Anatomical characterization of wood decay patterns in *Hevea brasiliensis* and *Pinus merkusii* caused by white-rot fungi: *Polyporus arcularius* and *Pycnoporus sanguineus*

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**Abstract.** Biological pulping is an environmentally friendly process for making pulp and paper whereby the wood raw material is pre-treated with selective delignifying white-rot fungi. Therefore, the purpose of this study is to determine the decay patterns of white-rot fungi *Polyporus arcularius* and *Pycnoporus sanguineus*, as well as its ability to decay *Hevea brasiliensis* (as hardwood) and *Pinus merkusii* (as softwood) by observing the anatomical characteristics. Fungal attack testing on wood was carried out by the Kolle-flask method with a variation of 6, 9, and 12 weeks incubation time. The structure of wood cells was analyzed using wood incision method, followed by a combination of safranin-picro aniline blue and safranin-astra blue, and maceration method. The results showed that *H. brasiliensis* wood has a higher percentage of weight loss than *P. merkusii* wood. Wood attacked by *P. sanguineus* showed a higher weight loss compared to the *P. arcularius*. The decay pattern of *P. sanguineus* infected wood was concluded as selective delignification in *P. merkusii* and simultaneous delignification in *H. brasiliensis* while those infected with *P. arcularius* both performed simultaneous delignification. In the early stages of decay, selective delignification is characterized by the formation of intercellular space due to degradation of lignin in the middle lamella, while simultaneous delignification is characterized by cell wall thinning. The anatomical structure of *H. brasiliensis* attacked by the white-rot fungi showed differences with *P. merkusii*, whereby the ray cells of *P. merkusii* wood was more degraded than in *H. brasiliensis*.

1. **Introduction**

The pulp and paper industry in Indonesia gets priority in its development because of the potential availability of wood raw material sources from industrial plantations and community forest [1]. Although the development has good prospects, the processing methods that were applied so far in the industry use a chemical process that produces waste which leads to adverse environmental impacts. The mechanical process also requires a lot of energy. The Ministry of Industry encourages the pulp and paper industries to use the latest sustainable technology [2]. Biological pulping is an environmentally friendly process for making pulp and paper whereby the wood chips are pre-treated with selectively delignifying white-rot fungi before entering chemical or mechanical processes [3]. When bio pulped chips are used to produce mechanical pulp, energy use was reduced by as much as 10% [4]. The pulp has good quality and significantly increased fiber strength [5].

Wood contains three natural polymers, including lignin, cellulose, and hemicellulose. White-rot fungi, from Basidiomycetes, are organisms that able to degrade lignin naturally by the enzymatic and
non-enzymatic mechanisms. White-rot fungi can recognize carbohydrate polymers (especially hemicellulose) in cell walls and have a specific enzyme system that can hydrolyze these polymers into digestible compounds [6]. The delignification processes by white-rot fungi are divided into selective and simultaneous delignification. In selective delignification, lignin is degraded earlier than cellulose and hemicellulose in the decay process. While in simultaneous delignification, decomposition of holocellulose and lignin occurs at nearly the same rate [7]. Some fungi can show both delignification processes in the same wood or in different wood species [8]. The decay pattern of white-rot fungi depends on wood species and environmental conditions. *Pycnoporus sanguineus* and *Polyporus arcularius* are species of white-rot fungi from the main group of wood-rotting fungi in the tropical forest, Polyporales.

*Hevea brasiliensis* is one of the hardwood species from angiosperm that frequently used for making pulp and paper as well as *Pinus merkusii* which belongs to of softwood from gymnosperm. The destructive behavior of white-rot fungi on wood has been studied with various methods, one of them is a wood staining method that can distinguish between simultaneously and selectively delignifying white-rot fungi [55]. The purpose of this study is to determine the decay patterns of white-rot fungi *Polyporus arcularius* and *Pycnoporus sanguineus*, as well as its ability to decay *Hevea brasiliensis* (as hardwood) and *Pinus merkusii* (as softwood) by observing the anatomical characteristics.

