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USING OF LOWLAND PEAT SUBSTRATE FOR CULTIVATION OF *Pichia anomala* IMB Y-5067 AND *Rhodotorula gracilis* IMB Y-5075 YEAST STRAINS

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The aim of the research was to study the accumulation of yeast lipids in the process of cultivation of strains *Pichia anomala* IMB Y-5067 and *Rhodotorula gracilis* IMB Y-5075 with the use of peat as growing substrate. The object of researches was strains *P. anomala* IMB Y-5067 and *R. gracilis* IMB Y-5075 from "Collection of strains of microorganisms and plant lines for food and agricultural biotechnology" of SE "Institute of Food Biotechnology and Genomics of the National Academy of Sciences of Ukraine". As a raw material, lowland peat which was preliminarily processed with the help of cavitation or explosive autohydrolysis was used. The accumulation of lipids over the course of cultivation *P. anomala* IMB Y-5067 and *R. gracilis* IMB Y-5075 on non-food substrate — lowland peat was shown. The effect of pulp explosive autohydrolysis and pulp cavitation processing on biomass accumulation and lipids synthesis by strains *P. anomala* IMB Y-5067 and *R. gracilis* IMB Y-5075 was researched. It was found that the maximum lipids accumulation by strains *P. anomala* IMB Y-5067 (9.7 g/dm^3) and *R. gracilis* IMB Y-5075 (8.9 g/dm^3) was over the course of cavitation processing of peat pulp and additional application of salts and yeast extract into cultivation environment.

Key words: growth substrate, peat, yeast lipids, *Pichia anomala* and *Rhodotorula gracilis* strains.

One of the possibilities of solving the problem of energy independence of the country can be using the alternative energy resources, in particular, biofuel. Biodiesel is a perspective alternative energy resource. Biodiesel is mainly received from agricultural oils or animal fat and used for substituting oil diesel fuel [1, 2].

Fats and oils of agricultural, animal and microbiological origin can be a raw material for biodiesel. The traditional technology of receiving biofuel for diesel engines is based on receiving methylic (ethanol) ethers by means of the reaction of oil etherification by methyl (ethanol) alcohol during which the process of oil triglycerides etherification by methanol (ethanol) with the use of base or acid catalysts is taking place [1].

Microbiological synthesis is one of the ways of receiving lipids as a potential raw material for biodiesel. The lipids of microbiological origin received in commercial scales will be able to substitute agricultural oils and animal fats. In order to receive microbiological lipids in industrial scales, it is necessary to have productive strains-producers and relatively cheap non-food substrate. Peat can be one of the kinds of such a substrate [2].

The aim of the work was to research the accumulation of yeast lipids in the process of cultivation of strains *P. anomala* IMB Y-5067 and *R. gracilis* IMB Y-5075 with the use of peat as growing substrate.

Materials and Methods

The subject of researches was yeast *P. anomala* IMB Y-5067 and *R. gracilis* IMB Y-5075 from "Collection of strains of microorganisms and plant lines for food and agricultural biotechnology" of SE "Institute of Food Biotechnology and Genomics of the National Academy of Sciences of Ukraine".

Inoculation material was received on malt wort with 8.0% dry substances content. In

order to receive inoculation material, the culture was reseeded from canted malt agar, yeast cells in quantity of 0.05 dm^3 were carried to 0.25 dm^3 volume flask with sterile fermentation medium and cultivated in shaker-incubator BIOSAN ES-20 (Lithuania) with 240 r/min mixing rate at temperature 31 ± 1 °C during 24 hours. Inoculation material in quantity 10% was brought into enzymatic medium.

Lowland and highmoor peat (Dnipropetrovsk region) in quantity of 50.0 g/dm^3 with the addition of nutritional and growth substances pursuant to relevant variants (g/dm^3): I — KH_2PO_4 — 2.0; $MgSO_4$ — 0.12; $(NH_4)_2PO_4$ — 10.0; yeast extract — 2.0; II — KH_2PO_4 — 3.0; $MgSO_4$ — 0.18; $(NH_4)_2PO_4$ — 15.0; yeast extract — 3.0; III — KH_2PO_4 — 4.0; $MgSO_4$ — 0.24; $(NH_4)_2PO_4$ — 20.0; yeast extract — 4.0, were used as a resource of carbon in enzymatic medium.

Explosive autohydrolysis [3, 4] and cavitation [5] were used for peat treatment processing. The medium acidity was defined with the help of pH-meter "pH-150M" (Belarus). The yeast was cultivated by deep-seated periodic way on shakers (240 r/min) at temperature 31 ± 1 °C during 72 hours.

