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Initial studies on the influence of *Aspergillus niger* on the wood components of *Populus sp*.

EWA LISIECKA[1], ANNA LESIAK[1], JAN SZADKOWSKI[2], DOMINIKA SZADKOWSKA[2], ANDRZEJ RADOMSKI[2], BOGUSŁAW ANDRES[2]

[1]Department of Wood Science and Wood Preservation, Faculty of Wood Technology, Warsaw University of Life Science – SGGW
[2]Department of Wood Science and Wood Preservation, Institute of Wood Sciences and Furniture, Warsaw University of Life Science – SGGW

Abstract: Initial studies on the influence of *Aspergillus niger* on the wood components of *Populus sp*. An attempt was made to determine the content of structural substances (cellulose, holocellulose, lignin) and non-structural substances in poplar wood (*Populus* sp.) infected with the mentioned fungus. For this purpose, wood chips were infected with spore suspension and then incubated under sterile and suitable growth conditions. After 7, 14 and 21 days the degree of poplar wood degradation was assessed by determining the substances contained in the wood by performing chemical analyses: Kürschner-Hoffer cellulose, holocellulose by the sodium chlorite method, lignin in accordance with PN-92/P50092 standard and non-structural components. The conducted analyses made it possible to determine the effect of *Aspergillus niger*'s presence time on the degree of degradation of individual wood components.

Keywords: *Aspergillus niger, Populus sp., wood degradation, cellulose, holocellulose, lignin*

INTRODUCTION

The growth of mould fungi belonging to e.g. *Ascomycotin* or *Deuteromycotin* appears on wood products commonly used by people (Ważny et al.1989). Wood elements treated or modified (particleboard, plywood, acylated wood) show less resistance to fungal infections than unmodified ones (Sumbramaniam et al. 2016). Fungi classified as mould cause surface discoloration of the wood influencing its aesthetic perception. It is assumed that elements infected by mould do not lose their mechanical properties and it is sufficient to mechanically clean the surface of the element in order to restore their previous functional parameters. (Rębkowski et al. 2016). However, some researchers indicate that mould fungi can cause the decomposition of wood-forming polysaccharides (Verdier et al. 2014).

Wood is a natural copolymer of complex structural compounds such as cellulose, lignin, hemicellulose and non-structural (mineral and extraction substances). These compounds are characterized by different resistance to biological and chemical factors (Hendriks and Zeeman 2009, Ralph et al. 2008).

Fungi produce enzyme complexes that allow the decomposition of wood compounds. Cellulose as a material is a very popular target for microorganisms. We count *Chaetomium, Aspergillus, Penicillium, Cladosporium, Alternaria, Stemphylium* and others among the most dangerous microorganisms decomposing cellulose (Karbowska-Berent J., Strzelczyk A. 2004).

Aspergillus niger is one of the most numerous *Aspergillus* species in nature. The reason for this phenomenon is its ability to grow on many different substrates, even with a very small number of available nutrients. The fungus produces allergens and can cause many serious human diseases, e.g. lung mycosis, hearing problems and even hearing loss, especially in people with reduced immunity. Despite its negative impact on human health, it is very often used in biotechnology. It produces extracellular enzymes and citric, glycolic and fumar acids. In 1919, a fermentation process was carried out using this mould, during which citric

acid was obtained. In the 1960s, it became a source of various enzymes which are used as technical acids in fruit processing. In nature, it is found in the ground, on litter, compost and decaying plants (Schuster et al. 2002). In 1986, Reiss examined the effect of temperature on *A. niger's* mould and found that it is able to grow between 6 and 47˚C.

