Contribution of Structural Modification to Enhanced Enzymatic Hydrolysis and 3-D Structural Analysis of Steam-Exploded Wood using X-Ray Tomography

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Steam explosion pretreatment modifies both the chemical and physical structures of a biomass. Chemical modifications are generated during the treatment of biomass with steam at high temperature. Physical modifications are created during the explosion step, which produces disintegrated and defibrillated biomass. In this study, the contribution of each modification to an increase in enzymatic hydrolysis has been studied. It was found that both physical and chemical modifications contributed to an increase in enzymatic hydrolysability. Additionally, high resolution X-ray tomography was performed to identify the structural modification created during the steam explosion process. Comparison of the 3-D micro-structure of a steam-exploded wood sample with an untreated wood sample revealed that several kinds of cracks were created during the explosion step, and the micro-structure of the wood sample was vigorously destroyed.

Keywords: Enzymatic hydrolysis; Steam explosion; Steam treatment; 3D structure; X-ray tomography

INTRODUCTION

With the aim of decreasing our oil dependency, wood and other biomass materials are being extensively considered as raw materials for the production of bio-polymers, bio-fuels, and chemicals. The conversion of cellulose and hemicelluloses to mono- and oligomeric sugars that can be further converted to ethanol and other valuable chemicals is typically carried out through enzymatic hydrolysis (Romání et al. 2013). However, the native wood is almost inert and indigestible by enzymes due to the strong chemical bonds between lignin and polysaccharides, and the inaccessible micro-structure of the wood (Grous et al. 1986; Rahikainen et al. 2013).

Currently, several pretreatment technologies are under investigation, and they can modify either the chemical or physical structure of the biomass to enhance the enzymatic hydrolysis process. Chemical pretreatments (e.g., acid, base, and hydrothermal treatments) hydrolyse the hemicelluloses and redistribute lignin in order to expose the cellulose to enzymes. Physical pretreatments (e.g., crushing and grinding) reduce the size of biomass and increase the available surface area at the cost of high energy requirement (Alvira et al. 2010). Steam explosion pretreatment combines the advantages of both chemical and physical pretreatments (Grous et al. 1986; Ramos 2003). This pretreatment process involves the treatment of wood chips with saturated steam under high pressure and at high...
temperature followed by rapid decompression and discharge of wood chips into a flash tank. The material obtained from steam explosion is chemically and physically modified biomass, which is easily digestible during enzymatic hydrolysis.

Chemical changes occurring in the wood chips are caused by the steam treatment during the steam explosion process (Kosikova et al. 1995). The main chemical reaction that takes place is an autohydrolysis reaction, which results in the breakage of glycosidic linkages. This reaction is primarily acid-catalysed by the acetic acid released from acetylated hemicelluloses (Ramos 2003). A decrease in molecular weight of cellulose has been observed with an increase in treatment severity (temperature and time) (Josefsson et al. 2002). In addition, several researchers have reported extensive degradation of hemicelluloses because of the steam treatment (Boussaid et al. 2000; Wang et al. 2009; Martin-Sampedro et al. 2011). At extreme pretreatment conditions, degradation of cellulose leads to hydroxyl-methylfufural and degradation of xylan leads to furfural (Li et al. 2005). Lignin acts as a physical barrier to enzymes and inhibits the hydrolysis reaction (Mooney et al. 1998; Rahikainen et al. 2013). During the steam treatment, lignin is predominantly degraded through the cleavage of β-O-4 ether linkages (Martin-Sampedro et al. 2011). Along with depolymerisation reaction of lignin a comprehensive repolymerization reaction also takes place which results in an increase in molecular size and formation of heterogeneous lignin structures (Li et al. 2007).

Physical modification involves the creation of microcracks in cell walls and the disintegration of wood chips into smaller fragments. The steam explosion pretreatment has the advantage that it requires 70% less energy for the increase in specific surface area by the same amount as compared to the conventional mechanical techniques such as attrition milling (Holtzapple et al. 1989). Several researchers have observed a remarkable increase in the glucose yield of steam-exploded wood as compared to untreated wood (Ballesteros et al. 2000; Jedvert et al. 2012; Martin-Sampedro et al. 2014). However, the contribution of physical structural modifications to an increased enzymatic hydrolysis has yet to be studied.

