EFFECT OF NITROGEN COMPONENTS ON GROWTH AND PIGMENT FORMATION OF MICROALGAE *CHLORELLA VULGARIS* 4 AND *SCENEDESMUS QUADRICAUDA* 32

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Abstract. *The utilization of microalgae in wastewater treatment leads to wastewater bioremediation, which can be used to produce algal by-products. However, to evaluate the growth of microalgae, many factors have to be considered, mainly the cultivation conditions. In this context, the components of the medium affect the direct growth of microalgae and consequently the biomass yield. In this work, the cultivation conditions of native microalgae strains Chlorella vulgaris 4 and Scenedesmus quadricauda 32 were optimized to obtain the highest biomass and pigments. It was shown that the formation of dry biomass of C.vulgaris 4 on the standard medium "Tamiya" is 358 mg/l, and on the medium with increased content of nitrogen components in 2 times - 424 mg/l of medium. Consequently, the increase in the nitrogen component of the medium increases the biomass formation of C. vulgaris 4 by 18.4%. Under the same conditions, the formation of dry biomass by the S. quadricauda 32 culture increases by 24.3%. When growing C. vulgaris 4 and S. quadricauda 32 on the medium "Chu-13" with increased content of nitrogen components in 2 times the formation of dry biomass exceeds the biomass formed when growing on standard medium by 16% and 14.3%, respectively. It was revealed that the content of chlorophyll and carotenoids in C. vulgaris 4 and S. quadricauda 32 increases in "Tamiya" and "Chu-13" media with increased content of nitrogen components 2 times. In particular, the content of chlorophyll a, chlorophyll b and carotene in C. vulgaris 4 grown on "Tamiya" medium increased by 12.5%, 18.9% and 20%, respectively. Thus, the analysis of the research results showed that the quality of nutrient medium influences the formation of biomass and pigments used in the cultivation of microalgae.*

Keywords: *microalgae, biomass, nitrogen components, Chlorella vulgaris, Scenedesmus quadricauda, chlorophyll a, chlorophyll b, carotene.*

INTRODUCTION. Integrated wastewater bioprocessing has recently attracted considerable attention. Algae have excellent ability to convert sunlight energy into biomass and absorb nitrogen, phosphorus and other organic toxins that accumulate in wastewater [1]. Also a significant advantage of bioprocessing based microalgae integrated in wastewater is that it simultaneously solves environmental problems and also produces biofuels as well as other value-added compounds such as pigments, trace elements, omega fatty acids, antioxidants and animal feed [2-4]. Many studies indicate that some wastewater (e.g., domestic, agricultural, and industrial wastewater) is rich in relevant nutrients, which can serve as a low-cost alternative source of crude nutrients for microalgae cultivation using atmospheric CO_2 and flue gases [5,6]. Consequently, microalgae have high growth rate, high CO_2 uptake capacity, high content of chlorophyll, proteins, vitamins, mineral salts, carbohydrates, antioxidant substances and fatty acids [7]. In the work of

Depra et al. (2020) showed that microalgae cells contain about 50% carbon and 1.8 kg of CO_2 is used to produce 1.0 kg of algal biomass [8]. Thus, CO_2 bioremediation using microalgae can be more economical, cost-effective and environmentally friendly when incorporated into wastewater treatment infrastructure [9,10]. Cultivation of microalgae in wastewater results in wastewater bioremediation, which can be utilized to produce algal by-products [11]. However, to evaluate the growth of microalgae, many factors need to be considered, mainly the cultivation conditions. In this context, the medium components affect the direct growth of microalgae and consequently the biomass yield.

The aim of this work is to optimize the cultivation of local microalgae strains *Chlorella vulgaris* 4 and *Scenedesmus quadricauda* 32 to obtain the highest biomass and carotene.