The optimum temperature for growth of this mould is 35–37˚C. It also features the growth potential in a very wide range pH values – from 1.4 to 9.8. These features and the high efficiency of spore production make it common in warm and humid environments (Rippel-Baldes 1955). However, its ability to break down cellulose is important. *A. niger* produces various types of hydrolytic enzymes. It should be noted that fungi are the main sources of industrial active enzymes (Carbohydrate-active enzymes) for the saccharification of lignocellulose during the production of second-generation biofuels (Pullan et al. 2014). A lot of knowledge has been gathered about the *Trichoderma* fungi and their cellulase system. The *Trichoderma* species can produce significant quantities of endoglucanase and exoglucanase but very low concentrations of β-glucosidase. *A. niger* is also studied in this regard. The parameters for the formation of endoglucanase and β-glucosidase product indicate that *A. niger* is capable of moderate to high levels of both endoglucanase and β-glucosidase when grown on another carbon sourses containing natural substrate, for example grass or corn cob. The highest production of cellulase was observed at pH 4.0 at 35°C. The growth and production of the enzyme was influenced by temperature and pH changes (Sohail et al. 2004).

AIM OF THE STUDY

The aim of the study was to determine the content of structural substances (cellulose, holocellulose, lignin) and non-structural substances on poplar wood (*Populus sp.*) infected with *Aspergillus niger* fungus. This topic is interesting to the production of biofuels.

MATERIALS AND METHODS

Aspergillus niger (Tiegh) strain 287 were obtained from collection of Department of Wood Science and Wood Protection, Warsaw University of Life Science SGGW in Warsaw, Poland. Mould was cultured on malt-agar medium until strong spores formation occured. Previously chipped and sterilized poplar wood chips were placed in sterile aluminium moulds and placed in specially prepared culture vessels with the medium. Spores of mould fungus were applied to testing samples prepared in this way by means of suspension in accordance with ITB 355/98 test procedure. Concentration of disputes in suspense has not been investigated, as this procedure does not require this type of investigation. The culture vessels with mycelium *A. niger* on *Populus* sp. chips were incubated for 7, 14 and 21 days at 37°C and 85% air humidity. Reference samples were prepared in the same way as test samples, but not treated with *A. niger* fungus. After this time the swarf was subjected to chemical analysis. An extraction of non-structural substances was carried out in the mixture of chloroform:ethanol (93:7 by weight) (Antczak et al. 2006). The cellulose content on the extracted material was determined by Kürschner-Hoffer method, holocellulose by sodium chlorite (Krutul 2002) and lignin according to PN-92/P-50092. Each chemical determination was performed three times.
The strains of fungi used in the study were obtained from the collection of the Department of Wood Science and Wood Protection, Warsaw University of Life Science - SGGW in Warsaw, Poland.

RESULTS

The results of the determinations obtained for the individual substances of which the wood is composed are presented below. Figure 1 shows the content of extraction substances.

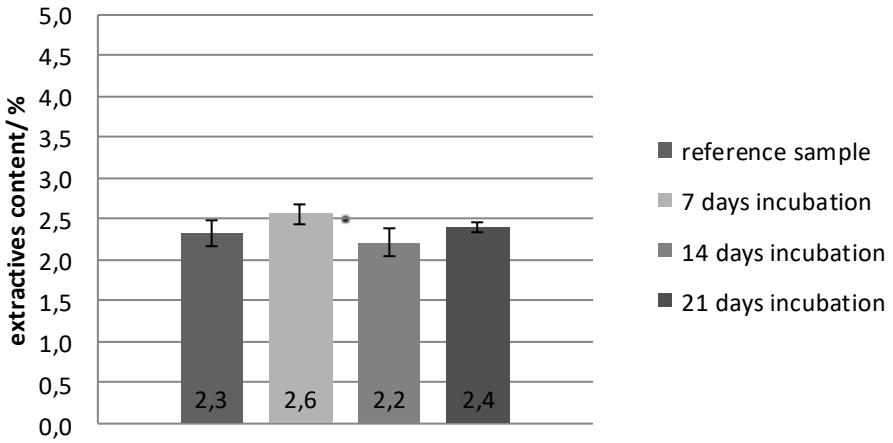

Figure 1. Extractives content after *Aspergillus niger* incubation on poplar wood

