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# Use of Post-Extraction Fir Wood Greenery Residues by the Bioconversion Method with the Production of Feed Additives

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**Abstract:** The effectiveness of forest resources depends on the comprehensiveness and rationality of their consumption and processing into finished products. This article discusses the problem of using solid fir wood greenery residues generated during the industrial production of essential oils. Bioconversion is considered to be the most promising use. The objective of this research was to study the chemical composition of bioconversion products of fir wood greenery-based substrates. The PP-3.2 strain of *Pleurotus pulmonarius* (Fr.) Quél was used as a biodestructor. In the process of bioconversion, the contents of polysaccharides and lignin substances is reduced to 38% and 28%, respectively. Up to 20% of protein accumulates in bioconversion products of fir wood greenery. The amount of nucleic acids is not more than 1.5 g per kg; the contents of heavy metals, such as mercury, cadmium, arsenic, and lead, do not exceed the maximum permissible concentration standards. The substrate weight loss reaches 15%. When fallen leaves and post-extraction poplar bud residues are added to the substrate, the substrate-destroying activity of fungi increases, and the protein content increases by 3%. The digestibility of products as a result of bioconversion increases 1.6–2.8 times depending on the substrate composition. The obtained data support the recommendation of post-fermented substrates based on fir wood greenery and balsam poplar biomass for use as a protein feed additive.

**Keywords:** post-extraction residues; fir wood greenery; bioconversion; protein feed additive; *Pleurotus pulmonarius*; chemical composition



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## 1. Introduction

Forests are distributed around the globe and are the dominant terrestrial ecosystem on the Earth (31% of the global land area). The global total forest area is now 4.06 billion hectares. According to the Food and Agriculture Organization of the United Nations (FAO, 2020), more than half (54%) of the world's forests are located in five countries: Russia (815), Brazil (497), Canada (347), the United States (310), and China (220 million hectares) [1].

In Russia, the Krasnoyarsk Territory is one of the leading forest regions, with 69.3% of its land area covered with forests. The forest area is 20.3% of the country's total forest fund, i.e., 164 million hectares [2]. The main forest-forming species of the Krasnoyarsk Territory are coniferous trees. They occupy more than 75.9% of the region's forest-covered areas that amount to 9.7 billion $m^3$. Spruce–fir plantations account for only 16.0% (1.6 billion $m^3$) [2,3].

For coniferous species, the rate of annual deforestation in the Krasnoyarsk Territory is 48.9 million $m^3$, of which 8.3 million $m^3$ are spruce and fir trees. The total weight of wood used for further processing amounts to 75% of the total volume of harvested wood. The share of bark is 15% and that of wood greenery is 10–20%. Parts such as crowns, trunk parts, tops, boughs, stumps, roots, and wood greenery parts are left on a harvesting area as logging waste. The amount of this waste ranges from 30% to 50% of the total biomass [3–5]. Most of the logging waste is not removed or processed; therefore, it is a large-scale source of environmental pollution [3–7]. A number of authors have shown the possibility of using

logging waste as a source of thermal energy [8,9] and a raw material for fiberboards and other valuable materials [10–12].

Coniferous greenery formed as a result of logging is a promising raw material for obtaining valuable marketable products [7,13]. Its chemical processing results in a number of products, including chlorophyll–carotene paste, sodium chlorophyllin, conifer saline extract, conifer therapeutic extract, and conifer wax [13–17].

Fir wood greenery contains a set of substances notable for their high biological activity. On an industrial scale, it is traditionally used to extract essential oils [18–20]. Various extracts containing a wide range of chemical compounds are obtained from fir wood greenery: from organic acids and microelements to flavonoid compounds and polyphenol complexes [13,21–26].

After obtaining extractive substances, fir wood greenery wastes are used to produce vitamin flour and protein–vitamin concentrates, which are important feed additives in the diet of farm animals [27–29]. Post-extraction fir wood greenery residues can be used as a substrate for bioconversion [30–32]. Strong wood-destroying fungi belonging to the class basidiomycetes are promising producers for the conversion of lignocellulosic plant raw materials [33–35]. Among wood-destroying basidiomycetes, representatives of the *Pleurotus* genus are notable. Oyster mushroom has a strong enzymatic system (cellulases and oxidases) that is able to deconjugate cellulose and lignin, while causing white rots of wood. The mushrooms of the *Pleurotus* genus are edible, non-toxic, and nonpathogenic, and rich in digestible protein [36]. These properties enable the use of mushrooms of the *Pleurotus* genus as a destroyer of vegetable wastes to produce both fruit bodies and protein feed products [37–41].

Other works [37–45] showed the possibility of using mushrooms of the *Pleurotus* genus for the biodestruction of vegetable wastes; for example, straw [36], wheat bran particles [43], sugarcane [44], cabbage vine [45], etc. However, the use of mushrooms of the *Pleurotus* genus, namely *Pleurotus pulmonarius* (Fr.) Quél, as a biodestructor of fir wood greenery-based substrates has not yet been studied.

The objective of this research was to study the componential composition of products obtained during the biodestruction of fir wood greenery-based substrates with the PP-3.2 strain of *Pleurotus pulmonarius* (Fr.) Quél. The use of bioconversion for the use of industrial wastes generated during the production of essential oils is a promising area using technology for obtaining protein feed products.

## 2. Materials and Methods

As a biodestructor, we used basidial mushrooms of the *Pleurotus pulmonarius* (Fr.) Quél (PP-3.2 strain). This strain is stored in the museum collection of cultures of the Department of Chemical Technology of Wood and Biotechnology of Reshetnev Siberian State University, Krasnoyarsk, Russia. The strain was isolated in a pure culture from commercial fruit bodies and is not pathogenic [38].

