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COMPLEX EVALUATION OF *NOSTOC LINCKIA* (ROTH.) BORN. ET FLAH. BIOMASS, CULTIVATED ON WASTE WATER FROM RECIRCULATING AQUACULTURE SYSTEM

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The paper considers the possibility of cultivating Nostoc linckia (Roth.) Born. et Flah. on waste water from Recirculating Aquaculture System (RAS). Nostoc is a genus of cyanobacteria found in various environments that forms colonies composed of filaments of moniliform cells in a gelatinous sheath.The investigated algae was grown on the Fitzgerald's artificial medium number 11 in the modification of Zehnder and Gorham and on the water from RAS, that was standardized with the indicators pH and total mineralization. It is noted that waste water contains a sufficient amount of biogenic elements for the cultivation of cyanobacteria.A complex scheme has been developed for the analysis of N. linckia biomass, taking into account the solubility of organic substances and the sequence of their extraction. The biomass dynamics, protein content, carbohydrates, lipids, chlorophyll a, carotenoids and phycobilin pigments in the culture of N. linckia are studied. The maximum content of total protein was noted on the 30th day of cultivation and was about 60% of the dry weight of algae. The content of chlorophyll a, and carotenoids in N. linckia cells was maximal at 16,2 mg/g and 9,5 mg/g dry weight, respectively. Also, the level of the main components of phycobilin pigments (phycoerythrin, phycocyanin and aloficocyanin) was determined.A comparative description of the productive indicators of periodic culture depending on its initial supply with basic elements of mineral nutrition has been carried out. When grown on waste water from RAS N. linckia culture, the content of basic nutrients does not significantly differ from the control values, but its biomass cost is much lower.

Keywords: Nostoc linckia, waste water from RAS, complex evaluation

Introduction. *Nostoc linckia* (Roth.) Born. et Flah. is a thallogen heterocyst cyanobacterium capable of nitrogen fixation (Fokina, 2011). Unlike most cyanobacteria, which in large quantities produce toxins with multidirectional effects, *N. linckia* remains an absolutely non-toxic species. This feature allows using *N. linckia* as animal feed (Lishchuk, 2014), including in aquaculture. Microalgae biomass is characterized by a high content of amino acids and proteins (Rosales-Loaiza, 2017). This property allows them to be used as a feed additive in aquaculture, since they have a positive effect on increasing the weight of industrially valuable species of fish (Ophilia, 2017). In addition, pigment-rich cyanobacterial biomass is used to impart bright colors to fancy fish (Gupta, 2007).

Like most thallogen species, *N. linckia* rapidly increases biomass, responds clearly to changes in the component composition of the nutrient medium, and does not require complex approaches in post-cultivation separation of biomass from the nutrient medium. All this makes this species a convenient and promising object for biotechnology. The main problem here is the choice of optimal nutrient media, which, for the most part, are multicomponent and quite expensive. Therefore, it is necessary to look for new alternative environments that will satisfy the need for cyanobacteria in nutritional factors.

As such an alternative medium, wastewaters of various origins can be used, for example, industrial wastewater from ethanol and citric acid production (Valderramaa, 2002), corn powder hydrolyzate (Xu, 2006), rice straw hydrolyzate (Penglin, 2011) and domestic droppings (Muzaffarov, 1984).

Promising is the use of waste water from a recirculating aquaculture system (RAS) (Khudyi, 2016). As it is known, the waste water from RAS in its composition contains a significant amount of nitrogen, which is formed as a result of the vital activity of fish, as well as other necessary mineral elements (carbon, phosphorus, sulfur and others). Due to this, cultures that are grown on waste water from RAS should be provided with a complex source of nitrogen and other mineral elements, which are also necessary for cyanobacteria.

The aim of our work was to show the possibility of cultivating *Nostoc linckia* (Roth.) Born. et Flah. on waste water from RAS and to carry out a comprehensive assessment of the biomass obtained under such conditions.

