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Original article

DEVELOPMENT OF A NEW METHOD FOR OBTAINING THE BIOPLASTICS BASED ON MICROBIAL BIOPOLYMERS AND LIGNIN

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Background. The ever-increasing demand for plastic polymer products with simultaneous depleting fossil fuels such as oil and natural gas, as well as the growing problem of waste disposal, creates a need to find alternative technologies that meet current trends in both environmental and economic development. Bioplastic materials that are synthesized from renewable sources and have the ability to biodegrade are considered as such an alternative. The main obstacle of modern bioplastics which makes it impossible to completely replace traditional plastics is the high cost of production. In order to reduce the cost of existing biopolymers, production waste is added to the polymer matrix. One such waste is lignin – the second most common biopolymer. An additional way to reduce the cost of production is to find more cost-effective producers. Thus, although the classical microbial synthesis has fairly high productivity, the source of carbon for the cultivation of microorganisms are sugars obtained from agricultural raw materials which could cause a threat for food industry. The new producer for production of polyhydroxyalkanoates (PHA) is cyanobacteria, the carbon source of which is carbon (IV) oxide or gas emissions from enterprises, which reduces the cost of the target product.
Objective. Development of a method for obtaining bioplastics using products of microbial synthesis and lignin.
Methods. Cyanobacteria *Nostoc commune* was grown using a nutrient medium BG-11 with subsequent limitation of Nitrogen for the synthesis of PHA. Hydrolyzed lignin from hardwoods was combined with polylactic acid (PLA) or cyanobacteria-synthesized PHA in different ratios with further casting of the solution to determine the ability of lignin and polymer matrix to form polymer films.
Results. The content of PHA in the cells of cyanobacteria *Nostoc commune*, when grown in a nutrient medium limited to Nitrogen, reached 7.8%. The synthesized polymer films based on PLA and lignin were not homogeneous, and films based on PHA and lignin were fragile.
Conclusions. The possibility of obtaining PHA by using cyanobacteria of the *Nostoc commune* species under environmental conditions that differ from the optimal ones for both cultivation and PHA production is shown. The possibility of obtaining a biopolymer based on lignin and PLA is shown. To form homogeneous films, it is necessary to change the standard conditions for obtaining a mixture of components. The interaction of lignin with PHA forms a homogeneous polymer mixture, which is fragile and requires the addition of plasticizers to obtain the necessary properties.

Keywords: lignin; cyanobacteria; polylactic acid; polyhydroxyalkanoates; bioplastic; industrial waste; renewable materials.

Introduction

The world's demand for plastic products is growing every year. Natural gas and oil are used for their production, which leads to the depletion of their reserves and pollution of the environment during production. The main disadvantage of plastics is the long duration of their decomposition processes in the natural environment. Therefore, there is a need to create alternative products – bioplastics, the production of which would be associated with renewable raw materials and the possibility of their disposal in the wild [1]. Today, bioplastics make up only a small part of the global plastic products market, due to their high cost of $ 2 to $ 6 per kilogram, compared to the average price of traditional plastics, which cost $ 1-2 per kilogram [2]. In addition, it should be noted that not all bioplastics are biodegradable or decompose rapidly under certain conditions, which in turn does not solve environmental problems.

The most common representative of bioplastics today is polylactic acid (PLA). It is similar in properties to polyethylene, terephthalate, and polystyrene [2, 3]. The monomer for the formation of polylactate is lactic acid, which is obtained by heterotrophic bacteria of the genus *Lactobacillus* and *Sporolactobacillus* [4].

Another group of bioplastics that is of commercial interest – polyhydroxyalkanoates (PHA) –

biopolymers that have the ability to biodegrade and, moreover, the mechanical properties are not inferior to polypropylene. The most studied among PHA is polyhydroxybutyrate (PHB), which today is mainly synthesized on an industrial scale by heterotrophic bacteria such as *Cupriavidus necator*, *Escherichia coli* [5]. Unlike PLA, PHA polymers are synthesized in the middle of the cell and do not require an additional polymerization step [5].

