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**ABSTRACT**

of the dissertation submitted for the scientific degree of  
Doctor of Philosophy

**BIOCONVERSION OF NON-FOOD WASTE IN SOLID  
FORM**

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## INTRODUCTION

**Relevance and degree of the completion of the topic.** Plant raw materials are plant organisms used by humans in various fields of production (whole plants or parts thereof). Depending on the purpose of use, they can be generally divided into 4 places: food, feed, medicine and technical. Regardless of the form of use, the plants used for this or that purpose consist of both cultivated and wild plants, and *"as a result of production processes aimed at their use, in all cases, a small or large amount of waste"*<sup>1</sup>, more specifically, materials that do not belong to the intended product is formed. The way they are treated today is almost the same all over the world and in many cases the same. So, they are either *"irregularly thrown into the environment, burned, or used in the form in which they were created, even if it is not effective"*<sup>2</sup>. All three forms of this relationship in all cases lead to environmental pollution and deterioration of the ecological situation. For this reason, waste recycling is one of the current problems that are in the focus of attention and are being studied almost everywhere in the world. It is true that, currently in several countries of the world there are production areas aimed at adding waste to recycling, but they

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<sup>1</sup> Сеницын А.П., Сеницына О.А. Биоконверсия возобновляемой растительной биомассы на примере биотоплива второго поколения: сырьё, предобработка, ферменты, процессы, экономика//Успехи биологической химии, т. 61, 2021, с. 347–414 34

<sup>2</sup> Muradov P.Z. Muradov P.Z. Bitki substrtlarının bioloknversiyasını əsasları.-Bakı: Elm, 2003, 114s.

cover a small part of the waste generated in these areas, and a large amount of them are not used today and become sources of environmental problems in the above-mentioned aspects.

For this reason, preparation of the scientific and practical basis of their recycling is one of the most urgent issues today.

It should be noted that in order to facilitate the use of waste generated after the use of plants for various purposes, they are systematized and various criteria are used for this. More precisely, either "*they are systematized according to their destination, the species they belong to, or their danger*"<sup>2,3</sup>. For example, vegetable waste, animal waste, food waste, non-food waste, etc. It is true that this systematization is mostly conditional, and sometimes waste from the same source is included in different systems. So, plants used for food purposes also have enough non-food parts. In addition to the parts of cultivated fruits, vegetables, melons and other plants that are used for food purposes, there are also enough parts that are not suitable for use for food purposes. In addition, waste is generated after the processing of raw materials used for feed, technical and medicinal purposes their quantity is measured in hundred thousand, million tons and depending on the state of the aggregate, they are both liquid, gas, and solid. In general, these materials, which can be combined under the name of non-food waste, in other words, those characterized as "*renewable plant waste*"<sup>1</sup>, have the same form of attitude as above, and their disposal is currently one of the issues in the focus of attention.

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<sup>3</sup>Хорошавин Л. Б. Беляков В. А., Свалов Е. А. Основные технологии переработки промышленных и твердых коммунальных отходов. Екатеринбург : Изд-во Урал. ун-та, 2016. – 220 с.

In the Republic of Azerbaijan, there is literature information about *"this type of waste is generated and their amount is in thousands of tons"*<sup>2</sup>. It is true that among them *"those related to the waste of food products (for example, edible vegetable oils) are more numerous"*<sup>4,5</sup>, and the research conducted on their disposal is both numerous and wide-ranging. Other wastes, especially non-food ones, are open for research today, although they contain a sufficient amount of both organic and inorganic substances, and it is possible to transform them into various products, especially food and feed. Realization of this is important both from the point of view of expanding the raw material base of a number of production areas, primarily microbiology and biotechnology, at the expense of non-traditional sources, as well as from the point of view of environmental pollution and improvement of the ecological situation.

**The aims and tasks.** The purpose of the presented work is the selection of a method that allows to realize the utilization of a number of non-food wastes generated in the conditions of Azerbaijan and the evaluation of a number of wastes corresponding to the selected method from this aspect.

In order to achieve the mentioned goal, it was considered appropriate to implement the following tasks:

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<sup>4</sup> Əttarhüseyni, M.Y. Bitki tullantılarının mikrobioloji konversiyasının fizioloji-biokimyəvi əsasları (Günəbaxan bitkisinin tullantılarının nümunəsində):/b.ü.f.d. dissertasiyasının avtoreferatı/-Bakı, 2016, -24s

<sup>5</sup> Musayeva, V.H. Bitki yağı istehsalı tullantılarının ulizasiyasının biotexnoloji əsasları:/b.ü.f.d. dissertasiyasının avtoreferatı/-Bakı, 2022, 32s.

- Determination of the chemical composition of a number of non-food wastes generated in Azerbaijan;
- Evaluation of a number of non-food wastes generated in Azerbaijan according to the numerical composition of the fungal biota;
- Microbiological conversion of non-food waste and process optimization;
- Enzymological conversion of non-food waste and process optimization;
- Evaluation of the products obtained from the biological conversion of non-food waste according to their biochemical composition

**Research methods.** As both chemical and biological points were clarified during the research, the methods used were chosen accordingly. Thus, during the chemical analysis of waste, were used known methods used for this purpose in chemistry, but during the biological analysis, were used various methods that are widely use in biotechnology, mycology, microbiology and biochemistry. Repetition of all experiments related to the dissertation work (4-6), the statistical performance of the obtained results, the accuracy of the used devices, the purity of the reagents also determine the honesty of the obtained results.

