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# Glycerol as a carbon source for the accumulation of black yeast Pseudonadsoniella brunnea biomass

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***ABSTRACT:*** *Research into determining the optimal conditions for biomass accumulation and melanin production by black yeast fungi Pseudonadsoniella brunnea is a pressing issue. The purpose of this study was to evaluate the possibility of glycerol application for biomass accumulation of the black yeast fungus P. brunnea. Purified glycerol (pharmaceutical) and glycerol (techn.) obtained in the production of biofuels were used as the carbon source in the nutrient media. Glycerol, 20 g/l, peptone enzymatic 10 g/l and yeast extract 5 g/l were introduced into the culture media. The liquid nutrient media malt extract broth (MEB), Sabouraud broth and barley malt extract with addition peptone enzymatic (10 g/l), and yeast extract (5 g/l) to it were the control variants of the study. P. brunnea cultivation was carried out at pH 4, at + 24 ± 2 ° C for 28 days. The highest increase in P. brunnea biomass was found on glycerol (techn.) medium, and barley-malt extract - 2.68 ± 0.054 and 2.71 ± 0.023, respectively. The analysis of the results of the conducted research shows that technical glycerol, which is a by-product in the production of biodiesel, can be used for the accumulation of biomass by the producer of melanin Pseudonadsoniella brunnea. This will make significant changes to the economic component of biomass production of melanin producer P. brunnea yeast fungus.*
***KEYWORDS****: black yeast-like fungi, melanin producer, technical glycerol, biomass*

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## I. INTRODUCTION

Currently, the production of biofuels is an urgent problem. As biodiesel production increases, the production of its main by-product, namely raw (technical) glycerol, increases. Utilization of crude glycerol is an environmental and economic problem, and the search for ways to use it is important and relevant. Furthermore pure glycerol can be used in the cosmetic, tobacco, pharmaceutical and food industries. Efficient use of crude glycerol will contribute to biodiesel production. One of the most important uses of crude glycerol is its bioconversion using microorganisms to produce 1,3-propanediol, organic acids (e.g. citric acid) and polyols [1-3]. Recent advances in the production of value-added chemicals and lipids using biodiesel have enabled crude glycerin to be obtained as a substrate.

The use of glycerol for the growth and biosynthetic activity of microorganisms is recognized as promising. The results obtained in this direction demonstrate the potential use of crude glycerol as a carbon source in yeast cultivation and the ability of yeast to convert crude glycerol into value-added products. There is a large amount of literature available, including several reviews that present data on the use of crude glycerol by the yeast Yarrowia lipolytica as a substrate for growth [3-6].

Out of 113 yeast strains tested, 45 grew on pure glycerol with growth rates ranging from 0.11 to 0.37 h(-1). Twenty-three strains showed specific growth rates (h(-1)), biomass production and biomass yields higher or comparable to those on glucose. A biomass concentration of 25.7 g/l and a biomass yield of 0.92 g/g (Y/Xglyc) was obtained with Yarrowia lipolytica DiSVA C 12.1 and with Rhodotorula mucilaginosa DiSVA C 7.1, respectively. From these results Taccari M. et al. [4] made conclusion that crude glycerol can be utilized as carbon source in yeast cultivation for biomass production.

Crude glycerol was used as a carbon source in the cultivation of forty wild yeasts, aiming for the production of microbial lipids and citric acid [7]. Four yeasts (Lidnera saturnus UFLA CES-Y677, Yarrowia lipolytica UFLA CM-Y9.4, Rhodotorula glutinis NCYC 2439, and Cryptococcus curvatus NCYC 476) were then selected owing to their ability to grow in pure (OD600 2.133, 1.633, 2.055, and 2.049, respectively) and crude (OD600 2.354, 1.753, 2.316, and 2.281, respectively) glycerol (10%, 20%, and 30%). Yarrowia lipolytica UFLA CM-Y9.4 was selected for its ability to maintain cell viability in concentrations of 30% of crude glycerol, and high glycerol intake (18.907 g/l). Y. lipolytica was submitted to lipid production in 30 g/l of crude glycerol, and therefore obtained 63.4% of microbial lipids. In the fatty acid profile, there was a predominance of stearic

(C18:0) and palmitic (C16:0) acids in the concentrations of 87.64% and 74.67%, respectively. It was also performed optimization of the parameters for the production of citric acid, which yielded a production of 0.19 g/l of citric acid in optimum conditions (38.4 g/l of crude glycerol, agitation of 184 rpm, and temperature of 30°C. These data may be used for production in large quantities for the application of industrial biodiesel.