The microstructure of spruce wood is mainly based on long hollow cells called tracheids. These tracheids are closed from all sides, and the interior surface is accessible only through pores in the walls. Wood material has high porosity, but that does not necessarily mean that it has high permeability since the void spaces are, or may be, isolated. Knowledge about the attributes of these pores enables the understanding of the interaction between wood and enzymes. In bioethanol production, the diffusion of enzymes determines the efficiency with which the cellulose hydrolysis can take place (Wu et al. 2009).

Most commonly the microstructure of steam-exploded wood is analysed with scanning electron microscopy (Donaldson et al. 1988; Zhang and Cai 2006). However, this technique provides only 2-D images of the material surface. The interior structure of the steam-exploded wood without disruption cannot be visualized through this technique. High-resolution X-ray tomography is a powerful technique to study the internal structure of wood without destroying it. This technique can be used to acquire 3-D images, which provide much insight into wood structure. Basically, the 3-D images of the material are reconstructed based on a set of two-dimensional projections taken from different angles by rotating the sample on a high precision stage (Bulcke et al. 2013). Over the years, X-ray tomography has been used for medical purposes. Recent developments of this technology and its improvement in resolution to sub-micron levels, have made it an excellent analytical tool for studying the anatomical features of many materials, including wood. Steppe et al. (2004) have analysed the network of vessels in beech and oak heartwood with a spatial
resolution of 10 µm³ using absorption based tomography. However, on this scale, small microstructural features such as individual tracheids and pits are poorly resolved. Trtik et al. (2007) have performed detailed analysis of the microstructure of spruce using synchrotron radiation phase-contrast X-ray tomography at the Swiss Light Source, PSI Villigen, Switzerland. With a voxel size of 0.7 × 0.7 × 0.7 µm³, they were able to efficiently capture the microstructural features of wood anatomy. X-ray tomography has been used to study the modifications in structural features caused by certain biomass treatments. Gilani et al. (2013) have performed the dynamic analysis of microcrack propagation in hardwood during heat treatment using synchrotron-based X-ray tomography. Bulcke et al. (2013) have performed dynamic tomography of wood and analysed the effect of thermal treatment on aspen wood at 160 °C for 1 h. The results presented by those authors showed that the wood shrinks because of thermal treatment, but the overall micro-structural features remain similar.

In this study, steam-treated wood chips that had undergone chemical modifications were compared with steam-explored wood chips that had both chemical and physical modifications. Any increase in enzymatic hydrolysis from steam-treated wood to steam-explored wood was expected to be the result of physical modifications. This study focused on the physical structural modifications caused by steam explosion pretreatment. The X-ray tomography analysis gave a detailed description of the micro-structural changes that took place during the explosion step, which contributed to an increase in enzymatic hydrolysis process.

EXPERIMENTAL

Sample Preparation and Steam Explosion

Wood sticks with the dimensions of 120 × 20 × 4 mm³ were produced from a trunk of Norway spruce (Picea abies) obtained from Södra (Värö, Sweden). These sticks were divided into six small pieces with the dimensions of 20 × 20 × 4 mm³. One piece was kept as a reference (untreated), and the others were used for pretreatment process. This facilitated a comparison as the chips to be compared were taken from the same annual ring in the trunk. In order to analyse the contribution of physical and chemical effects to an increase in enzymatic hydrolysis, three cases were compared:

1. Untreated wood (no chemical or physical modifications)
2. Steam-treated wood (only chemical modification)
3. Steam-explored wood (both chemical and physical modifications)

The steam-treatment and steam explosion experiments were performed in specially designed steam explosion equipment built at Chalmers University of Technology, Gothenburg (Muzamal et al. 2015). The steam-explored wood was obtained by treating the wood chips with saturated steam at 14 bar (198 °C) in a steam treatment vessel for 10 min. Then, the pressure was rapidly decompressed to atmospheric pressure by opening the steam exit valve. As a result, the steam and the wood chips escaped from the steam treatment vessel and collided with each other and the walls of the vessel. Eventually, disintegrated steam-explored wood was obtained (Fig. 1c). The steam treatment was performed in another vessel in which the wood chips were enclosed in a wire frame to prevent them from moving and colliding with the walls of the vessel. The pressure was...
slowly reduced to atmospheric pressure over a one min time span. In both treatments, the wood chips were heated with saturated steam and did not make contact with the condensed steam collected at the bottom of the vessel during the steam treatment. However, during the explosion step, when the wood chips left the steam treatment vessel, they were collected at the bottom of the flash tank along with condensed steam.