**The main provisions of the dissertation submitted for defense.**

- The processes applied during the obtain of various products from plants, including non-food products, do not allow production to be realized without waste, which causes gthe eneration of a significant amount of extraneous materials with a complex composition every year;

- The role of moisture is important in the increase of biological pollution in the environment by enriching the wastes from the use of coniferous and broad-leaved trees with toxic metabolites of fungi;
- The use of a substrate that is easily assimilated by fungi is one of the factors that ensure the effectiveness of biological conversion of non-food waste, especially those formed after the use of coniferous plants for various purposes;
- For biological processing of lignocellulose substrates with a complex polymer composition, it is advisable to use species of xylotrophic macromycetes that cause white rot in natural conditions, which is due to the inclusion of both hydrolases and oxidases in their enzyme system.

**Scientific novelty of the research.** The scientific and practical aspects of the microbiological and enzymological conversion of non-food products' solid aggregate waste generated in the conditions of Azerbaijan were studied in the conducted researches.

It has been determined that in the conditions of Azerbaijan, the amount of aggregate solid waste of non-food products is sufficient, and although they contain compounds that allow the expansion of the raw material base of the biotechnology industry, they also contain compounds that limit this process, and therefore, at present, their involvement in recycling and biotechnology the use of industry in terms of expanding the raw material base is not at a satisfactory level. It became clear that the waste of non-food substrates is also one of the food sources of fungi to one degree or another. This causes the waste to become one of the sources of accumulation of various metabolites with phytotoxic activity, which are formed as a result of the life activity of fungi, and

one of the factors that seriously affect the activation of this process, that is, the increase in the phytotoxic activity of the waste, is its initial moisture.

For the utilization of non-food waste containing cellulose, lignin, hemicellulose and other complex polymers are used fungi that cause white rot in natural conditions. At this time, the microbial conversion under solid-phase fermentation conditions is favorable for both environmental and economic considerations. For the effective flow of both processes, it was considered appropriate to use fungi such as *P.tigrinus* NU-32 vø *P.ostreatus* UN-118 as active producers.

By using *P. tigrinus* NU-32, and *P. ostreatus* UN-118 mushrooms selected as active producers was shown the possibility of conversion the waste belonging to broad-leaved trees turned into fodder, as well as a food product, which riches in protein and other biologically active substances, without toxic effect, digestibility increases up to 2 times. It was also determined that in order to obtain the maximum result of this process, it is necessary to use  $\text{NH}_4\text{NO}_3$  as an additional source of nitrogen, to carry out cultivation at 280C, and to use as planting material the biomass obtained as a result of 5-day cultivation in a medium consisting of 1% waste, and the growing period should be 10 days. Although it is impossible to effectively carry out the microbiological conversion of the waste of conifers due to the presence of compounds of a phytocidal nature in their contents, the possibility of carrying out their microbiological conversion using other substrates suitable for fungi has also been shown. As a result, optimal conditions were found that made it possible to significantly improve the amount of proteins, digestibility, and other indicators in the obtained product.

Using technical enzyme preparations obtained from *P. tigrinus* NU-32 and *P. ostreatus* UN-118 mushrooms as an



active producer for microbiological conversion in enzymological hydrolysis of non-food waste, it was obtained a hydrolyzate whose composition mainly consists of glucomannans which its use as a raw material in the purchase of biofuel is promising.

#### **Theoretical and practical significance of research.**

The results obtained in the research are actual material for expanding the source of raw materials of biotechnology at the expense of non-traditional substrates, primarily those related to coniferous plants.

The results obtained in the studies are also useful in terms of prevention of biological pollution of the environment, prevention of disposal of wastes that are not suitable for use into the environment.

The obtained results are a source of information that serves to expand the ideas about the biological conversion of plant wastes, whose biological degradation is extremely difficult.

**Publication, approval and application of dissertation.** 12 scientific works related to the subject of the dissertation have been published, 8 of which are articles. The materials of the dissertation were presented at the IV International scientific conference on "Innovation problems of modern biology" (Baku, 2014), at the scientific conference on "Actual problems of modern biology" (Baku, 2019), at the V International scientific conference on "Science in the era of imbalances" (Ukraine R., Kyiv, 2019), and at the I International scientific-practical conference on "Innovative biotechnologies for environmental protection: from theory to practice" (Republic of Belarus, Minsk, 2024).

**The organization where the dissertation was carried out.** The dissertation work was carried out in the microbiological biotechnology laboratory of the Institute of

Microbiology of the Ministry of Science and Education of the Republic of Azerbaijan.

**The structure and volume of the dissertation.** The dissertation consists of an introduction, 4 chapters (literature summary - I, materials and methods - II, experimental part - III and IV), final analysis of research results, conclusions, lists of literature and abbreviations used in the dissertation, which is a total of 210,500 signs.

## **CHAPTER I**

### **NON-FOOD WASTES: SOURCES OF FORMATION, RESERVES AND SUITABILITY FOR BIOCONVERSION**

Section 1.1 of the dissertation provides information on sources of non-food waste, and section 1.2 analyzes information on the chemical composition and reserves of non-food waste. In section 1.3 are analyzed the studies conducted on the bioconversion suitability of non-food waste and the current state of its implementation and also clarifies the issues that need to be resolved to achieve the set goal.