Classical microbial synthesis has a fairly high productivity, but the source of carbon for the cultivation of microorganisms are sugars obtained from agricultural raw materials. The new producer for PHA production is cyanobacteria, the carbon source of which is carbon (IV) oxide or gas emissions from enterprises, which reduces the cost of the target product.

In order to increase the efficiency of PHA synthesis by cyanobacteria, various methods are used: limiting the nutrient medium by nitrogen, changing the temperature, pH. The influence of factors that affect metabolism depends on the type and strain of cyanobacteria. Thus, the most intense effect of nitrogen limitation was found in *Calothrix scytonemicola* TISTR 8095 [6], *Chlorogloea fritschii* TISTR 8527 [7], *Synechocystis* sp. PCC 6714 [8], in which the content of PHB on dry cell mass increased almost 17, 13 and 9 times relative to cultivation under standard conditions. That is, to increase the yield of PHB, it is necessary to create conditions for the lack of nitrogen compounds in the environment.

It is known that the most cyanobacteria grow at the pH values from 7 to 9. This phenomenon is due to the fact that such conditions increase the efficiency of carbon uptake [9]. For *Nostoc muscorum* NCCU-442 [10] the highest PHB content was observed at pH 7.5 and was 7.6%, for *Scytonema geitleri Bharadwaja* [11] and *Synechocystis* sp. PCC 6803 [12] at pH 8.5 and was 3.98 and 4.55%, respectively, and for *Spirulina platensis* UMACC 159, 161, 162 [13] at pH 9.0, the PHB content was 7.1, 10.1, and 7.8%, respectively. Thus, for each species of microorganisms it is necessary to maintain the pH of the environment in which there is increased biosynthesis of PHB.

The temperature optimum for the cultivation of cyanobacteria is in the range from 20 to 35 °C. For PHB-producing microorganisms, the temperature optimum is 29 ± 1 °C, which coincides with the temperature optimum for PHB synthesis. Thus, for *Scytonema geitleri Bharadwaja* [11] and *Nostoc muscorum* NCCU-442 [10] the highest PHB content was observed at 30 °C and amounted to 3.18 and 7.6%, respectively, for *Synechocystis* sp. PCC 6803 [12] temperature optimum for PHB synthesis is slightly lower and is 28 °C with a PHB content of 4.55%.

The high cost of bioplastics production from the products of microorganism synthesis is a significant obstacle to their widespread distribution. Therefore, in order to reduce costs, it is proposed to use them in a mixture of natural biopolymers. One of such components may be lignin a by-product of hydrolysis of cellulose-containing raw materials, the cost of which is 0.27 USD per kilogram [14, 15]. The lignin market in 2019 was $ 954.5 million. However, the processing of lignin into useful products is only 5% of the total amount of lignin [16, 17].

Therefore, the use of lignin as a component of bioplastics, will simultaneously reduce the cost of bioplastics and dispose of lignin as a waste product.

The aim of the work is development of a method for obtaining bioplastics using products of microbial synthesis and lignin.

Materials and methods

Luminy brand PLA, a hydrolyzed lignin from hardwoods. The cyanobacterium *Nostoc commune* was used as a producer of PHA. To increase the biomass of cyanobacteria, BG-11 medium was used, followed by replacement of the medium with BG-0 (medium limited by nitrogen) in purpose to intensify the PHA synthesis process [18].

Cyanobacteria were grown in a 1 L photobioreater with the use of 2:1 red and blue LEDs as a light source at 16:8 light cycle (light:dark).

The growth rate of cyanobacteria was observed by varying the density of the medium with the use of the bio ULAB 102 spectrophotometer and the number of cells on the Countess II cell counter.

