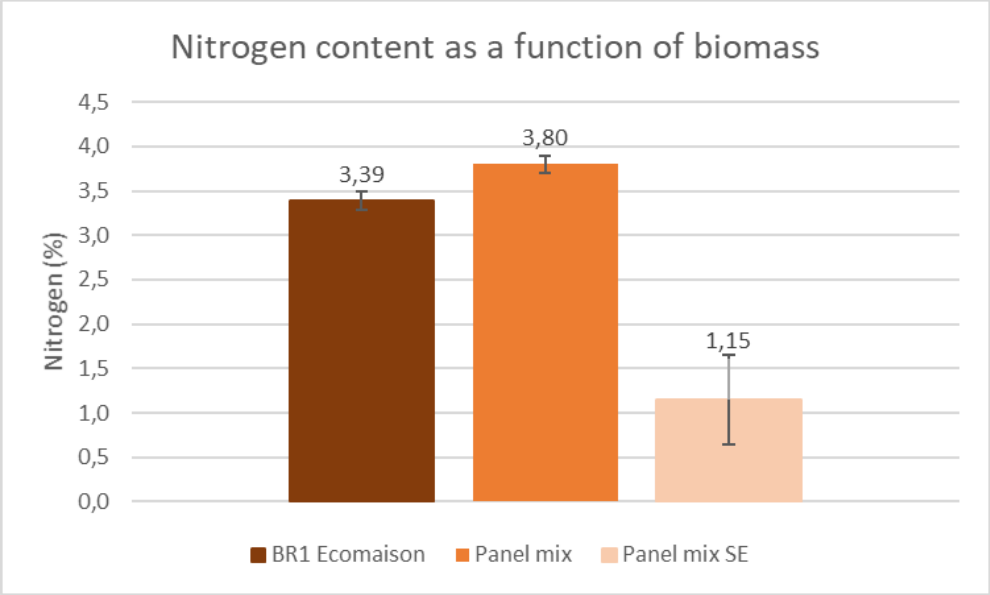


Figure 10. Nitrogen content measured by Kjeldahl analysis before and after steam explosion in BR1 wood and panel mix.

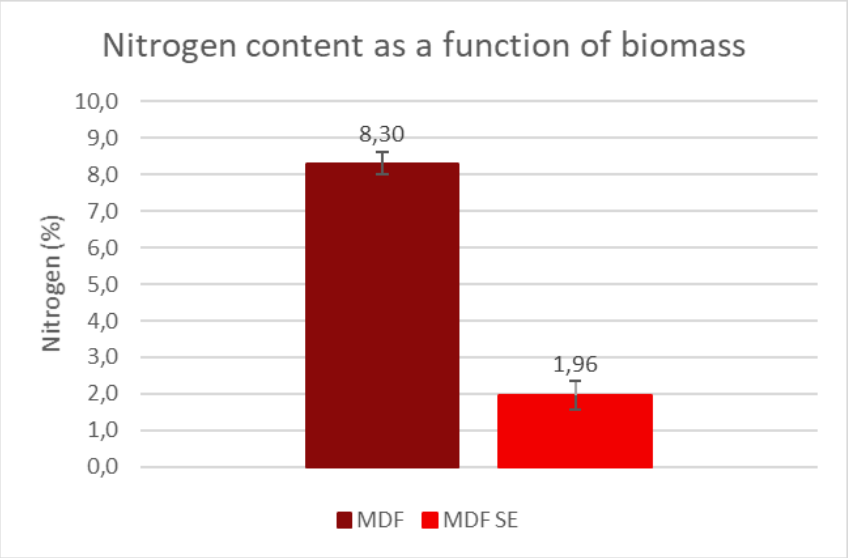


Figure 11. Nitrogen content measured by Kjeldahl analysis before and after steam explosion in MDF.

FTIR-NIR spectroscopy combined with Principal Component Analysis (PCA) can be effectively used to visualize the effects of steam explosion pretreatment on materials, compare different samples, and assess their chemical similarity. This approach also uncovers correlations between chemical parameters, such as the impact of pretreatment, nitrogen content, and fiber morphology. The characteristic wood fingerprint region lies between 6500 and 4500 cm^{-1} (Schwanninger et al., 2011).

Figure 12 presents the PCA analysis of MDF, panel mix, and particleboard samples after steam explosion treatment. The primary components are PC1, PC2, and PC3, with PC2 and PC3 providing

more refined insights. The method's validity is supported by the fact that the confidence ellipses do not overlap, indicating clear differentiation between sample groups. This analysis is both replicable and robust.

The PC1 axis primarily corresponds to glue content: MDF and panel mix samples cluster on the left side (negative PC1 values), while after steam explosion, the biomass shifts to the right (positive PC1 values). Among the most positive values, MDF ranks highest, followed by panel mix, then particleboard. This shift aligns with the observed decrease in nitrogen content, which is more pronounced in MDF than in panel mix (Figure 10 and Figure 11). Therefore, PC1 reflects the amount of glue remaining in the biomass.

The raw data clusters are very compact, suggesting the material is homogeneous before the steam explosion. After treatment, variability increases along PC2 and PC3, likely due to fiber defibration, glue loss, and moisture variation.

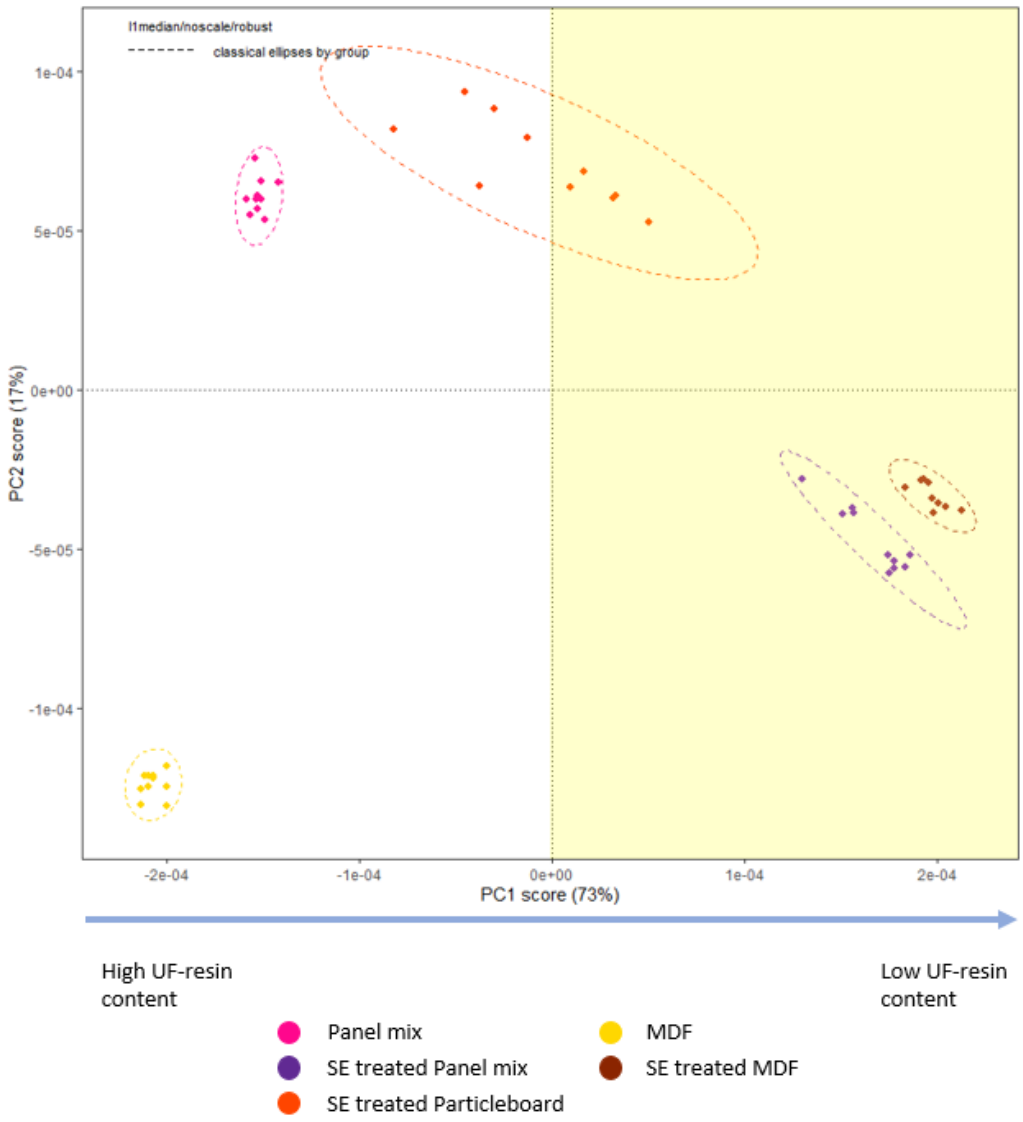


Figure 12. FTIR-NIR and PCA analysis of biomass before and after steam explosion. The yellow zone corresponds to a glue-free area.

The efficiency of the steam explosion (SE) process is strongly influenced by the particle size of the biomass. In the case of BR1 wood, the heterogeneity in particle size likely affected the quality and uniformity of the pretreatment. According to (Simangunsong et al., 2020), smaller particles tend to undergo more effective steam explosions due to increased surface area and better heat and pressure transfer. Consequently, future work will focus on identifying the optimal particle size of the biomass to maximize the effectiveness of the SE process.

Monitoring of the SE process efficiency at the microscopic level

To visualize the effect of the pretreatment, the fibers were stained with 0.1% safranin and observed using a fluorescence microscope as previously described (Troilo et al., 2023). Glue detection was achieved using an excitation wavelength of 450–490 nm and an emission wavelength of 515 nm. Wood autofluorescence was observed with an excitation wavelength of 515–560 nm and an emission range above 590 nm. The wood staining method made it possible to visualize the distribution of resin on wood particles with high sensitivity and specificity (Grigsby et al., 2005). Therefore, the glue appears in green and the wood in red in the fluorescence settings used. Before the steam explosion, glue clusters are clearly visible as green, fluorescent patches (Figure 13, A). They represent concentrated areas of adhesive that need to be removed during treatment. After steam explosion, some glue remains in the biomass (Figure 13, B). It was previously described that UF-resin is embedded within the wood cell walls, making it harder to extract (Xing et al., 2005). This explains the few residual traces still visible in orange-yellow on Figure 13, B. The change in color is due to the embedding of resin in the wood. Nonetheless, the visual comparison indicates a significant reduction in glue content following the pretreatment.

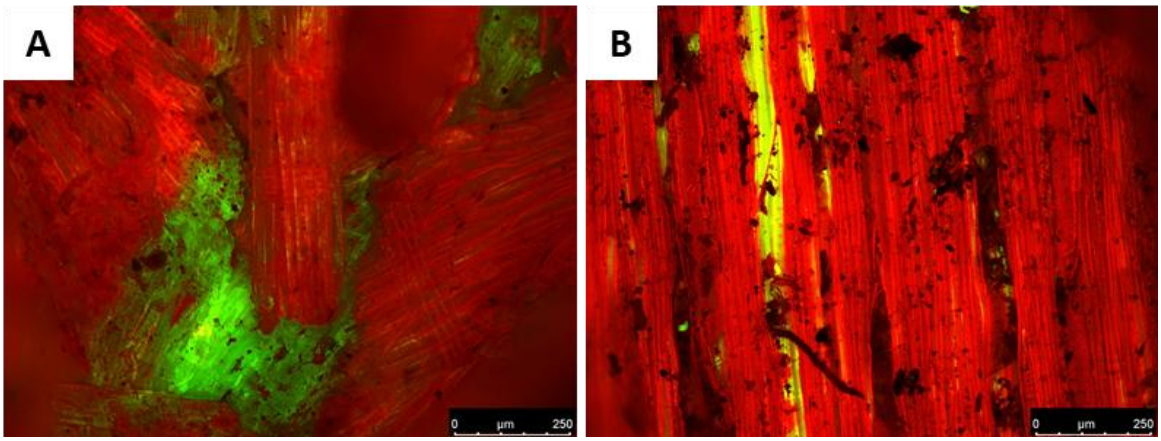


Figure 13. Fluorescence microscopy image of a panel mix particle A/before or B/ after steam explosion. Green areas indicate the presence of glue clusters.