Materials and Methods. Studies were conducted using algologically pure culture of *Nostoc linckia* (Roth.) Born. et Flah. HPDP-453 from the collections of the Institute of Hydrobiology of NAS of Ukraine. Cyanobacteria were cultivated under sterile conditions in waste water from recirculating aquaculture system (RAS). Water was autoclaved at 121 °C for 30 min and standardized in terms of pH (7.5–8) and total salt content (495 ± 5 ppm). As a comparison medium, the standard Fitzgerald No. 11 medium was used in the modification of Zehnder and Gorhem (Zolotareva, 2009).

Cultivation was carried out in Erlenmeyer flasks with a volume of 500 ml at a temperature of 21 ± 2 ° C, illumination with fluorescent lamps of about 2500 lux and a 16-hour photoperiod. The inoculation was carried out in the ratio of inoculum : nutrient medium – 1:10 (Marchenko, 2015).

All manipulations associated with the inoculating of culture, were carried out in a laminar box.

Cyanobacteria under these conditions were cultured for 50 days. Every 10 days density of the culture was determined by the amount of biomass using an optical indicator at 750 nm on CaryWin UV 60 (Agilent, USA). The transition from the units of optical density (D750) to the value of absolutely dry biomass (ADB) was carried out through the empirical coefficient k: ADB = k x D750 (Gudrilovich, 2005). According to the obtained indicators, growth curves were constructed for the culture of *N. linckia*.

At the end of the stationary phase of growth, the performance of algoculture was determined by the amount of total protein, carbohydrates, lipids and pigments in biomass.

The algae biomass was concentrated and washed from the rest of the nutrient medium with sterile distilled water. The selection of algae cells from the culture medium was performed by centrifugation at 6 thousand rpm within 15 min on Biofuga stratos "Herauses".

Extraction of hydrophilic components of the biomass was carried out with 0.1 M phosphate buffer solution with a pH of 7.4 (Musienko, 2001). Extraction of fat-soluble pigments was performed with 100% acetone. Lipids were extracted using the Folch method (Folch, 1957). Determination of total protein content was carried out according to the method of Lowry (Lowry, 1951). Lipids were determined by the presence of sulfuric acid and phosphorus-vanillin reagent (Anschau, 2017), carbohydrates by color reaction with anthrone reagent (Olennikov, 2006). The obtained indicators were calculated as a mass fraction (%) calculated on dry biomass.

Statistical processing of the obtained results was performed using Microsoft Excel software. Differences in the results obtained are probable at a significance level of $p \leq 0.05$ by the Student's criterion.

Results

It is known that the waste water from RAS contains in its composition the main mineral elements present in Fitzgerald's environment (Table 1).

Table 1.
Content (mg / l) of the main mineral elements

Mineral elements	Waste water from RAS	Fitzgerald's medium
NO_3^-	20,2±0,2	81,7 ± 0,2
NO_2^-	0,62 ± 0,03	-
NH_4^+	0,48 ± 0,02	-
PO_4^{3-}	0,031 ± 0,03	0,040 ± 0,03
CO_3^{2-}	0,094 ± 0,02	0,031 ± 0,01
pH	7,0-8,0	7,0-8,0
Total mineralization	371,0-477,0	232,0-547,0

In both cases, a sufficient amount of nitrogen, phosphorus, and carbon is observed (Malishchuk, 2015). The availability of these mineral elements has a significant impact on biomass growth and biosynthetic processes. Thus, with non-sufficient nitrogen content, a slowdown in growth rates and a decrease in protein synthesis is observed, and with a lack of phosphorus in cells, no accumulation of lipids occurs (Levtun, 2017). The pH of the medium is also important, since a decrease in its value leads to a decrease in the absorption of CO_2 by cyanobacteria, as well as a change in the charge on the cell surface, which can lead to their death.

The state of culture of cyanobacteria can be determined by estimating the rate of biomass growth. From the first day of the experiment on waste water from RAS, we observe a significantly lower growth activity than on the control medium (Fig. 1). A similar trend persisted during the first 10 days of cultivation. However, by the middle of the exponential growth phase, these differences were leveled and the same amount of biomass was observed on both media. This indicates that the waste water contains a sufficient amount of nutrients necessary for normal functioning of *N. linckia*. Active growth of biomass was observed up to 30-35 days of the experiment, after which the culture passed into the stationary phase. Starting from the 50th day of cultivation, we noted a decrease in the amount of biomass, due to the accumulation of metabolic products in the culture fluid and a decrease in the available nutrients in the medium. It is noted that under such culture conditions, a *N. linckia* culture is characterized by a rather long exponential phase of the growth curve. The maximum amount of biomass culture reached on the 30 day of cultivation. So, if the goal of accumulative cultivation is to obtain the maximum amount of biomass with desired properties, then cultivation should be carried out up to 30-35 days.