Fir wood greenery was the base of the substrates used for bioconversion. The fir wood greenery included branches covered with fir needles with a diameter of no more than 8 mm at their bottom. The fir wood greenery residues used in this work were obtained as a result of extracting essential oils by hydrodistillation using a small-sized pilot plant developed by Reshetnev Siberian State University [46]. The following additives were added to the mixed substrates: buds and fallen leaves of *Populus balsamifera* L., which were an additional source of carbohydrates, microelements, and protein substances necessary for the growth of microorganisms [47,48]. The added poplar buds gave looseness to the substrate. We used solid poplar bud residues after the removal of essential oils by hydrodistillation [47]. The buds were sampled in April from the trees growing in the vicinity of Krasnoyarsk, and the fallen leaves were sampled in September.

To prepare the substrate for biochemical processing, alcohol-soluble components were additionally extracted from the fir wood greenery and poplar bud residues, as such components have separate uses [13,49,50].

We used the following substrates: post-extraction residues (PER) of fir wood greenery as substrate 1; mixed substrate of PER of fir wood greenery, poplar bud, and fallen leaves (fall) in a 1:1:1 ratio by weight as substrate 2; mixed substrate of PER of fir wood greenery and poplar bud in a 1:1 ratio by weight as substrate 3.

To conduct the bioconversion process, the substrates were ground to 2–5 mm, moistened with water to 70%, and repeatedly sterilized in an autoclave under a pressure of $1.01 \times 10^5$ MPa. The cultivation was performed following the solid-phase technique in Petri dishes. The strain was cultivated with blocks of 14 mm in diameter. The cultivation process lasted for 15 days.

The chemical composition of the substrates and biodestruction products was studied using the methods adopted for the chemistry of raw plant materials [51].

The total moisture content of the studied samples was determined by the thermogravimetric method. The substrates were dried at 105 °C before and after biodestruction until moisture was completely removed. Alcohol- and water-soluble substances were isolated by 3 h extraction with ethyl alcohol and hot water, respectively. The easily hydrolyzable polysaccharides were hydrolyzed by boiling with 2% hydrochloric acid for three hours, and hardly hydrolysable polysaccharides with 80% sulfuric acid at room temperature for two hours. The amount of monosaccharides was determined by the ebuliostatic method. The amount of non-hydrolysable residues (lignin substances) was determined gravimetrically. The content of mineral substances (ashes) was determined by burning a suspension of plant material followed by calcining ashes in a muffle furnace [51].

The Spirin method was used to evaluate the content of nucleic acids. The method is based on measuring the difference in the optical density of hydrolysates in the ultraviolet area of the spectrum, which characterizes the content of nucleic acid phosphorus. Based on measuring the optical density of solutions at 270 nm (maximum nucleic acid uptake) and 290 nm (maximum impurity uptake), the nucleic acid content was determined [52].

The vitamin $B_1$ (thiamine) content was determined by titrating the 0.1 N NaOH solutions in the presence of the 1% brom-blue solution indicator [53]. The method of quantitative determination of $B_2$ (riboflavin) and its oxidized and reduced forms in plant tissues is based on the spectrophotometric method of riboflavin analysis in plants [54]. The optical density of the solutions was determined using an SF-26 spectrophotometer with a wavelength of 445 nm. The total content of reduced and oxidized riboflavin was found based on a calibration curve, and the resulting difference was considered when calculating reduced riboflavin.

The quantitative determination of the vitamin P (rutin) content is based on its ability to be oxidized by potassium permanganate. Indigo carmine, which reacts with potassium permanganate after rutin has completely oxidized, was used as an indicator [53]. The ascorbic acid content in the substrates was determined by the iodometric method [53].

The digestibility of biodestruction products was determined according to the method of Zhukov [55]. This method is based on the ability of a chlorophenol mixture to dissolve organic substances of a product under study to the same extent as in the stomach of an animal.

The mass concentration of elements in the substrate before and after biodestruction was determined using a mass spectrometer with an inductively coupled plasma (Agilent Technologies, Santa Clara, CA, USA). This method is based on the use of ions as a source and a mass spectrometer for their separation and detection. The samples were preliminarily mineralized using concentrated nitric and sulfuric acids.

The experiments were conducted in three replicates. The statistical processing of results was performed according to standard procedures [56]. The obtained results were considered significant within the limits of confidence probability ($p = 0.95$).

## 3. Results and Discussion

To assess the effects of the PP-3.2 strain of *P. pulmonarius* on the plant substrates, the chemical composition of the initial raw materials (Table 1) was studied. The contents of the components were calculated per unit of absolutely dry raw materials.

**Table 1.** Componential composition of the substrates prior to biodestruction.

| Component | Content, % a.d.m. * | | |
|---|---|---|---|
| | Substrate 1 | Substrate 2 | Substrate 3 |
| Substances extracted by hot water | 12.5 | 13.5 | 14.9 |
| Substances extracted by ethyl alcohol | 3.6 | 2.5 | 2.2 |
| Easily hydrolyzable polysaccharides | 17.8 | 12.1 | 14.2 |
| Hardly hydrolyzable polysaccharides | 23.8 | 17.9 | 19.5 |
| Lignin substances | 31.8 | 43.4 | 34.5 |
| Mineral substances | 5.9 | 9.2 | 9.0 |

* Absolutely dry raw materials.

The results showed that the substrates were a lignin–carbohydrate complex, with 30% to 42% attributed to polysaccharides. Carbohydrates in the plant substrates were mainly represented by hardly hydrolyzable polysaccharides, which accounted for 18% to 24%. The substrates contained extractive substances from 16% to 17%, of which 78–87% were water-soluble substances.

The content of lignin substances in substrate 2 was 1.3–1.4 times higher than in the other substrates. The increased content of lignin substances in substrate 2 is explained by the extractive substances from the residual green fir and poplar buds after distillation of essential oils being not completely removed during the preparation of the substrate, according to the method in [51].

Notably, the fir wood greenery residues after the removal of alcohol-extractive substances and essential oils were characterized by a high content of polysaccharides, up to 42%, which was 1.2–1.4 times higher than in other substrates.