SEM-EDS analysis was tentatively used to identify and quantify the chemical elements present locally on the surface observed by SEM. Elements such as C, O, N, Al, and Na can be detected. In this study, the technique was applied to compare the nitrogen content in the biomass before and after

steam explosion pretreatment to validate *in situ* the bulk quantification performed by NIRS and elemental analysis. The second objective was to validate the staining-based mapping of the glue in BR1 wood. Sub samples of the BR1 biomass, namely particle board and MDF boards were observed with SEM-EDS technology. Representative pictures obtained before the SE processing are shown in Figure 14. Melamine coating was included in the analysis as a positive control of nitrogen detection and quantification.

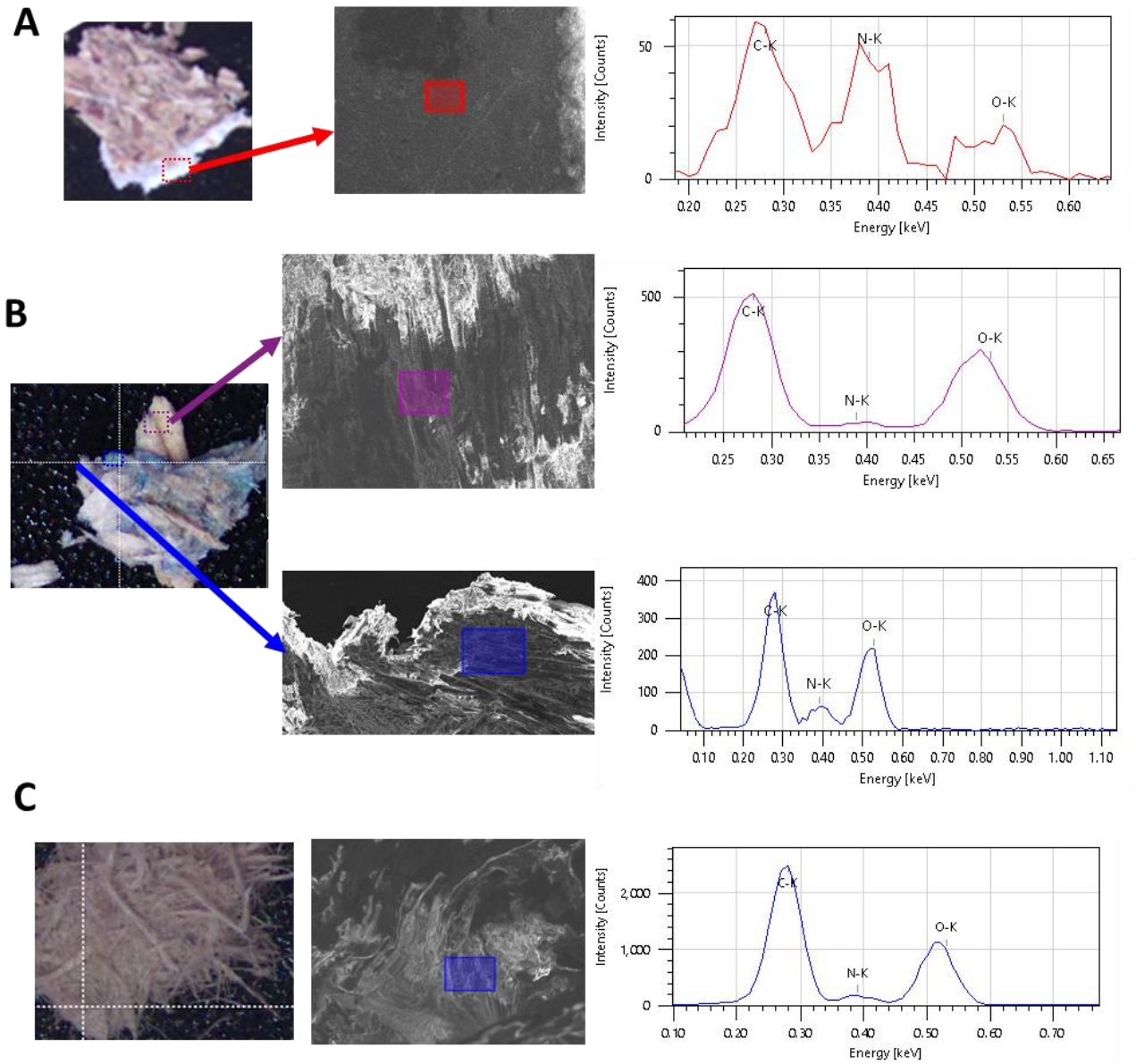
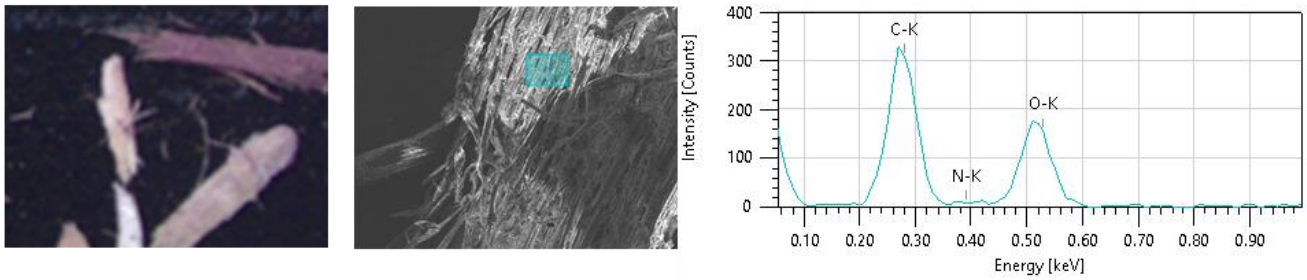


Figure 14. Microscopic scale evaluation of the UF-resin based on nitrogen detection by SEM-EDS on BR1 wood sample before SE. A/ Melamine coating, B/ Particle board with analysis of wood (pink) or glue containing area (blue), C/ MDF fibers. For each sample stereomicroscopic view of the sample, SEM (SE detector) observation and EDS spectra are given.

The same procedure was performed on samples after SE treatment. Representative pictures are shown in **Σφάλμα! Το αρχείο προέλευσης της αναφοράς δεν βρέθηκε.**.

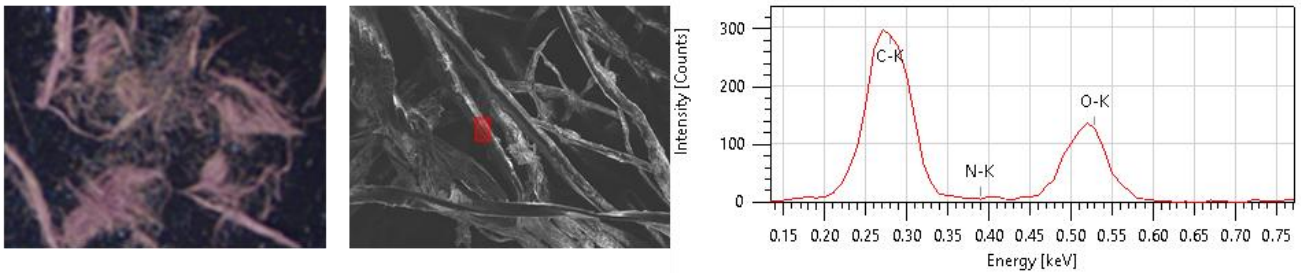
Figure 15. Microscopic scale evaluation of the UF-resin based on nitrogen detection by SEM-EDS on BR wood sample after SE. A/ Particle board with

A



B

analysis of wood, B/ MDF fibers. For each sample stereomicroscopic



view of the sample, SEM (SE detector) observation and EDS spectra are given.

Finally, a semi-quantitative analysis of the sample elemental composition was performed to evaluate the process efficiency. Data are reported in Table 3 and

Table 4.

Table 3. Semi-quantitative evaluation of C, N and O elements by SEM-EDS performed on sample before SE.

	Mass % (± 95% CI)		
Sample	**C**	**N**	**O**
Melamine	33.53 (±1.05)	45.66 (±2.4)	20.81 (±2.21)
UF-resin in particleboard	45.71 (±0.54)	14.71 (±0.71)	39.58 (±1.12)
Wood in particleboard	55.84 (±0.53)	0.53 (±0.04)	43.63 (±1.00)
MDF fiber	56.72 (±0,25)	7.75 (±0.25)	35.53 (±0.45)

Table 4. Semi-quantitative evaluation of C, N and O elements by SEM-EDS performed on sample after SE.

	Mass % (± 95% CI)		
Sample	**C**	**N**	**O**
BR1 wood particle	59.25 (±0.73)	0.91 (±0.39)	39.84 (±1.3)
MDF fiber	63.18 (±0.8)	n.d	36.82 (±1.35)

The SEM-EDS analysis of the sample validated the bulk quantification of nitrogen content. It clearly showed a significant reduction of nitrogen amount both in particle and MDF panel boards after SE. The SEM-EDS also validated the staining observations and brought semi quantitative data in complement. It suggested that safranin staining coupled with image computation could be a fast method to detect and quantify resin containing wood. It might also be a fast and cheap alternative to evaluate the SE process efficiency on UF resin removal. However, further work must be performed to evaluate the feasibility of the UF resin quantification by using florescence spectrometry. Additionally, SEM observations confirmed the fragmentation and defibrating effect on biomass.

Optimization of the SE and evaluation of the impact on particles and fiber recycling

The new objective is to reduce energy and water consumption. To achieve this, iterative steam explosion treatments were carried out at lower temperatures and pressures.

This part of the study focuses on glue removal under these milder conditions. After evaluating the different biomass types, we decided to continue the analysis using only panel mix.

The Kjeldahl analysis reveals a significant decrease in nitrogen content (58%) after the first steam explosion (SE1, b) compared to the initial panel mix (a) (Figure 16). Due to the mild explosion conditions, only a small amount of glue is removed during the second explosion (SE2, c), and 0.83% of nitrogen can be removed. The nitrogen content appears to stabilize after the third and fourth explosions (SE3 and SE4, cd). A minimum value is reached after the fifth explosion (EV5, d), with a nitrogen content of 0.35%, corresponding to a 60% reduction compared to the initial biomass with glue. Statistical significance was confirmed using one-way ANOVA followed by Tukey's post hoc test. Different letters indicate significant differences between treatments ($p < 0.05$).