2. Materials and methods

2.1 Wood decay test

For this experiment, inoculation procedures followed the Kolle-flask method by Indonesian National Standards (SNI 7207:2014). Woodblocks, measuring 5 cm × 2.5 cm × 1.5 cm, were prepared from *Hevea brasiliensis* and *Pinus merkusii* for each of 56 pieces. Some of the woodblocks were oven-dried at 103±2°C, then weighed to determine the initial weight (IW). The pure culture of *Polyporus arcularius* HHBI-371 and *Pycnoporus sanguineus* HHBI-448 used for decay tests were obtained from the Forest Products Research and Development Center. The two separate fungi were grown for 21 days on autoclaved Kolle-flask containing Malt Extract Agar. Two until three woodblocks from each species were kept in each Kolle-flask and incubated for 6, 9, and 12 weeks at 25±2°C and humidity 70-80%. After each incubation period, the blocks were removed from the flasks and brushed clean to remove superficial mycelia. Six blocks were oven-dried at 103±2°C until a constant dry weight was reached to determine the final weight (FW). Two blocks were reserved for microscopic observations. Uninoculated blocks were employed as controls.

2.2 Percentage of weight loss

The percentage of weight loss due to decay was calculated using the formula by Indonesian National Standards (SNI 7207:2014).

\[
\text{Weight loss} = \frac{IW - FW}{IW} \times 100
\]

The percentages of weight loss were analyzed using a complete randomized design with 3x2x2 factorial experiment (incubation time, wood species, mushroom type) with six replications. The data was processed using the *IBM SPSS Statistics* 25.

2.3 Wood Sectioning

Tested wood was cut into 1.5x1.5x1.5 cm³ in size. Wood slices were prepared by the Sass method [9]. Tested wood was soaked in 96% alcohol: glycerin (1: 1) for at least 2 hours. To eliminate glycerin and make it easier to slice, the wood sample was soaked in 70% alcohol or water. The wood sample was sliced using a microtome to a thickness of 15-25 μm each for transverse, radial, and tangential sections.

2.4 Mycelium staining

Wood slices staining was prepared using Cartwright & Findlay method [10] to stain the mycelium. The wood slices were stained with safranin for minimum of ½ hours then washed to prevent overstained. Picro aniline blue was dripped onto the slices while heated on a flame until the dye slight boil later washed with water to remove the dye. Stained wood slices were dehydrated with 70% and 96% alcohol and cleaned with clove oil and xylene each for five minutes. The best slices were
mounted on a glass slide with entellan and observed under a light microscope. Lignified cell walls appear red and hyphae appear blue.

2.5 Cell Wall Staining
Wood slices staining was undertaken using van der Werf et al method [11]. The wood slices were soaked in a combination of 40 mg safranin and 150 mg astra blue in distilled water with the addition of 2 ml acetic acid for 10 minutes, then washed with water to prevent overstained. Stained wood slices were dehydrated with a series of alcohol concentrations (30%, 50%, 70%, 96%, and absolute) each for five minutes later cleaned with xylene. The best slices were mounted on a glass slide with entellan and observed under a light microscope. Safranin stains lignin regardless of whether cellulose is present with red color, whereas astra blue stains cellulose only in the absence of lignin with blue color.

2.6 Maceration
Microscopic characteristics of individual fibers were observed with the wood maceration method according to the Forest Product Laboratory method [12]. Wood samples were cut into wood chips then macerated using a mixture of equal parts of 60% glacial acetic acid and hydrogen peroxide (20% by volume), heated at 80°C until the sample is colorless and soft. Macerated samples were washed with tap water until acid-free. The separated fibers were stained with safranin for 3 hours, then washed with distilled water, placed on a glass slide, and covered with a cover glass. Further observation on those individual fibers was done using \textit{Axioo Imager A1m (micro) Zeiss} with \textit{Axiovision LE software}.

3. Results
The white-rot fungi began to attacked wood after two weeks incubation, indicated by the beginning of mycelium growth on the wood surface. As the incubation time increases, the mycelium got thicker and turned dark brown. The attachment of mycelium on the wood surface was getting stronger. At this stage, the remaining layers of the medium were very thin and dry. Fungi-attacked wood turned to be brighter than the previous color. The presence of mycelium filling the pores in the \textit{Hevea brasiliensis} wood was observed macroscopically. The observations were strengthened by the mycelium staining method with picro aniline blue which the mycelium inside the tracheal cells appeared blue (Figure 1a). Based on Figure 1e, mycelium was observed inside resin canals in \textit{Pinus merkusii} wood. These results were also observed microscopically (Figure 1b).