## **CHAPTER II**

### **MATERIALS AND METHODS OF RESEARCH**

#### **2.1. General characteristics of waste used in research**

In the course of the researches were used the waste generated during the use of broad-leaved (BL) and coniferous (C) plants, the aerial part of some vegetable plants (tomatoes

and potatoes) cultivated in the agrarian area, and the above-ground part of the cotton plant, and wheat bran. The waste generated during the use of coniferous and broad-leaved plants was taken from the enterprises (From the Darnagul building materials market) engaged in the processing of wood materials in the Absheron peninsula, and the waste generated in the agricultural area was taken from the fields belonging to farms in Guba-Khachmaz (tomatoes), Ganja-Tovuz (potatoes) and Karabakh economic regions. The collected wastes were first air-dried and some of them (cotton, tomato and potato above-ground parts) were crushed in laboratory conditions and turned into a mass consisting of scraps with a size of 1-5 mm. Wood shavings and wheat bran were used in their original form.

## **2.2. Methods used during bioconversion of waste**

It was considered appropriate to carry out research of waste involved in bioconversion under solid-phase fermentation (SPF) conditions. In the research conducted for this purpose, air-dried wastes are wetted with ordinary water in a ratio of 1:1 and collected in Erlenmeyer flasks with a volume of 1L (up to 1/3 of the total height of the flask), the head is closed with a cotton plug, covered with paper and sterilized for 1 hour at 1 atmosphere. After sterilization, when the waste temperature drops to room temperature, it is inoculated with planting material made from biological agents and incubated at 28<sup>0</sup>C. The incubation period lasts for 3-20 days, depending on the nature of the experiments. After the expiration of the period, the obtained biomass is dried at a temperature not higher than 40<sup>0</sup>C and analyzed according to the criteria (weight loss, cellulose and lignin degradation, protein accumulation) used to evaluate the microbiological

conversion process, that "*known methods and approaches*"<sup>6,7</sup> were used during these works. The numerical composition and phytotoxic activity of the mycobiota of the wastes were determined according to the "*methods and approaches used in the work of various authors*"<sup>8,9</sup>. Enzymatic hydrolysis of waste was carried out according to "*known methods*"<sup>10</sup> based on accepted general principles and approaches.

All experiments conducted during the study were carried out in 4-6 replicates, and the results obtained were processed "*statistically*"<sup>11</sup>.

### **CHAPTER III**

#### **EVALUATION OF NON-FOOD PLANT WASTE BY PHYSICOCHEMICAL PARAMETERS AND MYCOBIOTIC CONTENT**

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<sup>6</sup> Muradov P.Z. Bitki tullantılarının konversiyası prosesində hidrolaza və oksidazaların aktivliklərinin dəyişilməsi/biologiya elmləri doktorluğu dissertasiyanın avtoreferatı/-Bakı, -2004, -48s.

<sup>7</sup> Оболенская А.В., Ельницкая З.П., Леонович А.А. Лабораторные работы по химии древесины и целлюлозы. М.: Экология, 1991. 321 с.

<sup>8</sup> Вахşəliyeva K.F. Azərbaycanda yayılan toksigen göbələklərin ekobioloji xüsusiyyətləri:/biologiya üzrə elmlər doktoru dissertasiyanın avtoreferatı./ -Bakı, 2017. -45s.

<sup>9</sup> Мирчинк, Т.Г. Почвенная микология. /Т.Г. Мирчинк М.: Издательство МГУ. -1988. -220 с.

<sup>10</sup> Andersen, N. Enzymatic Hydrolysis of Cellulose: Experimental and Modeling Studies:/ Ph.D. Thesis./DTU, 2007, 163p.

<sup>11</sup> Гланц, С. Медико-биологическая статистика / С. Гланц. – М. : Практика, 1999. – 334 с

### 3.1. Biochemical composition of waste

Firstly, the wastes used in the research were analyzed according to their chemical composition. From the obtained results, became clear that although the wastes sampled for analysis differ from each other mainly in terms of the quantitative indicators of their constituent components, in all of them, the specific gravity of difficult hydrolyzable polymers is greater than that of easily hydrolyzable ones (tab. 3.1). As it can be seen, this reaches its highest value in the

**Table 3.1.**

#### General characterization of wastes

Wastes	The amount of the constituent elements					
	1*	2	3	4	5	6
Scraps of trees (BL)	57,6	28,4	1,5	2,2	1,0	2,2
Scraps of trees (CP)	55,7	29,3	1,4	2,1	1,1	2,4
Scraps of trees (mixed)(BL+CP)	56,4	29,0	1,5	2,2	1,1	2,3
The above-ground part of vegetable plants (potatoes and tomatoes)(VP)	47,8	36,5	0,9	2,4	0,80	1,7
The above-ground part of the cotton plant(CP)	50,5	32,1	1,2	1,9	0,7	2,0

**Note:** \* 1 - Difficult hydrolyzed; 2 - Easily hydrolyzed; 3- Soluble sugars; 4-protein; 5-lipid and 6-ash.

wastes obtained from trees, but the presence of easily hydrolyzable and soluble sugars in their composition allows to note the possibility of involving the mentioned wastes in recycling.

As mentioned, any form of treatment of waste leads to environmental pollution. On the other side, these wastes also contain nutrients that are favorable for microorganisms, and at the same time, they are one of the sources of settlement and spread of microorganisms and can participate in biological pollution of the environment. In addition, it is also important in terms of ensuring the mycological safety of the future use of this type of waste. Thus, fungi use this type of substrate not only for food purposes, but also as a place where they secrete metabolites formed as a result of their life activity. Among these metabolites, there are many dangerous ones not only for humans, but also for various living things. For this reason, taking into account that the characterization of their mycobiota is important from both a scientific and a practical point of view, research was conducted in this regard too.