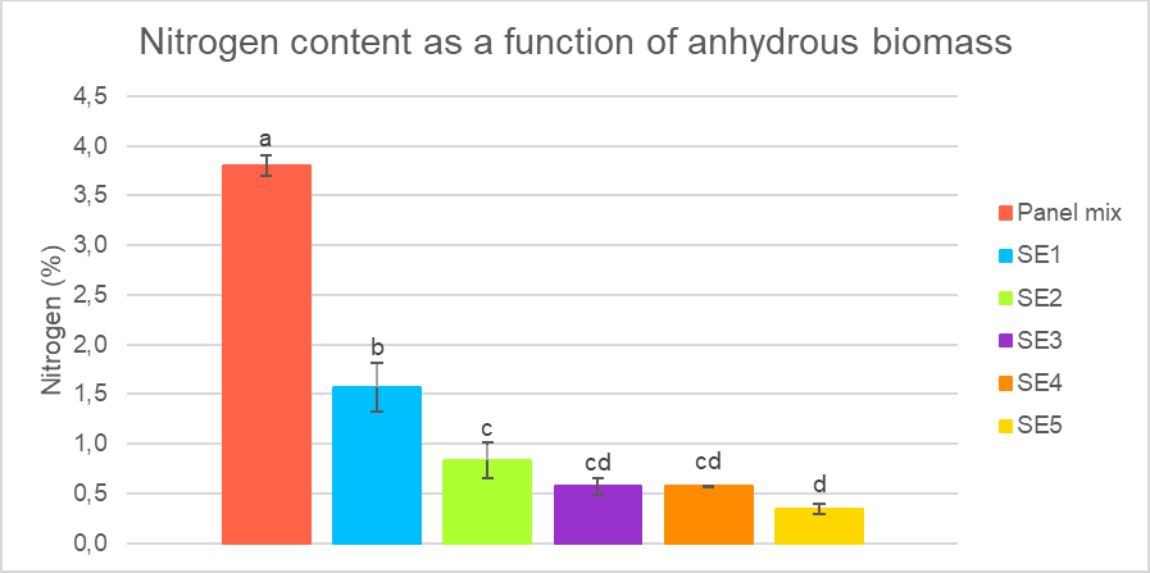


Figure 16. Nitrogen content measured by Kjeldahl analysis before and after multiple steam explosion treatments of panel mix. Statistical differences were assessed using one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$). Different letters indicate significant differences between groups.

Figure 17 presents the PCA results following the multiple steam explosions. As previously explained, the PC1 axis is associated with the presence of adhesive. SE1, which has the highest nitrogen concentration, is positioned on the left side of the plot (negative PC1 values). As the glue is progressively removed through pretreatment, the samples shift towards the right (positive PC1 values) along the PC1 axis.

In addition to decontamination, pretreatment also influences the defibration of the biomass. Steam explosions are known to affect fiber morphology. Since the initial material is heterogeneous, each type of fiber responds differently to the treatment. As a result, not all fibers are defibrated in the same way, which explains the increased variability observed within the large cluster after the fifth explosion.

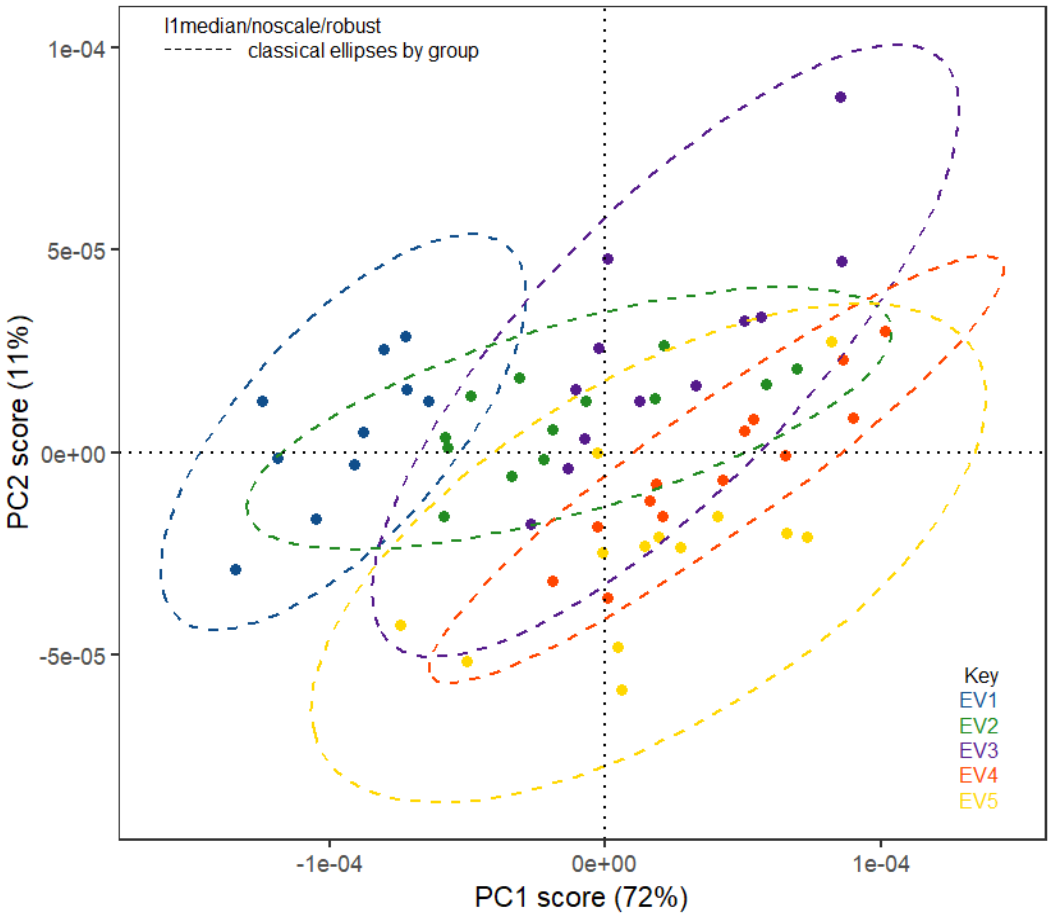


Figure 17. Non-destructive evaluation of successive multiple steam explosion treatments on the wood sample. PCA was performed on NIR spectra acquired on sample subjected to 5 iterations of SE treatments performed in mild conditions.

2.2. MORPHOLOGICAL CHARACTERIZATION OF PARTICLES AND FIBERS

Steam explosion with unground biomass, a study of the defibration

The steam explosion (SE) process is a well-established method for fiberizing wood and reducing particle size, commonly used in biomass pretreatment. In the work carried out within this task, we compared two batches of wood particles: one labeled Panel mix, supplied by the industrial partner CF2P, and another consisting of unground wood waste collected by Ecomaison. The objective was to evaluate the ability of the steam explosion process to replace conventional mechanical milling, by assessing its effectiveness in breaking down larger, untreated wood pieces into fine, processable material.

Pieces of particleboards and raw wood with dimensions between 10 cm and 15 cm were pretreated with a steam explosion. The biomass was processed with high severity process parameters. After the process, the harvested biomass was completely defibrated. The particleboards turned into fibers, and some large pieces of the raw wood were also extensively disrupted (Figure 18).

Following the steam explosion treatment of a raw wood sample approximately 10 cm in size, displayed marked structural breakdown, indicative of efficient fiber separation. Macroscopic observation revealed a significant breakdown of the lignocellulosic matrix.

The fibrous bundles appeared largely separated, with a clear dissociation of longitudinal fibers, characteristic of the disruption of bonds between lignin, hemicellulose, and cellulose. This structural breakdown results from the combination of high-pressure thermal treatment followed by a sudden depressurization, which induces a mechanical burst effect.

Morphologically, the resulting substrate consisted of:

- Long, coarse, and partially frayed fibers, reflecting an effective fragmentation of the primary wood structure (blue arrow).
- Irregular woody fragments with torn and uneven edges, suggesting violent mechanical rupture (blue arrow).
- Finer particles and fibrous debris originate from the exploded particleboards (red arrow).
- The melamine coating initially adhered to the surface of the particleboards is separated and removed as a result of the steam explosion treatment (white arrow).

A marked loss of cohesion was observed, with the initially dense wood material transformed into a loose fibrous mass suitable for subsequent processing steps. The brownish coloration is typical of the onset of thermal degradation of hemicelluloses, with no apparent signs of carbonization.

Figure 18. Particleboards and raw wood from the Ecomaison batch after steam explosion at high severity conditions. The biomass shows frayed fibers and ruptured wood fragments (blue arrows), fine fibrous particles from panel breakdown (red arrow), and detached melamine coating (white arrow).

Impact of the SE process on the recyclability of the Panel mix fibers

The effect of steam explosion onto the fibers is now demonstrated. For the rest of our study, we chose to work with the Panel mix from CF2P to avoid a time-consuming shredding step. The objective is now to do cascading steam explosions and to study the impact on fiber's recyclability, using less severe conditions for energy reduction. We are interested in the defibration process. Indeed, the morphology of fibers determines what materials can be produced with this pretreated biomass exploded one to five times.

Figure 19 presents the biomass after each sequential steam explosion. A progressive darkening of the material is observed, along with the formation of fiber clusters, suggesting increased fiber bonding and aggregation phenomena occurring with repeated treatments.

To further investigate this transformation, the morphological evolution of the biomass before and after a single steam explosion is illustrated in Figure 20, highlighting the transition from compact, angular particles (A), to partially defibrated material (B), and finally to a dense network of frayed fibers (C). This confirms the effectiveness of the process in disrupting the lignocellulosic structure and promoting fiber separation.

Figure 19. Macroscopic pictures of the panel mix after five cascading stem explosions in mild conditions.

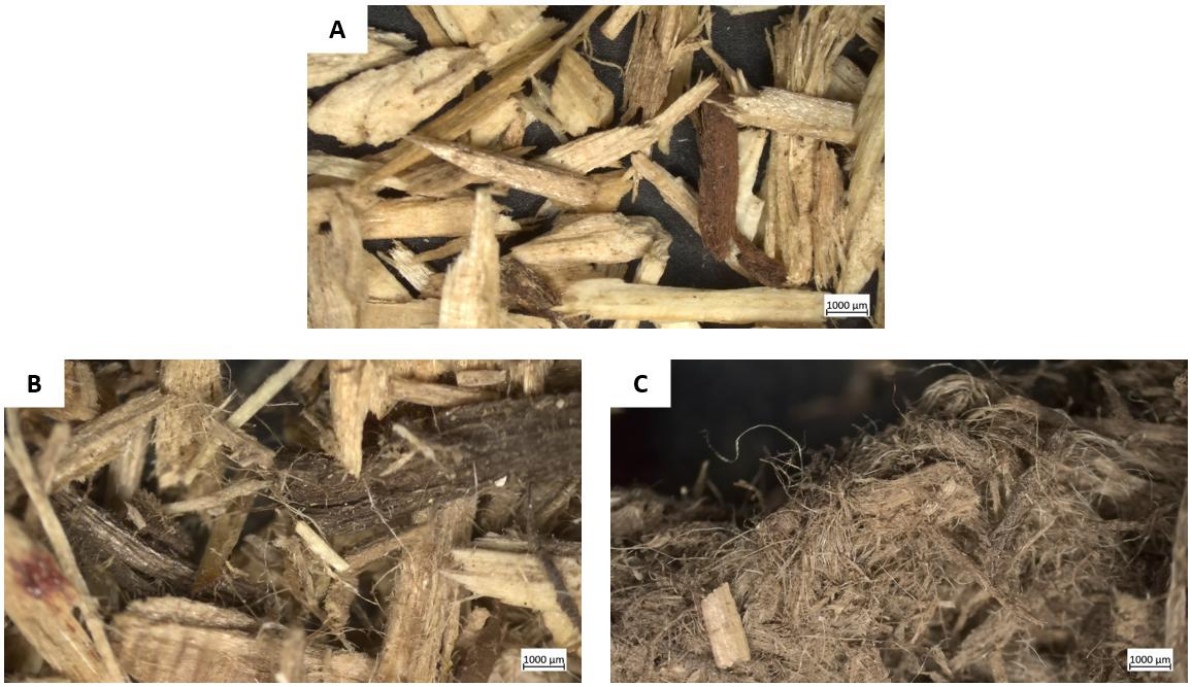


Figure 20. Binocular images of the Panel mix (A), steam-exploded sample SE1 (B), and steam-exploded sample SE5 (C).