![Figure 1. Microscopic observations of fungi-attacked wood](image)

(a) \textit{Hevea brasiliensis} and (b) \textit{Pinus merkusii}

Scale bar = 10 µm

The percentage of weight loss of \textit{Hevea brasiliensis} and \textit{Pinus merkusii} wood after being attacked by white-rot fungi \textit{Polyporus arcularius} and \textit{Pycnoporus sanguineus} at each incubation time are shown in Table 1.
The microscopic observation result of *Hevea brasiliensis* wood incubated at certain times with the *Pycnoporus sanguineus* and *Polyporus arcurarius* are shown in Figure 2. Figure 2 (d) shows a cross-section of 6-week incubated wood with *Pycnoporus sanguineus*. Hyphae growth was observed branching in the tracheal lumen and spread through the axial parenchyma cell wall. As a result, the parenchymal cell walls were thinning and partially degraded. The fibre tracheid adjacent to the trachea is degraded to form a large cavity. Some of the fibres have thinned cell walls. Figure 2 (e) shows a tangential section of 9-week incubated wood with *P.sanguineus*. Hyphae originating from the parenchyma and ray cells were spread, penetrated, and degraded the fibre tracheid wall. Around the hyphae colonized ray cell, the fibre tracheid stained in blue indicated delignification. Figure 2 (f) shows a radial section of 12-week incubated wood with *P.sanguineus*. The walls of fibre tracheid were thinning. The entrance of hyphae into adjacent cells was carried out through the pits by degrading the hole membrane or by direct penetration through the cell wall. Figure 2 (g) shows a cross-section of 12-week incubation wood with *P.sanguineus*. Delignification of S3 layer of fibre tracheid was more apparent. Axial parenchyma cells are completely degraded. Cavities are formed due to massive cell degradation.

Figure 2 (h) shows a tangential section of 6-week incubated wood with *Polyporus arcurarius*. Colonies of hyphae were observed in axial parenchyma cells and ray cells. A small portion of ray cells has been degraded by hyphae. Figure 2 (i) shows a radial section of 9-week incubated wood with *P.arcurarius*. Boreholes were observed in the fibre tracheid wall. Figure 2 (j) shows a cross-section of 9-week incubated wood with *P.arcurarius*. The fibre tracheid adjacent to the trachea was increasingly degraded and forming a cavity. Some of the fibre tracheid cell walls were thinning and all the lumen filled with hyphae colonies. Ray cells and axial parenchyma cells are fully degraded. Figure 2 (k) shows a tangential section of 12-week incubated wood with *P.arcurarius*. The walls of the fibre tracheid and parenchyma cells are getting thinner. Parenchyma cells were more degraded. Hyphae have been observed to spread on each fibre tracheid and penetrated the cell walls. Figure 2 (l) shows a radial section of 12-week incubated wood with *P.arcurarius*. The hyphae were thickening and spread to each fibre tracheid. Cell damage due to degradation by hyphae was increasingly widespread, especially in the cells of the rays and parenchyma cells.

The microscopic observation result of *Pinus merkurii* wood incubated at certain times with the *Pycnoporus sanguineus* and *Polyporus arcurarius* are shown in Figure 3. Figure 3 (d) shows a cross-section of 6-week incubated wood with *Pycnoporus sanguineus*. Concentric delignification was observed starting from the lumen surface of tracheid cells. In some parts, the separation of adjacent cells was observed as a sign of delignification in the middle lamella. An erosion canal was observed in the tracheid cell wall. Figure 3(e) shows a tangential section of the 9-week incubated week with *P.sanguineus*. At this stage, more boreholes and erosion canals observed in the tracheid cell walls. Figure 3 (f) shows a radial section of 12-week incubated wood with *P.sanguineus*. The tracheid cell walls were increasingly degraded and the number of boreholes and erosion canals increased. Fenestiform pit or window-like pit on the ray parenchyma cells were degraded and formed a large hole. The bordered pit opening was getting wider. Figure 3 (g) showed a cross-section of 12-week incubated wood with *P.sanguineus*. Tracheid cell walls were almost completely degraded.