During the treatment of wood chips with steam, chemical modifications took place. The steam treatment causes minor physical modifications, e.g. an increase in porosity of the material because of the removal of wood components (Muzamal et al. 2015) and shrinkage of the overall structure because of heat treatment (Bulcke et al. 2013). However major structural changes that involve creation of cracks in the cell walls and disintegration of the wood chip into small fragments does not happen because of the steam treatment. The physical structural modifications mainly take place during the explosion step. It is impossible to obtain only physically modified wood chips through the steam explosion process. Therefore, steam-exploded wood chips (with both chemical and physical modifications) were compared with steam-treated wood chips (with only chemical modification).

**Acid Hydrolysis**

The carbohydrate and lignin compositions of the untreated, steam-treated, and steam-exploded samples were determined according to the procedure presented by Theander and Westerlund (1986). Acid hydrolysis was performed to hydrolyse the wood carbohydrates. Monosaccharides thus obtained were analysed through sugar analysis (as described below). The remaining acid insoluble solid fraction was Klason lignin. The acid soluble lignin was determined by measuring UV absorbance values at a wavelength of 205 nm in a Specord 205 (Analytik Jena, Germany) (Lin and Dence 1992).

**Enzymes and Enzymatic Hydrolysis**

The untreated and pretreated wood materials were subjected to enzymatic hydrolysis using the cellulolytic complex Cellic® Ctec3 kindly provided by Novozymes A/S ( Bagsvaerd, Denmark). Cellic® Ctec3 is a cocktail consisting of cellulases, hemicellulases, and a high level of β-glucosidases for the conversion of carbohydrates into monosaccharides. The enzyme dose applied was 10% w/w (g Cellic Ctec3/100 g carbohydrate) calculated based on the dry weight of carbohydrates of each wood sample.

The hydrolysis reactions (in triplicates) were conducted in 50 mL Falcon tubes with a total volume of 15 mL. Incubation was performed in a rotary shaker (KS 4000 ic control, IKA, Germany) at 200 rpm, 45 °C and pH = 5 (using 50 mM sodium acetate buffer). The reactions were stopped after 30 and 72 h by boiling for 15 min at 100 °C. The solids were separated from the liquid by centrifugation, and supernatants were filtered through 0.2 μm sterile nylon filters (VWR, USA) prior to sugar analysis.

**Sugar Analysis**

Monosaccharides released during acid and enzymatic hydrolysis were analysed using a high performance anion exchange chromatography ICS3000 system equipped with 4 × 250 mm Dionex Carbopac™ PA1 column with a 4 × 50 mm guard column maintained at 30 °C, equipped with a pulsed amperometric detector (HPAEC-PAD), and the Chromelon software (Thermo Scientific, Sweden). The eluents prepared were; A: Milli-Q water; B: 300 mM sodium hydroxide, and C: 200 mM sodium hydroxide + 170 mM sodium acetate. The column was equilibrated for 19 min with 40% eluent A, 40% eluent
B, and 20% eluent C, and further equilibrated at 100% solvent A for 6 min prior to injection. The samples were eluted with 100% eluent A as the mobile phase for 30 min at a flow rate of 0.5 mL/min and detected with the post-column addition of 1 mL/min of solvent B.

**High-Resolution X-Ray Tomography**

X-ray tomography was performed at the Ångström laboratory Uppsala University (Uppsala, Sweden) using SkyScan 1172 (Bruker, Sweden). The X-ray detector was an 11 megapixel, 12-bit dynamic range cooled charge-coupled device (CCD) camera. The samples were rotated 192° in the X-ray beam at increments of 0.2°, yielding about 960 different 2-D images per sample. Raw 2-D tomographic projection images were reconstructed to obtain a stack of 2-D horizontal slices using NRecon 1.6.10.1 software (Bruker, Sweden). These images were post-processed to obtain 3-D internal structures using Avizo 9.0 (FEI, France). Image post-processing included image enhancement, noise removal, cropping to sub-volume, and rotation for the alignment of the sub-volume. The internal structures of one untreated and two steam-exploded samples (SE-1 and SE-2) were analysed. The cubical samples with the approximate dimensions of 1.4 × 1.4 × 1.4 mm³ were sliced from untreated and steam-exploded wood in wet conditions with a sharp razor blade. The settings used for the analysis are given in Table 1.