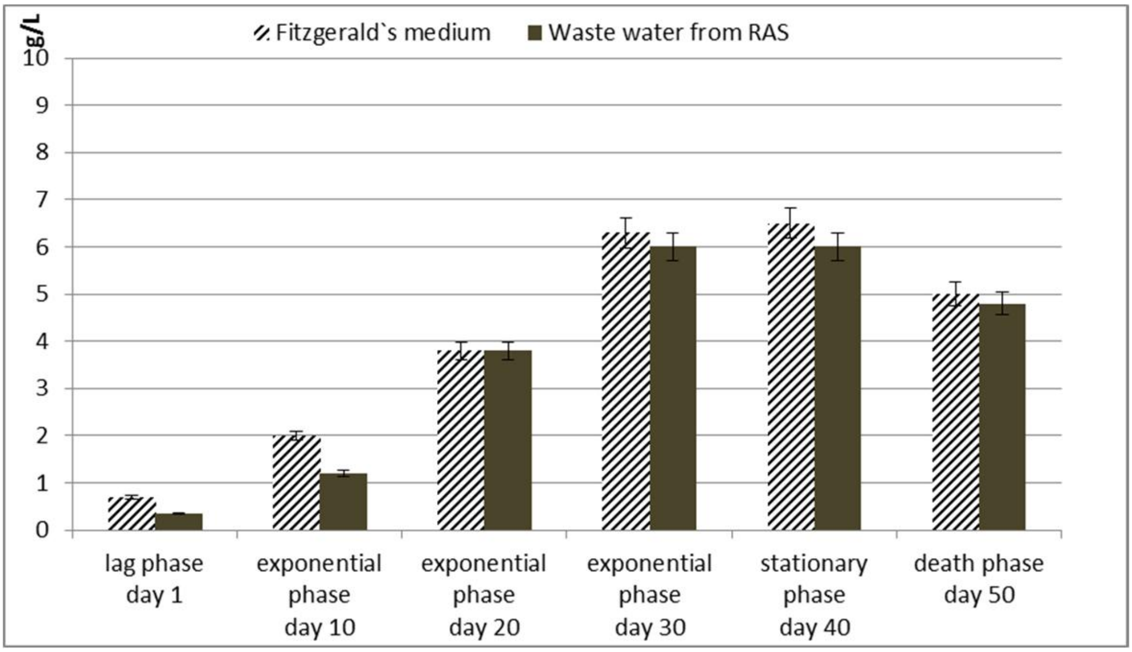


Fig. 1. Biomass of N. linckia under cultivation on waste water from RAS

The biotechnological value of cyanobacteria is determined by the content of proteins, lipids and carbohydrates in the cell. To efficiently assess the biomass productivity, we developed a scheme for the complex analysis of *N. linckia* biomass, since, for example, after extraction of proteins, hydrophobic compounds still remain in the cells (Fig. 2).