Carbohydrates and lignin can be a source of carbon for fungal feeding. The development of fungi requires the presence of macro- and microelement sources in the medium, which stimulate their growth. Table 2 shows the quantitative contents of certain minerals in the separate substrate components.

Table 2 shows that the main microelements of the substrates included zinc, iron, manganese, iodine, and aluminum. Microelements such as copper, iron, manganese, molybdenum, and zinc are known to be associated with enzyme production and the decomposition of the lignin–carbohydrate complex [57].

The contents of macroelements such as magnesium, sulfur, and potassium in the fallen leaves were higher than in the PER of fir wood greenery and poplar bud. Therefore, when poplar biomass is added to the PER of fir wood greenery, the contents of macroelements required for the growth and development of fungi increase. A high content of sulfur in substrates will contribute to the development of fungi, since fungi develop well in slightly acidic environments. Sulfur is also associated with stimulating proteolytic enzymes.

The contents of lead (0.009–0.02 μg/kg), mercury (0.002 μg/kg), cadmium (0.001–0.01 μg/kg), arsenic (0.003–0.03 μg/kg), and nickel (0.02–0.05 μg/kg) in the raw materials did not exceed maximum permissible concentrations [58].

Some vitamins, such as rutin, thiamine, riboflavin, and ascorbic acid, were identified in the substrates before cultivation. Rutin is a complex of bioflavonoids with a high antioxidant activity. In plants, it is contained in the form of glycosides, providing protection against ultraviolet radiation and contributing to the accumulation of vitamin C. The content of vitamin P in substrates 1 and 3 was 0.3–0.4 mg%, and 0.7 mg% in substrate 2.

**Table 2.** Mineral content in the initial raw materials.

| Name | Element Content, mg/kg | | |
|---|---|---|---|
| | PER * of Fir Wood Greenery Residues | Fallen Poplar Leaves | PER of Poplar Buds |
| Sodium | 0.4 | 0.6 | 0.9 |
| Magnesium | 1.9 | 6.6 | 3.3 |
| Phosphorus | 1.5 | 2.1 | 2.5 |
| Sulfur | 366.8 | 691.9 | 483.4 |
| Chlorine | 178.2 | 55.7 | 28.3 |
| Potassium | 6.3 | 10.4 | 5.6 |
| Calcium | <1.0 | <2.1 | <2.1 |
| Iron | 0.7 | 0.9 | 1.6 |
| Aluminum | 0.3 | 0.3 | 0.4 |
| Chromium | 0.04 | 0.03 | 0.04 |
| Manganese | 1.1 | 0.4 | 0.1 |
| Cobalt | 0.001 | 0.007 | 0.003 |
| Copper | 0.03 | 0.1 | 0.09 |
| Zinc | 0.4 | 2.3 | 4.6 |
| Selenium | <0.02 | <0.04 | <0.04 |
| Bromine | 0.02 | 0.04 | 0.3 |
| Strontium | 0.08 | 0.1 | 0.1 |
| Molybdenum | 0.001 | 0.006 | 0.004 |
| Iodine | 0.2 | 0.1 | 0.08 |

* Post-extraction residues.

Vitamins B and C are essential for the growth, development, and reproduction of fungi. As illustrated by the example of the oyster mushroom (*Pleurotus ostreatus*), a reliable increase occurs in growth rates when vitamin B1 is added [59]. We found that substrates 1 and 2 contained approximately the same amount of vitamin B1, 4.7–4.5 mg%, and in substrate 3, the vitamin content was less, 3.6 mg%. The amount of vitamin B2 in substrate 1 was 0.03 mg%, while it was 0.3 mg% in substrate 2, and 0.5 mg% in substrate 3.

The vitamin C content in substrates 1 and 3 was 0.7 mg%; in substrate 2, its content was 1.0 mg%. A need for ascorbic acid in fungi is not recognized, although vitamin C is found in the mycelium of many fungi and stimulates their fructification [59].

Therefore, the substrates based on the PER of fir wood greenery, PER of poplar buds, and fallen leaves are favorable for the solid-phase cultivation of the PP-3.2 strain of *P. pulmonarius* and can serve as sources of biogenic elements, microelements, and vitamins for fungi.

When the PP-3.2 strain of *P. pulmonarius* was cultivated, the radial growth rate on the PER of fir wood greenery was 2.8 mm/day; with PER of poplar buds added to the substrate, it was 3.8 mm/day; with fallen poplar leaves, it was 4.3 mm/day [30].

The results regarding the effects of the enzymic complex of the studied strain on plant substrates were estimated by changing the contents of the main polysaccharidic and lignin components (Table 3). To compare the chemical composition of the biodestroyed substrate to that of the initial one, the obtained data were recalculated considering the weight loss coefficient during fungal cultivation. The weight loss of substrates 1, 2, and 3 were 7.3%, 15.4%, and 11.6%, respectively.

When the PP-3.2 strain of *P. pulmonarius* was cultivated on substrate 2, lignin substances were maximally used. In the course of bioconversion, their content decreased by 28%. This can be explained by the high phenol oxidase activity of the strain when cultivated on the mixed substrate. The lignin of poplar buds and leaves is less methoxylated compared to that of wood and is close in its composition to that of herbaceous plants. The total of polysaccharides decreased by only 8%.

**Table 3.** Componential composition of the biodestroyed substrates.

| Component | Content, % a.d.m. | | |
|---|---|---|---|
| | Substrate 1 | Substrate 2 | Substrate 3 |
| Substances extracted by hot water | 18.6 | 10.8 | 13.9 |
| Substances extracted by ethyl alcohol | 3.3 | 2.3 | 2.1 |
| Easily hydrolysable polysaccharides | 9.6 | 11.8 | 11.7 |
| Hardly hydrolysable polysaccharides | 16.1 | 15.9 | 14.1 |
| Lignin substances | 37.2 | 31.2 | 34.7 |
| Mineral substances | 7.7 | 7.8 | 8.7 |

Alternatively, in the case of substrates 1 and 3, we observed an increase in lignin substances after biodestruction. We assumed that the weakening link of the lignin–carbohydrate complex through the destruction of celluloses plays an important role in the process of bioconversion of substrates. Therefore, the relative proportion of lignin increases [60] after carbohydrate use. The total polysaccharides in substrates 1 and 3 decreased by 38% and 23%, respectively.

