Transformation of plant substrates by fungi *Fomitopsis pinicola*

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Abstract. The results of chemical and thermal analysis of substrates based on the post-extraction residue of balsam poplar (*Populus balsamifera* L.) buds before and after bioconversion by Fp5-15 *Fomitopsis pinicola* fungi were compared. The thermal characteristics obtained using thermogravimetry (TG/DTG) methods are presented. The stages of thermal decomposition, their temperature intervals and the temperatures of the maxima on the DTG curves, as well as the weight loss of the samples, have been established. Studies have shown that under the action of the enzyme complex of fungi, easily and difficultly hydrolysable polysaccharides are utilized, and lignin substances also undergo changes.

1 Introduction

Fungi of the genus *Fomitopsis* have rich complexes of cellulose and lignolytic enzymes. They began to be widely used in the Middle Ages in European medicine and are still used [1, 2]. The most studied among all Fomitopsis species are *Fomitopsis pinicola*.

Fomitopsis pinicola is considered one of the promising xylotrophic basidiomycetes for biotechnology, including the bioconversion of plant waste, due to the formation of enzymes that allow the utilization of complex compounds that make up wood. Species *Fomitopsis pinicola* (Sw.: Fr.) P. Karst. (bordered tinder fungus) is a fairly common saprophyte fungus. Due to the efficient decomposition of cellulose-containing materials, this group of fungi can be used as biodestructors of fir needles, sawdust of *Abies sibirica, Larix sibirica, Pinus sibirica, Pinus sylvestris, Populus tremula*, etc. [3-5], as well as the vegetative part of balsam poplar (*Populus balsamifera* L.) [6, 7]. It is known that fungi of the genus Fp5-15 *F. pinicola* on substrates consisting of the solid residue of poplar buds after the removal of essential oils and alcohol-soluble substances (having independent use), grow with the following growth rates: growth rate - 6.8 mm/day, growth coefficient - 13.6. Under the influence of the enzyme complex of fungi, easily and difficultly hydrolysable polysaccharides are utilized, their content is reduced by 51-52%. Water-extractive and lignin substances undergo changes; in the process of destruction, their content decreased by 24 and 10%, respectively, while the weight loss of the substrate remains 4.4% [7]. The chemical composition of substrates before and after biodegradation by Fp5-15 fungi *F. pinicola* is mainly studied by methods adopted

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in plant chemistry and biochemistry [8], but for a deeper study of substrate biotransformation under the action of enzymes of wood-destroying fungi, it is necessary to conduct its thermal analysis, which and was the aim of this study. The kinetic characteristics obtained from the analysis of thermogravimetric data are necessary for understanding the process of plant substrate bioconversion.

2 Research methods

The balsam poplar buds collected on the territory of Krasnoyarsk served as a substrate for the cultivation of mushrooms. Essential oils and alcohol-extractive substances (p.e.o.) were previously removed from the buds, since these substances have independent use and are also capable of inhibiting the growth of fungi.

Fungi of the genus Fp5-15 *Fomitopsis pinicola (Sw.)* P. Karst were used as a biodestructor. The Fp5-15 strain of *F. pinicola* was isolated from the fruiting bodies of *Fomitopsis pinicola*, which grew on living trees of Siberian larch in a forest area on the territory of the Yemelyanovsky district of the Krasnoyarsk Territory [3].

The preparation of substrates for solid-phase cultivation was carried out as follows: the crushed plant substrate was brought to 70% moisture content with water, placed in Petri dishes, and sterilized for 30 min at a pressure of 1.01 105 Pa several times in a VK-75 autoclave. Solid-phase cultivation of *F. pinicola* was carried out at (25 ± 2) °C until the complete fouling of the substrate. In order to standardize the inoculations, blocks (diameter 8 mm) were used as an inoculum, cut out with a microbiological punch from the growth zone by a seven-day culture of the corresponding strain.

Biotransformation of substrates before and after biodegradation was studied using thermal analysis. Thermogravimetry (TG) of substrate samples was carried out using a TG 209 F1 instrument (NETZSCH, Germany). TG analysis is a thermal analysis method that measures the change in mass of a sample as a function of temperature. The method of derivative thermogravimetry (DTG) shows the rate of change - the first derivative of the TG curve with respect to temperature.

Thermogravimetry of experimental samples was carried out in air under the following conditions: heating rate 10 $^{o}C \cdot min^{-1}$ from 25 to 700 ^{o}C, flow rate of protective and purge gases 20 $ml \cdot min^{-1}$; sample weight 8.35–8.67 mg, cylindrical Al_2O_3 crucible. The measurement results were processed using the NETZSCH. Proteus Thermal Analysis. 4.8.4".

Analysis of the kinetics of thermal degradation of substrates was carried out based on thermogravimetric data using the Broido kinetic model:

$$\ln\left[\ln\left(\frac{1}{y}\right)\right] = -\frac{E_a}{RT} + \ln\left(\frac{ART_m^2}{\beta E_a}\right)$$

where y – mass fraction of undecomposed substance of the test sample;
E_a – apparent activation energy;
R – universal gas constant;
T – temperature;
A – pre-exponential factor (frequency factor);
T_m – temperature corresponding to the maximum on the DTG curve;
β – heating rate [9].