<table>
<thead>
<tr>
<th>Table 1. Settings of X-ray Tomography Analysis</th>
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<tr>
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<tr>
<td>Voxel size (µm³)</td>
</tr>
<tr>
<td>Untreated and SE-1</td>
</tr>
<tr>
<td>0.81 x 0.81 x 0.81</td>
</tr>
<tr>
<td>X-ray source voltage (kv)</td>
</tr>
<tr>
<td>31</td>
</tr>
<tr>
<td>X-ray source current (µA)</td>
</tr>
<tr>
<td>187</td>
</tr>
<tr>
<td>Exposure time (ms)</td>
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<td>4000</td>
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**RESULTS AND DISCUSSION**

In this section, the chemical compositions of the wood samples and the results from the enzymatic hydrolysis experiments are presented first. This is followed by 3-D images of untreated and steam-exploded wood obtained from High-Resolution X-Ray tomography.

**Sugar Composition of the Wood Samples**

Table 2 gives the lignin and carbohydrate compositions of untreated, steam-treated, and steam-exploded wood samples. The results show that hemicelluloses were degraded significantly because of the steam treatments. The degradation of hemicelluloses takes place through an autohydrolysis reaction, which cleaves the glycosidic linkages. Several researchers have observed a degradation of hemicelluloses because of the steam treatment (Wang et al. 2009; Martin-Sampedro et al. 2011). A plausible reason for the further decrease in hemicellulosic content from steam-treated to steam-exploded wood is that during the explosion step, when the wood chips left the steam treatment vessel, they were collected at the bottom of the flash tank along with condensed steam, which possibly removed additional hemicelluloses. Table 2 shows that the relative amount of lignin increased after pretreatment and no direct delignification (based on Klason lignin content) took place. The increase in lignin content was also observed by Martin-Sampedro et al.
(2014). However, the steam treatment has been reported to break the complex linkages between lignin and polysaccharides (Li et al. 2007; Martin-Sampedro et al. 2011).

**Table 2. Chemical Composition of Oven-Dried Solid Residue of Untreated, Steam-Treated, and Steam-Exploded Spruce Wood**

<table>
<thead>
<tr>
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<th>Untreated w/w% (SD)</th>
<th>Steam-treated w/w% (SD)</th>
<th>Steam-exploded w/w% (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klason lignin</td>
<td>27.52 (0.14)</td>
<td>29.58 (1.29)</td>
<td>33.23 (0.88)</td>
</tr>
<tr>
<td>Acid soluble lignin</td>
<td>0.48 (0.02)</td>
<td>0.61 (0.01)</td>
<td>0.45 (0.00)</td>
</tr>
<tr>
<td>Glucan</td>
<td>38.26 (2.00)</td>
<td>38.79 (0.66)</td>
<td>41.99 (0.28)</td>
</tr>
<tr>
<td>Xylan</td>
<td>5.01 (0.35)</td>
<td>4.55 (0.05)</td>
<td>3.29 (0.05)</td>
</tr>
<tr>
<td>Mannan</td>
<td>10.71 (0.81)</td>
<td>9.90 (0.13)</td>
<td>6.17 (0.13)</td>
</tr>
<tr>
<td>Arabinan</td>
<td>0.93 (0.05)</td>
<td>0.43 (0.01)</td>
<td>0.25 (0.01)</td>
</tr>
<tr>
<td>Galactan</td>
<td>1.32 (0.07)</td>
<td>1.11 (0.02)</td>
<td>0.40 (0.30)</td>
</tr>
</tbody>
</table>

**Enzymatic Hydrolysis**

Figure 1 illustrates untreated, steam-treated, and steam-exploded wood samples used for enzymatic hydrolysis process. It is clearly visible from the figure that the colour was darker for both the steam-treated and steam-exploded wood samples because of chemical modifications during the steam treatment. In addition to that, the wood chips were disintegrated into small fragments because of the explosion step.