In the process of bioconversion, the content of extractive substances also changes. For substrates 2 and 3, the proportion of extractive substances decreased by 18% and 6%, respectively. The total extractive substances increased (by 36%) against a decrease in the remaining substrate components during the cultivation of the PP-3.2 strain of *P. pulmonarius* on post-extraction fir wood greenery residues.

Therefore, the bioconversion process differs depending on the composition of the substrates. For substrate 1, the fungus destroyed polysaccharides, which resulted in an increase in water-extractive substances. When fallen leaves were added (substrate 2), the direction of conversion changed, as easily digestible carbohydrates were introduced with a water-extractive fraction. Therefore, the content of water-extractive substances firstly decreases in the process of biodestruction. When fallen leaves are added, the degree of substrate bioconversion changes: the weight loss increases 2.1 times compared to substrate 1 and 1.3 times compared to substrate 3.

In the course of *P. pulmonarius* PP-3.2 strain cultivation, the protein content in plant substrates increased approximately 2.5 times irrespective of the substrate composition. The largest amount of protein accumulated on substrate 2 (23%); on substrates 1 and 3, it was 20%.

To further assess the feeding value of plant raw material bioconversion products, we determined that they contain some vitamins that have high physiological activity and are not synthesized in a sufficient amount in the animal body.

During biodestruction, we observed a sharp decrease (70 times) in vitamin P (substrate 2), which was 30–40 times that for substrates 1 and 3. This occurred due to rutin being a glycoside combining the flavonol quercetin and the disaccharide rutinose. Its structure carries carbohydrates required by fungi for normal growth [59,61]. The thiamine content in the substrates also decreased (1.2–1.3 times), as basidiomycetes most often need this vitamin for growth and development [59]. Animals' need for thiamine is affected by their age, species, diet, and physiological state [62].

During cultivation, the content of vitamin B2 increases as fungi are able to synthesize it. So, the vitamin amount increased 4–7 times in substrates 2 and 3, and 40 times in substrate 1. In animal organisms, riboflavin is involved in the transformation of amino acids, from which protein is built [61]. When lacking riboflavin, the intensity of tissue respiration decreases, growth is delayed, and resistance to infectious dermatoses decreases [62].

During biodestruction, the ascorbic acid content increases 1.2–3.4 times compared to initial substrates. The fungi can synthesize vitamin C through the xylulose pathway of carbohydrate metabolism through intermediate stages of glucuronic and gluonic acids [59]. Vitamin C is involved in the formation of collagen in the reactions of hydroxylation of amino acids (proline and lysine). However, the vitamin consumed with animal feed is destroyed in the rumen, but its synthesis occurs in the liver [62].

When assessing the suitability of products obtained during the bioconversion process, the mineral composition of ashes was studied as a feed additive. Mineral substances do not have any energetic and carbohydrate nutritional value, but they play an important role in the feeding of farm animals. Their characteristic feature is that they are not synthesized in living organisms and must regularly be consumed with feedstuff and water. In addition, most essential macro- and microelements cannot be accumulated in animals, even with high contents in the external environment [62,63]. The main macro- and microelements required for the evaluation of protein feed additives are shown in Table 4.

**Table 4.** Elemental composition of the substrate ashes after bioconversion.

| Name | Element Content, mg/kg | | |
|---|---|---|---|
| | **Substrate 1** | **Substrate 2** | **Substrate 3** |
| Sodium | 0.6 | 0.7 | 1.3 |
| Magnesium | 2.7 | 5.8 | 3.1 |
| Phosphorus | 1.7 | 4.1 | 3.4 |
| Sulfur | 462.7 | 747.8 | 536.8 |
| Chlorine | 552.2 | 136.2 | 47.9 |
| Potassium | 8.1 | 12.9 | 10.1 |
| Calcium | 1.6 | <2.1 | <2.1 |
| Iron | 1.0 | 1.3 | 0.9 |
| Aluminum | 0.4 | 0.3 | 0.2 |
| Chromium | 0.05 | 0.04 | 0.03 |
| Manganese | 1.4 | 1.1 | 1.1 |
| Cobalt | 0.002 | 0.003 | 0.001 |
| Copper | 0.05 | 0.6 | 0.09 |
| Zinc | 0.6 | 1.7 | 0.7 |
| Selenium | <0.02 | <0.04 | <0.04 |
| Bromine | 0.02 | 0.04 | 0.1 |
| Iodine | 0.5 | 0.1 | 0.06 |

Microelements such as copper, zinc, iron, manganese, and iodine are most important for animal metabolism. They are associated with enzymes, hormones, and vitamins. Of the macroelements, phosphorus, calcium, potassium, magnesium, sulfur, and sodium [62,63] are of greatest importance for animals.

In terms of the content of sulfur included in glutathione, insulin, and other substances, all substrates after biodestruction correspond to such feedstuffs as corn (0.4 g/kg), meadow grass (0.8 g/kg), spring wheat (0.5 g/kg), etc. [64].

The chlorine content in bioconversion products corresponds to, for example, that of feedstuffs such as oat hay and corn [64].

By analyzing these tables, we observed the highest copper and zinc contents in substrate 2. In the biodestruction process of substrates 1 and 2, the iron content increased 1.4 times. In terms of the copper content, substrate 2 was comparable to corn and exceeded winter rye (7.5 times); its zinc content exceeded that of waxy corn by 1.9 times [64].

In terms of the iodine content, the biodestroyed product obtained from substrate 1 exceeded that of feedstuffs such as clover hay and pea straw (1.7 times) and corn (17 times) [64].