In recent years, the Broido method has been widely used to calculate the kinetic characteristics of thermal degradation proceeding according to the first-order reaction mechanism of cellulose, wood, and other materials [10–11]. It follows from the Broido equation that the activation energy E_a is determined from the slope of the straight line plotted in the coordinates: ln [ln(1/y)]–1/T.

3 Research results

Using the method of thermal analysis, it is possible to evaluate the effect of the biodegradation process on the plant substrate. The analysis makes it possible to obtain physicochemical indicators of the weight loss of the substrate at individual stages of thermal destruction, temperature intervals of individual stages, the rate of thermal decomposition, and the magnitude of thermal effects in the studied temperature range.

Figure 1 shows the TG and DTG curves for the thermal decomposition of p.e.o. poplar buds before and after bioconversion.

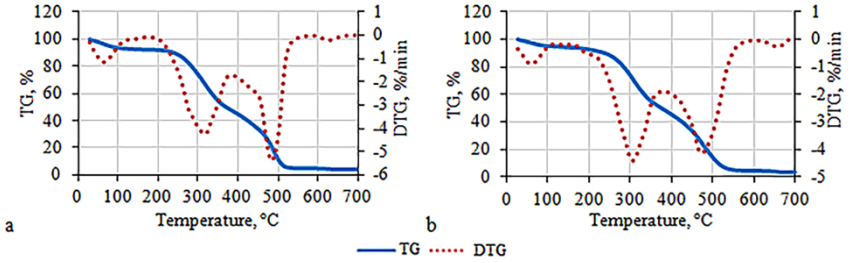
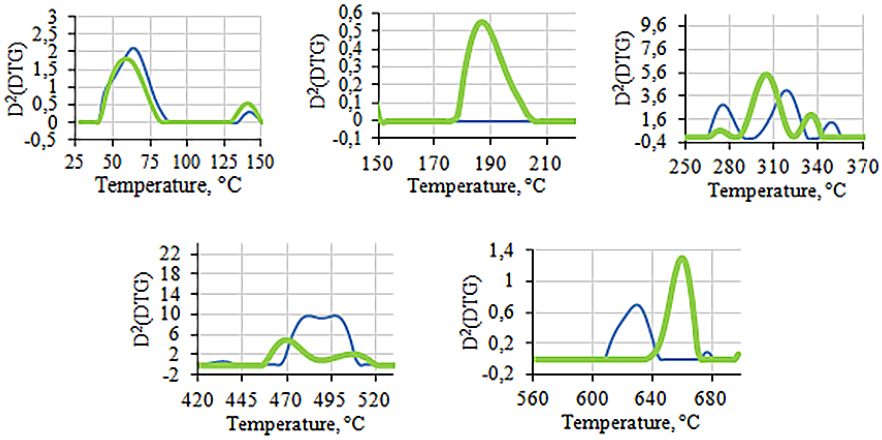


Fig. 1. TG, DTG curves of the thermal decomposition of the substrate before (a) and after (b) bioconversion.

The type of TG and DTG curves and, as a result, the values of the kinetic parameters of thermal destruction are determined by the content of extractives, hemicelluloses, cellulose, and lignin in the substrate under study.

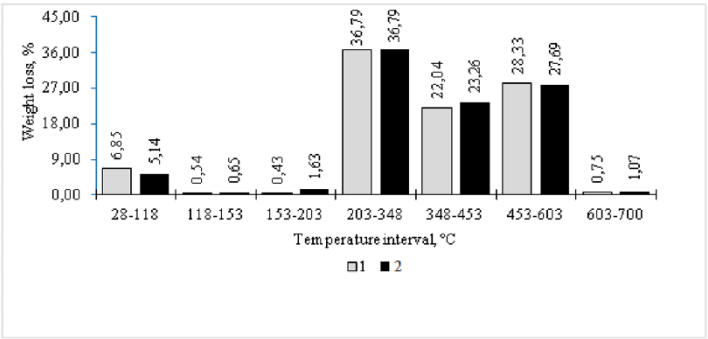
It follows from the results of the analysis that the biodegradation of the substrate under the action of Fp5-15 fungi *F. pinicola* leads to a change in the width and amplitude of the DTG peaks, as well as to a shift in the temperature maxima t_{max}.

Figure 2 shows the second derivative of the DTG contour with respect to the s.e.o. temperature. poplar buds before and after bioconversion. The dependence of the weight loss of the substrate before and after biodegradation on temperature is shown in Figure 3.



1 – substrate before biodegradation; 2 – substrate after biodegradation

Fig. 2. The second derivative of the DTG contour with respect to temperature in various temperature ranges.



1 – substrate before biodegradation; 2 – substrate after biodegradation

Fig. 3. Weight loss of substrates.