In the graph we note the percentage values for the reference sample and 7, 14 and 21 days after *A. niger* was introduced. In the initial phase of the test (after 7 days) the proportion of extraction substances increases and then decreases. It should be noted that the variation in the extractive matter content determined on samples of wood degraded by *A. niger* is small and within the error limits for the reference sample. It can be assumed that the slight increase in the extraction substances was due to the transition of *A. niger* from primary to secondary metabolism. This is accompanied, among others, by *A. niger's* production of a number of secondary metabolites which were extracted from wood during the determination. As we notice, the distribution of extractive substances in these four variants is quite similar, ranging from 2.2 to 2.6%, and is of a fluctuating nature. Such differences may result from the wood material itself, which is heterogeneous.

Figure 2 shows the results of the Kürschner-Hoffer method. It was noted that the percentage of cellulose increases with the duration of wood exposure to *A. niger*. Initially, the increase in the percentage of cellulose in the tested samples was insignificant, and it cannot be clearly stated whether it is a result of *A. niger's* effect on wood or the non-homogeneity of the wood material. After 21 days there is a distinct increase in the proportion of cellulose in relation to the previous increments.

This trend may result from the fact that *A. niger* decomposes primarily hemicellulose. In addition to polysaccharides, moulds also have the ability to decompose lignin. Therefore, with the loss of wood mass caused by the fungus, an apparent increase in the proportion of cellulose in the sample may occur.

Figure 3 shows the percentage of holocellulose. We note that the proportion of holocellulose in samples treated with *A. niger* decreases with respect to the reference sample after the first two exposure periods reference. This confirms that *A. niger* degrades hemicellulose in the first place. However, after 21 days this trend is changed and a large increase in holocellulose content is observed. This is determined at the level of holocellulose content in the reference sample, i.e. native wood. The increase in holocellulose content in the last stage of the test is most likely an increase in the share of holocellulose in relation to the mass of the wood.

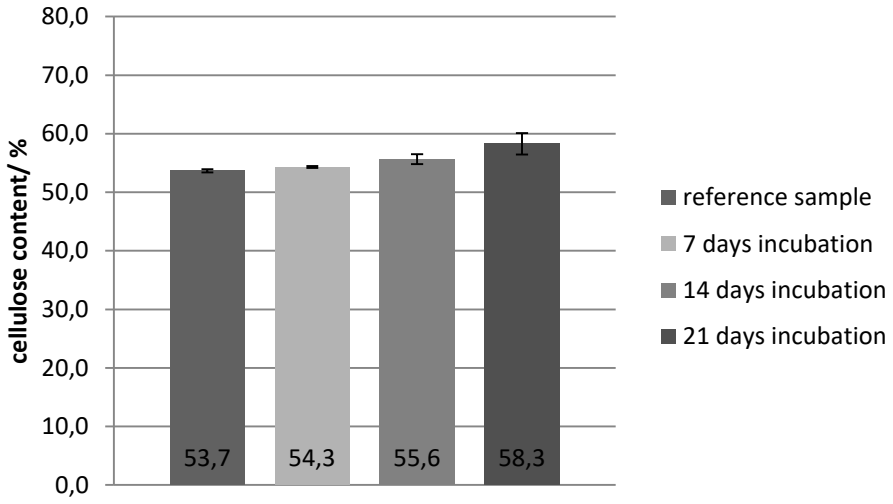

Figure 2. The content of cellulose after incubation of *Aspergillus niger* on poplar wood

