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PRODUCTION OF PROTEIN-CONTAINING PREPARATIONS OF NATURAL ORIGIN

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Abstract. *The possibility of increasing the yield of mycelial biomass and protein substances of higher edible mushroom Pleurotus ostreatus in the conditions of submerged cultivation, as well as the productivity increase of the culture Eisenia foetida as producer of protein biomass on the nutrient substrate mixture of sunflower and rice husk modified to 200–500 microns have been studied. The greater amount of deep Pleurotus ostreatus biomass was found to accumulate on media containing hydrolysates of buckwheat, corn or wheat flour combined with gluten. It is on these media that the highest protein content of biomass is observed. The presence of soy milk as part of nutrient medium provided pleasant mushroom smell specific to Pleurotus ostreatus of natural growth. Fermented substrate of sunflower and rice husk mixture was established to increase vermiculture yield at densities ranging between 10–15 thousand per m^2 and to optimize biochemical composition of the Eisenia foetida biomass tissue.*

Keywords: *higher edible mushroom, biomass, vermiculture, fermented substrate, culture media.*

1. Introduction

Protein-containing products are widely used in medical, veterinary, food, pharmaceutical industry, animal husbandry and in the research area. Today's society shows a special interest to the protein products of natural origin [1].

Industrial production of protein-containing natural products is possible by extraction from plant, animal or microbial raw materials [2, 3]. However, this method does not allow to produce sufficiently large quantities of protein-containing preparations, because the method is limited by the deficiency of vegetable and animal raw materials, crop areas, weather and climatic conditions. In addition, to extract proteins from a corresponding raw material it is necessary to use chemical reagents (acids, bases) having negative influence on the native properties of proteins in finished product. Thus, the industrial production of protein biopreparations by this method appears to be not profitable.

The production of protein-containing preparations with appropriate producer organisms turns out to be the most expedient both from the economic and technological point of view. Various commercial protein products produced industrially in various countries are known [2]. Among the producers of such preparations yeast are the most common (bacteria less common). Thus, preparations of food yeast, therapeutic beer yeast are produced by *Saccharomyces cerevisia*. The basis for such preparations as paprin (yeast feed protein) and torutin is *Candida maltosa* and *Candida utilis*, respectively. Protein preparation gaprin

(bacterial feed protein) is produced on the basis of *Methylophilus methylotropus* bacteria. There are a few examples of using organisms from other taxonomic groups as producers of protein-containing products. The technologies for producing protein-containing preparations of mycelial origin are also developed. For example, digitatin and food mycoprotein are prepared using fungi *Penicillium digitatum* and *Fusarium graminearum*, respectively. Saprophytic fungi *Paecilomyces varioti* are used to prepare the mushroom feed mass. Mycelial organisms as industrial producers have some advantages. In particular, it is easier to separate mycelium from the culture liquid by filtration; the amount of protein in the cells of many fungi is higher than that of yeast or bacterial organisms [1].

Organisms from other taxonomic groups as producers of protein products are not studied enough. So, with the help of representatives of higher edible mushrooms (Pleurotus ostreatus, champignon, etc.) it is possible to produce not only fodder products, but also quality foodstuffs with high protein content possessing racy flavour and aroma of natural fungi [4–6].

Vermiculture *Eisenia foetida* can be used as a basis for protein products of medical, pharmacological and fodder purpose [3]. However, the use of these organisms as industrial producers of protein-containing products requires comprehensive research including selection and optimization of culture media and substrates for growing them *in vitro*. Thus, this problem is urgent and requires further investigations. The aim of this work is selection and optimization of liquid media for submerged cultivation of *Pleurotus ostreatus* as well as modification of solid-state nutrient substrate for increasing biomass of *Eisenia foetida* culture.