To separate the PHA, cyanobacterial cells were filtered off and dried in an Labexpert 3050 oven (drying temperature 30 °C). Extraction was performed using chloroform at a temperature of 60 °C in the ratio of cell mass to mass of chloroform 1:1 for 36 h. The hot extract was filtered off on a vacuum filter followed by precipitation of the polymer in two volumes of diethyl ether. The resulting polymer was filtered off using filter paper (red ribbon) and washed with acetone and diethyl ether [19].

Before obtaining of bioplastics lignin, PHA, and PLA was dried to constant weight in an oven. Lignin was ground with a coffee grinder to a state of dust.

The selected polymer was dissolved in chloroform in a ratio of 1:10 for two hours, then created mixture was homogenized using stirring with a magnetic mixture for 15 min, then heated to 135 °C and add lignin in the amount of 1, 10, 20, 30, and 40% in a mixture with PLA (for PHA only 10%) of the amount of polymer. The resulting mixture was stirred for 10 minutes. After homogenization, the mixture was formed in Petri dishes and dried at room temperature for 24 hours.

To conduct statistical processing and data visualization Python's language modules Pandas, Seaborn, Matplotlib, and NumPy were used.

Results

Growing cyanobacteria

Typically the standard growth curve corresponds to the sigmoid shape curve that consists of four phases: lag, log, stationary and death phases (20). As can be seen from Fig. 1, which shows the change in density and number of cells in the process of growing cyanobacteria *Nostoc commune*, the growth of cyanobacteria corresponds to the standard growth curve of bacteria. The lag phase was quite long and amounted to 13 days, the period of exponential growth was 17 days, and steady state was observed during the last 8 days of the experiment. This long lag phase can be explained by a change in temperature (15 ± 2 °C), which differs from the optimal growing temperature (25 ± 2 °C) and lighting (did not use sunlight). This choice of LEDs is due to the absorption spectrum of chlorophyll and cyanobacteria. Due to the lack of yellow-green LEDs, the use of carotenoids for photosynthetic processes of cyanobacterial cells was absent. It is the rearrangement of the photosynthetic apparatus of cells that can explain the duration of the lag phase. Nitrogen-depleted medium was used during the stationary growth phase. In this cultivation, the final PHA content was 7.8%.

The biopolymer synthesis

The biopolymer was prepared using a crushed mixture of natural polymers (lignin and PHA or PLA) according to the method [20]. The synthesized mixture of PLA and lignin is not homogeneous. Lignin particles are contained in the PLA in the form of inclusions ranging in size from 0.5 mm to 4 mm (Fig. 2). This can be explained by their large particle size to obtain a biopolymer or the adhesion of smaller particles during the synthesis process. This is typical for a mixture with a lignin content of 10 to 40%. The formed polymer films are quite elastic and do not break during assembly, however dents remain at the assembly site, but when the formed film is heated with warm air, the dents are smoothed.

During storage of the formed film for 30 days, the appearance of brittleness of the material was observed, which may indicate the beginning of the process of destruction of the polymer due to the low level of homogeneity of the polymer mixture.

In contrast to the bioplastic of lignin from PLA in the synthesis of bioplastic from 10% lignin and PHA, the resulting mixture had a homogeneous composition. However, after drying, a uniform film was not formed, there were bulges on the surface. The polymer was brittle and under the action of applied force disintegrated into pieces (Fig. 3).

Thus, to obtain biopolymers based on lignin and PLA, it is necessary to change the conditions of polymer synthesis to achieve its homogeneity. In the case of using lignin and PHA to achieve plasticity, it is necessary to use plasticizers that will achieve plasticity in the formed polymer.