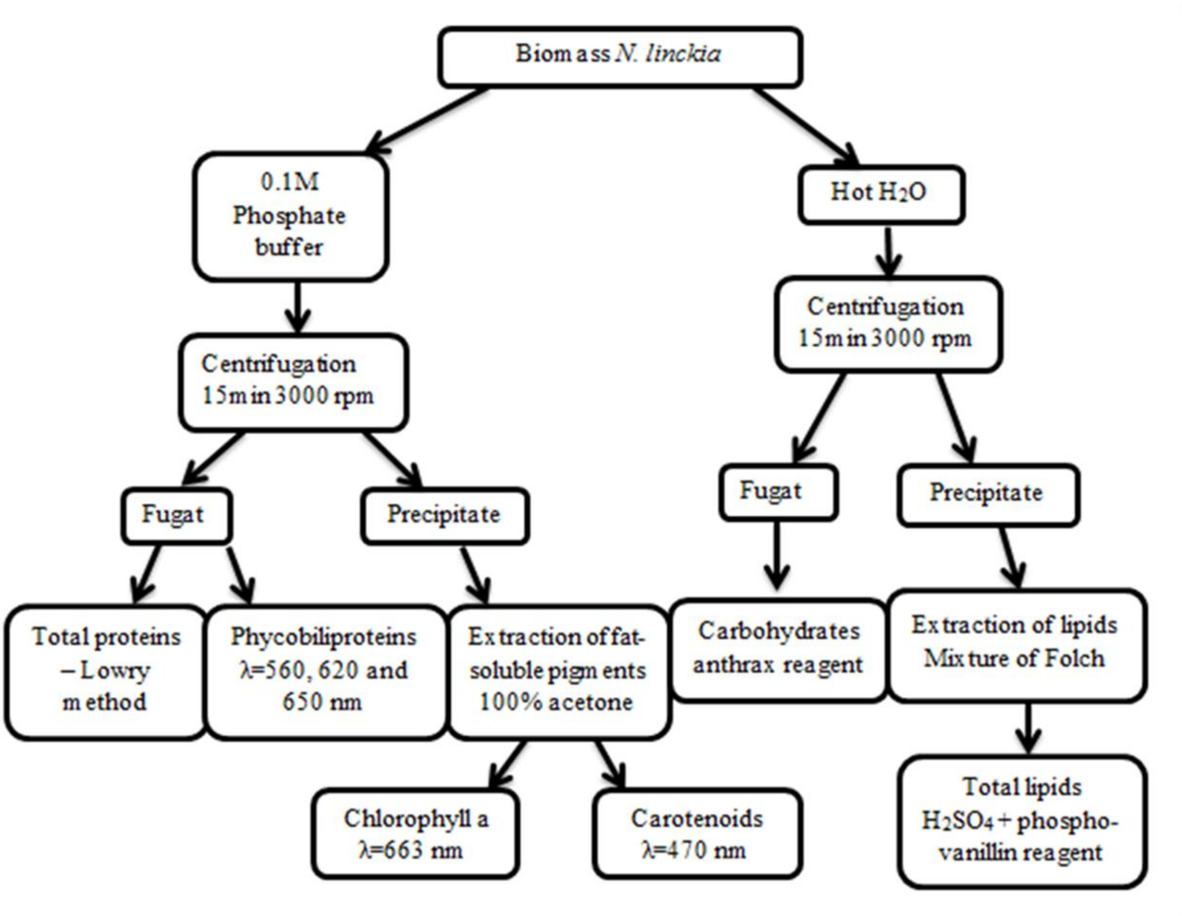


Fig. 2. Scheme of biomass N. linckia complex analysis

The use of this scheme provides a reduction in the cost of biomass for research. Such an approach to integrated assessment can reduce the time of the experiment.

Our studies have shown that the ratio of the main components in the biomass of *N. linckia* practically does not differ in both the applied media (Fig. 3). In cells cultivated in waste water, the amount of total proteins, lipids and carbohydrates was slightly less than when using the control medium and amounted to 55.1% of proteins, 14.1% of lipids and 9.3% of carbohydrates. Analyzing the level of accumulation of these compounds in biomass grown on both nutrient media, there was no significant difference in these indicators. Consequently, when grown on waste water from RAS, *N. linckia* culture is characterized by the same content of basic nutrients compared to the control, however, its biomass cost is much lower than when using standard nutrient medium.

As is known, the protein content in the biomass of microalgae can vary from 20 to 70%, depending on the cultivation conditions. For example, in green algae *Chlorella* and *Scenedesmus*, cells contain about 50% of proteins (Bai, 200), whereas the protein content in cyanobacterial biomass can reach 70% (Tartie, 2008, Cheban, 2014; Rosales-Loaiza, 2017). So, a significant content of proteins in the biomass of *N. linckia* allows using these cyanobacteria as a feed additive for the needs of aquaculture.