### **3.2. Numerical composition of waste mycobiota**

From the obtained results, it became clear that the mycobiota of each of them is characterized by a quantity that carries specificity in a certain sense, and this finds its highest expression in the samples taken from the scraps of broad-leaved trees, and the indicator characteristic of each material varies depending on the season of the year (tab. 3.2). As it can be seen, the indicators characterizing the numerical composition of mycobiota of all materials reach their highest form of expression in summer, more precisely in June. In the

studied samples, the lowest indicators are found in the winter season. It is more appropriate to explain the reason

**Table 3.2**

**Changing the number composition( $\times 10^3$  CFU/g) of mycobiota of the analyzed materials by seasons**

Analyzed products	Winter	Spring	Summer	Autumn
BL	1,72	2,82	3,17	3,15
CP	0,31	0,53	0,71	0,70
BL+CP	0,93	1,43	1,54	1,49
VP	1,1	1,7	1,9	1,8
Packaging containers (Cardboard)	0,43	0,74	0,94	0,90
Packaging containers (Paper)	0,39	0,64	0,76	0,73

for this with the temperature factor, as the vast majority of fungi are mesophilic and the temperature range favorable for their growth and development is between 20-30<sup>0</sup>C. The temperature range in June in Azerbaijan is exactly in this range.

From the results obtained with the numerical composition of fungi, it is necessary to touch on one point, which is related to the chemical composition of the waste sampled for research. Thus, the used wastes differ from each other according to their chemical composition, which is confirmed by both the literature data and some indicators that we determined in our research. For example, the main constituent elements of scraps, both they belong to broad-leaved or coniferous plants are mainly consists cellulose and

lignin, that is polysaccharides that are difficult to hydrolyze, and the amount of which varies between about 30-50% in both. Nevertheless, the mycobiota of scraps stored under the same conditions and formed as a result of almost the same process differ significantly in terms of their numerical composition. As can be seen (tab. 3.2), the number of fungi in the scraps belonging to conifers is 4.5-5.5 times less than that from broad-leaved trees in all seasons. Compared to other wastes, i.e. those used for packaging, the number of mycobiota of scraps belonging to coniferous plants is much lower. In the composition of the mentioned materials (cardboard and paper used for packaging), cellulose is more abundant.

As is known, phytoncides are among the metabolites produced by coniferous plants, on the side, they also contain resin compounds that have antimicrobial activity.

This also ensures that there are few fungi in the waste of coniferous plants.

In the research has also been set as a task the analysis of the numerical composition of mycobiota of the same waste, which differs according to the umidity content and samples for analysis were taken only in one season (summer), and from trees scraps. From the obtained results, it became clear that the number of fungi also changes depending on the humidity, and the humidity content above 10% is accompanied by a corresponding increase in the number of fungi(tab. 3.3). As can be seen, an increase in humidity content up to 20% increased the amount of fungi in trees scraps of broad-leaved trees by 4.3 times compared to the lowest value (<10). Analogously, respectively this increase is 7.1 and 4.7 times in coniferous and mixed scraps. In short, humidity plays an important role in the formation of mycobiota of the mentioned wastes.



**Table 3.3****Changes in the number composition of the mycobiota of the analyzed materials ( $\times 10^3$  CFU/g) by seasons**

Analyzed products	Humidity, %			
	<10	15	20	>20
BL	0,92	3,2	4,0	4,6
CP	0,10	0,53	0,71	0,79
BL+CP	0,37	1,48	1,74	1,92

The toxicity of the mycobiota of the wastes was also studied and this issue was carried out with wastes that differed in their humidity content. It was clear from the results that the toxicity of non-food waste products with an initial humidity content above 10%, increases with humidity content. Thus, the germination capacity of wheat and peas obtained from the extract obtained from trees scraps with a moisture content of up to 10% is 1.2 times higher than those with a moisture content of 15%, and 1.4 times higher than those with a moisture content of 20%. This is accompanied by comparison with both broad-leaved and conifers, as well as the above-ground parts of cotton and vegetable plants. Therefore, the moisture factor should be taken into account when using these wastes.

## CHAPTER IV EVALUATION OF SUITABILITY OF NON-FOOD WASTE FOR BIOLOGICAL CONVERSION

### 4.1. Microbiological conversion of waste

The waste contains a considerable amount of complex polymers and they differ both in their quantity and

biodegradation mechanisms. Among the active producers of the biodegradation process, fungi are of particular importance and in most studies have confirmed the expediency of using species of fungi that cause white rot in natural conditions has been confirmed. For this reason, in the course of research, we first time assessed the possibilities of using fungal strains in the process of microbiological conversion, which are the causative agents of white rot in natural conditions, which were presented us by the microbiological biotechnology laboratory of the Microbiology Institute of the Ministry of Science and Education of the Republic of Azerbaijan. From the obtained results, it became clear that all the fungal strains used in general can significantly degrade the polymers included in the composition of the waste, and the difference between them is manifested only at the level of the indicators characterizing this or that component(tab. 4.1). As it can be seen, the strain characterized by the highest index in terms of cellulose and lignin degradation, as well as weight loss and protein enrichment of the substrate is not found. Thus, although the highest indicator of lignin degradation during the use of scraps obtained from broad-leaved plants (BL) belongs to the *Trametes hirsuta* NU-17 strain, for cellulose this indicator belongs to the *Bjerkandera adusta* PM-1 strain. Although the mass loss was highest in mushroom *Pleurotus ostreatus* NU-18, the *Panus tigrinus* NU-32 strain differs by enriching the substrate with protein. As for the degree of degradation of individual substrates, although there are certain differences in this case, the microbiological conversion of conifer waste in the form in which it is formed is extremely weak, and in any case, the degraded amount of lignin and cellulose together does not reach 10%. In other substrates, namely cotton bolls and above ground part of