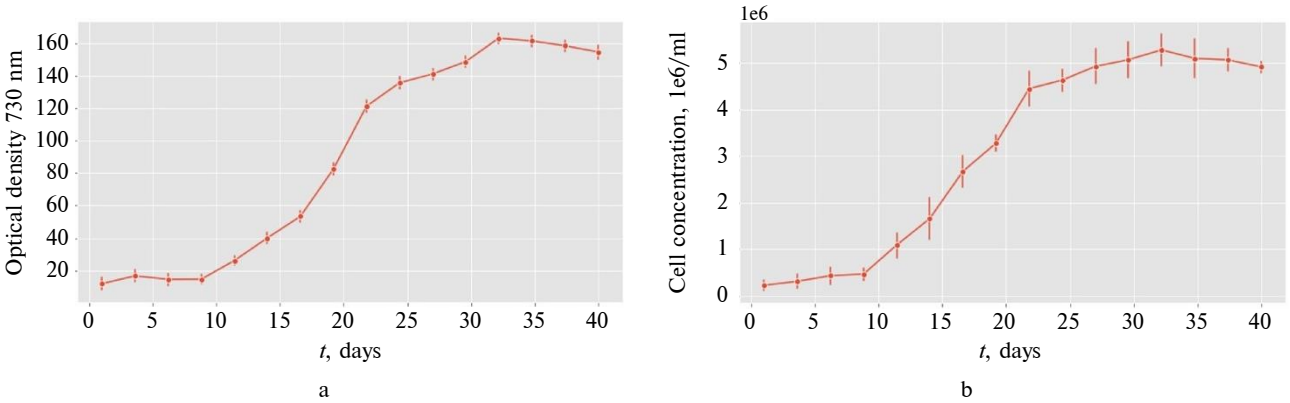

Figure 1: Change in optical density (a) and number of cyanobacteria *Nostoc commune* cells (b) (cell concentration – 10^6 cells per ml) with time of the cyanobacteria *Nostoc commune* growth

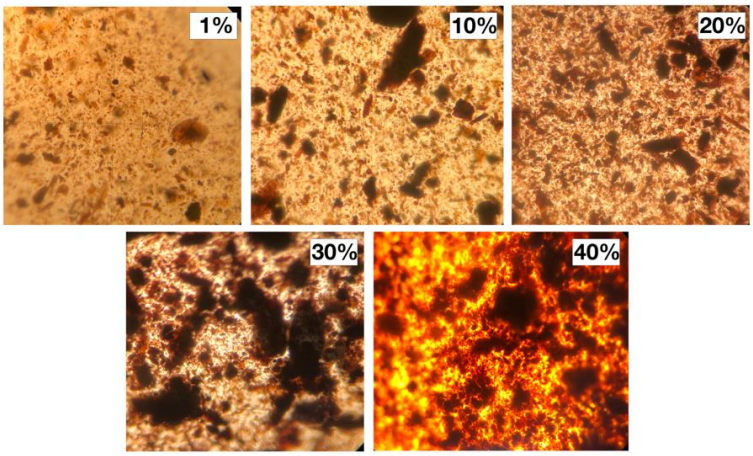

Figure 2: PLA-lignin bioplastic with lignin content of 1, 10, 20, 30, 40% (×15)

Figure 3: Bioplastic based on PHA and 10% lignin

Discussion

The results obtained in this paper are slightly different from those shown by other studies. For example (21), bioplastics based on PHB and lignin (with a lignin content of 40%) had a fairly high homogeneity, while improving the mechanical properties. This can be explained by the low lignin content, which led to delamination between the components of the composite, as well as the inadequate quality of the obtained PHA. In order to solve the existing problem, it is necessary to study the synthesized PHA, in order to establish qualitative characteristics, as well as to change the composition of the composite, either by increasing the amount of lignin or by adding compatibilizers that will increase system homogeneity.

Bioplastics based on lignin and PLA, obtained by solution casting, confirmed the data of works in which other methods of obtaining a polymer mixture were used [22, 23]. This may indicate that the method of creating a polymer mixture has little effect on the final product, and in order to increase the homogeneity of the mixture, the addition of compatibilizers is a necessary step.