MATERIALS AND METHODS. Local strains of microalgae *Chlorella vulgaris* 4 and *Scenedesmus quadricauda* 32 isolated in pure culture from samples of water bodies of Tashkent region [12,13] were used in this work. To optimize biomass and pigment formation, unicellular green algae of the genus *Chlorella* and *Scenedesmus* were cultured on standard "Tamiya" medium, g/l: KNO_3 – 5.0; $MgSO_4 \times 7H_2O$ – 2.5; KH_2PO_4 – 1.25; $FeSO_4 \times 7H_2O$ – 0.009; trace element solution, 1 ml. Micronutrient solution (g/l): H_3BO_3 – 2.86; $MnCl_2 \times 4H_2O$ – 1.81; $ZnSO_4 \times 4H_2O$ – 0.222; MoO_3 – 17.64 mg/l; NH_4VO_3 – 22.96 mg/l, and "Chu-13" mineral standard medium, (g/l): KNO_3 – 0.2, K_2HPO_4 – 0.04, $MgSO_4 \times 7H_2O$ – 0.1, $CaCl_2 \times 6H_2O$ – 0.08, ferric citrate – 0.01, citric acid – 0,1, boron – 0.5 mg, $MnSO_4$ –$\times 7H_2O$ – 0.5 mg, $CuSO_4 \times 5H_2O$ – 0.02 mg, $CoCl_2 \times 2H_2O$ – 0.02 mg, $Na_2MoO_4 \times 2H_2O$ - 0.02 mg, pH 7.5 [14,15]. Also in this work, the same media with increased content of nitrogen components by 2 times were used. The prepared media were inoculated with microalgae culture in the amount of 10% of the volume and density of $0.8\text{-}10^6$ cells/ml. Illumination was carried out by daylight lamps. The pH was determined using a Metlerr Toledo pH meter (China). Cultivation of microalgae was carried out under aeration conditions using an air compressor BP-800, 5 W power. Cultivation was carried out for 14 days. To determine the dry weight of cell biomass, glass bouquets were placed in a desiccator and dried for 2 h at a temperature of 110°C. Then, the bouquets were removed from the desiccator with tweezers and transferred to the desiccator with anhydrous $CaCl_2$. After 1 h, the bouquets were weighed to the nearest 0.1 mg. Drying and weighing were repeated in the specified sequence of operations until the mass reached a constant value, i.e. fluctuations in its determinations did not exceed ± 0.1 mg.

Chlorophyll and carotenoid content of microalgae grown under the same conditions was determined by spectrophotometric method [16]. For this purpose, a 50 ml sample of microalgae suspension was centrifuged at 5000×g for 10 minutes. The supernatant was removed and the pigments were extracted from the sediment with 100% acetone with treatment for 3 times one minute each with an ultrasonic dispersant. After treatment, the extract mixture was centrifuged once more (5 min at 3000 g) to separate the white cell debris from the pigment extract. The light transmission of the clear extract was measured on a V-5000 VIS spectrophotometer (Shanghai Metash Instruments CO., LTD, China) at the following wavelengths (440.5, 644, and 662 nm in 0.1 nm steps) in quartz cuvettes. The pigment density in the sample was calculated according to the following equations (by Holm-Wettstein).

$C_{chl.a} = 9{,}784D_{662} - 0{,}990\ D_{644}$;

$C_{chl.b} = 21{,}426D_{644} - 4{,}650D_{662}$;

$C_{chl.a+chl.b} = 5{,}134D_{662} + 20{,}436D_{644}$;

$C_{car} = 4{,}695D_{440{,}5} - 0{,}268\ C_{chl.a+chl.b}$

In this case, the concentration of pigments in the extract in μg/ml is calculated by the formula Holm - Wettstein.

The Student's t-criterion and computer programs STATISTICA 6.0 were used in establishing the reliability of the results of experiments in calculating the linear deviation, mean deviation, in calculating the intervals of reliability.

