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SELECTION OF CONDITIONS FOR SUBMERGED CULTIVATION OF *PLEUROTUS OSTREATUS*

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Abstract

The object of our research was the culture of *Pleurotus ostreatus*. In our previous studies hypolipidemic, hypocholesterolic, immunomodulating, hypoglycemic actions of *P. ostreatus* were demonstrated. Also *P. ostreatus* is known for its: antitumour, anti-inflammatory, antioxidant, antibiotic and radioprotective actions. A special attention should be paid to beta-glucans, which are structural polysaccharides of fungal cell walls and are demonstrating significant biological activity. They are suitable candidates for research and development of new functional foods and nutraceuticals. Beta-glucans, isolated from basidiomycetes, are of considerable interest due to their various useful preventive and functional properties to widely used ordinary food products. The aim of this study was to select the conditions for submerged cultivation to obtain the highest yield of biomass of *P. ostreatus*.

The submerged cultivation was carried in Erlenmeyer flasks on rotary shaker. Various sources of carbon: rye whole grain flour, medium rye flour, light wheat flour, dark wheat flour, corn flour, potato starch, soy flour and sources of nitrogen: carbamide, NaNO3, NH4NO3, (NH4)2SO4, soy flour were used in the semisynthetic nutrient medium. Also, the growth of P. ostreatus was studied on various initial pH values and for different dissolution rates of oxygen in the culture medium. Aeration rate was determined by the sulfite method.

As a result, the high aeration levels (3.3-4.0 g O_2/(L·h);) were selected for cultivation. The initial pH value of the culture medium 7.5 is preferable to the lower values. Medium rye flour or dark wheat flour as a source of carbon and soy flour as source of nitrogen provided the highest yield of *P. ostreatus* biomass were selected.

Submerged biomass of *P. ostreatus* can be used for obtaining beta-glucan preparations as functional supplement for fortification of dairy products and other ordinary food products.

Key words: *Pleurotus ostreatus, β-glucans, Submerged cultivation, Sources of carbon, Sources of nitrogen, nutrient medium.*

1. Introduction

Pleurotus ostreatus or oyster mushroom is a valuable mushroom whose fruiting bodies are used for food and are grown industrially in many countries around the world. In addition to its taste and nutritional properties, this fungus also has a wide range of biological activity.

In our previous studies hypolipidemic and hypocholesterolic, actions of *P. ostreatus* were demonstrated (Shamtsyan *et al.*, [1]). Also *P. ostreatus* is known for its: immunomodulating, anti-allergic, antitumour, anti-inflammatory, antioxidant, antibiotic, antiproliferative, prebiotic and radioprotective actions (Bashir and Choi, [2]; Friedman, [3]; Kozarski *et al.*, [4]; Facchini *et al.*, [5]; Du *et al.*, [6]; Llauradó *et al.*, [7]; and Radzki *et al.*, [8]). A special attention should be paid to beta-glucans, which are structural polysaccharides of fungal cell walls and demonstrate significant biological activity. They are suitable candidates for R&D of new functional foods and nutraceuticals. Beta-glucans, isolated from basidiomycetes, are of considerable interest due to their various useful preventive and functional properties to widely used ordinary food products. Beta-glucans

preparations can be used in medicine as immunostimulating agents. For example some studies showed that beta-glucans from *P. ostreatus* can be helpful in reducing respiratory tract infections in children with chronic respiratory disorders (Bashir and Choi, [2]; Jesenak *et al.*, [9]; and Pasnik *et al.*, [10]).

Such preparations from *P. ostreatus* could provide additional preventive and functional properties to widely used ordinary food products, such as: immunostimulating, hypoglycemic, antitumour, anti-inflammatory and hypoglycemic properties, as it was mentioned in Giavasis [11]. Polysaccharides isolated from *P. ostreatus* fungus culture can be used for development of new functional products and nutraceuticals (Gargano *et al.*, [12]). In our previous study we tested the possibility of using fungal polysaccharides containing beta-glucans to create functional foods based on fermented milk products (Antontceva *et al.*, [13]). Frioui *et al.*, [14], explored rheological influence of mushrooms beta-glucans, used as flour substitution in bakery industry.