Figure 3 (h) shows a tangential section of 6-week incubated wood with *Polyporus arcurarius*. In the tracheid cell walls, large erosion channels and boreholes were observed. In the lumen of tracheid cells, hyphae colonies branched and connected with boreholes. The ray parenchyma cells were completely degraded. Epithelial cells that surround resin canals were degraded. Figure 3 (i) shows a radial section

| Type of wood       | Weight loss (%)       | 6 weeks | 9 weeks | 12 weeks |
|--------------------|-----------------------|---------|---------|----------|
| *Hevea brasiliensis* | 13.49 ± 2.16          | 10.06 ± 4.04 | 16.18 ± 3.14 | 20.41 ± 1.05 | 26.45 ± 7.80 | 28.99 ± 5.70 |
| *Pinus merkurii*    | 4.20 ± 0.75           | 4.19 ± 0.81 | 5.71 ± 2.30 | 9.13 ± 1.42 | 10.10 ± 5.25 | 12.21 ± 4.69 |

Table 1. Percentage of weight loss of treated wood
of 9-week incubated wood with *P. arcularius*. Several boreholes were observed in the adjacent tracheal cell walls. Figure 3 (k) shows a tangential section of 12-week incubated wood with *P. arcularius*. Erosion canals and bore holes were located close to each other in the tracheid cell walls. The area which was originally a degraded ray cell was filled with hyphae colonies. Figure 3 (l) shows a radial section of 12-week incubated wood with *P. arcularius*. Many boreholes observed in the tracheid cell walls. Some cell walls were getting thinner. The opening of a simple pit in the tracheid cell walls were getting bigger. The opening of the bordered pit in the tracheid cells was getting wider due to degradation.

4. Discussion

The percentage of wood weight loss has increased along with the incubation duration. The lowest weight loss percentage occurs in *Pinus merkusii* wood in the range of 4.20-12.21%, so the wood likely has a higher resistance to the attacked of fungi. On the contrary, the different results shown by *Hevea brasiliensis* wood with the highest weight loss in the rage of 13.39-28.99%. Thus, it might be concluded that the wood’s resistance to fungal attacked is quite low. Statistical analysis showed a significant difference (p<0.05) in the percentage of weight loss attacked by each type of fungus and at each incubation time. It can be argued that there is a relationship between the percentage of weight loss for both wood species with a variation of incubation time. In contrast, the percentage of weight loss between different types of wood did not show a significant difference. This is probably due to the classification of wood resistance classes, which *Hevea brasiliensis* and *Pinus merkusii* are in the range of class IV-V according to Indonesian International Standards (SNI 7207:2014), indicated that both types of wood have a very low resistance to wood-decay organisms [13].

According to Hunt and Garrat [14], factors that supported the growth of white-rot fungi were environmental temperature, availability of oxygen, and moisture content of the wood. The tested wood was incubated in a room with a temperature of 25±2°C and humidity 70-80%. This condition is suitable to support the optimal development of fungi, with temperatures ranging from 24.4°C-30°C [15]. According to Lilly and Bernett [16], fungi obtain energy from the oxidation of organic or carbon-containing compounds that important for fungal nutrition because carbon forms more than half of the dry weight of fungal cells. About 40% of organic carbon in plant biomass is estimated to be bound with cellulose and 30% with lignin [17]. Fungi also need nitrogen especially for enzyme production. The addition of organic or inorganic nitrogen is needed to accelerate the decomposition of wood by decay-fungi because wood only contains 0.01-0.03% nitrogen [17].

Malt extract agar contains malt extract, mycological peptone, and agar Nad. Mycological peptone is a source of nitrogen for fungal growth. The final acidity (pH) of the media at 25°C is 5.4±0.2 which is suitable for wood-rot fungi to grow optimally C [15]. Polysaccharides contained in malt extracts are not only useful as a source of nutrition but also make the media in acidic conditions. According to Nadir et al [19], temperature and pH in culture conditions will affect metabolism, germination of spores, and fungal growth.

The tested wood incubated with white-rot fungi have a brighter appearance than the previous color. According to Panshin et al [15], the wood color attacked by white-rot fungi becomes whiter like bleaching effect. White-rot fungi can reconstruct the structure of lignin and cellulose so that the color of the wood becomes brighter than the initial color. In this study, mycelium was observed inside the resin canal in *Pinus merkusii* wood and inside pores in *Hevea brasiliensis* wood. Trachea and resin channels that are parallel to the axis can facilitate fungus access into the wood and facilitate the distribution of hyphae within the xylem [20].
Figure 2. The microscopic observation on anatomical characteristics of *Hevea brasiliensis* wood. The observations showed control wood (a-c), attacked wood by *Pycnoporus sanguineus* (d-g), and attacked wood by *Polyporus arcularius* (h-l). - a: Cross-section - b: Tangential section. - c: Radial section. - d: Cross-section of 6-week incubated wood. - e: Tangential section of 9-week incubated wood. - f: Radial section of 6-week incubated wood (orange arrow showed pit degradation). - g: Cross-section of 12-week incubated wood. - h: Tangential section of 6-week incubated wood. - i: Radial section of 9-week incubated wood (red arrowed indicated bore hole). - j: Cross-section of 12-week incubated wood. - k: Tangential section of 12-week incubated wood (black arrowhead showed erosion canal and red arrowhead indicated cell wall thinning). - l: Radial section of 12-week incubated wood.