The quantity of lipids was defined pursuant to [6]. The quantity of dry biomass was defined by weight method [7]. The concentration of sugar in the medium and cultural liquid was defined by spectrochemical method [8].

All the researches were held in three repetitions, statistical processing of results was held pursuant to [9] with the help of Microsoft Excel program. The difference between two average values was considered to be true at $P < 0.05$.

Results and Discussion

Peat is a rather compound substrate for proximate consumption by microorganisms, and that is why it requires treatment (processing) [10]. In order to learn the accessibility of peat pulp macrocomponents for microorganisms without treatment, the strains *R. gracilis* IMB Y-5075 and *P. anomala* IMB Y-5067 were cultivated on fermentation medium — pulp with lowland and highmoor peat. The cultivation of strains R. *gracilis* IMB Y-5075 and P. *anomala* IMB Y-5067 on the pulp of lowland peat is presented on the Fig. 1.

The Fig. 1 shows that strains *P. anomala* IMB Y-5067 and *R. gracilis* IMB Y-5075 have the ability to accumulate reducing substances during cultivation. It is shown that the most accumulation of reducing substances was during the third day of cultivation for both cultures, herewith strain *P. anomala* IMB Y-5067 accumulated 1.5 g/dm^3 of reducing substances and strain *R. gracilis* IMB Y-5075 — accumulated 1.6 g/dm^3, further cultivation led to decrease of reducing substances levels.

In order to compare accumulation of reducing substances on the pulp of highmoor peat, the cultivation of strains *R. gracilis* IMB Y-5075 and *P. anomala* IMB Y-5067 was held. The results of the research were presented on the Fig. 2.

The Fig. 2 shows that accumulation of reducing substances in cultural liquid over the course of cultivation of strains *P. anomala* IMB Y-5067 and *R. gracilis* IMB Y-5075 on fermentation medium with highmoor peat is less than the one over the course of cultivation on breeding environment with lowland peat. The source of energy necessary for life-sustaining activity of yeast-producers of lipids

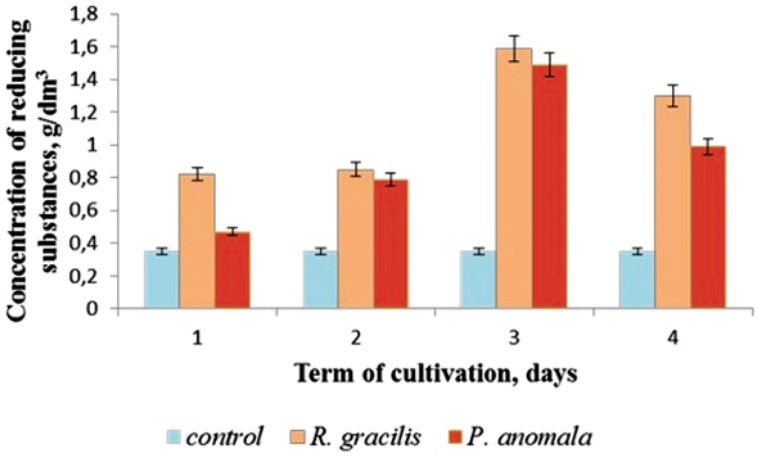

Fig. 1. **Using the pulp of lowland peat for *P. anomala* IMB Y-5067 and *R. gracilis* IMB Y-5075 cultivation**
Hereinafter: as control was a peat without culture

can be a different carbohydrate containing raw material in which the main component is carbonhydrates. Accumulation of reducing substances (monosugars) in fermentative mixture says about the capability of yeast to peat polysaccharides hydrolysis.

In further researches, lowland peat was chosen as fermentation medium, and the cultivation of strains *P. anomala* IMB Y-5067 and *R. gracilis* IMB Y-5075 on fermentation medium with lowland peat was held. The received results are presented on the Fig. 3.

The Fig. 3 shows that strains *P. anomala* IMB Y-5067 and *R. gracilis* IMB Y-5075 accumulated lipids on the substrate with lowland peat, although the concentration of lipids was less than 2.0 g/dm^3. This can be conditioned by the fact that peat consists of inhibitors of microorganisms growth and development — humic acids and humic substances [10].

In order to decrease their influence and improve the accessibility of substrate, preliminary processing of peat pulp by explosive autohydrolysis was held. The received product after explosive autohydrolysis was used as strains cultivation substrate (Fig. 4).