2. Materials and methods

In these experiments, carried out at the Institute of Biotechnology of the Ukrainian State University of Chemical Technology and in the research laboratory at the Department of Microbiology, Virology and Biotechnology of Dnipropetrovsk National University n.a. O. Honchar, we used as objects of study: higher edible mushroom *Pleurotus ostreatus* strain NC-35 (Department of Mycology, Institute of Botany, Kiev) as a producer of protein substances and vermiculture *Eisenia foetida* (Research institute "Biotechnology" of IHE "USCTU" Dnipropetrovsk Tech. Specs. TU 3336406.002-95) as a producer of protein biomass, sunflower and rice husk (Tech. Specs. TU-18 USSR6280).

For storage of museum culture *Pleurotus ostreatus* large microbiological test-tubes with wort-agar media (6 °C) were used. Subsequent fermentation was performed in 300 ml capacity shake flasks containing 50 ml of nutrient medium. The composition of the fermentation media was different. Test media contained (%): corn steep liquor – 6, hydrol (high-green syrup) – 6, potassium dihydrogen phosphate – 0.05, thiamine chloride – 0.0002, sunflower oil – 4. Experimental nutritive media included enzyme hydrolysates of different types of flour or husking bran and gluten or soya milk at different pH concentrations (6.8–6.9).

The main raw material of the experimental culture media (different types of flour) was subjected to enzymatic prehydrolysis using amylolitic enzyme preparations of domestic production. Hydrolysis conditions were as follows: temperature 57–60 °C, 10–15 minutes duration, concentration of alpha-amylase preparation 1:1900 and that of glucoamylase – 1:2000. The prepared nutrient media were autoclaved for 45 minutes at 120 °C [7]. Fungus cultivation was carried out in submerged conditions for 120 hours at the temperature of 26–28 °C with stirring at 240 r/min. in the shaker of FHC-12-250 type. The resulting culture medium (CM) was analyzed for protein and

biomass content [7, 8] after its separation in the centrifuge K-23 at 3000 rpm for 10 minutes. Experiments were carried out in 7 replications. The results were processed by mathematical statistics methods [9].

Modification of sunflower and rice husk was carried out in a mill with rotary beater having cylindrical elements to fractions 200–500 microns. Cultivation was carried out on *Eisenia foetida* fermented substrate of sunflower and rice husks in the ratio 1:0.5 at a temperature of 20–25 °C; pH 6.5–7.5; stocking density – 10–15 thousand worms per 1 m^2 (to speed up the growth of biomass), 15-30 thousand worms per 1 m^2 (to increase the number of *Eisenia foetida* population); frequency of making fresh substrate – once every 10 days. Biological value of *Eisenia foetida* biomass was determined by amino-acid analyzer N1200E, by Bernstein, Lowry spektrometric method. Potassium was defined by the weight of potassium tetraborate which was formed in acidic medium; phosphorus – by colorimetric Briggs' method; calcium – by conductormetric method [7, 8, 10–12]. Gross energy and digestible energy of *Eisenia foetida* biomass was determined by V.I. Ivanov's formula [13].

3. Results and discussion

Increasing of fungus productivity at the basic parameters was possible by optimizing the qualitative and quantitative composition of the culture media. For the growth of the producer, media of different composition were proposed. The extract molasses medium containing starch production waste was corn extract (a source of nitrogen and growth factors) and hydrol (carbon and energy source). For comparison, culture media including other sources of producer development (starchy flour production waste as a source of carbon and energy as well as gluten or soya milk as a nitrogen supply source of the fungus) were studied. Starchy components were subjected to enzymatic prehydrolysis. The concentration of the investigated components in the medium was calculated by the content of sugar (0.15 %) and nitrogen (0.22–0.24 %), respectively. The results on the accumulation of biomass and protein by edible higher mushroom of *Pleurotus ostreatus* (strain TC-35) which were identified in the conditions of this experiment are presented graphically in Fig. 1 and 2.