In general there is huge variability among yeasts with regard to their efficiency in utilizing glycerol as the sole source of carbon and energy. Certain species can show growth rates with glycerol comparable to those reached with glucose as carbon source; others are virtually unable to utilize glycerol, especially in synthetic medium. Most of our current knowledge regarding glycerol uptake and catabolic pathways has been gained from studying laboratory strains of the model yeast Saccharomyces cerevisiae. The growth of these strains on glycerol is dependent on the presence of medium supplements such as aminoacids and nucleobases. In contrast, there is only fragmentary knowledge about S. cerevisiae isolates able to grow in synthetic glycerol medium without such supplements as well as about growth of nonSaccharomyces yeast species on glycerol. Thus, more research is required to understand why certain strains and species show superior growth performance on glycerol compared with common [8].

A glycerol obtained as a by-product of biodiesel production can be used as a substrate for the growth of Saccharomyces cerevisiae yeast [9]. When comparing the kinetic growth indices, it was revealed that the growth of S. cerevisiae yeast culture using glycerol as a substrate after biodiesel production is higher than the analogous indices for growth on chemically pure glycerol, and is close in kinetic parameters to growth on glucose. This phenomenon can be explained by the presence in glycerol as a by-product of biodiesel production of residues of unreacted transesterification products, as well as trace elements trapped in oil from plant materials, which more fully satisfy the nutrient requirements of a growing culture. At the same time, deep cleaning of glycerol from unreacted components is not required, with the exception of removing excess ethanol by evaporation and neutralizing alkali, which makes it beneficial to utilize technical glycerin. Thus the results of this study confirm that glycerin can be used in the production of fodder yeast.

The production of microorganism pigments is one of the new areas of research as industry's interest in safer, more environmentally friendly products is increasing. The use of microorganisms producing pigments can provide a cost-effective biotechnological process, since the synthesis of pigments by microorganisms can be carried out using inexpensive culture media, does not depend on seasonal restrictions, etc. Depending on the culture conditions, the microorganisms are able to produce various pigments and other biologically active compounds in the required amount [10-14].

Among the pigments of microorganisms, particular attention is paid to melanins which are promising for widespread use in the pharmaceutical industry. Melanin acts as a "fungal armor" due to its ability to protect the fungal cell from adverse conditions. Usually, this pigment is in the outer layer of chitin-bound cell wall (called "cell wall-bound melanin"), but in some fungi, melanin can be found outside the fungal cell, usually in the form of granules in the culture medium. The composition of the environment and the conditions of cultivation affect the synthesis of pigment by fungi, and manipulation of these parameters can lead to increased pigment output. This will allow its further large-scale production for use in various biotechnological fields, biomedicine, dermocosmetology, materials science, nanotechnology [15].

Melanins are characterized by a broad spectrum of biological action: antioxidant, immunomodulatory, antimicrobial, antifungal, antiviral, anti-inflammatory, antistress, antitumor, dermatotropic, cyto-, photo- and radioprotective. Melanins of microorganisms can also act as sorbents of radionuclides and heavy metals. Fungal melanins are actively used to create a new class of biologically active high-tech materials [15-17].

Black yeast mushrooms, promising for use in the medical field, occupy a special place among melanin producers. The black yeast fungus Pseudonadsoniella brunnea T.O. Kondratyuk et S.Y. Kondr. (Basidiomycota, Meripilaceae) isolated from Antarctic rock samples of Galindez Island [18] is the object of our many years research. The main feature of this black yeast is its ability to synthesize not only intracellular melanin but also to excrete melanin into the culture medium. In our previous studies, it was also shown that melanin produced by the strain Pseudonadsoniella brunnea 470 FCKU exhibits antioxidant, anti-phytopathogenic [19], dermatotropic, wound healing, antibacterial [20, 21], stress-adaptogenic effect [22], which, accordingly, makes it promising for use in the pharmaceutical industry.