An important parameter used to assess the quality of the obtained feed additives is their safety in terms of the content of heavy metals, which may be toxic for animals and may reduce the nutritional value of feed products [63,65]. According to veterinary and sanitary standards and requirements for the quality of animal feed [58], the contents of mercury, cadmium, arsenic, lead, copper, and zinc in the bioconversion products did not exceed the maximum permissible concentrations.

One more important parameter used to assess the quality of resultant feed additives is their digestibility. We found that the digestibility of products before cultivation was 23.4% (substrate 1), 33.7% (substrate 2), and 32.0% (substrate 3). In the process of destruction, the

digestibility index increased 1.6–2.8 times. The highest digestibility was observed for the product of post-extraction fir wood greenery residues (substrate 1), which amounted to 66.0%. The obtained parameters correspond to digestibility standards applicable to the dry feed matter [66].

The content of nucleic acids per 1 kg of dry matter in the diet of animals should not exceed 9 g as they, when in excess, are dangerous for animals [66]. We found that the content of nucleic acids in biodestroyed substrates was not more than 1.5 g per 1 kg of the product.

Coniferous feed flour is the official feed additive that is currently used in Russia. The products obtained in the process of bioconversion of substrates based on the post-extraction fir wood greenery residues according to their characteristics correspond to TS (technical specifications) 477-15-147-80 "Coniferous feed flour": humidity, 8–12%; mass fraction of fiber, not more than 30%; digestibility, not less than 30%.

The product obtained as a result of bioconversion was 2–4 times higher in protein content than coniferous feed flour (6–12%). Additionally, the bioconversion product contained a low amount of nucleic acids—no more than 0.2%—while coniferous flour contains up to 12%.

From the results of analysis of the chemical composition of substrates after bioconversion based on fir wood greenery—including the quantitative protein content, the low nucleic acid content, and the absence of heavy metals—the bioconversion products of plant substrates are recommended for use as protein feed additives.

## 4. Conclusions

In this study, we established the componential composition of the bioconversion products obtained in the process of cultivation of the PP-3.2 strain of *Pleurotus pulmonarius* on substrates formed from fir wood greenery. During bioconversion on post-extraction fir wood greenery residues, the fungus used up to 38% of the polysaccharides. When post-extraction poplar bud and fallen leave residues were added to the substrate, lignin and extractive substances decreased by 28% and 18%, respectively.

The pretreatment of substrates with *P. pulmonarius* strain PP-3.2 is an advantageous option not only for reducing the content of lignin substances in lignocellulosic wastes (fir wood greenery), but also for increasing their protein content (up to 20%) due to the growth of fungal biomass. When cultivated on a mixed substrate formed from the PER of fir wood greenery, poplar buds, and fallen leaves, the protein content in the substrate increased to 23%, and the weight loss increased to 15% (two times higher than on fir wood greenery).

The product digestibility was 54–66%; the content of nucleic acids was not more than 1.5 g per 1 kg of the product. The content of heavy metals in the bioconversion products did not exceed the maximum permissible concentration standards.

Therefore, the results demonstrate the possibility of using post-extraction fir wood greenery residues both as an independent substrate and in combination with the poplar biomass for bioconversion by *P. pulmonarius* (PP-3.2) mushrooms to produce protein feed additives for farm animals.