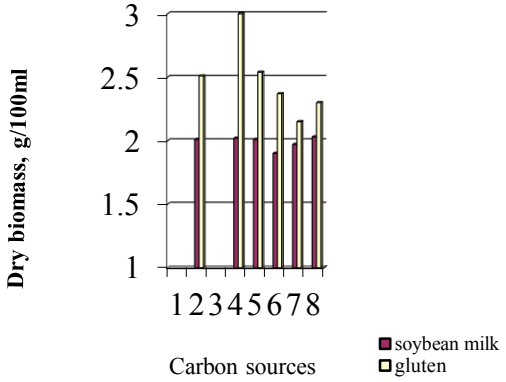

Fig. 1. Pleurotus ostreatus biomass yield depending on the quality of carbon and nitrogen sources in culture media: 2, 4, 5, 6, 7, 8 – hydrolysates of corn, buckwheat, wheat flour, wheat husking bran, oats and rye flour, respectively

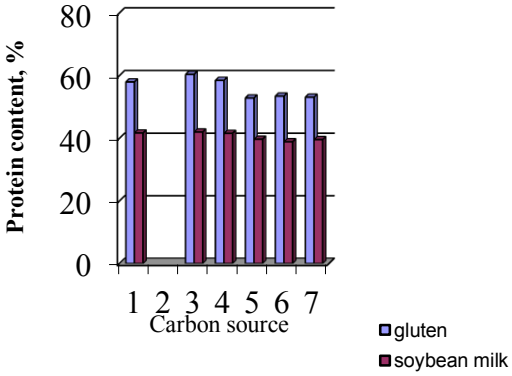

Fig. 2. Protein yield of *Pleurotus ostreatus* depending on culture media quality: 1, 3, 4, 5, 6, 7 – hydrolysates of corn, buckwheat, wheat flour, wheat husking bran, oat and rye flour, respectively

These charts indicate that more of depth *Pleurotus ostreatus* biomass was accumulated on the media containing hydrolysates of buckwheat, corn or wheat

flour combined with gluten. The highest protein content in the biomass was observed on these particular media. The combination of these hydrolysates and soya milk provided obtaining of smaller amount of biomass by a factor of 1.5; 1.3 and 1.4 times respectively than that produced on gluten. Similar pattern was observed in the analysis of protein content in the deep fungal biomass. Thus, obtaining *Pleurotus ostreatus* biomass with high protein content (up to 60%) is possible.

To do this, enzymatic hydrolysates of various starch-containing materials could be used. But the use of buckwheat, corn or wheat flour hydrolysates seemed to be optimal. The nitrogen source was found to be more important as a component of the culture medium for the accumulation of *Pleurotus ostreatus* biomass and protein, in the conditions of the experiment involved. Thus, gluten provided a greater accumulation of both biomass and protein therein as compared with soya milk. It was also noted that the quantitative values for biomass and protein on all hydrolysis media in combination with soya milk have no significant differences between themselves and as compared with the control (the extract-molasses medium). But in the presence of soya milk as a part of the culture medium, an important feature of mushroom mycelium, namely the emergence of a pleasant mushroom smell characteristic of natural growth *Pleurotus ostreatus*, was revealed. The presence of the appropriate taste and smell were indispensable features of deep fungal mycelium as biotechnological product of food quality.

It was also found that the cultivation of these mushrooms on the extract-molasses medium did not provide the highest yield of the fungus protein-containing biomass. The concentration of the mycelium in liquid culture reached 1.82±0.091 g/100 ml and the protein content was 45.99±2.01 %, which is 1.5 and 1.3 times lower than that on the enzymatic hydrolysate of buckwheat flour in combination with gluten.

The analysis of the obtained data indicated a need for further research on the optimizing of culture media composition, which would ensure the yield increase of protein-containing biomass having characteristic aroma of natural *Pleurotus ostreatus* mushroom. To do this one must probably combine nitrogen sources under investigation in one nutrient medium, but it is necessary to optimize quantitatively media for nitrogen-containing components.

During the investigations it was established that the mixture of natural polymers of sunflower and rice husks crushed on a rotary mill to 200–500 microns appeared to be optimal medium for breeding *Eisenia foetida*, met the international requirements for the content of the necessary materials and components to ensure the nutritional value of the substrate: crude protein – 5–6 %; crude fat – 4–4.5 %; crude fiber – 40–45 %; calcium – 4.5 g/kg; phosphorus – 0.8 g/kg.