Therefore, research into determining the optimal conditions for biomass accumulation and melanin production by black yeast fungi Pseudonadsoniella brunnea is a pressing issue. The purpose of this study was to evaluate the possibility of glycerol application for biomass accumulation of the black yeast fungus P. brunnea.

## II. MATERIALS AND METHODS

The strain of black yeast fungi Pseudonadsoniella brunnea T.O. Kondratyuk et S.Y. Kondr. 470 FCKU (Basidiomycota, Agaricomycotina, Agaricomycetes, Polyporales, Meripilaceae) isolated from Antarctic rock specimens from Galindez Island was used within this study. This strain is stored in the Microscopic Fungal

Collection of the Educational and Scientific Centre 'Institute of Biology and Medicine' of the Taras Shevchenko National University of Kyiv (FCKU, an international acronym of the collection). In order to accumulate biomass, the Pseudonadsoniella brunnea 470 FCKU strain was cultured on liquid culture media. Purified glycerol (pharmaceutical, Germany) and technical glycerol (obtained from biofuels, Ukraine) were used as the carbon source in the nutrient media. Glycerol, 20 g/l, peptone enzymatic 10 g/l, and yeast extract 5 g/l were introduced into the nutrient media in these studies.

The liquid nutrient media malt extract broth (MEB), Sabouraud broth (HiMedia Laboratories, India), barley malt extract (BME, 6.2% sugars by the AST-2 areometer-sugar meter) with the addition of peptone enzymatic (10 g/l), yeast extract (5 g/l) were control variants of the study. Our earlier obtained results [23, 24] were used when choosing the variants of experiments. The ability of P. brunnea to grow at low acidity was considered. The pH of the culture media is adjusted by the addition of 1M hydrochloric acid. P. brunnea cultivation was carried out at pH 4, at + 24 ± 2 ° C for 28 days. P. brunnea biomass was separated from the culture medium by centrifugation (2800 rpm) for 15 min (CM-6M Centrifuges, ELMI).

Statistical processing of the results was carried out by conventional methods of variational statistics. The results obtained were checked for normality using the W Shapiro-Wilk test. As the obtained results were normally distributed, comparison of difference between control and experimental variants was performed by means of Anova analysis for independent samples, significance level $p < 0.05$. The data obtained are presented as mean (M) and standard deviation (SD).

## III. RESULTS AND DISCUSSION

The analysis of the results shows that glycerol can be used as a carbon source for the cultivation of black yeast fungi P. brunnea. Our results shown, that the both purified and technical glycerol can be used as a carbon source contributing to the accumulation of P. brunnea biomass (Table).

The growth rates of P. brunnea biomass when glycerol is used (both purified and technical) are correlated with those in the control variants (using MEB, Sabouraud Broth, barley-malt extract with peptone and yeast extract additions) (Fig.)

**Table. Pseudonadsoniella brunnea biomass accumulation on different nutrient media, M ± SD**

| | Indicators of biomass Ps. brunnea, g/l | |
|---|---|---|
| Name of culture medium | initial | after 28 days of cultivation |
| Sabouraud Broth | 21,34± 0,22 | 23,98±0,31 |
| Malt extract broth | 22,12 ±0,18 | 24,72±0,27 |
| Barley-malt extract + enzymatic peptone (10 g/l) + yeast extract (5 g/l) | 22,41±0,23 | 25,12±0,42 |
| Glycerol, pharmaceutical (20 g/l)+ enzymatic peptone (10 g/l) + yeast extract (5 g/l) | 21,77 ±0,33 | 24,38±0,37 |
| Glycerol, technical (20 g/l) + enzymatic peptone (10 g/l) + yeast extract (5 g/l) | 22,26±0,28 | 24,94±0,48 |