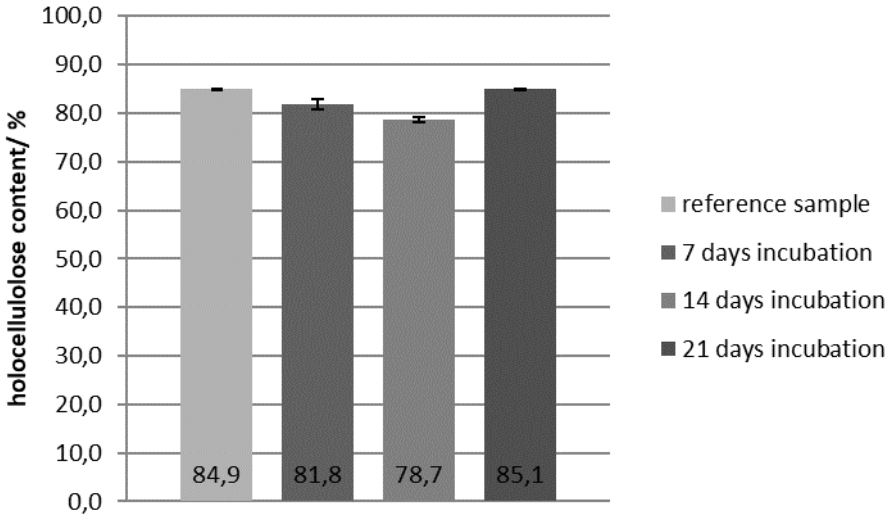

Figure 3. Holocellulose content after incubation of *Aspergillus niger* on poplar wood

Figure 4 shows the results of the determination obtained for lignin. The lignin content of the reference material is approx. 16%. In the wood infected with *A. niger*, the lignin content increases to approx. 17%. The lignin content determined in the wood after the incubation period of 7, 14 and 21 days does not change. This may be due to changes in the content of other wood components, such as a decrease in the content of extractive substances (especially for 7 and 14 days) and the content of holocellulose at the same incubation times.

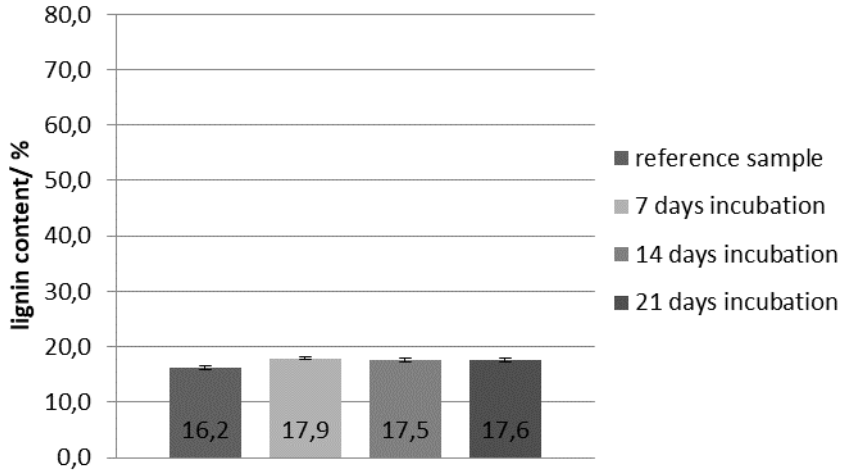

Figure 4. Lignin content after incubation of *Aspergillus niger* on poplar wood

The results were statistically analyzed by performing an analysis of variance for a single classification using the Snedecor statistic (F statistic). Statistical inference was performed for the significance level $\alpha = 0.05$. Then, the means of the individual sample groups were compared using the multiple comparison test (*post hoc* test). For this purpose, the Tukey test was used. Statistical analyzes were developed using Statistica version 13.3 software. The analysis of the variance showed the significance of the differences between the individual experimental variants (Tables 1-3).

Table 1**.** Cellulose content after incubation of *Aspergillus niger* on poplar wood - analysis of Variance and Division of Sample Groups in Tukey Test.

Variability	$F_{emp.}$	p-value	$F_{0.05}$
Factor *A.niger*	2983,27	0,00000001	4,07
Samples	Average	Sample Groups According to Tukey Test	
reference samle	53,6731	a	
7 day incubation	54,3285	a,b	
14 day incubation	55,6496	b	
21 day incubation	85,0769	c	

Table 2**.** Holocellulose content after incubation of *Aspergillus niger* on poplar wood - analysis of Variance and Division of Sample Groups in Tukey Test.