**Table 4.1.**

**Microbiological conversion of non-food plant waste (10 days)**

Mushroom	Weight loss,%	Degradation (%)		Protein(%)	
		Cellulose	Lignin	initially	final
1	2	3	4	5	6
BL					
<i>Bjerkandera adusta</i>	22,1	37,9	37,4	2,7	7,9
<i>Cerrena unicolor</i>	21,2	35,1	37,5		7,8
<i>Fomes fomentarius</i>	19,2	34,5	34,8		7,5
<i>Ganoderma lipsence</i>	20,1	35,2	35,1		7,9
<i>G.lucidum</i>	19,3	34,7	33,8		7,7
<i>Phellinus igniarius</i>	19,9	35,6	34,7		7,4
<i>Panus tigrinus</i>	22,1	37,5	37,7		8,4
<i>Pleurotus ostreatus</i>	23,2	37,0	37,8		8,0
<i>Trametes hirsuta</i>	21,0	33,7	38,5		7,5
<i>T.versicolor</i>	20,6	31,7	38,0		7,3
CB					
<i>Bjerkandera adusta</i>	4,1	4,7	5,0	2,5	3,1
<i>Cerrena unicolor</i>	4,0	4,5	4,2		3,0

**Continuation of Table 4.1**

1	2	3	4	5	6
<i>Fomes fomentarius</i>	3,2	3,4	3,4	2,5	2,9
<i>Ganoderma lipsence</i>	2,9	3,2	3,1		2,8
<i>G.lucidum</i>	2,7	3,4	3,3		3,0
<i>Phellinus igniarius</i>	2,3	3,1	2,9		2,8
<i>Panus tigrinus</i>	3,1	3,5	3,2		3,6
<i>Pleurotus ostreatus</i>	4,1	3,7	3,4		3,7
<i>Trametes hirsuta</i>	2,8	3,1	3,3		2,9
<i>T.versicolor</i>	2,6	3,1	3,0		2,8
CP					
<i>Bjerkandera adusta</i>	20,1	34,7	35,0	2,1	7,0
<i>Cerrena unicolor</i>	19,0	34,5	34,9		6,9
<i>Fomes fomentarius</i>	18,2	33,0	33,8		6,0
<i>Ganoderma lipsence</i>	17,9	32,7	33,8		6,1
<i>G.lucidum</i>	17,2	34,0	34,9		6,6
<i>Phellinus igniarius</i>	18,2	35,1	36,9		6,9
<i>Panus tigrinus</i>	20,1	36,5	37,0		7,0
<i>Pleurotus ostreatus</i>	21,2	36,3	37,4		7,4
<i>Trametes hirsuta</i>	2,8	33,1	38,3		6,6

**Continuation of table 4.1**

1	2	3	4	5	6
<i>T.versicolor</i>	2,6	32,1	38,0	2.1	6,3
VP					
<i>Bjerkandera adusta</i>	24,1	36,7	36,0	2,5	7,2
<i>Cerrena unicolor</i>	24,0	35,5	37,2		7,0
<i>Fomes fomentarius</i>	23,2	31,5	32,1		6,0
<i>Ganoderma lipsence</i>	22,9	32,5	33,8		6,4
<i>G.lucidum</i>	22,7	33,1	33,6		6,5
<i>Phellinus igniarius</i>	22,3	33,4	33,9		7,1
<i>Panus tigrinus</i>	23,1	37,5	38,2		7,7
<i>Pleurotus ostreatus</i>	24,1	37,7	38,4		7,8
<i>Trametes hirsuta</i>	22,8	34,2	39,8		7,0
<i>T.versicolor</i>	22,6	33,8	39,0		6,8

potatoes, degradation occurs at a high level and is formed biomass rich in protein. As a conclusion of this stage, trees scraps typical of broad-leaved plants and above ground part of potato plant (PP) were selected as substrates for further research.

As a biological agents were selected strains *P.tigrinus* NU-32 and *P.ostreatus* NU-118. In the selection of these strains, it was taken as a basis that all indicators are generally high and that they differ little from those with high

specific indicators, as well as that they belong to the category of edible mushrooms.

Studies were conducted on the optimization of the process of microbiological conversion of non-food plant (broad-leaf, potato and cotton) waste of mushrooms selected as active producers, and the conditions ensuring the effectiveness of the process were found, which were as follows: Substrate humidity– 60,4-62,5%; Additional nitrogen source –  $\text{NH}_4\text{NO}_3$ - 0,037-0,038%( according to nitrogen); initial pH -6.5-6.7; planting material - consisting of 1% trees scraps, 5-days biomass and cultivation time - 240 hours. As a result of the optimization(optimized for additional mineral nitrogen source - $\text{NH}_4\text{NO}_3$ , preparation and duration of the inoculum), the efficiency of the conversion process was increased by 7-11% compared to the initial one. Biochemical analysis of the product (biomass) obtained from broad-leaved plants under such optimized conditions allowed us to note the possibility of using them primarily as fodder in the future (tab.4.2). As a basis for this, can be mentioned it is possible to mention the non-toxicity of the obtained biomass, the fact that its digestibility is 1.7-2.0 times higher than the original substrate, low specific gravity of difficult hydrolyzable components of the lignocellulose complex, its richness in other biologically active substances, and other results.