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## References

1. FAO. *Global Forest Resources Assessment 2020–Key Findings*; FAO: Rome, Italy, 2020; Available online: http://www.fao.org/documents/card/en/c/ca8753en (accessed on 15 November 2020).
2. Decree of the Governor of the Krasnoyarsk Territory No. 332-ug Dated 21 December 2018 "On the Approval of the Forest Plan of the Krasnoyarsk Territory" Introd. 01 January 2019. Krasnoyarsk. 2018, p. 73. Available online: http://docs.cntd.ru/document/550303431 (accessed on 15 November 2020). (In Russian).
3. Morozov, V.I.; Petrusheva, N.A. The Use of Potential Wood Greenery Resources of Coniferous Forests of the Krasnoyarsk Territory. *Tendentsii Razvitiya Nauki i Obrazovaniya [Trends in the Development of Science and Education]* **2019**, *50–2*, 38–41. (In Russian)
4. Gubanenko, G.A.; Rechkina, E.A. Influence of pectin of wood greens of scots pine on the quality of wheat flour gluten. In Proceedings of the International Scientific and Practical Conference Technologies of Food Production and Examination of Goods, Kursk, Russia, 2–3 April 2015; pp. 58–60. (In Russian).
5. Chistova, N.G.; Mironov, G.S. *The Integrated Use of Wood*; SibGTU: Krasnoyarsk, Russia, 2011; p. 226. ISBN 978-5-8173-0503-6.
6. Korpachev, V.P.; Peregrin, A.I.; Andrias, A.A.; Vladykin, E.A.; Sukhov, A.I. Potential of unclaimed resources of wood raw materials for bioenergy. *Khvoinye Borealnoi Zony [Conifers of the Boreal Zone]* **2019**, *37*, 295–300. (In Russian)
7. Forest Plan of the Krasnoyarsk Territory, Krasnoyarsk, Russia. 2009, p. 210. Available online: http://docs.cntd.ru/document/422436834 (accessed on 15 November 2020). (In Russian).
8. Nurek, T.; Gendek, A.; Roman, K. Forest Residues as a Renewable Source of Energy: Elemental Composition and Physical Properties. *BioResources* **2019**, *14*, 6–20. [CrossRef]
9. Nurek, T.; Gendek, A.; Roman, K.; Dąbrowska, M. The effect of temperature and moisture on the chosen parameters of briquettes made of shredded logging residues. *Biomass Bioenergy* **2019**, *130*, 105368. [CrossRef]
10. Khanturgaev, A.G.; Shiretorova, V.G.; Kotova, T.I.; Khanturgaeva, G.I.; Zalutsky, A.V.; Alekseev, G.T. Method for Obtaining Decorative Products from Coniferous Tree Waste. RU Patent 2442697 C1, 20 February 2012. (In Russian).
11. Afanasyev, A.S.; Kiseleva, I.G. Modern construction materials based on softwood logging waste. In *Problems of Economics and Construction Management in the Conditions of Environmentally Oriented Development, Proceedings of the Fifth International Scientific and Practical Online Conference, Tomsk, Russia, 12–13 April 2018*; Tomsk State University of Architecture and Civil Engineering: Tomsk, Russia, 2018; pp. 45–48. (In Russian)
12. Alberg, N.I.; Sanzhieva, S.E.; Salhofer, S. *Integrated Sustainable Waste Management. Woodworking and Pulp and Paper Industry*; Publishing House of the Academy of Natural Sciences: Moscow, Russia, 2016; pp. 162–218. ISBN 978-5-91327-432-8. (In Russian)
13. Ushanova, V.M. The Processing of Siberian Fir Wood Greenery and Bark with the Production of Biologically Active Products. *Khvoinye Borealnoi Zony [Conifers of the Boreal Zone]* **2013**, *31*, 138–142. (In Russian)
14. Nikitin, V.Y. The Method of Production of Chlorophyll-Carotene Paste. Patent No. SU 233165 A1, 25 January 1976. (In Russian).
15. Jēgina, S.; Kukle, S.; Šutka, A. Potential of Nanofibers Webs with Sodium Chlorophyllin for the Skin Care. In Proceedings of the 16th International Multidisciplinary Scientific GeoConference SGEM 2016, Vienna, Austria, 2–5 November 2016; pp. 127–134.
16. Uus, E.G.; Tearo, E.N.; Siirde, E.K.; Strizhak, V.I.; Vorotinsky, S.Y.; Kapten, V.I.; Augas, R.P.; Keerov, I.A. Method for Production of Conifer Saline Extracts. Patent No. SU 1279634 A1, 6 April 1986.
17. Popov, S.A.; Shevtsov, S.A.; Kozlova, L.P.; Klimenko, L.I.; Kolesnikova, I.V. Method of Fir Wood Greenery Processing with the Production of Chlorophyll-Carotene Paste, Fir Oil and Water Fir Extract. Patent No. RU 2530656 C1, 19 February 2013.
18. Stepen, R.A.; Repyakh, S.M. *Readily Volatile Terpenoids of Pine Forests*; Siberian State Technological University: Krasnoyarsk, Russia, 1998; p. 404. (In Russian)
19. Pankiv, O.G.; Miroshnichenko, V.V.; Parshikova, V.N.; Stepen, R.A. Yield and Composition of Fir Oil Extracted by Various Methods. *Khimiya Rastitelnogo Syriya [Chemistry of Plant Raw Materials]* **2009**, *3*, 95–98. ISSN 1029-5151. (In Russian)
20. Hmaied, M.; Bouafif, H.; Magdouli, S.; Braghiroli, F.L.; Koubaa, A. Effect of Forest Biomass Pretreatment on Essential Oil Yield and Properties. *Forests* **2019**, *10*, 1042. [CrossRef]
21. Kostesha, N.Y. *Siberian Fir (Abies Sibirica) Extracts and Their Use in Medicine: Clinical-Experimental Research*; "Polden" Publishing House: Tomsk, Russia, 1997; p. 152. ISBN 5-7137-0055-0. (In Russian)
22. Sidelnikova, V.N.; Patrushev, Y.V.; Sizova, N.V.; Petrenko, T.V. Comparative Analysis of the Composition of Fir Oil Produced by Water-Steam Distillation and of the Ethereal Oil Fraction of the $CO_2$ Extract of Siberian Fir Twigs. *Khimiya Rastitelnogo Syriya [Chemistry of Plant Raw Materials]* **2003**, *1*, 79–85. ISSN 1029-5151. (In Russian)
23. Kozlova, L.P.; Kukina, T.P.; Malykhin, E.V.; Popov, S.A.; Salnikova, O.I.; Chibiryaev, A.M. Extractive Substances of Florentine Water, Organic Composition of Hydrodistillated Ether Extracts of Fir Twigs. *Khimiya rastitelnogo syriya [Chemistry of Plant Raw Materials]* **2004**, *2*, 39–46. ISSN 1029-5151. (In Russian)
24. Khasanov, V.V.; Ryzhova, G.L.; Kuryaeva, T.T.; Dychko, K.A. Study of the Composition and Antioxidative Activity of Water-Steam Distillation Siberian Fir Products. *Khimiya Rastitelnogo Syriya [Chemistry of Plant Raw Materials]* **2009**, *4*, 83–88. ISSN 1029-5151. (In Russian)