Scale bar = 10 µm
According to Kirk and Moore [21], the decay rate of conifer wood by white-rot fungi is generally slow. Schwarze and Fink [20] suggested that white-rot fungi have high potential to degrade the complex structure of angiosperm wood. The difference is related to the lignin component contained in a different type of wood cells. The presence of lignin components, which are complex polymers, strengthen cell walls and become a hydrophobic defense to protect the wood from invading organisms that use an enzymatic system [6]. Lignin content in softwood (25-33% of dry weight) is relatively higher than in hardwood (20-25% of dry weight) [22]. The percentage of lignin content in Pinus merkusii wood is 24.3% of dry weight [13], while in Hevea brasiliensis wood is 20.78% of total dry weight [23].

Figure 3. Microscopic observation on anatomical characteristics of Pinus merkusii wood. The observations showed control wood (a–c), attacked wood by Pycnoporus sanguineus (d–g), and attacked wood by Polyporus arcularius (h–l).

- a: Cross-section
- b: Tangential section
- c: Radial section
- d: Cross-section of 6-week incubated wood (black arrowhead showed erosion canal and black arrow indicated separation of adjacent cell).
- e: Tangential section of 9-week incubated wood (red arrow showed borehole)
- f: Radial section of 12-week incubated wood.
- g: Cross-section of 12-week incubated wood.
- h: Tangential section of 6-week incubated wood.
- i: Radial section of 9-week incubated wood.
- j: Cross-section of 9-week incubated wood (red arrowhead indicates cell wall thinning).
- k: Tangential section of 12-week incubated wood.
- l: Radial section of 12-week incubated wood.

Scale bar = 10 μm
In addition to the amount of lignin content, lignin composition also plays an important role in the defense of wood cell walls against decay activity. Lignin is a polymer composed of guaiacyl (G) units of trans-coniferyl-alcohol precursors, syringil units (S) of trans-syringyl-alcohols, and p-hydroxyphenyl (H) units of trans-p-coumaryl alcohol precursors. The composition of lignin varies for each species which is classified in two main groups, namely guaiacyl lignin and guaiacyl-syringyl lignin [17]. The ratio of each monomer varies between each cell type and each cell wall. This affects the wood’s resistance to fungal attack. Lignin in softwood (Gymnosperms) is composed of guaiacyl subunits that make the structure denser and more resistant to microbial degradation than lignin in hardwood (Angiosperms) consisting of guaiacyl and syringyl subunits with a balanced ratio [24], [25], [26], [27]. Wood that has a high content of lignin syringyl generally experiences greater weight loss [7]. This is related to the arrangement of the guaiacyl subunits that produce lignin which is more condensed than the syringyl subunits [6].

Tyloses is formed as one of the self-defense reactions in the Angiosperms of the Euphorbiaceae family against pressure from the outside (infection or attack of organisms) in nature. Tracheal blockage by tylosis plays an important role in slowing down and preventing the spread of pathogens. Tyloses can contain organic components and minerals such as resin, starch, crystals and phenolic components. Tyloses originate from the development of living parenchymal cells into dead tracheal cells through pit aperture [28], [29]. Successful prevention of pathogens depends on the speed of tyloses formation and in the formation of the sap that may be an accumulation of anti-microbial compounds such as tannins, catechols, flavonoids, and coumarin which prevents the spread of pathogens in the infected part by different mechanisms [30].