The Fig. 4 shows that after explosive explosive autohydrolysis on the third cultivation day accumulation of reducing substances increased in comparison with accumulation of reducing substances onmedium with unprocessed peat pulp to 2.5 g/dm^3 for *P. anomala* IMB Y-5067 and to 1.5 g/dm^3 for *R. gracilis* IMB Y-5075. Accumulation of lipids and biomass after explosive autohydrolysis of lowland peat pulp on the third cultivation day is shown on the Fig. 5.

The Fig. 5 shows that after explosive autohydrolysis accumulation of biomass by strain *R. gracilis* IMB Y-5075 in comparison

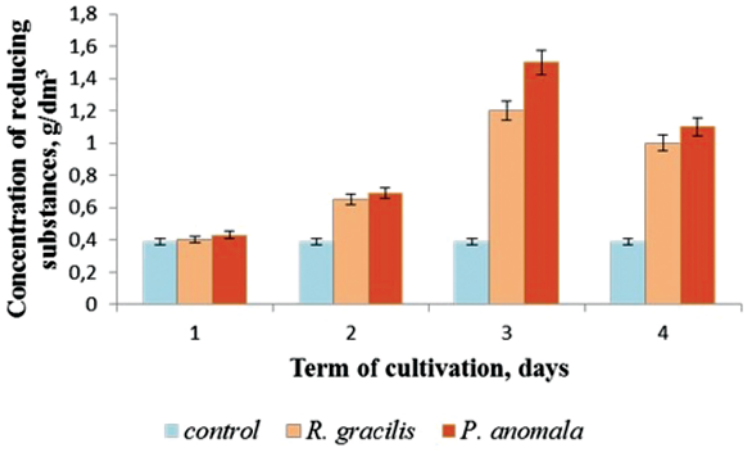

Fig. 2. **Using the pulp of highmoor peat for *P. anomala* IMB Y-5067 and *R. gracilis* IMB Y-5075 cultivation**

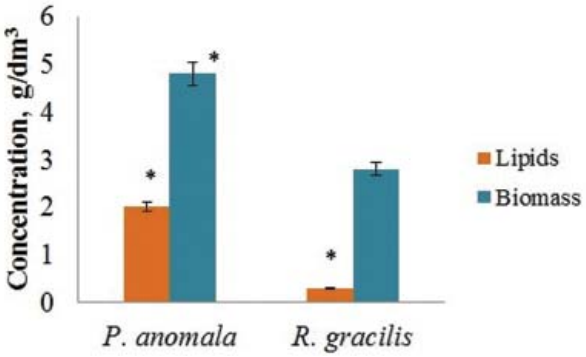

Fig. 3. **Lipids and biomass accumulation by stains *P. anomala* IMB Y-5067 and *R. gracilis* IMB Y-5075 during cultivation on non-treatment lowland peat**

Hereinafter: * — $P < 0.05$ as compared to control; in Fig. 3, 5, 7 there is no control, because control is a native peat pulp, where there is no biomass and lipids. For the remaining Figs. control is the amount of reducible pulp materials (water-soluble sugars)

with accumulation of reducing substances on the medium of unprocessed peat pulp increased from 2.6 to 7.0 g/dm^3 and accumulation of lipids increased from 0.5 to 1.0 g/dm$^{3.}$ Accumulation of biomass by strain *P. anomala* IMB Y-5067 over the course of cultivation on the medium with lowland peat pulp after explosive autohydrolysis was 2 times higher than the one on the medium with unprocessed pulp. Accumulation of lipids by strain *P. anomala* IMB Y-5067 with the use of lowland peat pulp before explosive autohydrolysis and after it was the same (2.0 g/dm^3).

In order to raise accumulation of reducing substances in cultivating medium, strains *P. anomala* IMB Y-5067 and *R. gracilis* IMB Y-5075 were cultivated on medium with a different content of salts, growth factors and peat pulp which went through cavitation treatment (Fig. 6).

You can see from the Fig. 6 that addition of salts and yeast extract influenced over accumulation of reducing substances in e medium depending on the strain. The culture *R. gracilis* IMB Y-5075 (2.2 g/dm^3) over the course of using medium pursuant to the variant I accumulated the most quantity of reducing substances. In return, the culture *P. anomala* IMB Y-5067 accumulated 1.3 g/dm^3 over the course of using medium pursuant to the variant II. Accumulation of lipids by strains *P. anomala* IMB Y-5067 and *R. gracilis* IMB Y-5075 on medium with a different content of salts, yeast extract and peat pulp which went through cavitation processing was studied (Fig. 7).

After peat cavitation processing and addition of salts and yeast extract accumulation of lipids was intensified on the 72-nd cultivation hour. The most accumulation of lipids over the course of cultivation of *P. anomala* IMB Y-5067 (9.7 g/dm^3) and *R. gracilis* IMB Y-5075 (8.9 g/dm^3) was received over the course of using processed pulp of lowland peat by cavitation and addition of salts and yeast extract in concentrations pursuant to the variant III. Over the course of using other concentrations of salts and yeast extract accumulation of lipids was less.