Besides cultivation of oyster mushroom fruit bodies, its submerged culturing is also possible. Submerged cultivation method permits to obtain homogenic biomass, which simplifies product isolation, as well as the stability of the properties of the finished product and its relative standardization (Biliaderis, [15]). One of the most common nutrient media for the cultivation of basidiomycetes is a semisynthetic glucose-peptone medium. Composition of the nutrient medium has significant importance for submerged cultivation. The yield of biomass, the biosynthetic activity of the producer and the final cost of the product can be strongly influenced by media composition. Zhang *et al.*, [16], studied effect of carbon source on properties and antioxidant potential of exopolysaccharides produced by *Trametes robiniophila* cultures. Hoa and Wang, [17], observed the effects of temperature and nutritional conditions on mycelium growth of two oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*). From the literature, it is known that organic nitrogen compounds are best used for the cultivation of *P. ostreatus* fungus. The most widely used nitrogen source is peptone and on the second place is carbamide (Bisko *et al.*, [18]).

The aim of this work is to select the composition of the medium and the aeration rate that ensure the highest yield of biomass. Obtained submerged biomass of this fungus was used as a source of polysaccharides. Extraction of polysaccharides from mycelia biomass was carried out as it was mentioned in Giavasis, [11].

2. Materials and Methods

P. ostreatus (Jacq.) P. Kumm culture was taken from the museum of the Department of Technology of Microbiological Synthesis of the Saint-Petersburg State Institute of Technology.

The inoculum of *P. ostreatus* was first incubated in test tubes on agarised Saburo medium at the temperature of 28 - 30 ^{0}C for 7 - 10 days. Then, few pieces of mycelium measuring were transferred with the agar medium to conical flasks with glass beads and the nutrient medium of the following composition (Table 1):

Table 1. Composition of nutrient medium

Component of nutrient medium	Concentration, g/L
Glucose	15
Peptone	2.5
Yeast extract	2.0
KH_2PO_4	0.6
K_2HPO_4	0.4
$MgSO_4$	0.5
NaCl	0.5
$CaCl_2$	0.05

Initial pH value of the medium, before sterilization was 6.5.

Inoculate has been farther grown under steady-state conditions at the temperature of 28 - 30 ^{0}C for 7 - 10 days until the surface of the medium completely overgrew with mycelium. The raised surface inoculum was crushed with glass beads by shaking the flasks. The resulting suspension was used as inoculate for the fermentation step.

To determine the effect of aeration of the medium on the synthesis of biomass, the mushroom was cultured in 750 mL Erlenmeyer flasks with different quantity of liquid medium - 50, 100, 150 and 200 mL of medium on a rotary shaker with a rotation frequency of 230 min^{-1} at the temperature of 28 - 30 ^{0}C. The inoculum volume was 10% of the volume of liquid medium in Erlenmeyer flasks. The fungus was cultured for 7 days in all cases.

Aeration rate was determined by the sulfite method (Yegorov, [19]). The dissolution rates of oxygen in the flasks were: 4.4, 3.0, 1.8 and 1.2 g/(L·h) for the volume of medium: 50, 100, 150 and 200 mL respectively.

After cultivation of *P. ostreatus*, the mycelium was separated from the native liquid by filtration through a paper filter under vacuum. Wet biomass was dried in an oven at the temperature of 50 ^{0}C. The weight of dried biomass was determined gravimetrically.

To study the effects of various initial pH values *P. ostreatus* was cultured in Erlenmeyer flasks with 100 mL of liquid medium (composition is indicated in Table 1) with initial pH values of culture medium: 5, 6, 6.5, 7 and 7.5. Initial pH values of nutrient mediums were set up to the desired values by adding to the basic medium definite amounts of HCl or NaOH.

To study the growth of *P. ostreatus* culture on the nutrient medium with various carbon sources the medium from

the Table 1 was used as a base. In the experiment: rye whole grain flour, medium rye flour, light wheat flour, dark wheat flour, corn flour, potato starch and soy flour were taken as carbon sources instead of glucose. The concentrations of carbon sources in the media were equivalent to the content of glucose 15 g/L.