To further characterize the defibration process, particle size measurements were conducted using a Camsizer, a device based on Dynamic Image Analysis. In this method, particles flow through an illuminated measurement zone, where high-speed cameras capture their images in motion. These images are processed by dedicated software to analyze size and shape parameters such as length, width, and equivalent diameters. This optical technique allows for precise, individual-based measurements and yields detailed particle size and shape distributions.

Figure 21 presents the particle size distribution of the Panel mix before and after one to five successive steam explosion treatments (SE1 to SE5). A clear shift in particle morphology is observed across the treatments. The blue area highlights the near disappearance of fine wood dust particles (< 0.63 mm) after the second explosion (SE2), which could be attributed to losses during handling. This also indicates a cleaning effect, where fine residues are no longer generated or removed.

In contrast, the yellow area denotes an increase in the proportion of large fiber clusters (> 8 mm), particularly after SE4 and SE5. This suggests a progressive agglomeration of individual fibers as defibration advances.

From CF2P (Panel mix) to SE2, a notable reduction in average particle size is observed, confirming effective fragmentation. However, from SE4 to SE5, the distribution values become more uniform, indicating a stabilization of particle morphology. This homogenization trend may reflect a limit in the defibration process, where most fibers have already been separated.

Overall, the data confirm that increasing the number of steam explosions enhances fiber individualization and promotes defibration. With higher severity, fibers are more clearly separated,

but also begin to reassociate into fiber aggregates, as shown by the growing fraction of large-sized particles.

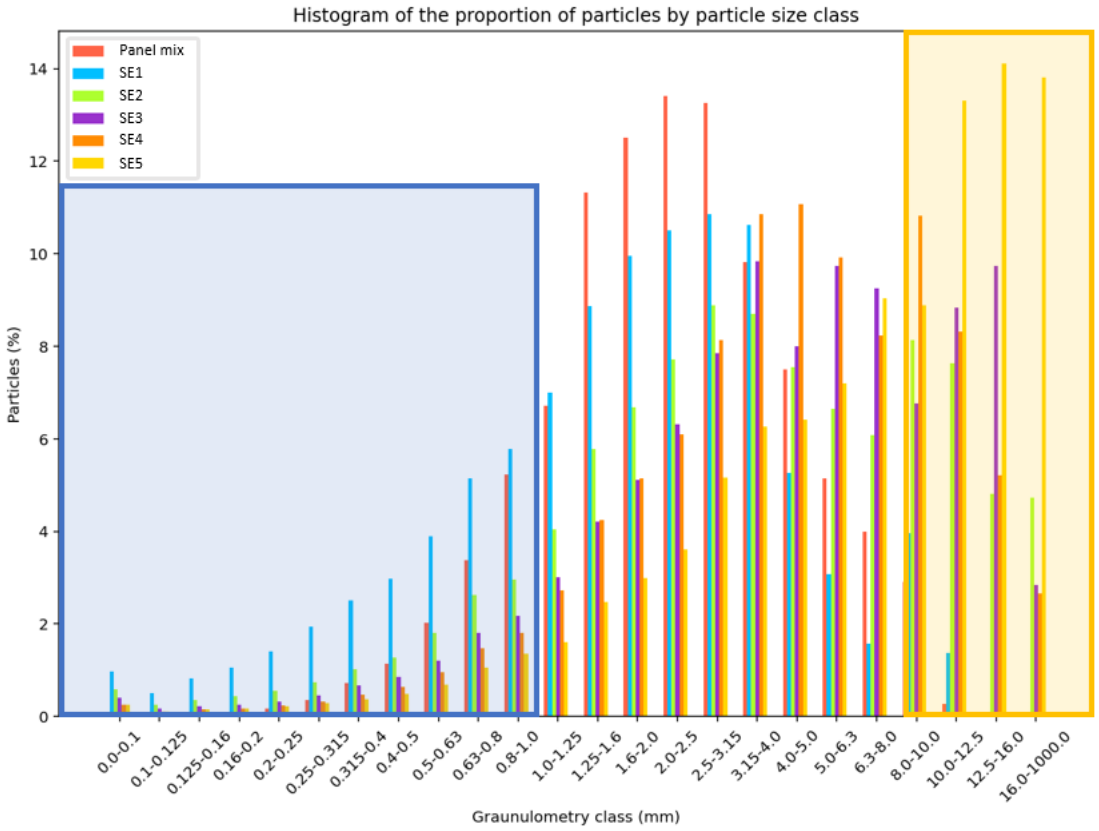


Figure 21. Histogram of particle size distribution for the Panel mix and after successive steam explosion treatments (SE1 to SE5). The blue area highlights the disappearance of fine wood dust particles (< 0.63 mm) after the second explosion (SE2). The yellow area corresponds to the presence of large fiber clusters (> 8 mm).

In conclusion, wood fibers can be subjected to multiple recycling cycles through successive steam explosions. However, the morphological changes induced by these treatments must be carefully considered, as they directly impact the subsequent transformation processes and the quality of the final material.

3. CHAPTER 2 MYCOREMEDIATION AND SUBMERGED FERMENTATION

In the context of recycling processes, our objective is to decontaminate pollutants potentially present in SE effluents (Figure 22), with a particular focus on formaldehyde. To initiate the decontamination protocol, we opted to work with a synthetic effluent, prepared by dissolving varying concentrations of formaldehyde in water. Although other compounds are also present in real effluents, they have not yet been characterized. As their presence could interfere with the decontamination mechanisms, we chose to use a simplified model solution to eliminate potential confounding factors.

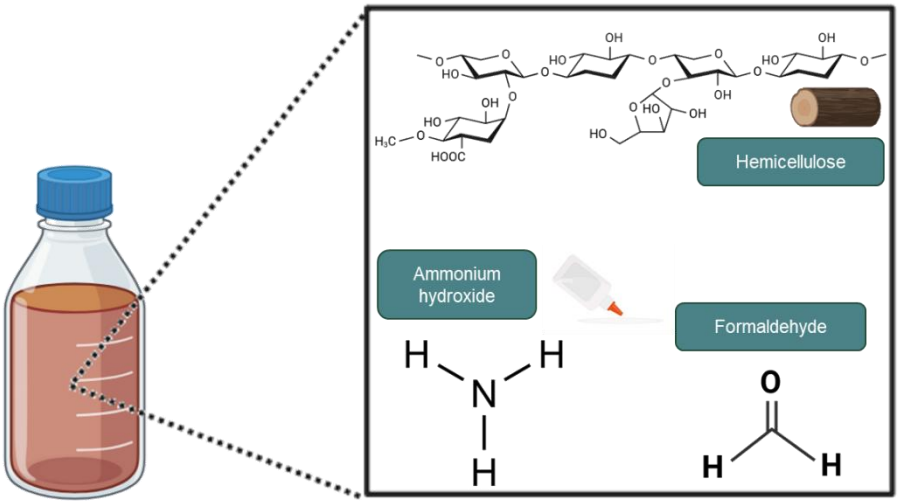


Figure 22. Diagram of molecules that may be present in steam explosion effluents.

3.1. ISOLATION OF THE FUNGAL STRAIN

The isolation process began using various fungal strains available in our laboratory. Previous study performed in the lab showed that pure MDF contains between 13 to 25 mg of formaldehyde per gram of biomass (Gibier et al., 2022). To assess their resistance to formaldehyde, each strain was cultured on Petri dishes containing 3.9% PDA (Potatoes Dextrose Agar) supplemented with 600 mg/L of formaldehyde (CH_2O). Among all the strains tested, only one was able to grow under these conditions. Consequently, this Ascomycota strain was selected for further investigation.

Several formaldehyde concentrations were tested during the screening process, and the isolation required considerable time and effort. Further work is still necessary, including DNA extraction, additional decontamination assays, strain adaptation, and optimization of growth parameters such as growth rates.

Notably, formaldehyde concentrations in MDF (medium-density fiberboard) can reach up to approximately 1500 mg/L (Troilo et al., 2023), highlighting the relevance of identifying resistant fungal strains.

The isolated organism is a filamentous fungus with septate hyphae. Morphological features suggest that it belongs to the phylum Deuteromycota (the asexual form of Ascomycota), as it produces asexual spores (conidia) and exhibits phialides arising from the hyphae (Figure 23).

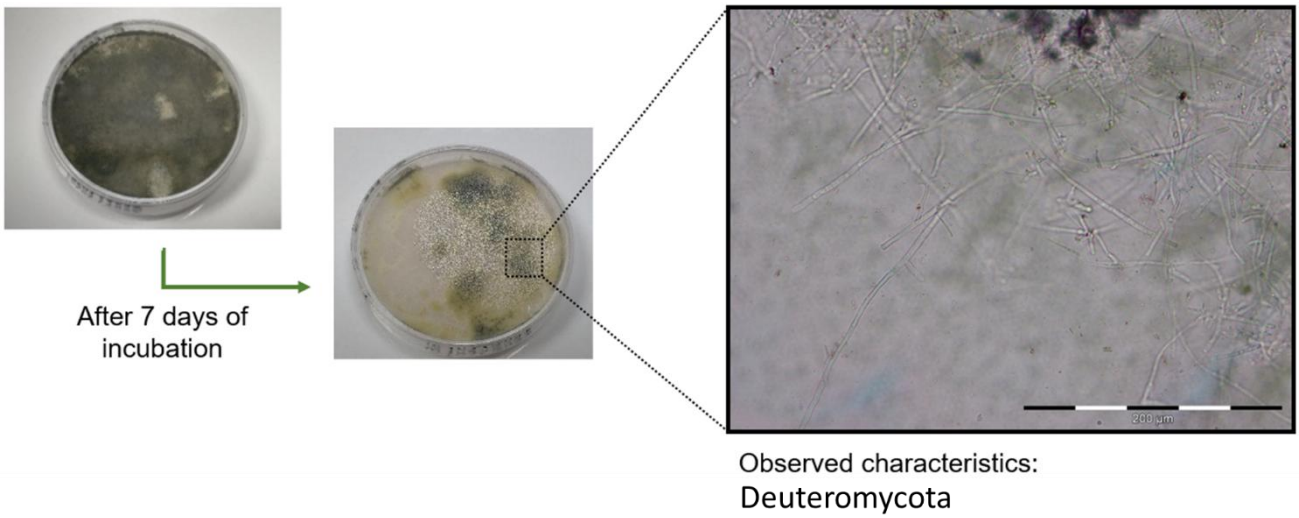


Figure 23. Evaluation of formaldehyde resistance in an isolated fungal strain. Macroscopic view of the strain growing on 3.9% PDA medium supplemented with 1500 mg/L formaldehyde (CH_2O) after 7 days of incubation (left and center). The microscopic image (right) shows the fungal hyphae and reproductive structures characteristic of filamentous fungus belonging to Deuteromycota.