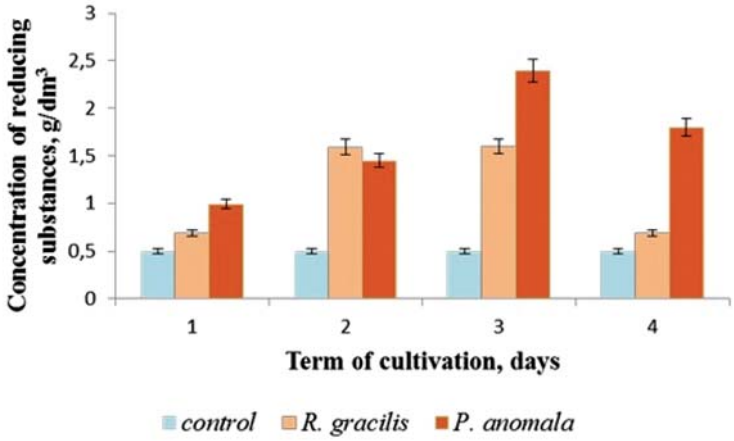

Fig. 4. **Using of pulp of lowland peat as a substrate after explosive autohydrolysis**

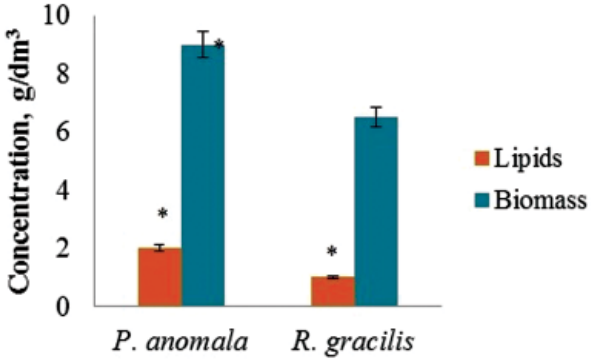

Fig. 5. **Lipids and biomass accumulation by stains *P. anomala* IMB Y-5067 and *R. gracilis* IMB Y-5075 during cultivation on lowland peat after explosive autohydrolysis**

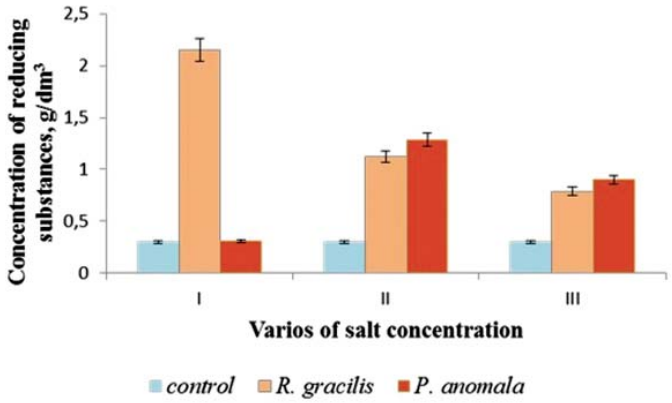

Fig. 6. ***P. anomala*** **IMB Y-5067 and** ***R. gracilis*** **IMB Y-5075 cultivation using different salt concentration and yeast extract**

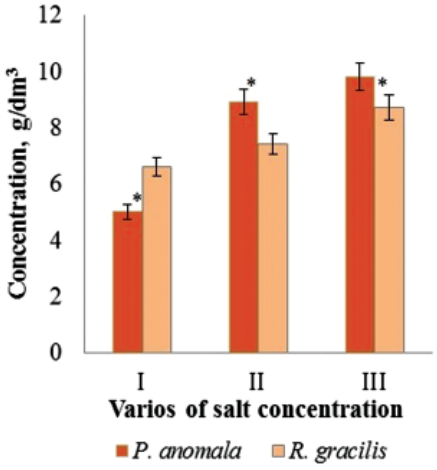

Fig. 7. **Lipids accumulation by yeasts** ***P. anomala*** **IMB Y-5067 and** ***R. gracilis*** **IMB Y-5075 using cavitation peat as a medium**

Therefore, the results of the research testify about the opportunity of using lowland peat as a resource of carbon for receiving microbic lipids. Using hydrodynamic cavitation as a way of preliminary processing of water-peat mixture and addition of salts and yeast extract led to increase of accumulation of lipids by strains *P. anomala* IMB Y-5067 and *R. gracilis* IMB Y-5075 more than in 5 times. The conducted researches confirmed one more time that the advantage of the lipids received by means of microbiological synthesis [11–13] is comparatively fast capacity of their content change by means of producer directed cultivation, use of cheap raw material and processability of producer cultivation process.