Wood attacked by *Pycnoporus sanguineus* experienced a higher weight loss (4.19-28.99%) than that attacked by the fungus *Polyporus arcularius* (4.20-26.45%). Based on the research conducted by Suprapti et al [31], *Pycnoporus sanguineus* has a high wood-decay ability, followed by *Polyporus arcularius*. Both types of fungi are included in the group of white-rot fungi that can remodel cellulose and lignin [32]. *Pycnoporus sanguineus* produces extracellular laccase among its main ligninolytic enzymes. The laccase enzyme consists of copper-containing glycoproteins that are involved in the lignin degradation process. Laccase requires oxygen to carry out its function as a catalyst and can oxidize phenolic compounds. This causes the enzyme laccase often used in biotechnology applications, such as for pulping, pulp bleaching, dye decolorization, and phenolic degradation [33]. *Polyporus arcularius* is known to have peroxidase to decompose cellulose and lignin in wood [34].

The penetration of white-rot fungi hyphae usually begins with enzyme secretion that can convert insoluble substances in wood into solutes form. The enzyme diffuses through the lumen and cell wall, then degrades lignin into simpler compounds, which are H₂O and CO₂. The complex enzymes released mainly consist of lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase (Lac) along with other enzymes. Wood-decay fungi also can recognize carbohydrate polymers (especially hemicellulose) in cell walls and have a specific enzyme system that can hydrolyze these polymers to be more easily digested [6]. The sub-apical hyphae zone functions to carry out biochemical processes and can produce the necessary enzymes as a catalyst for biochemical processes to penetrate the cell walls of wood and the acquisition of nutrients needed for fungal growth. Enzymatic activity will damage the structure of cell walls so that hyphae can be bypassed [15], [35], [36]. The fibre strength decreases as the polymer and cell wall matrix are increasingly degraded by fungi [6].

In this study, the decay patterns of the white-rot fungus *Pycnoporus sanguineus* on *Hevea brasiliensis* and *Pinus merkusii* wood showed different results. The pattern observed in *Pinus merkusii* wood is selective delignification shown by its characteristic which is cell separation. This result is consistent with the *Pycnoporus sanguineus* study conducted by Luna et al [37] in *Populus deltoides* and Singh et al [38] in oil palm biomass. However, other features such as boreholes, erosion canals, and openings in pits are also found in *Pinus merkusii* wood. According to Anagnost [39], types of fungi with selectively decay patterns can show the same anatomical features as simultaneously decay patterns. However, the cell wall thinning and overall degradation of cell walls that characterize simultaneous weathering patterns are not present.

Conflicting results occur in *Hevea brasiliensis* wood attacked by *P.sanguineus* where simultaneously decay patterns are observed. Colonization of fungal hyphae starts from inside of the
trachea is a special characteristic of the simultaneously decay pattern of white-rot fungi [40]. At the beginning of the decay process in the second to the fourth week, many fungal hyphae occupy the trachea then spread to adjacent cells such as the ray parenchyma cells, axial parenchyma cells, and fiber tracheid until they reach 6 weeks incubation time. The same result occurred in the decay pattern of *Shorea gibbosa* wood (Angiosperms) by the fungus *Phlebia brevispora* [41]. According to Ferraz et al [42], Levin et al [43], Rohr et al [44], and van Heerden et al [45], decay patterns by *Pycnoporus sanguineus* showed simultaneous degradation of lignin and cellulose. Based on research conducted by Luna et al [37], the decay pattern of white-rot fungi is strongly influenced by the type of fungal isolate and the type of wood used so that a possible combination of simultaneous and selective degradation can occur. According to Bari et al [46], rot fungi decay patterns cannot easily be categorized as simultaneous or selective delignification because mushroom species may change modes of action depending on environmental conditions. Kirk and Moore [21] found that the rate of lignin/carbohydrate removal by fungi varied depending on the wood used as a substrate.

The results for *Pycnoporus sanguineus* observations showed selective delignification in angiosperms wood and simultaneous delignification in gymnosperms wood, which can be caused by the lignin component in each species. Many species that cause simultaneously decay in angiosperm wood rarely show the same pattern in gymnosperms wood. This is probably related to the S3 layer of the tracheid cell wall which is very elastic potential to prevent degradation by hyphae from the inside of cell lumen. Conversely, low molecular weight compounds that cause selectively decay can easily diffuse through the S3 layer in the secondary wall [7].