Carbamide, $NaNO_3$, NH_4NO_3, $(NH_4)_2SO_4$ and soy flour were tested as a source of nitrogen instead of peptone. The other components were the same as in the Table 1. The concentrations of nitrogen sources in the media were equivalent to the nitrogen content in the medium from Table 1.

All experiments were carried out at least in three replicas. Standard deviations were calculated and included in the graphical representation of the data. The results were processed statistically with program Microsoft office Excel.

3. Results and Discussion

The concentration of oxygen in the medium has a significant effect on the biosynthesis of biomass by fungi (Wang *et al.*, [20]). Therefore, synthesis of beta-glucans depend on dissolution rates of oxygen in the culture medium. The effect of different dissolution rates of oxygen in the culture medium on the yield of *P. ostreatus* culture was studied. The results of these studies are shown in Figure 1.

From the presented in Figure 1 data it is obvious that the highest dissolution rate of oxygen gives the best yield of *P. ostreatus* biomass.

The biomass concentration accumulated during the growth of *P. ostreatus* culture on a semisynthetic glucose-peptone medium with different initial pH values is presented in Figure 2.

From the obtained results it is clear that when the pH value of the medium is less than 6, the accumulation of biomass by the culture of *P. ostreatus* decreases; in the range of pH values of the medium from 6 to 7, the culture demonstrates good growth and the best growth was shown at pH value of 7.5.

The choice of the source of carbon is very important for the selection of the nutrient medium. The source of carbon affects the metabolism of microorganisms, their biosynthetic activity. As in our case the target product is the cell wall polysaccharides, accumulation of fungal biomass which depends primarily on the carbon source is important. In addition, the task was to replace glucose with a cheaper carbon source, which would increase the economic efficiency of the process. Growth of *P. ostreatus* culture on the nutrient medium with different sources of carbon is demonstrated in Figure 3. Biomass concentration obtained from the basic nutrient medium (Table 1) is marked on the Figures 3 and 4 by yellow colour.

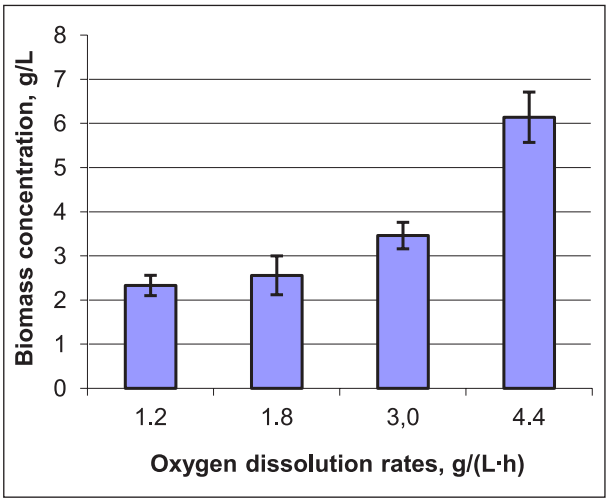


Figure 1. Concentrations of *P. ostreatus* biomass at different dissolution rates of oxygen