25. Sirše, M.; Fokter, S.K.; Štrukelj, B.; Zupan, J. Silver Fir (*Abies alba* L.) Polyphenolic Extract Shows Beneficial Influence on Chondrogenesis In Vitro under Normal and Inflammatory Conditions. *Molecules* **2020**, *25*, 4616. [CrossRef]
26. Royer, M.; Houde, R.; Stevanovic, T. Non-wood forest products based on extractives—A new opportunity for Canadian forest industry Part 2—Softwood forest species. *J. Food Res.* **2013**, *2*, 164–189. [CrossRef]
27. Prytkov, Y.N.; Kistina, A.A. Use of Conifer-Carotene Additives in Egg Poultry Farming. *Agrarny Nauchny Zhurnal [Agricultural Scientific Journal]* **2016**, *8*, 52–55. ISSN 2313-8432. (In Russian)
28. Kirgintsev, B.O.; Belenkaya, A.E.; Yarmots, G.Y. Use of Needles in the Feeding of Farm Animals. In Proceedings of the Integration of Science and Practices for the Development of the Agro-Industrial Complex: All-Russian Scientific Conf, Tyumen, Russia, 10 November 2017; pp. 229–234. (In Russian).
29. Vorobyov, A.L.; Kalachev, A.L.; Zalesov, S.V. The Use of Logging Wastes as Raw Materials for the Production of Feed Additives. *Lesa Rossii i Khozyaistvo v Nikh [Forests of Russia and Related Economy]* **2018**, *3*, 65–72. ISSN 2218-7545. (In Russian)
30. Lekontseva, I.V.; Mamaeva, O.O.; Isaeva, E.V. Fir Wood Greenery as a Substrate for the Bioconversion of Basidial Fungi. *Khvoinye Borealnoi Zony [Conifers of the Boreal Zone]* **2019**, *37*, 265–270. ISSN 1993-0135. (In Russian)
31. Bondar, P.N.; Sadykova, V.S.; Ushanova, V.M.; Savitskaya, A.G. Biotechnological Aspects of Selection of the *Trichoderma* Strains for the Production of Preparations for Plant Protection. In Proceedings of the International Scientific and Practical Conference on Modern Means, Methods and Technologies for Plant Protection, Novosibirsk, Russia, 10–11 July 2008; pp. 30–34. (In Russian).
32. Zaika, N.A. Ecological-Biological Rationale for the Screening of Fungi of the *Trichoderma* genus for the Production and Use of Biological Products on Plant Substrates. Ph.D. Thesis, Siberian State Technological University, Krasnoyarsk, Russia, 2006. (In Russian).
33. Brischke, C.; Grünwald, L.K.; Bollmus, S. Effect of size and shape of specimens on the mass loss caused by *Coniophora puteana* in wood durability tests. *Eur. J. Wood Wood Prod.* **2020**, *78*, 811–819. [CrossRef]
34. Civardi, C.; Grolimund, D.; Schubert, M.; Wick, P.; Schwarze, F.W.M.R. Micronized copper-treated wood: Copper remobilization into spores from the copper-tolerant wood-destroying fungus Rhodonia placenta. *Environ. Sci. Nano* **2019**, *6*, 425–431. [CrossRef]
35. Litovka, Y.A.; Pavlov, I.N.; Ryazanova, T.V.; Gazizulina, A.V.; Chuprova, N.A. Wood-destroying properties of *Fomitopsis Pinicola* (SW.) P. Karst. from middle Siberia. *Khimiya Rastitelnogo Syrya [Chemistry of Plant Raw Materials]* **2018**, *1*, 193–199. (In Russian)
36. Solomko, E.F.; Sirenko, I.P.; Fedorov, O.A. *The Analysis of the Growth Kinetics of the Mycelium of the Higher Basidial Fungus of Pleurotus Ostreatus in the Deep Culture. Production of Higher Edible Fungi in the USS*; Naukova Dumka: Kiev, Ukraine, 1985; pp. 124–126. (In Russian)
37. Tarnopolskaya, V.V.; Alaudinova, E.V.; Mironov, P.V. Prospects for the Use of Basidial Fungi for the Production of Feed Products. *Khvoinye Borealnoi Zony [Conifers of the Boreal Zone]* **2016**, *37*, 338–341. ISSN 1993-0135. (In Russian)
38. Melnikova, E.A. The Processing Technology for Plant Biomass with the Production of Protein Feed Products. The Microbiological Conversion of *Pleurotus Pulmonarius*. Ph.D. Thesis, Siberian State Technological University, Krasnoyarsk, Russia, 2016. (In Russian).
39. Dhaliwal, R.P.S.; Garcha, H.S.; Khanna, P.K. Regulation of lignocellulotic enzyme system in *Pleurotus ostreatus*. *Indian J. Microbiol.* **1991**, *31*, 181–184.
40. Singh, M.P. Biodegradation of Lignocellulosic Wastes through Cultivation of *Pleurotus sajor-caju*. In Proceedings of the 15th International Congress on the Science and Cultivation of Edible Fungi, Maastricht, The Netherlands, 15–19 May 2000; pp. 517–521. Available online: https://www.researchgate.net/publication/236535751_Biodegradation_of_lignocellulosic_wastes_through_cultivation_of_Pleurotus_sajor-caju (accessed on 15 November 2020).
41. Van Kuijk, S.J.A.; Sonnenberg, A.S.M.; Baars, J.J.P.; Hendriks, W.H.; Cone, J.W. Fungal treated lignocellulosic biomass as ruminant feed ingredient: A review. *Biotechnol. Adv.* **2015**, *33*, 191–202. Available online: https://doi.org/10.1016/j.biotechadv.2014.10.014 (accessed on 15 November 2020). [CrossRef]
42. Pandey, V.K.; Singh, M.P. Biodegradation of wheat straw by *Pleurotus ostreatus*. *Cell. Mol. Biol.* **2014**, *60*, 29–34.
43. Marques de Souza, C.G.; Peralta, R.M. Purification and characterization of the main laccase produced by the white-rot fungus *Pleurotus pulmonarius* on wheat bran solid state medium. *J. Basic Microbiol.* **2003**, *43*, 278–286. [CrossRef]
44. Pandey, V.K.; Singh, M.P.; Srivastava, A.K.; Vishwakarma, S.K.; Takshak, S. Biodegradation of Sugarcane bagasseby *Pleurotus citrinopileatus*. *Cell. Mol. Biol.* **2012**, *58*, 8–14. [PubMed]
45. Singh, M.P.; Pandey, V.K.; Srivastava, A.K.; Vishwakarma, S.K. Biodegradation of Brassica haulms by white rot fungus *Pleurotus eryngii*. *Cell. Mol. Biol.* **2011**, *57*, 47–55. [PubMed]
46. Ryazanova, T.V.; Loskutov, S.R.; Aniskina, A.A. The Effect of Storage of Wood Green of Fir to the Composition of the Essential oil. In Proceedings of the International Conference on Scientific Research of the SCO Countries: Synergy and Integration, Part 1: Participants' Reports in English, Beijing, China, 25–26 March 2019; pp. 163–168. Available online: https://kpfu.ru/staff_files/F_1183896347/Scientific_research_of_the_SCO_countries___English_Reports___March_25_26___Part_1__2_.pdf (accessed on 15 November 2020).
47. Isaeva, E.V.; Ryazanova, T.V. The Composition, Properties and Processing of Vegetative Poplar Wastes after Extraction of Extractive Substances. Message 1. The Chemical Composition of Solid and Liquid Wastes. *Khimiya Rastitelnogo Syriya [Chemistry of Plant Raw Materials]* **2012**, *3*, 59–65. eISSN 1029-5143. (In Russian)
48. Isaeva, E.V.; Mamaeva, O.O.; Ryazanova, T.V. The Bioconversion of Fallen Poplar Leaves with Balsamic Mycelial Fungi of the Genus *Trichoderma*. *SFU Chem.* **2017**, *10*, 381–389. (In Russian)