**Fig. 1.** Wood samples used for enzymatic hydrolysis; (a) untreated, (b) steam-treated, (c) steam-exploded

Results presented in Fig. 2 show that the enzymatic hydrolysis of untreated wood resulted in very low glucose yield. The percentage of glucose released (w/w%) was calculated by dividing the amount (mg) of glucose released during enzymatic hydrolysis with the amount of glucose initially present in the dry solid mass. The glucose yield increased with the steam treatment of the wood and increased further when the wood chips were steam-exploded. The increase in glucose yield from 5.1% in steam-treated wood to 9.8% in steam-exploded wood is a result of physical structural modification. Pielhop et al. (2016) also observed 90% increase in digestibility of cellulose in steam exploded wood as compared to steam-treated wood. The chemical changes during the steam-treatment step (i.e., the hydrolysis of hemicelluloses and redistribution of lignin) increased the accessibility of enzymes to celluloses and resulted in greater cellulose hydrolysis. However, the complex and tight physical structure of wood hindered the transportation of...
enzymes (with a typical size of 5 to 8 nm) into the inner part of the wood chips. During the explosion step, the vapour expansion inside the wood cells (tracheids) increased the pore size of the chips, and the collisions and impact of the wood chips with each other and with the walls of the steam explosion equipment caused them to disintegrate into smaller fragments (Muzamal and Rasmuson 2016). As a result, the available surface area increased and the diffusion distance decreased, thus improving the mass transport of enzymes. The glucose release observed in steam-exploded wood was 9.8 %, which is in the same range as obtained by Ballesteros et al. (2000) at similar steam explosion conditions (190 °C and 8 min) and feed chip size of 8 to 12 mm. The results of the present study show that both chemical and physical modifications contributed synergistically to the increase in enzymatic hydrolysis during steam explosion.

Fig. 2. Glucose released after enzymatic hydrolysis. Standard deviations of triplicates are presented as error bars.

High-Resolution X-Ray Tomography

Figure 3 shows ortho-slices of untreated and steam-exploded samples. The region shown is approximately 1-mm in size obtained from a larger sample after alignment and cropping. It can be seen in the figure that the untreated wood was fairly intact and free from damage. During the steam treatment of wood, chemical changes in cellulose, hemicelluloses and lignin at high temperature and moisture contents caused the wood material to become soft and easily deformable. This facilitates the structural changes that take place during the subsequent step (Muzamal and Rasmuson 2016).

The steam explosion pretreatment created large structural changes which are visible in steam-exploded samples (Fig. 3b, c). The steam-exploded wood attained large variations in the size of and damage to the fragments. Some wood chips were totally defibrillated while others remained intact to some extent.

For X-ray tomography, two samples of steam-exploded wood were selected. Sample SE-1 was relatively little damaged compared to sample SE-2. To study the internal structure in detail, 3-D sub-volumes (SV) were created at random locations, which is also shown in Fig. 3.
Fig. 3. Ortho-slices and location of 3-D sub-volumes (SV) and cell walls (W): (a) Untreated; (b) steam-exploded sample SE-1; and (c) steam-exploded sample SE-2.

Fig. 4. Internal structure of untreated spruce wood. The locations of sub-volumes SV-1, SV-2, and tracheid radial wall W-1 are shown as in Fig. 3.
The sub-volumes extracted from the untreated wood with a short section of 10 tracheids (SV-1) and a long section of two tracheids (SV-2) are presented in Fig. 4 along with a section of the radial wall (W-1). To avoid pixel visualization, the surface was smoothed using the Avizo surface smoothing algorithm with scale 3 (FEI, France). It can be seen in the figure that the tracheids in the untreated wood were completely intact, and the only passage between tracheids was through pits. These bordered pits (with openings approximately 3 µm wide) are visible on some tracheid walls in Fig. 4. Each bordered pit has a membrane with a thickness of less than 1 µm, which controls transport through the pit. However, in the images, they seem open since the X-ray tomographic resolution could not capture the membrane inside the bordered pits. This is because the thickness of the membrane is equal to the size of an individual pixel. The cross-sectional shape of the tracheids was mainly rectangular, pentagonal, or hexagonal. These untreated spruce wood tracheids resemble those presented by Trtrik et al. (2007). Figure 4 also shows the radial wall (W-1) of the tracheids. It is clearly visible in the figure that the tracheid wall of the untreated wood did not have any micro cracks. Ray parenchyma cells visible on the long section of two tracheids (SV-2) exist in the perpendicular direction. The ray parenchyma cells are responsible for the transport of fluids perpendicular to the tracheids and are connected to tracheids through cross-field pits (Brändström 2001). The ray parenchyma cells were not captured nicely by the X-ray tomography resolution because they had thin walls and several cross-field pits. A complete tracheid in the longitudinal direction is not presented here, since the length of the tracheid is longer than the sample size (tracheid length > 1 mm) (Herman et al. 1998).