Due to the lack of direct effect of microbiological conversion of waste from conifers, studies aimed at their complex use were also conducted. For this purpose, the fungus *P.ostreatus* NU-18, selected as an active producer, was used, and waste from conifers was mixed with other waste in different proportions, as well as with wheat bran (WB), and involved in the microbiological conversion

**Table 4.2**

**Biochemical analysis of the products obtained during the microbiological conversion (10 days) of waste (obtained from broad-leaved trees) under SPF conditions (%)**

Component composition	<i>P.tigrinus</i> NU-32	<i>P.ostreatus</i> NU-118	Control
Cellulose	23,4	23,2	34,3
Lignin	22,7	23,9	32,5
Soluble sugars	2,1	2,0	1,7
Protein	7,8	8,1	2,3
Lipids	2,7	3,0	1,0
Nucleic acids	0,76	0,82	0,2
Ash	2,3	2,4	2,1
Ability to digest	58,4	56,0	27,8

process. From the obtained results, it was clear that the applied approach accelerates the microbiological conversion of coniferous plant waste (tab.4.3). As seen the highest effect is observed when using WB (in a ratio of 2:1), and in this case all indicators are high compared to other options. This, in turn, shows that the methods and approaches related to the use of solid waste from broad-leaved plants are not exhausted and creates additional opportunities for their reuse.

#### **4.2. Enzymological conversion of wastes obtained from non-food plants**

In the studies conducted on enzymological conversion of the studied wastes, it became clear that it is possible to use

**Table 4.3.**

**The effect of different additives on the microbiological conversion of waste obtained from coniferous plants (CPW) (10 days)**

Substrates (ratio)	Weight loss, %	Degradation(%)		Protein(%)	
		Cellulose	lignin	initially	final
Waste obtained from broad-leaved plants					
CP+BL (1:1)	18,7	26,9	27,0	2,6	7,9
CP+BL(2:1)	16,0	20,7	20,5	2,5	5,7
CP+BL(3:1)	10,5	12,5	12,7	2,4	4,2
CP+VP(1:1)	19,6	28,2	27,4	2,2	7,9
CP+ VP (2:1)	17,0	21,6	20,6	2,3	5,8
CP+ VP (3:1)	11,6	14,1	14,0	2,4	4,0
CP+ WB (1:1)	21,4	31,1	30,9	8,7	14,3
CP+ WB (2:1 )	21,5	32,3	33,4	6,5	10,5
CP+ WB (3:1 )	16,0	24,0	23,8	4,4	6,1
CP+BL+VP+WB (1:1:1:1)	18,9	29,8	29,7	4,0	9,2

fungus strains selected as active producers as a source of enzymes. Thus, both fungi can actively synthesize all enzymes that degrade polymers in lignocellulosic substrates, that is both oxidases and hydrolases, during cultivation in a nutrient medium containing 1% waste, which has been confirmed by studies conducted in other centers. From the results obtained during the enzymatic hydrolysis of waste using the technical enzyme preparation (TEP) obtained from these fungi according to known methods, it was clear that the solid waste of coniferous plants undergoes the hydrolysis



process like other waste, in this case, the total sugar output can even be slightly higher in some cases than in others (tab. 4.4). As can be seen, the total sugar yield varies depending on both the substrate and the source of used TEP. The reason of this is that both the quantity indicator of the chemical elements of the waste and the activity ratios of those included in the enzyme system synthesized by the fungal strains, as well as the kinetic parameters (adsorption capacity, inhibition coefficient, thermostability, etc.) characterizing the catalytic activity of individual enzymes included in the system, are different.

**Table 4.4**

**Enzymatic hydrolysis of plant waste**

Substrates used	Substrate density (g/l)	Amount of added TEP (g/l)	T <sup>0</sup> C	Period (hour)	Total sugar extraction (g/l)
<i>Panus tigrinus</i> NU-32					
CP	100	0,04	40 <sup>0</sup> C	24	13,4
BL					14,5
CP					12,1
VP					12,3
<i>P.ostreatus</i> NU-18					
CP	100	0,04	40 <sup>0</sup> C	24	14,6
BL					15,8
CP					11,7
VP					12,6

The indicators given in the table, that is, the density, temperature and duration of the substrate and technical

enzyme preparation, were selected based on the information obtained as a result of optimization in the work of researchers conducting research in this field.

Whether these indicators are optimal for the process of enzymatic hydrolysis of all substrates has been clarified according to some indicators. The obtained results are summarized in table 4.5. As it can be seen, the solidity index of substrates and technical enzyme preparations is generally

**Table 4.5**

**Conditions that allow obtaining the maximum indicator of sugar extraction during enzymatic hydrolysis of non-food plant waste**

Substrates used	Substrate density (g/l)	Amount of added TEP (g/l)	Temperature	Period (hour)	Total sugar extraction (g/l)
<i>Panus tigrinus</i> NU-32					
CP	100	0,04	42 <sup>0</sup> C	21	13,3
BL					14,5
CP					12,0
VP					12,2
<i>P.ostreatus</i> NU-18					
CP	100	0,04	42 <sup>0</sup> C	19	14,6
BL					15,7
CP					11,7
VP					12,5

universal, but it shows its difference in temperature and duration of fermentation, for example, increasing the temperature by 2<sup>0</sup>C, shortening the fermentation time by 3

hours when using TEP from *Panus tigrinus* NU-32, and 5 hours when using TEP from *P.ostreatus* NU-18. The reduction in time allows the optimization process to produce essentially the same amount of sugar to still be considered a somewhat customized approach.