Thus, accumulation of lipids over the course of cultivation of strains *P. anomala* IMB Y-5067 and *R. gracilis* IMB Y-5075 at non-food substrate — lowland peat is shown. The influence of treatment of peat pulp by explosive autohydrolysis and cavitation to accumulation of biomass and lipids by strains *P. anomala* IMB Y-5067 and *R. gracilis* IMB Y-5075 was researched. It is stated that the maximum accumulation of lipids by strains *P. anomala* IMB Y-5067 (9.7 g/dm^3) and *R. gracilis* IMB Y-5075 (8.9 g/dm^3) was in case of cavitation processing of peat pulp and additional application of salts and yeast extract to cultivation medium.

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ВИКОРИСТАННЯ СУБСТРАТУ НИЗИННОГО ТОРФУ ДЛЯ КУЛЬТИВУВАННЯ ШТАМІВ ДРІЖДЖІВ *Pichia anomala* IMB Y-5067 ТА *Rhodotorula gracilis* IMB Y-5075

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Метою роботи було дослідити накопичення дріжджових ліпідів штамами *Pichia anomala* IMB Y-5067 та *Rhodotorula gracilis* IMB Y-5075 з використанням торфу як ростового субстрату. Об'єктом досліджень були штами *P. anomala* IMB Y-5067 і *R. gracilis* IMB Y-5075 із «Колекції штамів мікроорганізмів та ліній рослин для харчової і сільськогосподарської біотехнології» ДУ «Інститут харчової біотехнології та геноміки НАН України». Як сировину використовували низинний торф, який попередньо обробляли за допомогою кавітації або вибухового автогідролізу. Було показано накопичення ліпідів за культивування *P. anomala* IMB Y-5067 та *R. gracilis* IMB Y-5075 на нехарчовому субстраті — низинному торфі. Досліджено вплив вибухового автогідролізу пульпи та кавітаційного оброблення пульпи на накопичення біомаси і синтез ліпідів штамами *P. anomala* IMB Y-5067 та *R. gracilis* IMB Y-5075. Встановлено, що максимальне накопичення ліпідів штамами *P. anomala* IMB Y-5067 (9,7 г/дм^3) та *R. gracilis* IMB Y-5075 (8,9 г/дм^3) відбувалося за кавітаційного оброблення пульпи торфу і додаткового внесення солей та дріжджового екстракту в середовище культивування.

Ключові слова: ростовий субстрат, торф, ліпіди дріжджів, штами *Pichia anomala*, *Rhodotorula gracilis*.

ИСПОЛЬЗОВАНИЕ СУБСТРАТА НИЗОВОГО ТОРФА ДЛЯ КУЛЬТИВИРОВАНИЯ ШТАММОВ ДРОЖЖЕЙ *Pichia anomala* IMB Y-5067 И *Rhodotorula gracilis* IMB Y-5075

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Целью работы было исследовать накопление липидов штаммами дрожжей *Pichia anomala* IMB Y-5067 и *Rhodotorula gracilis* IMB Y-5075 с использованием в качестве ростового субстрата торфа. Объектом исследований были дрожжи *P. anomala* IMB Y-5067 и *R. gracilis* IMB Y-5075 из «Коллекции штаммов микроорганизмов и линий растений для пищевой и сельскохозяйственной биотехнологии» ГО «Институт пищевой биотехнологии и геномики НАН Украины». В качестве сырья использовали низовой торф, который предварительно обрабатывали с помощью кавитации или взрывного автогидролиза. Было показано накопление липидов при культивировании *P. anomala* IMB Y-5067 и *R. gracilis* IMB Y-5075 на непищевом субстрате — низинном торфе. Исследовано влияние взрывного автогидролиза и кавитационной обработки пульпы на накопление биомассы и синтез липидов дрожжами штаммов *P. anomala* IMB Y-5067 и *R. gracilis* IMB Y-5075. Установлено, что максимальное накопление липидов *P. anomala* IMB Y-5067 (9,7 г/дм^3) и *R. gracilis* IMB Y-5075 (8,9 г/дм^3) происходило при кавитационной обработке торфа и дополнительном внесении солей и дрожжевого экстракта в среду культивирования.

Ключевые слова: ростовой субстрат, торф, липиды дрожжей, штаммы *Pichia anomala*, *Rhodotorula gracilis*.