3.2. IMPACT OF THE CULTURE MEDIUM AND FORMALDEHYDE ON THE FUNGAL GROWTH

To test the fungal tolerance to formaldehyde, fungal growth tests were performed on different culture media which differed by their carbon to nitrogen (C/N) ratio. From the highest to the lowest C/N ratio the media can be ranked as follows: PDA+YE+Malt, PDA+YE, Agar+YE. Representative picture of the fungal growth after 5 days on different media is illustrated in Figure 24. In the absence of formaldehyde, a gradient of fungal growth was observed depending on C/N balance in the media. The strain was growing better on culture media with High C/N ratio. On the optimal culture media (PDA+YE+Malt), the fungal growth was slightly decreased above 600 mg/L formaldehyde concentration. Interestingly, when the fungus was cultivated with formaldehyde as the only carbon source, its growth followed the formaldehyde concentration gradient. This would suggest that the strain was using formaldehyde as a carbon source in its primary metabolism. This is particulary interesting in the framework of an ecofriendly myco-remediation process implementation. The radial fungal growth speed was measured by image computation as described previously (Charpentier-Alfaro et al., 2023).

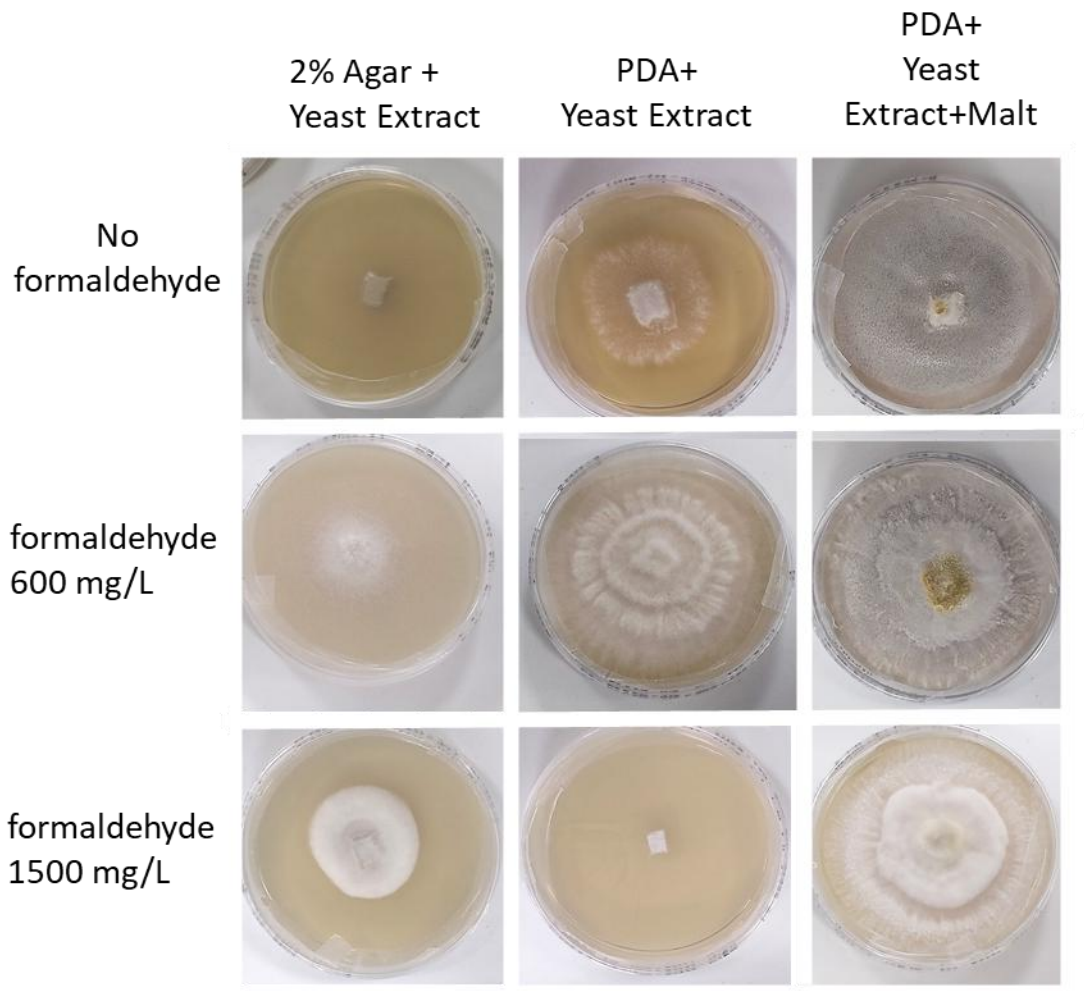


Figure 24. Fugal growth on different culture media in presence or absence of formaldehyde. The strain was cultivated for one week in the dark at 28°C. Picture after 5 days of growth are represented. Growth was done in triplicates for each condition.

The results are plotted in Figure 25. On optimal culture medium, a 1.5-day lag phase was visible when 1500 mg/L was added to the medium (Figure 25, A). A similar trend was observed in the other culture medium tested. The lag phase is probably due to the detoxification reaction triggered by the fungus to allow its subsequent growth. The growth curve was showing successive exponential and plateau phases suggesting metabolic switches of the fungus. No significant differences could be observed between the growth curves obtained for 0 and 600 mg/L of formaldehyde. This points toward a acquired tolerance to formaldehyde with a sub lethal concentration at 1500 mg/L. The comparison of the mean growth speed calculated on the linear exponential phase are plotted on Figure 25, B. In the PDA + YE + Malt media the carbon source is in excess. The intermediate (600 mg/L) formaldehyde concentration did not significantly affect growth. When the carbon source is limited (PDA+YE) or sub-optimal (Agar + YE) the supplementation with this formaldehyde concentration boosted the fungal growth. This confirmed the visual observation and strongly suggests that the fungal strain was using formaldehyde as a carbon source to fuel his primary metabolism. Further work is needed to shed light on this phenomenon. The occurrence of malt seems to be required to allow fungal growth at high (1500 mg/L of formaldehyde).

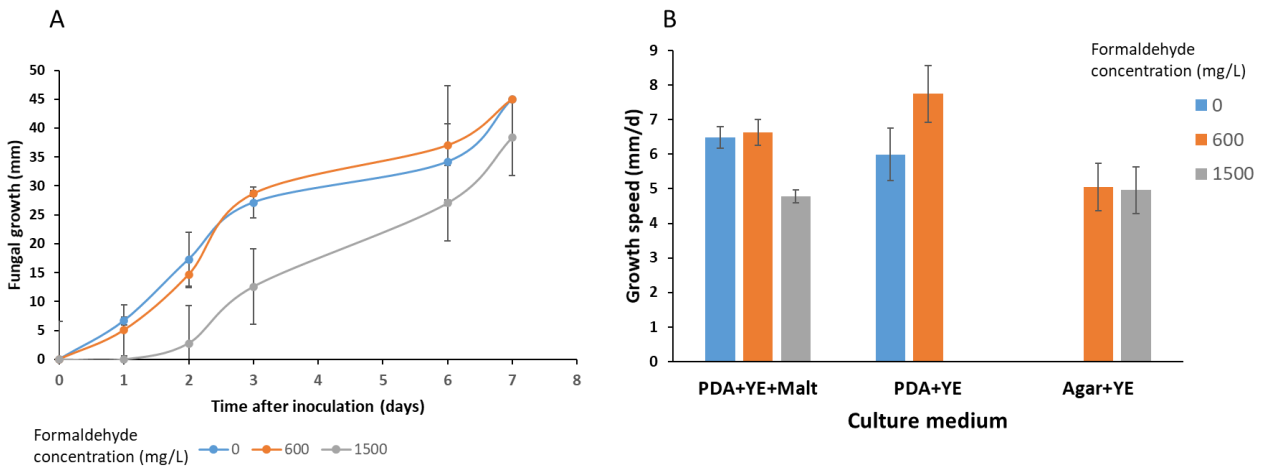


Figure 25. Growth speed of the fungal strain on different culture media supplemented or not with formaldehyde. A/ Growth of the fungal strain on PDA + YE + Malt supplemented with 0.600 mg/L or 1500 mg/L of formaldehyde. B/ Growth speed of the fungus calculated on linear growth phase.

3.3. LIQUID CULTURE

Liquid fermentation represents a promising and scalable approach for potential industrial applications in effluent treatment. The subsequent phase of this study involves cultivating the formaldehyde-resistant fungal strain in a bioreactor to process larger volumes of contaminated liquid. Prior to bioreactor scale-up, preliminary liquid culture experiments were conducted in Erlenmeyer flasks using Potato Dextrose Broth (PDB) supplemented with Yeast Extract (YE). Formaldehyde tolerance was evaluated at concentrations of 600 mg/L and 1500 mg/L.

The visual progress of the cultures was monitored over time. Figure 26 illustrates the appearance of the flasks containing 1500 mg/L formaldehyde compared to formaldehyde-free control, at the initiation of the experiment (t = 0 hours) and after 137 hours of incubation. A decrease in culture volume was observed over the incubation period, primarily due to the periodic collection of samples for analysis. The culture medium underwent a noticeable color change during growth, transitioning from an initial clear yellow/orange hue to a darker brown as the incubation progressed. This change might be due to oxidation status of the media in correlation with fungal development. To validate this hypothesis, the monitoring of the redox potential and the measurement of laccase enzymatic activity of the culture media will be performed in future experiments.

