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Microbiological conversion of solid waste generated during the production of vegetable oil

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Abstract

Every year during the processing and use of green biomass produced by photosynthesis forms a large amount of plant waste containing polysaccharides. One of the sources of such wastes is the production of vegetable oil. For the effective utilization of wastes generated from the production of sunflower, olive and corn oil has been determined the effectiveness of the microbiological conversion process. It became clear that solid waste produced during the production of vegetable oil is a suitable substrate for microbiological conversion. Thus in the condition of liquid phase fermentation, the use of them during cultivation of producers (*Pleurotus ostreatus* and *Schizophyllum commune*) hydrolase and oxidase allows achieving high results. Their use for this purpose opens great prospects for the effective utilization of plant waste in the future.

Keywords: photosynthesis, green biomass, waste, fungi, microbiological conversion, hydrolase, and oxidase

Introduction

As known plants were indispensable sources to meet the need of living things in the earth, especially of humans and still holds that role [11]. Continuous increase in the world population within a fixed territory leads to an increase of anthropogenic load on the environment. As a result of this process, deficiencies in food, energy and raw materials for industry become apparent. This also makes necessary efficiently use of resources, first of all, plants.

It should be noted that every year, through the process of photosynthesis are produced millions of tons of green biomass [14] and about 10% of it is used for the purposes of meeting the needs of the energy of the world population. While in use certain part of the green biomass of plants turns to the materials which are not suitable for use in that form or its use ineffective. The amount of this type of materials as commonly called "waste" is determined depending on the nature of the used production process, of applied technologies, as well as the properties of the plant material[13]. Although it is not possible to present a specific figure on the amount of such waste produced on Earth today, there is no doubt that its amount millions and even billions of tons.

In the background of these tasks, the use of non-traditional sources, such as wastes, from the point of solving the tasks emerging from the modern era has long been the subject of ongoing research in various aspects and continues to this day [1, 3, 6]. Despite a large number of conducted studies and the results that promise some serious perspectives, the production processes based on waste recycling in terms of practical requirements are extremely low. There are several reasons for this, among most important ones of

this are the less of strains-producers with high biological activity effectively making waste useful from the point of view of practical needs, the high cost of obtained products, etc. As a result of these shortcomings, today hundreds of millions of tons of materials are either or burned or thrown into the environment in a disordered form. Some of the wastes are indeed used in the obtained form, and some involved in recycling and used in practice but in this case, also arise some problems. Thus, the efficacy of waste in the obtained form is not so high and results in the formation of waste from waste. The problem today is open for research and keeps its relevance.

As noted, the obtained wastes cover all taxonomic groups of living things and, as noted among them, plant origin differs from others by their size and composition[13]. Thus, the main components of this type of plant waste are polysaccharides which the amount of polysaccharides in them can be up to 90%. Acquisition of various products, including biogas from plant wastes having such potential has a significant perspective from the theoretical point of view[1, 16]. Taking this into consideration, in the presented work search for strains-producers to making practical use of plant wastes which formed in the agrarian sector of Azerbaijan has been put forward as a task. The need to solve such a task is related to the following.

First, the agrarian sector plays an important role in the economy of the country, and each year, the amount of the product produced in this area is estimated at millions of tons. This type of production is also observed by the formation of waste, and their total quantity is estimated in millions of tons. İt should be noted one fact that according to the calculations of experts the production of 1 kg of wheat is followed by the formation of 1 kg of waste products [13]. The amount of produced wheat in Azerbaijan has already exceeded 2 million tons [8] for several years.

Second, quantitative indicators of the chemical composition of wastes formed in one or another area have a certain specificity depending on the natural climatic-soil characteristics of the area on which it formed that in the solution of the problem still keeps their importance of the principle of individuality.

Third, in recent years, products obtained from natural sources are considered more valuable in terms of environmental value, in the production of certain products, especially in food products special attention paid to the role of environmental factors. Considering some of the waste generated by various manufacturing processes is also a natural source makes it necessary to direct them to practical use.

Finally, ineffective treatment to the formed plant wastes in any country of the world, including in Azerbaijan may also lead to environmental problems which prevention of this is one of the urgent tasks of the modern era.

Materials and Methods

In research, were used from the wastes generated during processing raw materials used during the production of vegetable oil and from macro-micromycetes strains, provided by the Microbiological Biotechnology Laboratory of the Institute of Microbiology of ANAS.