X-ray tomography of the steam-treated wood was not performed. The structure was expected to be similar to untreated wood except that the degradation of hemicelluloses and redistribution of lignin might have increased the porosity of the material to some extent, and thermal treatment might have caused some shrinkage (Bulcke et al. 2013; Muzamal et al. 2015).

![Fig. 5. Internal structure of steam-exploded wood sample SE-1. The locations of sub-volumes SV-3, SV-4, SV-5, W-2, and W-3 are marked as in Fig. 3.](image-url)
The treatment of wood chips with saturated steam at high temperature caused the wood to soften and become easily deformable. Subsequently, the rapid decompression of steam inside the tracheids and collisions between wood chips and steam explosion equipment walls caused large structural deformation. Figure 5 shows three sub-volumes of the steam-exploded wood sample SE-1. It can be seen in the figure that the cross-sectional shape of the tracheids has completely changed, and microcracks were clearly visible in all the sub-volumes. In sub-volume SV-3, microcracks existed in the tracheid walls. However, no large cracks were visible. On the other hand, the sub-volume SV-4 had large cracks between the tracheids, and in sub-volume SV-5, the structure had been destroyed completely. Microcracks are also visible on the radial walls (W-2 and W-3) in the Fig. 5. In sub-volume SV-4, the cross-sectional shapes of the tracheids resemble the tracheids obtained after applying combined compression and shear load (De Magistris and Salmén 2008). This indicates that deformation in these cells might have been caused by a combination of compression and shear. The sub-volume SV-5 was taken from close to the edge of the disintegrated steam-exploded wood, and the sub-volume SV-3 was taken from deep inside the same sample. In other words, the wood was vigorously ruptured close to the edge because of impact and collisions, while tracheids deep inside the wood were less affected. Consequently, it would be easier for enzymes to penetrate into the tracheids close to the edge than to the tracheids deep inside.

In the steam-exploded wood sample SE-2, considerable ultra-structural rearrangements are clearly visible in Fig. 6. The shape and connections between the tracheids had changed as a result of steam explosion. Tracheids visible in all of the sub-volumes in the figure possess a completely destroyed structure. According to Grethlein and Converse (1991), untreated wood has a considerable surface area, but most of it is accessible through very small pores, which results in the low rate of enzymatic hydrolysis. Numerous cracks and pores in the tracheid walls are evidence of increased accessibility to the internal structure of the wood sample, which was not readily accessible when untreated.

![Fig. 6. Internal structure of steam-exploded wood sample SE-2. The locations of sub-volumes SV-6, SV-7, SV-8, and SV-9 are marked as in Fig. 3.](image-url)
CONCLUSIONS

During the enzymatic hydrolysis of untreated spruce wood, a negligible amount of glucose was released. However, the release of glucose was significantly increased due to chemical modification caused during steam treatment of wood. In addition to this, the explosion step further increased the release of glucose by creating physical structural changes in the wood. The structural changes during the explosion step play a vital role in an increase in enzymatic hydrolysis. Without the explosion step complete benefit of the steam explosion pretreatment cannot be obtained.

X-ray tomography of untreated and steam-exploded wood samples revealed details about the micro-structural modifications that took place during the steam explosion process. The explosion of wood created several cracks in the tracheid walls and the cross-sectional shape of the tracheids had been completely altered. The structure of wood tracheids was destroyed closer to the striking edges of the wood chips and in defibrillated fragments, while it remained intact in the inner part of the wood sample.

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REFERENCES CITED


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