Variability	$F_{emp.}$	p-value	$F_{0.05}$
Factor *A.niger*	60,53	0,0000077	4,07
Samples	Average	Sample Groups According to Tukey Test	
14 day incubation	78,6880	a	
7 day incubation	81,8397	b	
reference samle	84,8810	c	
21 day incubation	85,0770	c	

Table 3. Lignin content after incubation of *Aspergillus niger* on poplar wood - analysis of Variance and Division of Sample Groups in Tukey Test.

Variability	$F_{emp.}$	p-value	$F_{0.05}$
Factor *A.niger*	195,56	0,0000001	4,07
Samples	Average	Sample Groups According to Tukey Test	
14 day incubation	12,6264	a	
7 day incubation	16,1889	b	
reference samle	17,5106	c	
21 day incubation	17,8850	c	

Publications on the influence of *Aspergillus niger* on the chemical composition of wood are very difficult to obtain. The authors focus on the change of appearance (discoloration), and the surface of mycelium occurring on the surface of wooden samples (Rębkowski et al. 2016, Sumbramaniam et al. 2016).

The results obtained from the determination of changes in the chemical composition of poplar wood as a result of A. niger's action confirm the reduction of hemicellulose content in the analyzed samples. This may result from *A. niger's* secretion of hydrolytic enzymes such as β-xylosidase, α-arabinofuranosidase, and β-galactosidase (Hu et al. 2011, Wang 2018).

The lignin content of the tested material practically does not change. As indicated by Wang et al. (2018), an important issue in the application of *A. niger's*. for the hydrolysis of polysaccharides is the removal or reduction of the lignin content and the reduction of the degree of crystallinity of the polysaccharides in the material exposed to this mould. This can enable a reduction of up to 33 % in sugars in the biomass (Chinedu et al, 2008, Wang et al.2018, Galas et al. 1997).

CONCLUSIONS

1. *A. niger's* affects the change in the chemical composition of the examined poplar wood (*Populus* sp.).
2. As a result of *A. niger's* mould, the hemicellulose content decreases.
3. After seven days of infection, the lignin content increases, which then remains at a similar level.
4. As a result of *A. niger's* action, an apparent increase in cellulose occurs.
5. *A. niger's* decomposes low polymerization polysaccharides.
6. It is necessary to carry out further research on *A. niger's* effect on the structure of wood biomass.
7. The use of A. niger's as one of the methods of lignocellulosic biomass pre-treatment in liquid biofuel technology requires further research

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Streszczenie: Wstępne badania w*pływu Aspergillus niger na zawartość składników drewna Polupus sp.* W pracy podjęto próbę oznaczenia zawartości substancji strukturalnych (celulozy, holocelulozy, ligniny) oraz substancji niestrukturalnych na drewnie topoli (*Populus sp.*), zainfekowanym wspomnianym grzybem pleśniowym. W tym celu porażono wióry przy pomocy suspensji zarodnikowej, a następnie w sterylnych i odpowiednich warunkach wzrostowych całość inkubowano. Po 7, 14, 21 dniach określano stopień degradacji drewna topoli przez wyznaczanie substancji zawartych w drewnie, wykonując analizy chemiczne: celulozę Kürschnera-Hoffera, holocelulozę metodą chlorynu sodowego, ligninę według normy PN-92/P50092 oraz składniki niestrukturalne. Przeprowadzone analizy pozwoliły określić wpływ czasu porażenia przez *Aspergillus niger* na stopień degradacji poszczególnych składników drewna.

Corresponding author:

Dominika Szadkowska
Department of Wood Sciences and Wood Preservation,
Faculty of Wood Technology,
Warsaw University of Life Sciences – SGGW
166 Nowoursynowska St.
02-776 Warsaw, Poland
email: dominika_szadkowska@sggw.edu.pl