During the study of enzymatic activity cultivation of fungi was carried out under liquid phase fermentation (LPF). As a cultivated material were used biomass of micromycetes cultivated in the Czapek medium for 2-3 days, which as a carbon source in the medium was used glucose (10 g / l). For the receiving cultivated materials from macromycetes, they are firstly cultivated in the glucose peptone medium for 3-5 days and the resulting biomass crushed in a sterile magnetic mixer and the obtained suspension is used as cultivated material.

During LPF to the prepared appropriate medium (1 g of waste + 100 ml of ordinary water) adds 1 ml cultured material, sterilized at 0,5 atm for 0,5 h, and incubated for the required period (3-10 days) in 26-28^{0}C. After the end of the period with the help of centrifuges (5000 cycle/min, for 10 min) formed biomass is separated from the solution. The cultured solution is used to determine the exo-form activity of the enzyme.

The activity of hydrolase (endoglucanase, xylanase, amylase, pectinase, lipase, protease) and oxidase (lactase and peroxidase) in the course of research determined according to the methods currently used for this purpose[4, 9, 12]. The activity was expressed by iu /ml and iu /mg protein when the amount of protein was determined according to the spectrophotometric method [7].

Studies for optimizing the medium for strains selected as active producers were carried out according to the scheme and method used in the work of some authors[2, 5].

All the experiments were performed at least 4-6 times, the results were statistically analyzed and used only honest data[10].

Results and Discussion

There are many approaches to conversion of plant waste and among them, the microbiological conversion is considered effective for several reasons. For the effectiveness of this process, during the selection of producer the microorganism was used directly therefore, in studies microorganisms firstly were evaluated for their enzymatic activity.

Initial screening of macromycetes and micrometers used for selection of producer were carried out according to the extracellular activity of enzymes catalyzing the degradation of major components of waste. In this case, cultivation was carried out only in a medium consisting of 1% of one or another waste for 5 days. The results revealed that the strains involved in the screening process could synthesize one or the other enzymes. However, not of all have enzyme system at the level that will lead to deep conversion of compounds of waste, primarily cellulose and lignin,(difficult hydrolyzed components) intended suitable for the use in terms of practical demand and some are not even able to synthesize phenoloxidases involved in degradation of the lignin(tab. 1). As see, the enzyme system of macromycetes is much broader than that of micromycetes and some of them have the ability to intensive synthesize all of the enzymes which are necessary for bioconversion.

According to the table, the enzyme system of fungi such as *Pleurotus ostreatus* and *Schizophyllum commune* has been considered more favorable, thus these fungi are more balanced due to the enzyme's activity of the major polymers in the waste, primarily catalyzing the degradation of lignin and cellulose that hydrolyzed difficultly. İt is true that in some cases due to the activity of a specific enzyme observed higher indicator. For example, the strains of *Trametes hirsuta* for the activity of lactase and peroxidase, *T. versicolor* for the activity of amylase are characterized by higher quantitative indicators but, as mentioned, their other activities are not so high as those strains selected as active producers. For this reason, as active producers, they were selected to the next level.

İt should be noted that synthesis of one or another enzyme is a feature associated with the genome of strain-producers, however, the formation of quantitative indices of this enzyme is also determined by environmental factors [15, 17]. Taking this into consideration, in the next stage of research was also clarified the effect of environmental factors to the quantitative indicators of enzyme synthesis in the selected producers, in other words, it was implemented optimization of the medium. As a rule, research like this to the parameters selected for optimization belongs to carbon and additional nitrogen sources and their quantity, initial pH, cultivation temperature, method and time for preparation of cultured material.

From the carried out of research became clear that all of the noted parameters to some degree, influence the synthesis of both hydrolases and oxidases. The quantitative indicator of this effect is formed by both the nature of checked factors and the biological characteristics of the producers. This can be seen in table 2, where the results are summarized. As seen, the highest indicator in both mushrooms was obtained during used corn flour as a source of carbon which their amount added to the medium slightly differs from each other. This difference is evident both in nitrogen sources, during cultivation, as well as at cultivation temperatures. Despite these differences, the comparison of results obtained during the cultivation of selected mushroom under optimized conditions with known strain-producer and the use of waste such as carbon source in the nutritive medium gives an extra advantage to them.