Note: ± is the standard error of the mean M ± SD

The Pseudonadsoniella brunnea biomass gain on glycerol medium, was similar to the biomass gain on one of the barley malt malt (BME + enzymatic peptone + yeast extract) control variants. In these media, the greatest increase of P. brunnea biomass was found to be 2.68 ± 0.054 and 2.71 ± 0.023, respectively. It was also found that with the addition of peptone (1%) and yeast extract (0.5%), the accumulation of P. brunnea biomass increases by 7.9% compared to barley-malt extract similarly to that we noted in our previous studies [24]. Peptone and yeast extract are an additional source of nitrogen for yeast in this embodiment. Our data confirm previously known in literature data that malt contains a wide range of important growth ingredients - proteins, essential amino acids (lysine, leucine, isoleucine, tyrosine, tryptophan, phenylalanine, valine, methionine, threonine), carbohydrates (dextrin, sucrose, fructose, xylose), other low molecular weight starch hydrolysis products, fiber, minerals, vitamins (ascorbic acid, thiamine, riboflavin, pantothenic acid, pyridoxine, niacin, biotin), polyphenolic compounds, plant enzymes and hormones [25]. Barley-malt extract is also characterized by a high content of trace elements (Ca, K, Fe, Zn, P, Mg), as well as B vitamins.

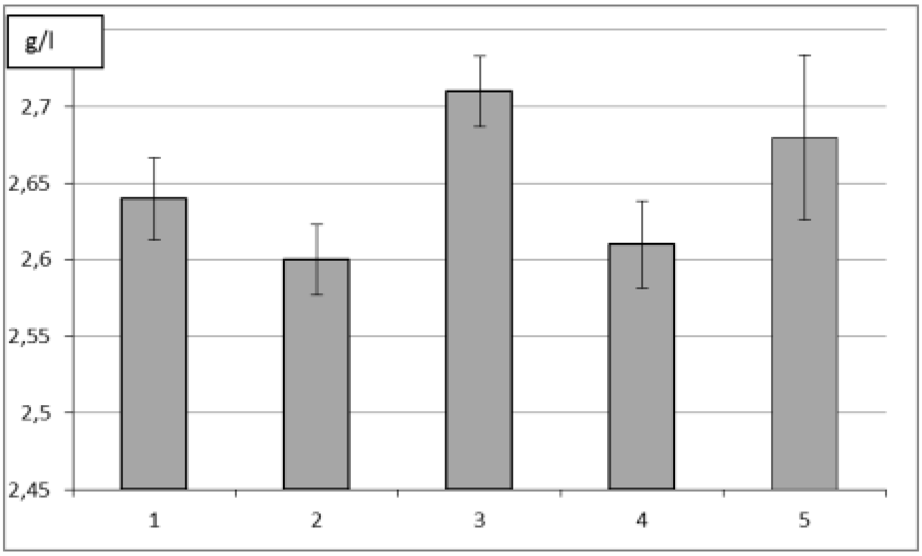


**Figure.** Pseudonadsoniella brunnea biomass growth on different nutrient media, M ± SD
1 - Sabouraud Broth, 2 - Malt extract broth, 3 - Barley-malt extract + enzymatic peptone (10 g/l) + yeast extract (5 g/l), 4 - Glycerol, pharmaceutical (20 g/l)+ enzymatic peptone (10 g/l) + yeast extract (5 g/l), 5- Glycerol, technical (20 g/l) + enzymatic peptone (10 g/l) + yeast extract (5 g/l)

The data obtained by us regarding the accumulation of P. brunnea biomass in the culture medium with technical glycerol indicate that the various impurities in it do not inhibit the growth of P. brunnea black yeast studied. Impurities in technical glycerol (as a by-product of biofuel production) contain unreacted transesterification residues, as well as trace elements from vegetable raw materials. That is, these impurities in the technical glycerol can provide more fully the needs of the growing yeast culture in nutrients. Therefore, the data obtained by us confirm the literature that technical glycerol can be used in yeast cultivation processes for biomass accumulation [9].

## IV. CONCLUSION

As biodiesel production currently increases, the production of its main by-product, namely raw (technical) glycerol, increases. Utilization of crude glycerol is an environmental and economic problem, and the search for ways to use it is important and relevant. Efficient use of crude glycerol will contribute to biodiesel production. One of the most important uses of crude glycerol is its bioconversion using microorganisms. Black yeast fungi, promising for use in the medical field, occupy a special place among melanin producers.

The analysis of the results of our research shows that for the accumulation of biomass by the producer of melanin Pseudonadsoniella brunnea, technical glycerol, which is a by-product of biodiesel production, can be used. This will allow making significant changes to the economic component when receiving the biomass of the producer of melanin of black yeast fungi P. brunnea.

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