At the end of the research, the chemical composition of the hydrolyzates obtained under optimal conditions was analyzed. Although the chemical composition of the obtained hydrolyzates is different, it was determined that glucomannans predominate in all of them, and the amount of glucose among them was determined to be 41-72%(tab. 4.6).

**Table 4.6**

**Chemical composition of hydrolyzate formed during enzymatic hydrolysis of non-food plant waste (%)**

Substrates used	<i>Panus tigrinus</i> NU-32			
	Glucose	Mannose	Galactose	Others
CP	71,0	12,1	9,3	5,8
BL	69,8	12,5	8,3	5,4
CP	41,0	21,9	19,7	17,4
VP	62,9	15,9	11,5	9,7
	<i>P.ostreatus</i> NU-18			
CP	70,3	15,7	6,2	7,8
BL	72,0	16,6	10,3	11,1
CP	43,2	22,4	16,2	18,2
VP	56,7	19,2	14,3	9,8

## FINAL ANALYSIS OF THE RESULTS OBTAINED IN THE RESEARCH

Despite the fact that the researches dedicated to the effective utilization of renewable wastes or making them useful in practical demand care have been carried out since the second half of the 20th century, today, the utilization of these wastes has not been solved on a large scale and a significant part of the wastes generated every year remains unused and mainly serves as one of the sources of environmental pollution. Among this type of waste, those generated after the use of non-food plants, primarily woody plants, differ from others in terms of both their quantity and areas of use, and this is due to the fact that they contain "*a supramolecular structure that is relatively resistant to both the amount of complex polymers and external influences, including the influence of microorganisms*"<sup>12</sup>.

The problem of recoverable plant waste, including non-food waste, is not alien to the Republic of Azerbaijan, and the amount of this type of waste generated annually in the country is "measured in hundreds of thousands of tons", and the attitude towards this type of waste is not only different from the ones in the world, but also somewhat complicated. Thus, the Republic of Azerbaijan is among the countries with few forests, and for this reason, the materials used for the production of wood are brought to the country mainly from abroad, primarily from the Russian Federation,

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<sup>12</sup> Obeng, E.M., Adam, S.N.N., Budiman, C. et al. Lignocellulases: a review of emerging and developing enzymes, systems, and practices. //Bioresour. Bioprocess, 2017., 4, 16. <https://doi.org/10.1186/s40643-017-0146-8>

and most of the materials imported from there are typical of coniferous plants. The possibility of their use also becomes a little more difficult due to the resinous compounds in their composition.

Taking into account the above, the goal of the presented work is to involve the waste generated during the use of mainly non-food plant materials in the territory of the Republic of Azerbaijan into bioconversion.

In order to achieve the set goal, it became clear from the obtained results that fulfilled the defined tasks, that in the Republic of Azerbaijan, after the use of non-food plants, as well as the waste generated from the parts of food plants that are not suitable for that purpose, there is a sufficient amount of waste in the conditions of Azerbaijan and their involvement in recycling or manufacturing is not so encouraging. Although there are a lot of promising compounds, primarily polysaccharides, in the chemical composition of the waste generated from the use of coniferous or broad-leaved plants, or from the above-ground parts of plants such as cotton, tomatoes and potatoes. Nevertheless, the fact that these wastes are one of the feeding grounds of microorganisms, including fungi, and that these wastes have phytotoxic activity, as a result of which they cause biological pollution, have been confirmed experimentally in our research, and this fact itself is one of the serious factors that show that the involvement of those wastes in bioconversion is relevant for environmental reasons.

In this issue, which is relevant for both economic and environmental reasons, it has been recognized that it is advisable to use xylotrophic species of macromycetes that cause white rot for the bioconversion of waste generated during the use of coniferous plants containing resinous

compounds. There are several reasons, the first of which is related to "*the enzyme system of white rot species is stronger and more colorful*"<sup>2</sup>. Thus, they are "*considered to be one of the organisms that carry out the most intense degradation of lignin*"<sup>13</sup> among living things. Second, a number of species of xylotrophic macromycetes that cause white rot are edible, and some are not edible due to their hard consistency, but have medicinal value, which may allow the products obtained when they are used for food, feed, and even medicinal purposes.

Due to the presence of resinous compounds in some of the recovered non-food plant wastes, certain problems arise in the realization of their bioconversion process, primarily of the microbiological type, and in order to eliminate this, it was considered appropriate to add other wastes that accelerate the growth of biological agents.

On the basis of the obtained results, have been developed the scientific and practical basis of biological conversion with favorable indicators of renewable vegetable wastes, including those belonging to conifers. These are expressed in the form of 6 final results and 3 practical recommendations mentioned below.

## **RESULTS**

1. Although there is a large amount of solid aggregate waste of non-food products generated

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<sup>13</sup> Di Lella S, La Porta N, Tognetti R, Lombardi F, Nardin T, Larcher R. White rot fungal impact on the evolution of simple phenols during decay of silver fir wood by UHPLC-HQOMS. //Phytochem Anal. 2022; 33(2):170-183.

in Azerbaijan, and they also contain enough components to be involved in the bioconversion process, but currently their use in terms of recycling and expanding the raw material base of the biotechnology industry is not at a satisfactory level[4].