At the first stage of heating the samples (28-118°C), they are dried and volatile components are removed. The change in the weight of the samples is 5-7% (Figure 3). With a further increase in temperature to 348 °C, a large weight loss is observed, in this area polysaccharides (oligosaccharides, starch, cellulose, hemicellulose) and partially lignin undergo changes, the weight loss of the samples is 37%. In the temperature range from 348-453 °C, the thermal decomposition of lignin is completed and the formed coal is burned. The proportion of the residual mass of substrates before and after biodegradation is 4.27 and 3.75%, respectively.

It was found that the weight loss profiles of the substrate components before and after biodegradation are very similar (Figure 3). This is due to the fact that along with the depolymerization of polysaccharides (cellulose and hemicelluloses), as well as the demethylation of lignin, occurring under the action of cellulolytic enzymes of the fungus Fp5-15 *F. pinicola*, the accumulation of fungal biomass occurs, which contains protein (up to 24%) and glucans. It is known that thermal degradation of α-glucan side chains occurs in the temperature range of 120–250°C [12, 13]. The main amino acids of the Fp5-15 protein of *F. pinicola* are asparagine (19.4%), alanine (15.4%), and glycine (14.9%). Amino acids, polysaccharides (except cellulose), some polyphenols and proteins decompose in the temperature range from 170 to ~310 °C. In this temperature range, the DTG profile is a superposition of overlapping DTG peaks of individual compounds, as well as in the range from 400 to 600°C. Visually differentiating by thermal characteristics (hence, by the difference in chemical composition) substrate samples before bioconversion and after fungal transformation, allows the second derivatives of DTG by temperature, which are shown in Figure 2.

Table 1 shows the general trend of change in the activation energy E_a of thermal decomposition for the substrate before and after biodegradation, calculated using the Broido kinetic model.

Table 1. Activation energy of thermal decomposition of the substrate before and after bioconversion.

Sample	Temperature interval, °C	Activation energy, kJ/mol
Substrate before bioconversion	178-378	94.0
	398-573	137.3
Bioconversion product	173-203	160.2
	208-378	71.7
	398-593	113.9

4 Conclusion

Thus it was found that the microbiological conversion of solid post-extraction residues of poplar buds by fungi of the Fp5-15 genus *F. pinicola* leads to a change in the temperature and amplitude of the peaks of the DTG curves of the thermal destruction of polysaccharides and lignin substances. During the study of the biodegradation product, a peak was found in the temperature range of 120-250 °C, which is due to the fact that in the process of cultivation a protein biomass of the fungus is formed, the cell walls of which contain glucans. The data obtained by thermal and chemical analysis are consistent with each other.

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References

1. N. E. Kostina, Zh. B. Ibragimova, M. A. Protsenko, Modern problems of science and education **2**, 25-26 (2014)
2. A. Chlebicki, V. A. Mukhin, N. Ushakova, A. Chlebicki, Mycologist **17**, 116-120 (2003)
3. Yu. A. Litovka, I. N. Pavlov, T. V. Ryazanova, A. V. Gazizulina, N. A. Chuprova, Chemistry of plant raw materials **1**, 193-199 (2018)
4. I. N. Pavlov, Yu. A. Litovka, E. A. Litvinova, S. M. Petrenko, Chemistry of plant raw materials **4**, 385-394 (2020)
5. Yu. A. Litovka, I. N. Pavlov, P. V. Makolova, A. A. Timofeev, E. A. Litvinova, A. A. Vasil'eva, A. V. Shabanov, Chemistry of plant raw materials **4**, 371-383 (2020)
6. O. O. Mamaeva, E. V. Isaeva, Conifers of the boreal zone **38(5-6),** 259-264 (2020)
7. O. O. Mamaeva, I. V. Lekontseva, E. V. Isaeva, *Biodegradation of the post-extraction residue of poplar buds with the Siberian strain Fp5-15 Fomitopsis pinicola New achievements in chemistry and chemical technology of plant raw materials* Proceedings of the VIII All-Russian Conference with international participation (Barnaul, October 05-09, 2020) Ed N G Bazarnova and V I Markin (Barnaul: Altai State University) 299-300 (2020)
8. T. V. Ryazanova, N. A. Chuprova, E. V. Isaeva, *Chemistry of wood* (LAP Lambert Academic Publishing GmbH & Co.KG, Germany, 2012)
9. U. Wendlandt, *Thermal methods of analysis* (Mir, Moscow, 1978)
10. O. Yu. Fetisova, N. M. Mikova, N. V. Chesnokov, Kinetics and Catalysis **60**, 3 (2019)
11. E. A. Tyutkova, O. A. Shapchenkova, S. R. Loskutov, Chemistry of plant raw materials **2**, 89-100 (2017). https://doi.org/10.14258/jcprm.2017021389
12. K. Werner, L. Pommer, M. Brostro, J Anal Appl Pyrol **110**, 130-137 (2014). https://doi.org/10.1016/j.jaap.2014.08.013
13. C. Girometta, D. Dondi, R. M. Baiguera, F. Bracco, D. S. Branciforti, S. Buratti, S. Lazzaroni, E. Savino, Cellulose **27**, 6133-6148 (2020). https://doi.org/10.1007/s10570-020-03208-4