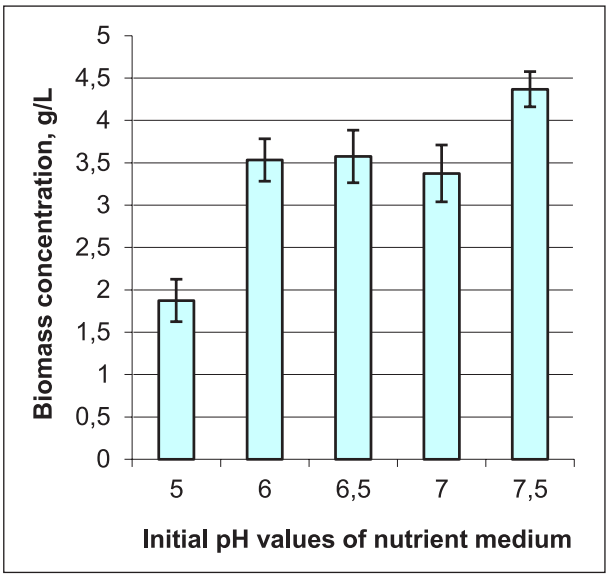
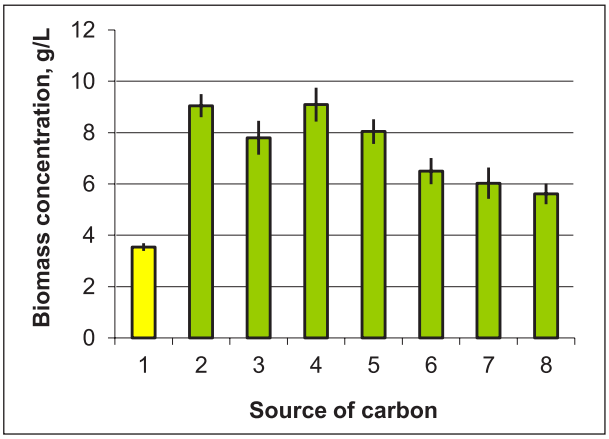


Figure 2. Correlation of the yields of *P. ostreatus* biomass and initial pH values of nutrient medium

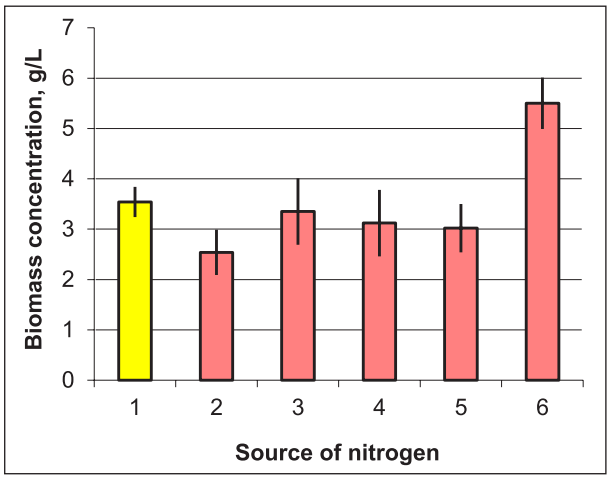


1 - glucose, 2 - rye whole grain flour, 3 - medium rye flour, 4 - light wheat flour, 5 - dark wheat flour, 6 - corn flour, 7 -potato starch, 8 - soy flour

Figure 3. Concentrations of *P. ostreatus* biomass at different sources of carbon in the nutrient medium

All carbon sources, which were used to substitute glucose demonstrated much better results. Based on these data, glucose can be replaced by a cheaper carbon source, which will also increase the yield of the product.

The second most important component in the nutrient medium is a nitrogen source. Growth of *P. ostreatus* culture on the nutrient medium with different sources of nitrogen is shown in Figure 4.



1 - peptone, 2 - carbamide, 3 - $NaNO_3$, 4 - NH_4NO_3, 5 - $(NH_4)_2SO_4$, 6 - soy flour

Figure 4. Correlation of the yields of *P. ostreatus* biomass and different sources of nitrogen used in the nutrient medium

From the data obtained, it can be seen that peptone can be replaced by inorganic sources of nitrogen. Carbamide as a source of nitrogen did not show the expected good results. Particular attention should be paid to soy flour as a source of nitrogen, it showed the best results.

4. Conclusions

- For the culturing of *P. ostreatus*, the following cultivation conditions are recommended:

a. Cultivation period 7 days;

b. Aeration rate 3.3 - 4.0 g/(L·h);

c. The initial pH value of the culture medium 7.5 is preferable to the lower values.

- For the cultivation of *P. ostreatus*, we recommend to use a nutrient medium of the following composition, (g/L): medium rye flour or dark wheat flour - 20, soy flour - 4.4, yeast extract - 2, KH_2PO_4 - 0.6, K_2HPO_4 - 0.4, $MgSO_4$ - 0.5, NaCl - 0.5, $CaCl_2$ - 0.5.

- Submerged biomass of *P. ostreatus* can be used for obtaining beta-glucan preparations as functional supplement for fortification of dairy products and other ordinary food products.

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