Also important for assessing the productivity of culture are indicators of the accumulation of photosynthetic pigments, in particular chlorophyll a, carotenoids and phycobilin proteins. Their number in the cell depends on many factors: pH, temperature, mineral and carbon nutrition, light and other cell culture conditions (Finenko, 2003). It is known that the efficiency of photosynthesis depends on the elements of mineral nutrition that form the photosynthetic apparatus (Sanchez, 2008). The waste water from the RAS has a sufficient amount of nitrogen, phosphorus and carbone, therefore, it is possible to assume a sufficient accumulation of photosynthetic pigments in the biomass of *N. linckia*.

So, in cells cultivated in waste water from RAS, we detected: phycocyanin - 12.2 mg / g, phycoerythrin - 23 mg / g, alophycocyanin - 25.7 mg / g, chlorophyll a - 16.2 mg / g and 9 , 5 mg / g carotenoids (Fig. 4). Thus, the average values of the studied parameters in *N. linckia* biomass did not significantly differ in the combination of both nutrient media. Almost the same content of these pigments in both biomasses is most likely due to the same growing conditions, pH of the medium and almost the same amount of basic nutrients.

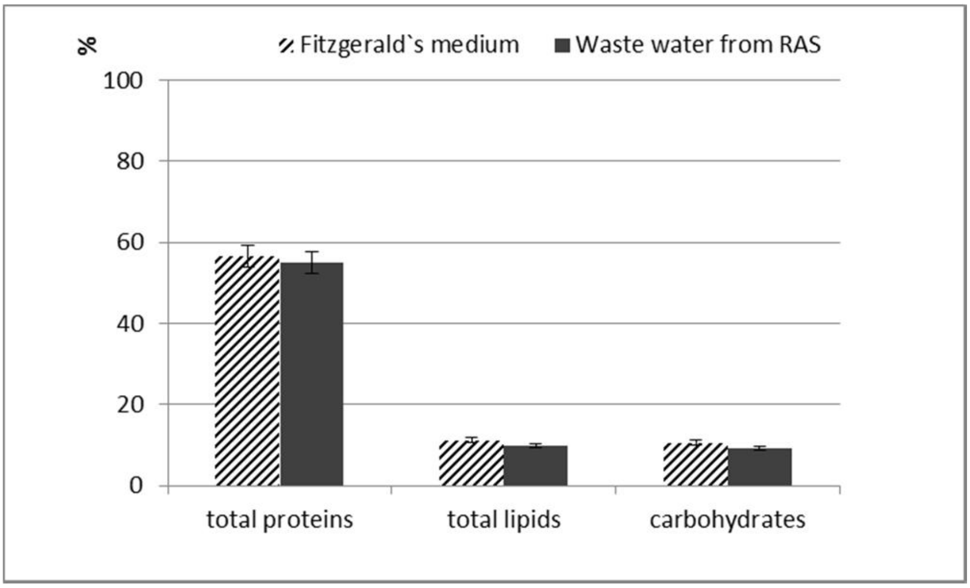
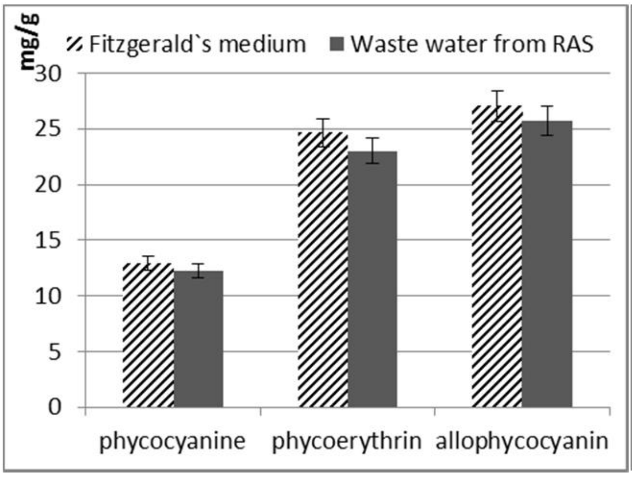
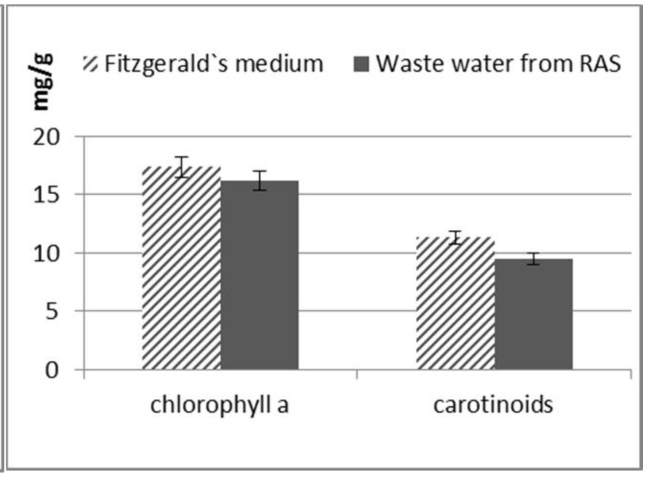