Conclusions

The possibility of obtaining PHA by using cyanobacteria of the *Nostoc commune* species under environmental conditions that differ from the optimal ones for both culture and PHA production is shown.

The possibility of obtaining a biopolymer based on lignin and PLA is shown. To form homogeneous films, it is necessary to change the standard conditions for obtaining a mixture of components.

The interaction of lignin with PHA forms a homogeneous polymer mixture, which is fragile and requires the addition of plasticizers to obtain the necessary properties.

In order to improve the combination of lignin and selected polymer matrices, the addition of compatibilizers or the use of other bioplastic synthesis techniques will be considered in further studies.

Interest disclosure

Nataliia Golub is the member of the Editorial Council of *Innovative Biosystems and Bioengineering* and was not involved in the editorial evaluation or decision to accept this article for publication. The other authors have no conflicts of interest to declare.

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РОЗРОБКА НОВОГО СПОСОБУ ОТРИМАННЯ БІОПЛАСТИКІВ НА ОСНОВІ МІКРОБНИХ БІОПОЛІМЕРІВ І ЛІГНІНУ

Проблематика. Постійне зростання попиту на пластичні полімерні вироби з одночасним виснаженням запасів викопних джерел, таких як нафта і природний газ, а також посилення проблеми утилізації відходів створюють потребу в пошуку альтернативних технологій, що відповідали б сучасним тенденціям як екологічного, так і економічного курсу світового розвиту. Як така альтернатива розглядаються біопластичні матеріали, що синтезуються з поновлюваних джерел і мають здатність до

біорозкладу. Основна перепона сучасних біопластиків, які унеможливлюють повну заміну традиційних пластмас, – висока вартість виробництва. З метою зниження собівартості існуючих біополімерів до складу полімерної матриці додають відходи виробництва. Одним із таких відходів є лігнін – другий найпоширеніший біополімер. Додатковим шляхом зниження собівартості виробництва є пошук більш економічно вигідних продуцентів. Так, класичний мікробний синтез хоч і має досить високу продуктивність, але джерелом карбону для вирощування мікроорганізмів є цукри, які отримують із сільськогосподарської сировини. Новим продуцентом для одержання полігідроксиалканоату (ПГА) є ціанобактерії, джерелом карбону для яких є карбон (IV) оксид або газові викиди підприємств, що знижує собівартість цільового продукту.

Мета. Розробка методу отримання біопластику за використання продуктів мікробного синтезу та лігніну.

Методика реалізації. Ціанобактерії *Nostoc commune* вирощували при використанні живильного середовища BG-11 із подальшим лімітуванням за нітрогеном з метою синтезу ПГА. Гідролізний лігнін із твердих порід дерев поєднували з полілактидом (ПЛА) або синтезованим ціанобактеріями ПГА в різних співвідношеннях методом лиття розчину з метою виявлення здатності лігніну та полімерної матриці утворювати полімерні плівки.

Результати. Вміст ПГА у клітинах ціанобактерії *Nostoc commune* при вирощуванні у живильному середовищі, лімітованому за нітрогеном, досяг 7,8 %. Синтезовані полімерні плівки на основі ПЛА та лігніну не були гомогенними, а на основі ПГА є крихкими.

Висновки. Показано можливість отримати ПГА за використання ціанобактерій виду *Nostoc commune* за умов навколишнього середовища, що відрізняються від оптимальних як для вирощування культури, так і для отримання ПГА. Показано можливість отримати біополімер на основі лігніну та ПЛА. Для утворення гомогенних плівок необхідно змінити стандартні умови отримання суміші компонентів. При взаємодії лігніну з ПГА утворюється гомогенна полімерна суміш, яка є крихкою і потребує для набуття необхідних властивостей додавання пластифікаторів.

Ключові слова: лігнін; ціанобактерії; полілактид; полігідроксиалканоат; біопластик; промислові відходи; відновлювані матеріали.