49. Polyakov, V.V.; Adekenov, S.M. *Biologically Active Compounds of the Populus, L. Plants and Related Preparations*; Gylym: Almaty, Kazakhstan, 1999; p. 160. (In Russian)
50. Lozhkina, G.A.; Isaeva, E.V.; Ryazanova, T.V. A study of the alcohol extract from balsam poplar buds. *Russ. J. Bioorganic Chem.* **2010**, *36*, 83–88. (In Russian)
51. Ryazanova, T.V.; Chuprova, N.A.; Isaeva, E.V. *Chemistry of Wood*; LAP Lambert Academic Publishing GmbH & Co.KG: Saarbrucken, Germany, 2012; p. 428. ISBN 978-3-8473-7148-9.
52. Spirin, A.S. The Spectrophotometric Determination of the Total Amount of Nucleic Acids. *Biochemistry* **1958**, *23*, 656.
53. Devyatkina, V.A. *Methods of Determation of Vitamins*; Pischepromizdat: Moscow, Russia, 1954; p. 135. (In Russian)
54. Chupakhina, G.N. *Physiological and Biochemical Methods of Plant. Analysis*; Kaliningrad Un-ty: Kaliningrad, Russia, 2000; p. 59. (In Russian)
55. Zhukov, A.P. The Method for Determining the Digestibility of Feedstuffs in vitro. *Trudy Saratovskogo Zoovetinstituta [Works of the Saratov Zoological Veterinary Institute]* **1961**, *10*, 109–124. (In Russian)
56. Lakin, G.F. *Biometry*; Higher School: Moscow, Russia, 1990; p. 351.
57. Koutrotsios, G.; Danezis, G.; Georgiou, C.; Zervakis, G.I. Elemental Content in *Pleurotus ostreatus* and *Cyclocybe cylindracea* Mushrooms: Correlations with Concentrations in Cultivation Substrates and Effects on the Production Process. *Molecules* **2020**, *25*, 2179. [CrossRef] [PubMed]
58. Standards and Requirements No. 13-7-2/174. The Veterinary and Sanitary Standards and Requirements for the Quality of Feedstuffs for Unproductive Animals. Available online: http://docs.cntd.ru/document/1200102836 (accessed on 15 November 2020). (In Russian).
59. Sashenkova, S.A.; Ilyina, G.V. The influence of vitamins and their precursors on the growth and development of the mycelia cultures of basidiomycetes xylotrophic mushrooms. *XXI Century Results Past Probl. Present* **2014**, *1*, 41–46. Available online: https://vek21.penzgtu.ru/wp-content/uploads/2020/04/2014_17.pdf (accessed on 15 November 2020). ISSN 2221-951X. (In Russian).
60. Tenkanen, T.M.; Siika-aho, M. Cellulases and the mechanism of cellulose degradation. In Proceedings of the Tricel 97: Carbohydrases from Trichoderma Reesei and Other Microorganisms, Ghent, Belgium, 28–30 August 1997; p. 19.
61. Velichko, N.A.; Berikashvili, Z.N. The Chemical Composition of the Fruit Body of the *Pleurotus Ostreatus* (Fr) Kumm Fungus. *Vestnik KRAGAU, Krasnoyarsk [Krasnoyarsk State Agrarian University Bulletin]* **2008**, 274–278. ISSN 1819-4036. (In Russian)
62. Kalashnikova, A.P.; Fisinina, V.I.; Shcheglova, V.V.; Kleimenova, N.I. *The Norms and Diets for Feeding Farm. Animals. Reference Book*, 3rd ed.; Rossel'hozakademiya: Moscow, Russia, 2003; p. 456. ISBN 5-94587-093-5. (In Russian)
63. Kosolapov, V.M.; Chuikov, V.A.; Khudyakova, K.K.; Kosolapova, V.G. *Mineral. Elements in Feedstuffs and Related Analysis Methods*; OOO Ugreshskaya Tipografiya: Moscow, Russia, 2019; p. 272. ISBN 978-5-91850-037-8. Available online: https://www.vniikormov.ru/pdf/mineralnye-elementy-v-kormah-i-metody-ih-analiza.pdf (accessed on 15 November 2020). (In Russian)
64. Chichaeva, V.N.; Komissarova, T.N.; Vorobyova, N.V.; Loginova, T.P. *Reference Tables on Feeding Farm. Animals*; FSBOU VO NGSKHA Zootekhniya: Nizhny Novgorod, Russia, 2017; p. 67. (In Russian)
65. Feshchenko, V.N. The Content of Heavy Metals in Feed Crops of Novosibirsk Oblast. *Agroecology, Vestnik Altaiskogo Gosudarstvennogo Agrarnogo Universiteta [Altai State Agricultural University Bulletin]* **2014**, *10*, 33–36. ISSN 1996-4277 (In Russian)
66. Ryadchikov, V.G. *The Fundamentals of Nutrition and Feeding of Farm. Animals*; KGAU: Krasnodar, Russia, 2014; p. 616. ISBN 978-5-94672-671-9. (In Russian)