Fig. 3. The amount of total proteins, lipids and carbohydrates in N. linckia culture grown on waste water from RAS



a



b

Fig. 4. The content of phycobilin proteins (a), chlorophyll a and carotenoids (b) in N. linckia cells cultivated on the waste water from RAS.

So, the waste water from RAS can be used as an alternative medium for the cultivation of *N. Linckia*. The resulting biomass in terms of total protein, lipids, carbohydrates, and basic photosynthetic pigments does not significantly differ from that grown on Fitzgerald control medium. The applied approach makes it possible to obtain *N. linckia* biomass with virtually the same as in the control series, the content of basic nutrients. However, biomass grown on waste water from RAS will be much cheaper.

Conclusions:

1. The amount of basic mineral elements in the waste water from RAS is sufficient for the normal cultivation of algocultures, so it can be used as a nutrient medium for cultivating *N. linckia*.

2. A scheme for the integrated analysis of biomass *N. linckia* has been developed which allows to reduce the amount of material for analysis and shorten the duration of the experimental study.

3. When grown on waste water from RAS the *N. linckia* culture, the content of basic nutrients is almost the same as compared to the control, but its biomass cost is much lower than when using standard nutrient medium.

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КОМПЛЕКСНА ОЦІНКА БІОМАСИ *NOSTOC LINCKIA* (ROTH.) BORN. ET FLAH., КУЛЬТИВОВАНОЇ НА СКИДНІЙ ВОДІ ІЗ РИБОВОДНОЇ УСТАНОВКИ ЗАМКНУТОГО ВОДОПОСТАЧАННЯ

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В роботі розглядалася можливість культивування Nostoc linckia (Roth.) Born. et Flah. на скидній воді із установки замкнутого водопостачання (УЗВ). Nostoc - це рід ціанобактерій, які зустрічаються в різних середовищах існування. Вони утворюють колонії, що складаються з ниток мономорфних клітин у полісахаридній оболонці.Досліджувані водорості вирощували на штучному середовищі Фітцджеральда № 11 в модифікації Цендера і Горхема та на воді із УЗВ, стандартизованій за показниками pH та загальної мінералізації. Відмічено, що скидна вода містить достатню кількість біогенних елементів для культивування ціанобактерії. Розроблено комплексну схему аналізу біомаси N. linckia з урахуванням розчинності органічних речовин та послідовності їх екстракції. Досліджено динаміку біомаси, вміст білка, вуглеводів, ліпідів, хлорофілу а, каротиноїдів та фікобілінових пігментів в культурі N. linckia. Максимальний вміст загального білку був відмічений на 30 добу культивування та становив близько 60 % сухої маси водорості. Вміст хлорофілу а і каротиноїдів у клітинах N. linckia були 16,2 мг/г і 9,5 мг/г сухої маси відповідно. Також було визначено рівень основних компонентів фікобілінових пігментів: фікоеритрину, фікоціаніну та алофікоціаніну.Проведено порівняльну характеристику продуктивних показників періодичної культури в залежності від її початкової забезпеченості основними елементами мінерального живлення. При вирощуванні на скидній воді із УЗВ культури N. linckia вміст основних нутрієнтів достовірно не відрізняється від контрольних значень, але вартість її біомаси значно нижча.

Ключові слова: Nostoc linckia (Roth.) Born. et Flah., скидна вода із УЗВ, комплексна оцінка

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