The decay patterns of *Polyporus arcularius* showed simultaneous delignification in both types of wood. The characteristics of simultaneously decay white-rot fungi were observed, such as the formation of boreholes, erosion canals, cell wall thinning, and large pit opening [39]. The hyphae penetrate the cell wall transversely through pit or by the formation of a borehole and then branch off along the cell cavity. The penetration of white rot fungus hyphae involves enzymatic activity that will produce a hole in the cell wall as the entry point. Hyphae will shrink when passing through a borehole, then return to its normal size when it reaches the lumen [15].

The same result has occurred in the decay pattern of *Polyporus versicolor* L.ex Fries which showed cell wall thinning from the lumen to the middle lamella [47]. Depletion of cell walls can be caused by erosion of cell wall material by extracellular enzymes released by hyphae that grow in the lumen [46]. According to Panshin et al [15], simultaneously white-rot fungi use every main polymer component at a constant rate at each decay stage. The depolymerization is equivalent to its conversion to a volatile compound as a product of respiration.

Based on the observations, it appeared that ray cells in *Pinus merkusii* wood are more degraded than in *Hevea brasiliensis* wood. According to Anagnost [39], the ray parenchyma of hardwood is less often attacked with white-rot fungi than softwood. According to Schwarze et al [20], the ability of secondary xylem degradation is related to the lignin composition of each cell. The composition of lignin from different types of wood cells facilitates the interpretation of the damage and predicts whether xylem in a wood species is resistant to decay [48]. The ratio of each monomer varies between each cell type and each cell wall. This affects the resistance of wood to rot fungi. According to Syafii and Yoshimoto [49], angiosperms wood with high levels of guaiacyl lignin are more resistant to damage than wood with high levels of lignin syringyl. In angiosperms wood, damage on each type of wood cell shows different results.

Tracheal cells are more resistant to damage than fiber tracheid cells and ray cells. Maceration observations showed a tracheal cell structure as shown in Figure 4. These results are following the statement by Sanghvi et al [50] which states the trachea is more resistant to decay than the fiber tracheid. The secondary wall of the tracheal cell has a higher concentration of guaiacyl lignin than the tracheid cell and parenchyma cell which is composed of a mixture of guaiacyl and syringil units with more dominating lignin syringil [51]. The composition of lignin in tracheal cells can withstand strong pressure during water transport due to the guaiacyl subunit which produces a more condensed lignin structure [6].
Axial parenchyma cells and ray cells showed more obvious damage compared to fiber tracheid cells. According to Iiyama and Pant [52], ray parenchyma has a relatively high syringyl monomer content, in contrast to the fiber tracheid which has a high guaicyl monomer content. In anatomical observations of wood slides, libriform fibers and tracheid cells are difficult to distinguish. The two cell types can be more easily distinguished in the maceration observations shown in Figure 5. The pits are clearly observed in fiber tracheid but not clearly observed in libriform fibers. According to Sutrian [53], fibre tracheid has bordered pits, whereas libriform fibers have simple pits. Baum [51] states that the syringyl monomer in libriform fibers is higher than that of tracheal fibers.

In *Pinus merkusii* wood, tracheid cells are less damaged than fiber cells in *Hevea brasiliensis* wood. The observations of tracheid cells in *Pinus merkusii* wood are shown in Figure 6. According to Blanchette [25], lignin in softwood is composed by guaiacyl alcohol subunits that make its structure denser and more resistant to microbial degradation. The distribution of lignin between the secondary wall and middle lamella in hardwood fiber cells are like in softwood. The difference is the secondary wall of the hardwood fiber cells less lignified than the softwood, which is related to the natural guaicayl-syringyl content of the angiosperm’s fiber.

5. Conclusion

The anatomical structures of *Hevea brasiliensis* wood cells attacked by white-rot fungi *Pycnoporus sanguineus* and *Polyporus arcularius* showed different result from *Pinus merkusii* wood, which the ray parenchyma cells of *P. merkusii* wood were more degraded compared to the ray parenchyma cells in *H.*
brasiliensis wood. The decay pattern of the P. sanguineus in P. merkusii wood was determined into selective delignification, whereas in H. brasiliensis wood was categorized as simultaneous delignification. The decay pattern of P. arcularius showed simultaneous delignification on both wood species. The H. brasiliensis and P. merkusii wood which were attacked by the fungus P. sanguineus experienced a higher weight loss compared to the fungus P. arcularius. Further study needs to be carried out a chemical analysis to obtain quantitative data of total loss of lignin, cellulose, and hemicellulose compounds due to decay by fungi and characterize the enzymes to determine the enzymes that work in the decay processes.

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