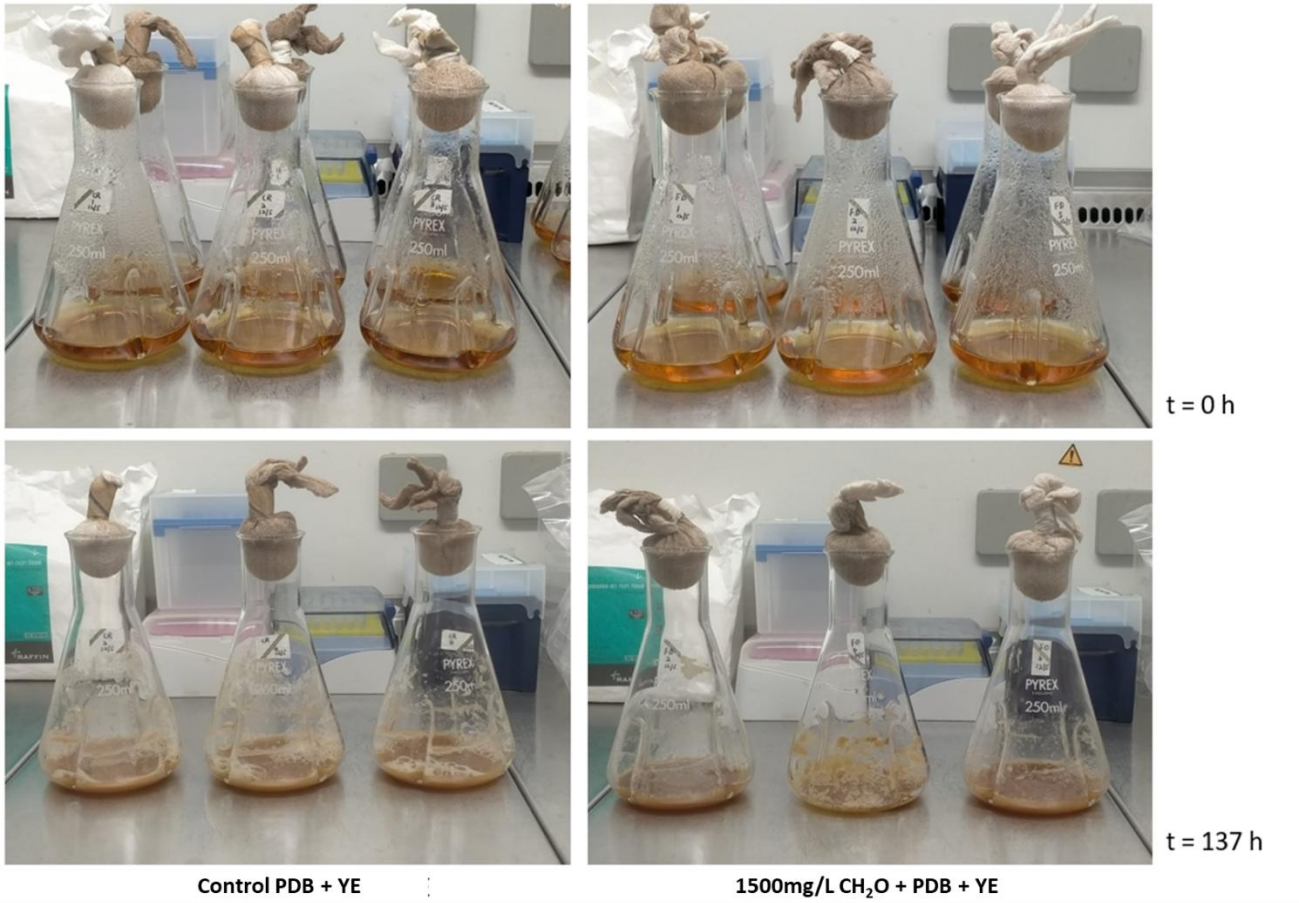


Figure 26. Visual progress of liquid cultures of the formaldehyde-resistant fungal strain. The figure shows Erlenmeyer flasks containing PDB + YE medium at the start of incubation (t = 0 h, top pictures) and after 137 hours (t = 137 h, bottom pictures). The left column shows the control condition (0 mg/L CH_2O), while the right column shows cultures supplemented with 1500 mg/L CH_2O.

3.3.1. Quantification of formaldehyde using HPLC

High-Performance Liquid Chromatography (HPLC) is used to separate and quantify formaldehyde. Because formaldehyde itself lacks absorbance properties for sensitive detection by standard HPLC detectors (like UV-Vis), it must first undergo a process called derivatization. This involves reacting formaldehyde with a chemical agent to form a stable, new compound (a derivative) that is easily detectable. The derivatized sample is then injected into the HPLC system, where the formaldehyde derivative is separated from other components on the column and subsequently detected, typically using a UV-Vis detector. The concentration of formaldehyde is then determined based on the signal from its corresponding derivative peak.

3.3.2. Monitoring of the formaldehyde removal by fungi

The Figure 27 and Figure 28 illustrate the dynamic process of formaldehyde detoxification by an isolated fungal strain cultured in liquid medium (PDB and yeast extract). Data obtained at initial formaldehyde concentrations of 600 mg/L and 1500 mg/L reveal a biphasic response.
The first phase is characterized by a slow or negligible decrease in formaldehyde levels, corresponding to an acclimation period. This latency phase is clearly dependent on concentration: it lasts approximately 0-20 hours at 600 mg/L but extends to nearly 60 hours at 1500 mg/L. This suggests that higher formaldehyde concentrations require longer metabolic adaptation times, likely due to the induction of detoxification pathways and deeper primary metabolism changes.

Once this acclimation phase is overcome, a strong and significant reduction in formaldehyde concentration is observed in both conditions, indicating the activation of effective detoxification mechanisms. Notably, the large error bars - particularly during the transition between the latency and active detoxification phases (*e.g.*, 20-30 hours at 600 mg/L) - reflect the inter-replicate variability in time and the pace of this metabolic change.

Despite this variability, the overall trend confirms the fungus's ability to adapt and efficiently eliminate formaldehyde, even under high stress conditions, highlighting its strong potential for bioremediation applications.

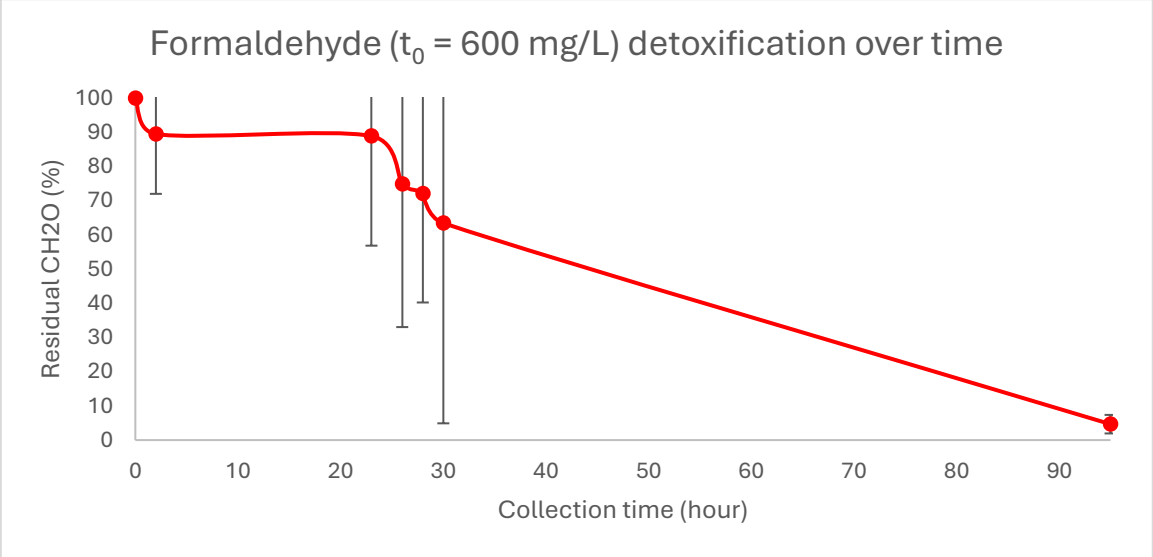


Figure 27. Percentage of formaldehyde assimilated by the fungal strain as a function of incubation time in hours. Cultures were performed in PDB and Yeast extract medium at an initial formaldehyde concentration of 600 mg/L.

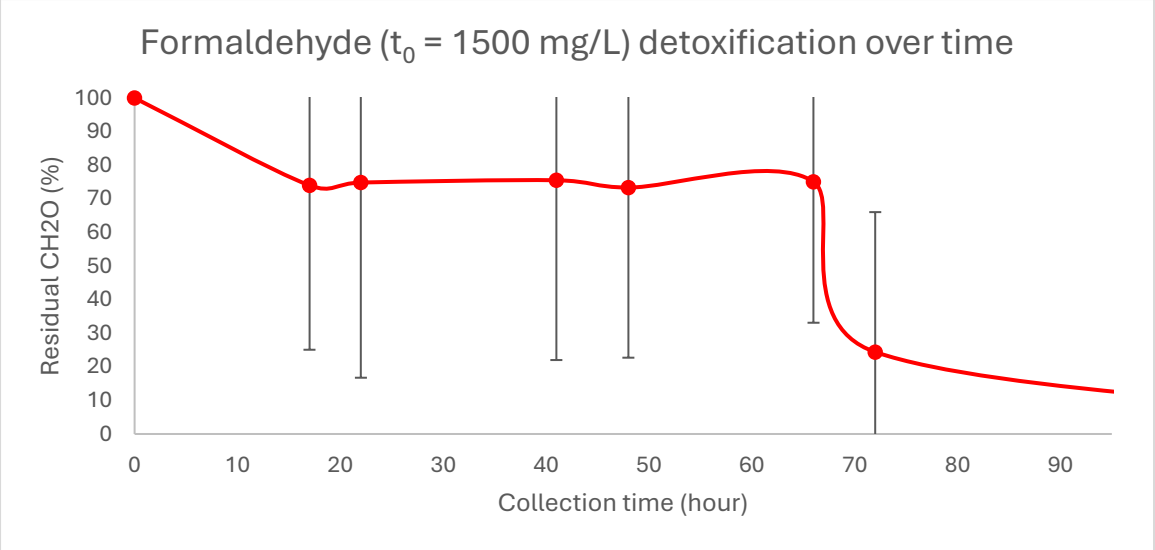


Figure 28. Formaldehyde detoxification by fungal strain as a function of incubation time in hours. Cultures were performed in PDB and Yeast extract medium at an initial formaldehyde concentration of 1500 mg/L.

3.3.3. Following the viability and the fungal growth during the decontamination process

Measurement of the dry mass, a viability quantification

Measurement of fungal dry weight in detoxification cultures serves as a critical parameter for evaluating the strain's performance. It provides a direct quantitative assessment of fungal growth and viability in the presence of the pollutant, indicating the strain tolerance to the toxic compound. Crucially, determining biomass allows for the correlation between the amount of active fungal material and the overall detoxification capacity, enabling the calculation of specific removal rates (*e.g.*, pollutant removed per unit of biomass). This data is essential for understanding the metabolic state of the fungus, assessing the inhibitory impact of the pollutant on growth, and ultimately optimizing culture conditions and scaling up the process for potential bioremediation applications.

To assess the fungal strain's tolerance and growth characteristics, liquid cultures were established with initial formaldehyde concentrations of 0 mg/L (control), 600 mg/L, and 1500 mg/L. Figure 29 visually depicts the cultures at the start (t = 0 h) and end (t = 137 h) of the experiment. At 137 hours, the control culture exhibited grey-colored biomass, suggesting limited viability or a transition to a senescent phase under these conditions. Conversely, the culture maintained with 1500 mg/L formaldehyde displayed significantly more abundant, lighter-colored biomass, indicative of active growth. This visual evidence demonstrates that the fungal strain not only tolerates a high concentration of 1500 mg/L formaldehyde but appears to grow well in its presence.

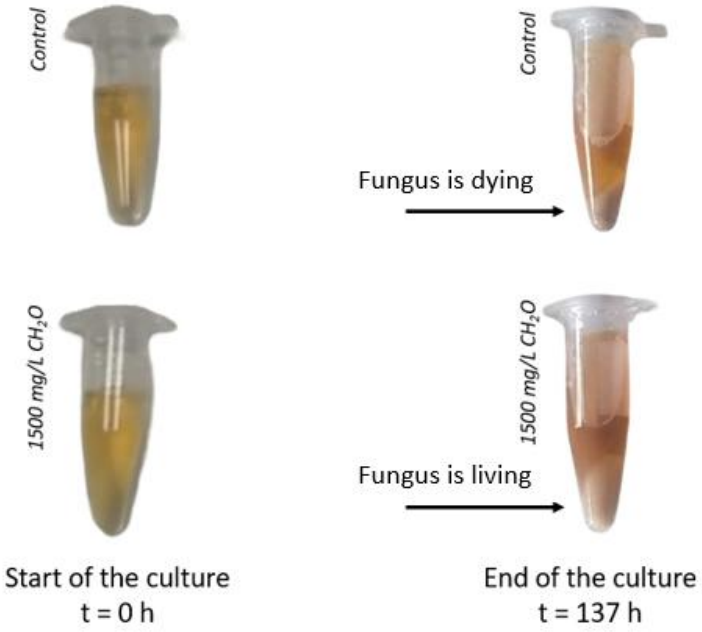


Figure 29. Differential growth of the fungal strain in liquid culture in the presence and absence of formaldehyde at the start of the incubation (t = 0 hours, left samples) and after 137 hours of incubation (right samples).