To determine the optimal conditions for keeping and breeding *Eisenia foetida* investigations concerning effect of humidity, temperature, worms stocking density on the fruitfulness and growth of the *Eisenia foetida* population biomass were carried out. Optimum conditions for keeping and breeding *Eisenia foetida* worm on the substrate of modified sunflower and rice husk were as follows: 70 % to 80 % humidity, the ecological valence of humidity 40–98 %, the normal life activity area 65–90 %. The optimum temperature for growth and functioning of *Eisenia foetida* biomass was 20–25 °C; normal activity area was 15–30 °C, the environmental temperature valence was 5–35 °C. The acidity of the substrate should be within 6.2–7.8.

Observations on the activity and survival of *Eisenia foetida* showed that the modified substrate consisting of natural polymers mixture was suitable as a habitat and a growth medium. The first cocoons were found a month later, larvae hatched out two weeks later. Microorganisms (mould) not

typical for a living environment appeared on the substrate surface during composting process in the first days after the introduction of worms, but they disappeared in two weeks. These results showed that during the processing of *Eisenia foetida* substrates mouldy microflora was destroyed, thereby disinfecting vermicompost.

We conducted the experiments to determine the effect of worm introduction density on the increase in *Eisenia foetida* population biomass. Introduction density during three experiments was 10000/m^2, 15000/m^2 and 30000/m^2, with an average weight of worms 210 mg. The experiment has been conducted for three months. Data on the effect of introduction density on the growth of *Eisenia foetida* population biomass is given in the table.

Table 1.

The impact of introduction density on the growth of *Eisenia foetida* population biomass

Indicator	Research Period	The number of worms per 1 m^2		
		10000	15000	30000
The average weight of a single worm, mg	Initiation of study	210±1.47	210±1.47	210±1.47
	After 1 month	427±5.12	425±5.52	369±6.27
	After 2 months	756±13.60	661±13.88	581±12.78
	After 3 months	1048±24.10	979±22.52	876±20.58
The gain since study initiation, mg	After 1 month	217±2.60	215±2.79	159±2.22
	After 2 months	546±9.83	451±9.47	371±8.16
	After 3 months	838±19.27	769±17.69	666±15.65

Thus, we can conclude that with greater introduction density the number of *Eisenia foetida* vermiculture and substrate processing speed increased. To intensify the *Eisenia foetida* biomass yield, introduction density may be in the range of 10–15 thousand / m^2. The intensity of the substrate processing by worms is 3.2–3.6 kg per day per 1 m^2; the yield of the protein additive is 50–60 %. As a result of processing of organic substances by vermiculture, the mixture of plant substrate extracted from the worms' digestive tract – coprolites – appeared to be nutritious: the total amount of humic acid constituted 60,36–60,95 %, that of nitrogen – 1.5–2.7 %, of phosphorus – 1.25–1.71 %, potassium 2,18–2,41 %; there were 17 amino acids, including essential ones – lysine, methionine, tryptophan; macro- and trace elements – magnesium, iron, copper, manganese and zinc, vitamins B_1, B_2, PP.

It was determined that dry matter in the *Eisenia foetida* biomass tissue, adapted on the modified sunflower and rice husks, constituted 17–23 %, crude protein up to 60 %, lipids 6–9 %, nitrogen extractives from 7 to 16 %. The criterion of gross and digestible energy served to assess quality of *Eisenia foetida* biomass for animal feed . 1 kg of *Eisenia foetida* biomass produced by culturing on sunflower and rice husk contained 1.03 of feed units and 14.4 MJ of digestible energy.

4. Conclusions

Thus, the selection and optimization of liquid nutrient media for submerged cultivation of *Pleurotus ostreatus* provided increased yield of both biomass and protein in it; modification of solid-phase nutrient substrates based on sunflower and rice husks for growing *Eisenia foetida* allowed to obtain a high quality protein containing mass with quality biochemical composition comprising all essential amino acids, microelements, vitamins, enzymes. Consequently, the above said organisms can be used as effective producers of protein-

containing products of natural origin.

5. References

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