2. It was determined that the waste of non-food products is also one of the food sources of fungi, which allows these wastes to play a role as one of the sources of accumulation of various metabolites with phytotoxic activity, which are formed as a result of the life activity of fungi. One of the factors that seriously affect the growth of phytotoxic activity of waste is its initial humidity content, when it higher than 10%, the phytotoxic activity increases, and as a result, it can cause an additional environmental problem due to the increase in biological pollution[10].
3. Due to the presence of complex polymers in their composition, such as cellulose, lignin, hemicellulose and others, for the utilization of non-food waste using fungi cause white rot in natural conditions in solid-phase fermentation is favorable from both an ecological and economic point of view. In order for both processes to take place effectively, it is appropriate to use *P. tigrinus* NU-32 and *P. ostreatus* NU-118 mushrooms selected as active producers[1-3?, 7].
4. Using the mushrooms *P. tigrinus* NU-32 and *P.ostreatus* NU-118 selected as active producers,

it is possible to transform waste belonging to broad-leaved trees into a product rich in protein and other biologically active substances, which does not contain toxicity, and the digestibility increases up to 2 times. To achieve this, adding  $\text{NH}_4\text{NO}_3$  as an additional nitrogen source, culturing at 28°C, using biomass obtained as a result of culturing for 5 days on a medium containing 1% waste as planting material, and culturing for 10 days allows achieving the maximum result[5-6, 11].

5. The waste of non-food products includes the waste of conifers, and their microbiological conversion cannot be carried out effectively due to the presence of compounds of a phytocidal nature in their chemical composition, however, mixing them with other substrates suitable for fungi, primarily with wheat bran, allows their effective use. As a result, conditions were found that made it possible to significantly improve the amount of proteins, digestibility and other indicators in the resulting product[8-9].
6. As an active producer for microbiological conversion, technical enzyme preparations obtained from *P. tigrinus* NU-32 and *P. ostreatus* NU-118 mushrooms are also suitable for enzymological hydrolysis of non-food waste. Thus, the composition of hydrolyzate obtained at this time mainly consists of gluco-mannans, and the amount of glucose is between 41-72%[6, 11].



## **PRACTICAL RECOMMENDATIONS**

1. Renewable plant waste, including those generated after the use of coniferous plants, are characterized by favorable indicators for the expansion of the raw material bases of production areas based on biotechnology and microbiology and it is important to establish production areas based on their use for these purposes. In order to prevent pollution of the environment, including contamination with biological agents of renewable plant waste, measures should be taken to ensure that they do not remain wet for a long time in the places where they form.
2. It is important for the effective completion of the process to implement the biological, primarily microbiological conversion process of recovered non-food plant waste using other plant wastes that are relatively easily absorbed by biological agents.

## **LIST OF PUBLISHED WORKS RELATED TO THE TOPIC OF THE DISSERTATION**

1. Huseynova A.A., Musayeva V.H., Neymatova U.V. "Biological methods of disposal of various wastes of plant origin/Proceedings of the IV International Scientific Conference on "Innovation Problems of Modern Biology" (May 16-17).Baku: BSU, 2014, p.184
2. Musayeva V.H., Neymatova U.V., Huseynova A.A., Bakshaliyev A.Y. "Prospects of using xylotrophic species of basidiomycetes in the bioconversion of lignocellulose-containing plant

- substrates// Scientific works of the Institute of Microbiology of ANAS, Baku, 2016, v.14, № 1, p. 325-329
3. Gakhramanova F.Kh., Ragimova M.M., Neymatova U.V., Bahshaliyev A.Y. Bioconversion as an effective method for the rational use of various wastes of plant origin// Scientific works of the Institute of Microbiology of ANAS, Baku., 2017, v. 15, № 1, p.312 – 316
  4. Bakshaliyev A.Y., Musayeva V.H., Neymatova U.V., Axundova S.M. Waste: sources of processing, composition and perspectives of use// Scientific works of ANAS Institute of Microbiology, 2018, v. 16, № 1, p.200 – 205
  5. Neymatova U.V. Bioconversion of solid waste of non-food plants// Scientific works of ANAS Institute of Microbiology, 2018, v. 16, № 2, p.89-96
  6. Neymatova U.V. Non-food waste and its disposal options // Materials of the scientific conference on "Actual problems of modern biology", 2019, p.85-87
  7. Bakshaliyeva K.F., Bakshaliyev A.Y., Alieva B.N., Musayeva V.H., Neymatova U.V., Bioconversion as an effective method for rational use of plant waste from the agricultural sector./V International Conference "Science in the Age of Imbalances", Kyiv, 2019, p.13-18
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9. Bakshaliev A.Y., Musaeva V.H., Huseynova A.E., Neymatova U.V., Gasanova A.R. Biochemical composition of products obtained by microbiological conversion of lignocellulosic substrates by mycelial fungi // Modern science: current problems of theory and practice. Series: Natural and Technical Sciences, -2020. -№04. -p. 7-11
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11. Neymatova U.V., Tomuyeva G.A., Agayeva Z.T., Bakshaliyeva K.F. Bioconversion of non-food waste//Proceedings of the I International Scientific and Practical Conference "Innovative Biotechnologies for Environmental Protection: From Theory to Practice" (Belarus, Minsk, April 23-25, 2024). -Minsk,-2024, p.173-174.



The defense will be held on 25 December 2024 at 11.00 at the meeting of the Dissertation Council BFD 1.07/1 of Supreme Attestation Commission under the President of the Republic of Azerbaijan operating at the Institute of Microbiology, the Ministry of Science and Education of the Republic of Azerbaijan.

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Electronic versions of the dissertation and its abstract are available on the official website of the Institute of Microbiology, the Ministry of Science and Education of the Republic of Azerbaijan (<https://azmbi.az/index.php/az/>).

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