Comparative analysis of fungal growth kinetics in the presence and absence of formaldehyde, correlated with formaldehyde detoxification

This study investigates the growth kinetics of a fungal strain in standard culture medium (Control) and in the presence of 0, 600, and 1500 mg/L of formaldehyde, in correlation with the evolution of formaldehyde concentration. The results highlight the significant impact of formaldehyde on fungal growth dynamics, particularly on the duration of the lag phase and subsequent growth rate, as well as the strain's ability to adapt and detoxify its environment. Figure 27 and Figure 30 illustrate these effects for the 600 mg/L condition, while Figure 28 and

Figure 31 present the corresponding data at 1500 mg/L.

Formaldehyde-treated culture (medium with 600 mg/L CH_2O)

Figure 27 and Figure 30 highlight the significant impact of 600 mg/L formaldehyde on fungal growth kinetics. The presence of formaldehyde notably extends the lag phase, indicating a delayed adaptation. The subsequent growth phase follows a linear trend (expressed in $mg.mL^{-1}$/hour), with a slightly reduced rate compared to the control culture. Interestingly, this linear growth phase is temporally correlated with the effective assimilation of formaldehyde, suggesting that the fungus's capacity to metabolize or tolerate residual formaldehyde may directly influence its biomass production rate. These findings emphasize the critical role of adaptation and detoxification

mechanisms — not only for fungal survival in contaminated environments but also for sustaining active growth under stress.

Control culture (formaldehyde-free medium)

The growth curve of the control culture (

Figure 31, blue curve) shows a typical batch microbial growth profile. A first acclimation phase or lag phase (Zone A, approximately 0-22 hours) is observed, during which biomass remains stable. During this period, the fungus adapts to medium conditions. This phase is followed by an exponential growth phase (Zone B, approximately 22-48 hours), characterized by a rapid and linear increase (on a logarithmic scale) of fungal dry mass. The maximum growth rate is determined during this phase. It is 0.12 $mg.mL^{-1}/h$. After reaching a peak biomass (approximately 12.5 mg/mL around 48 hours), the culture enters a stationary or decline phase (Zone C, approximately 48-168 hours), where biomass decreases, likely due to nutrient depletion or accumulation of toxic metabolites.

Formaldehyde-treated culture (medium with 1500 mg/L CH_2O)

The initial presence of 1500 mg/L of formaldehyde has a drastic effect on growth. A prolonged initial phase (Zone D, approximately 0-66 hours) is observed, with no detectable increase in fungal dry mass. This phase corresponds to an extended acclimation period, during which the fungus does not proliferate but exhibits essential metabolic activity. Figure 28 shows that during this period (0-66 hours), the formaldehyde concentration, although slightly decreasing, remains high (around 600-700 mg/L between 20 and 60 hours), indicating that the fungus is subjected to severe toxic stress. The absence of growth suggests that cellular energy and resources are primarily devoted to survival and potentially to the activation of detoxification mechanisms.

Following this prolonged acclimation and detoxification phase, the fungus initiates growth (Zone E, approximately 66-140 hours). This growth resumption coincides with a significant decrease in formaldehyde concentration, particularly marked between 60 and 70 hours (a drop from 650 mg/L to 200 mg/L), followed by a gradual reduction to levels close to zero around 140 hours (Figure 28). Growth during this phase (Zone E) can be described as "stressed", as, although effective, its rate is visibly lower than that observed during the exponential phase of the control culture (Zone B). The fungus appears to metabolize or tolerate the residual formaldehyde while initiating its growth.

In the subsequent phase (approximately 144-168 hours), when formaldehyde is practically eliminated from the medium (Figure 28), an acceleration of the growth rate is observed compared

to the previous phase (Zone E). Nevertheless, this late growth allows the fungus to eventually reach a biomass comparable to that of the control at 168 hours.

These data clearly demonstrate the capacity of this fungus to survive and grow in the presence of formaldehyde, a toxic compound. The initial response is a prolonged acclimation phase without apparent cellular growth but likely associated with intense metabolic detoxification activity. Growth only resumes when the formaldehyde concentration decreases significantly, occurring initially under stress at a reduced rate, then accelerating when the toxic compound is practically eliminated. After detoxification, growth may be lastingly affected, highlighting the profound physiological impact of formaldehyde-induced stress. This study underscores the importance of adaptation phases and detoxification mechanisms in microbial tolerance to inhibitory compounds.

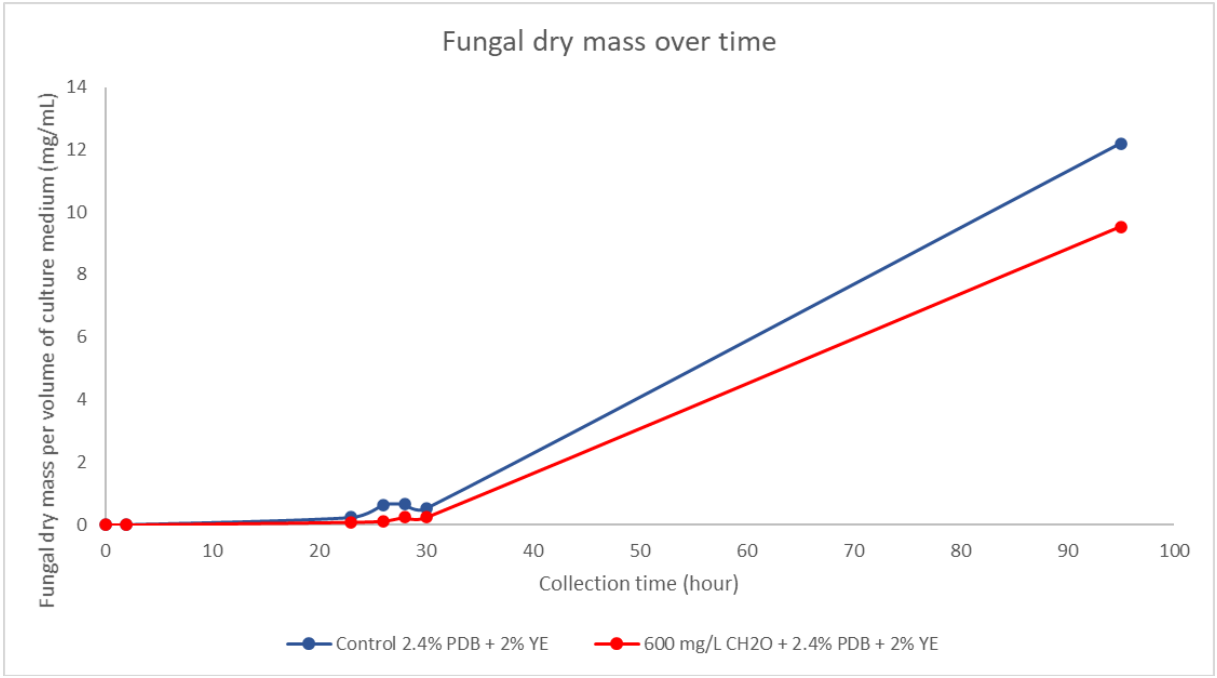


Figure 30. Kinetics of fungal dry mass (mg/mL) in PDB and YE culture medium. The graph shows the evolution of fungal dry mass in mg per mL of PDB and YE medium over time, comparing a formaldehyde-free control to a condition with 600 mg/L CH_2O.

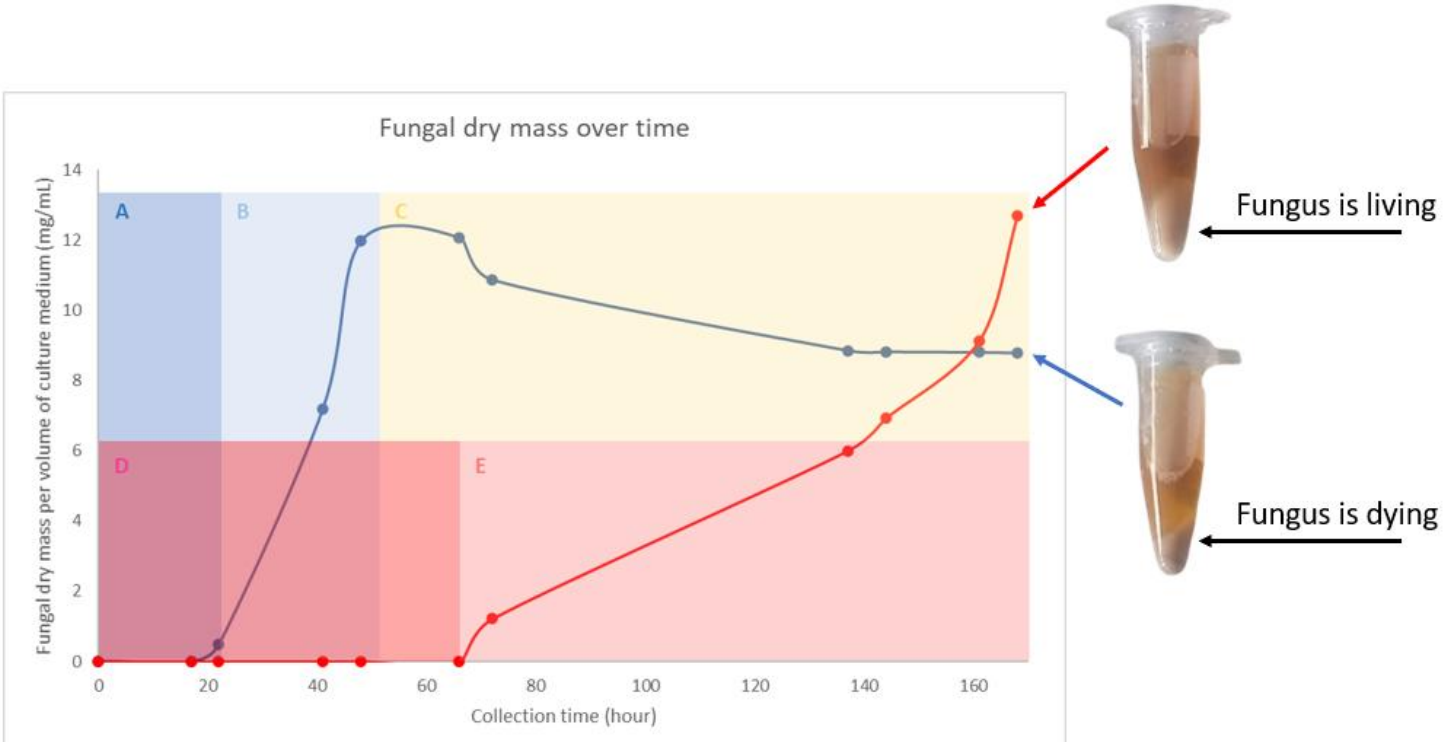


Figure 31. Kinetics of fungal dry mass (mg/mL) in PDB and YE culture medium. The graph shows the evolution of fungal dry mass in mg per mL of PDB and YE medium over time, comparing a formaldehyde-free control to a condition with 1500 mg/L CH_2O. The zones represented correspond to different growth steps.

4. CONCLUSION

Deliverable 9.1 of the Wood2Wood (W2W) project represents a key milestone in demonstrating the technical and scientific feasibility of cascade valorization of post-consumer wood, particularly through glue separation and bioremediation processes. Deliverable 9.1 demonstrates that the combination of thermomechanical (steam explosion) and biological (mycoremediation) technologies represents a promising approach for cascade valorization of post-consumer wood. By addressing technological barriers related to the presence of glues and pollutants, the W2W project contributes to the establishment of more sustainable, efficient, and integrated wood recycling systems within the dynamics of the European circular economy.