This is also in future research as an active producer promise serious perspectives in the use both in the direct and enzymological conversion process as a source of enzymes that allow it to be used.

Thus, from the carried out of research became clear that microbiological conversion is a more effective approach for the utilization of solid waste generated during the production of vegetable oils and the use of xylotrophic macromycetes as a producer in this process is more purposeful. Became clear that solid waste generated during the production of vegetable oil is a suitable substrate for microbiological conversion. Thus in the condition of liquid phase fermentation, the use of them during cultivation of producers (*Pleurotus ostreatus* and *Schizophyllum commune*) hydrolase and oxidase allows achieving high results. This opens great prospects for the effective utilization of plant wastes in the future.

Table 1 Screening of micromycetes and macromycetes strains by the hydrolase and oxidase (activity, iu /mg protein)

Fungi species(number of strains)	Cellulose	Xylanase	Pectinase	Protease	Amylase	Laccase	Peroksidaza	Ligninases
Alternaria alternata(3)	0,42-0,71	17-28	3,7-6,1	4,5-7,1	0,6-1,4	1,7	0,5	0,3
Aspergillus niger(3)	0,75-1,32	34-45	9,7-11,8	4,7-7,4	3,7-5,2	2,1	0,5	0,6
Botrytis cinerea(3)	0,35-0,58	22-27	1,3-2,7	0,8-1,1	0,01-0,03	1,1	0,2	0
Cladosporium herbarium(3)	0,42-0,63	20-28	2,6-4,8	1,5-2,0	0,4-0,9	0	0	0
Fuzarium oxysporium(3)	0,45-0,77	22-31	2,5-4,4	0,02-0,11	2,4-3,6	1,6	0	0,3
Mucor mucedo(3)	0,31-0,48	20-27	2,3-3,4	6,2-7,5	0,8-1,4	0	0	0
Penicillium chryzogenum(3)	0,68-0,95	19-24	2,0-4,4	2,8-4,7	0,5-1,5	2,4	0,7	0,6
Penicillium cyclopium(3)	0,32-0,71	27-35	3,3-7,2	1,2-2,1	1,3-2,5	2,1	0,4	0,4
Rhizopus nigricans(3)	0,42-0,65	17-22	2,2-4,0	4,4-5,5	1,2-1,5	0	0	0
Trichoderma viride(3)	1,05-1,94	31-43	1,2-1,7	3,1-5,3	0,3-0,5	4,2	2,7	2,4
Bjerkandera adusta(3)	0,54-0,92	80-109	2,3-4,7	2,3-5,6	1,9-3,5	10,2	5,9	5,3
Cerrena unicolor(3)	0,50-0,85	73-102	2,1-4,3	3,1-5,4	2,2-3,8	11,1	7,4	7,0
Ganoderma lucidum(3)	0,36-0,64	65-87	2,3-3,9	2,4-5,0	1,6-3,5	8,4	6,4	6,3
Hircshioporus pergamenus(3)	0,30-0,58	74-90	2,1-4,1	1,2-5,1	2,4-3,2	7,6	5,4	5,5
Phellinus igniarus(3)	0,30-0,43	56-72	1,9-3,5	1,4-3,2	1,2-3,1	7,1	6,1	5,1
Pleurotus ostreatus(3)	0,55-0,97	70-120	5,7-8,5	3,7-6,6	2,3-4,1	12,4	7,7	7,9
Schizophyllium commune(3)	0,52-0,99	76-124	7,0-8,2	4,0-6,8	2,6-4,5	11,6	7,8	7,5
Trametes hirsuta(3)	0,52-0,72	67-89	5,2-7,1	3,6-4,5	2,6-4,0	13,1	8,1	7,7
T.versicolor(3)	0,50-07,6	65-83	5,4-7,4	3,2-4,7	3,2-5,1	12,6	7,9	7,5

Table 2 Main parameters of optiamal conditions for the synthesis of enzymes in fungi selected as active producer

Producer	Carbon source and amount (g/l)	Nitrogen source, amount by the nitrogen %-lə	Cultivation temperature	Initial acidity	Cultivation period(Hours)
P. ostreatus	Corn waste (9,5)	$NH_4 NO_3$ (0,038)	28^0C	5,7	144
Sch.commune	Corn waste (9,6)	$NH_4 NO_3$ (0,036)	30^0C	5,7	130

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