The results obtained at this stage of the project confirm the relevance of technological and methodological choices, while emphasizing the need to continue research and optimization efforts. Ultimately, the ambition is to propose scalable solutions capable of transforming wood waste into valuable resources for the industries of the future.

The work carried out under WP9 has validated several fundamental project hypotheses while opening new perspectives for optimizing waste wood treatment processes, with the goal of reintegrating it into circular production cycles.

Major advances in glue separation via steam explosion

One of the central objectives of this deliverable was to demonstrate the effectiveness of the steam explosion (SE) process for decontaminating post-consumer wood, particularly for removing urea-formaldehyde (UF) and melamine-urea-formaldehyde (MUF) resins, which are widespread in particleboard and MDF from the furniture industry. Results obtained at various technology readiness levels (TRL3 to TRL5) confirmed the process's effectiveness, while highlighting several critical parameters:

- Particle size: Tests showed that finer particles allow better steam penetration and thus more effective glue degradation. Industrial panel mixes showed higher decontamination efficiency than raw BR1/BR2 fragments.

- Temperature and residence time: Optimal conditions identified enabled significant reductions in nitrogen content (used as a proxy for UF glue), with removal rates reaching up to 86% in some cases.

These results were validated through a multi-scale and multi-technique approach, combining elemental analyses (CHON, Kjeldahl), NIR/FTIR spectroscopy, fluorescence microscopy, and scanning electron microscopy coupled with EDS analysis. This combination of tools enabled not only quantification of decontamination but also visualization of residual glue distribution at the microscopic scale.

Toward energy and environmental optimization of the SE process

Beyond demonstrating feasibility, WP9 also explored ways to optimize the SE process, particularly by reducing temperature and pressure to lower energy consumption. Successive treatments under milder conditions achieved decontamination levels comparable to those obtained at higher temperatures, while reducing the environmental impact of the process. Fiber morphology analyses after treatment revealed a progressive evolution toward a more homogeneous and fibrous structure, favorable for reuse in composite materials or reconstituted panels. However, special attention must be paid to fiber aggregation after multiple treatment cycles, which could affect the mechanical properties of final materials.

Morphological characterization and recycling potential

In-depth study of the morphology of fibers and particles resulting from SE confirmed the potential of this process as an alternative to mechanical grinding. Macroscopic and microscopic observations revealed effective biomass fragmentation, with clear separation of longitudinal fibers and breakdown of lignin-hemicellulose-cellulose bonds.

Particle size analyses showed a significant reduction in average particle size after treatment, followed by stabilization after several cycles. This evolution suggests that SE can be used not only for decontamination but also as a fiber preparation step for further applications, particularly in the production of panels or bio-based composite materials.

Promising initial results on effluent bioremediation

The second part of the deliverable, dedicated to bioremediation of SE effluents, led to the identification of a fungal strain from the Trichoderma genus capable of tolerating and metabolizing formaldehyde, the main targeted pollutant. This strain demonstrated remarkable growth capacity in the presence of high formaldehyde concentrations (up to 1500 mg/L), potentially using it as a carbon source.

Liquid culture experiments revealed a two-phase dynamic: a latency phase linked to fungal acclimation, followed by an active detoxification phase. HPLC analysis confirmed the progressive

reduction of formaldehyde concentration, with significant removal rates after 60 to 100 hours of incubation.

These results pave the way for integrating biological processes into the SE effluent treatment chain, complementing physico-chemical approaches. They also highlight the importance of continuing the isolation and characterization of fungal strains adapted to polluted environments, with a view to pilot- and industrial-scale applications.

Perspectives

The work presented in this deliverable provides a solid foundation for the next stages of the Wood2Wood project. Several development areas can be identified:

- Optimization of SE conditions based on biomass types, with particular attention to particle size, initial moisture content, and chemical composition of post-consumer wood.
- Development of rapid and non-destructive methods for real-time decontamination monitoring, particularly using NIR spectroscopy coupled with image analysis.
- Integration of biological processes into a complete treatment chain, including glue separation, fiber recovery, and effluent detoxification.
- Evaluation of the quality of recycled materials, in terms of mechanical properties, durability, and compliance with standards in the construction and furniture sectors.

5. REFERENCES

*Aguilar, K., Figel, L., Saker, S., Soufflet, L., Brosse, N., Besserer, A., 2024. Steam Explosion: A Booster for Fungal Growth in a Myco-composite. ACS Sustain. Chem. Eng. 12, 11650–11659. https://doi.org/10.1021/acssuschemeng.4c03099

Altgen, M., Gedde, K.B., Ross, L., Larnøy, E., 2025. Physical and chemical characterization of post-consumer wood chips for recycling. Waste Manag. 200, 114762. https://doi.org/10.1016/j.wasman.2025.114762

Besserer, A., Troilo, S., Girods, P., Rogaume, Y., Brosse, N., 2021. Cascading Recycling of Wood Waste: A Review. Polymers 13. https://doi.org/10.3390/polym13111752

Bozzolan, N., Grassi, G., Mohren, F., Nabuurs, G., 2024. Options to improve the carbon balance of the harvested wood products sector in four EU countries. GCB Bioenergy 16, e13104. https://doi.org/10.1111/gcbb.13104

Charpentier-Alfaro, C., Benavides-Hernández, J., Poggerini, M., Crisci, A., Mele, G., Della Rocca, G., Emiliani, G., Frascella, A., Torrigiani, T., Palanti, S., 2023. Wood-Decaying Fungi: From Timber Degradation to Sustainable Insulating Biomaterials Production. Materials 16, 3547. https://doi.org/10.3390/ma16093547

Chum, H.L., Johnson, D.K., Black, S.K., Overend, R.P., 1990. Pretreatment-Catalyst effects and the combined severity parameter. Appl. Biochem. Biotechnol. 24–25, 1–14. https://doi.org/10.1007/BF02920229

European Commission, 2024. EU construction & demolition waste management protocol including guidelines for pre-demolition and pre-renovation audits of construction works: updated edition 2024. Publications Office, LU.

Fulvio, F.D., Snäll, T., Lauri, P., Forsell, N., Mönkkönen, M., Burgas, D., Blattert, C., Eyvindson, K., Caicoya, A.T., Vergarechea, M., Antón-Fernández, C., Klein, J., Astrup, R., Lukkarinen, J., Pitzén, S., Primmer, E., 2025. Impact of the EU biodiversity strategy for 2030 on the EU wood-based bioeconomy. Glob. Environ. Change 92, 102986. https://doi.org/10.1016/j.gloenvcha.2025.102986

Gibier, M., Sadeghisadeghabad, M., Girods, P., Zoulalian, A., Rogaume, Y., 2022. Furniture wood waste depollution through hydrolysis under pressurized water steam: Experimental work and kinetic modelization. J. Hazard. Mater. 436, 129126. https://doi.org/10.1016/j.jhazmat.2022.129126

Grigsby, W.J., Thumm, A., Kamke, F.A., 2005. Determination of resin distribution and coverage in mdf by fiber staining. Wood Fiber Sci. 37, 12.

Hagel, S., Saake, B., 2020. Fractionation of Waste MDF by Steam Refining. Molecules 25, 2165. https://doi.org/10.3390/molecules25092165

Jacquet, N., Maniet, G., Vanderghem, C., Delvigne, F., Richel, A., 2015. Application of Steam Explosion as Pretreatment on Lignocellulosic Material: A Review. Ind. Eng. Chem. Res. 54, 2593–2598. https://doi.org/10.1021/ie503151g

Jonsson, R., 2024. Strategic wood availability in Europe (No. 128). Department of 480 Energy and Technology, Swedish University of Agricultural Sciences.

Kral, I., Piringer, G., Saylor, M.K., Gronauer, A., Bauer, A., 2016. Environmental Effects of Steam Explosion Pretreatment on Biogas from Maize—Case Study of a 500-kW Austrian Biogas Facility. BioEnergy Res. 9, 198–207. https://doi.org/10.1007/s12155-015-9676-0

Kral, I., Piringer, G., Saylor, M.K., Lizasoain, J., Gronauer, A., Bauer, A., 2020. Life Cycle Assessment of Biogas Production from Unused Grassland Biomass Pretreated by Steam Explosion Using a System Expansion Method. Sustainability 12, 9945. https://doi.org/10.3390/su12239945

Kratky Lukas, Azizov Salman, Jirout Tomas, 2018. Lignocellulosic waste treatment in biogas biorefinery with reduced co2 production: a techno-economic study. Chem. Eng. Trans. 70, 1975–1980. https://doi.org/10.3303/CET1870330

Lerink, B.J.W., Schelhaas, M.-J., Schreiber, R., Aurenhammer, P., Kies, U., Vuillermoz, M., Ruch, P., Pupin, C., Kitching, A., Kerr, G., Sing, L., Calvert, A., Ní Dhubháin, Á., Nieuwenhuis, M., Vayreda, J., Reumerman, P., Gustavsonn, G., Jakobsson, R., Little, D., Thivolle-Cazat, A., Orazio, C., Nabuurs, G.-J., 2023. How much wood can we expect from European forests in the near future? For. Int. J. For. Res. 96, 434–447. https://doi.org/10.1093/forestry/cpad009

Overend, R.P., Chornet, E., 1987. Fractionation of lignocellulosics by steam-aqueous pretreatments. Philos. Trans. R. Soc. Lond. Ser. Math. Phys. Sci. 321, 523–536.

Qian, H., Hou, Q., Hong, L., Lu, X., Ziegler-Devin, I., Chrusciel, L., Besserer, A., Brosse, N., 2022. Effect of highly efficient steam explosion treatment on beech, poplar and spruce solid wood physicochemical and permeable performances. Ind. Crops Prod. 182, 114901. https://doi.org/10.1016/j.indcrop.2022.114901

Schmitz, N., Krause, A., Lüdtke, J., 2023. Critical review on a sustainable circular bio-economy for the forestry sector = Zirkuläre Bioökonomie in der Forst- und Holzwirtschaft für eine nachhaltige Entwicklung - Eine wissenschaftliche Einordnung. Johann Heinrich von Thünen-Institut, DE.

Schwanninger, M., Rodrigues, J., Fackler, K., 2011. A review of band assignments in near infrared spectra of wood and wood components. J. Infrared Spectrosc. 19, 287. https://doi.org/10.1255/jnirs.955

Simangunsong, E., Ziegler-Devin, I., Chrusciel, L., Girods, P., Wistara, N.J., Brosse, N., 2020. Steam Explosion of Beech Wood: Effect of the Particle Size on the Xylans Recovery. Waste Biomass Valorization 11, 625–633. https://doi.org/10.1007/s12649-018-0522-4

Troilo, S., Besserer, A., Rose, C., Saker, S., Soufflet, L., Brosse, N., 2023. Urea-Formaldehyde Resin Removal in Medium-Density Fiberboards by Steam Explosion: Developing Nondestructive Analytical Tools. ACS Sustain. Chem. Eng. 11, 3603–3610. https://doi.org/10.1021/acssuschemeng.2c05686

Xing, C., Riedl, B., Cloutier, A., Shaler, S.M., 2005. Characterization of urea–formaldehyde resin penetration into medium density fiberboard fibers. Wood Sci. Technol. 39, 374–384. https://doi.org/10.1007/s00226-005-0294-4

Ziegler-Devin, I., Chrusciel, L., Brosse, N., 2021. Steam Explosion Pretreatment of Lignocellulosic Biomass: A Mini-Review of Theorical and Experimental Approaches. Front. Chem. 9, 705358. https://doi.org/10.3389/fchem.2021.705358

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