RESULTS AND DISCUSSION

Local strains of microalgae *Chlorella vulgaris* 4 and *Scenedesmus quadricauda* 32 stored in the collection of the laboratory of "Environmental Biotechnology" of the Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan were used in the experiments. To study biomass formation, unicellular green microalgae *C. vulgaris* 4 and *S. quadricauda* 32 were cultured on standard nutrient media "Tamiya" and "Chu-13" and on the same media with increased content of nitrogen components in 2 times during 14 days. As can be seen from Figure 1, the formation of dry biomass by *C. vulgaris* 4 culture on standard medium "Tamiya" is 358 mg/l, and on medium with the increased content of nitrogen components was 2 times higher - 424 mg/l of medium. Consequently, the increase in the nitrogen component of the medium increases the biomass formation of *C. vulgaris* 4 by 18.4%. It should be noted that under the same conditions, dry biomass formation by *Scenedesmus quadricauda*

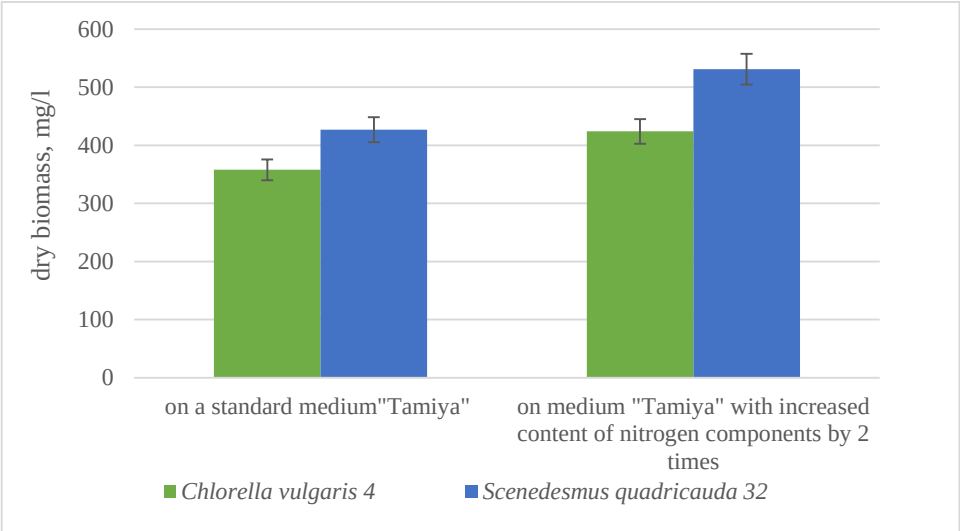

Figure 1. Biomass formation in Chlorella vulgaris 4 and Scenedesmus quadricauda 32 on "Tamiya" medium

Analysis of the results of the studies showed that the quality of the nutrient medium influences the biomass formation used in the cultivation of microalgae. The nutrient media used for cultivation of *Chlorella vulgaris* 4 and *Scenedesmus quadricauda* 32 contain macro- and microelements that ensure normal cell viability.

The source of nitrogen affects the biochemical composition of microalgae. Not only growth but also biochemical content depends on the source and amount of nitrogen. As is known, interest in phototrophic microorganisms is determined by the higher rate of biomass accumulation (20-30 times) compared to traditional agricultural crops. By changing the cultivation conditions, it is possible to obtain biomass of phototrophic microorganisms with different content of carbohydrates, proteins and lipids. It is necessary to provide a combination of a sufficiently large number of factors affecting the level of cell biomass accumulation and its bioorganic component composition, which include: the selected strain of phototrophic microorganism, the initial concentration of cells in the medium, the composition of the cultivation medium, the intensity of

illumination, the temperature of the process. For algae production, macronutrients must contain nitrogen and phosphorus, which make up about 10-20% of the microalgae biomass. Many species of microalgae utilize inorganic sources of nitrogen (nitrate, nitrite, and ammonium). Some microalgae (*Dunaliella tertiolecta* and *Botryococcus braunii*) prefer nitrate [17], while some *Chlorella* species prefer ammonium for growth [18].

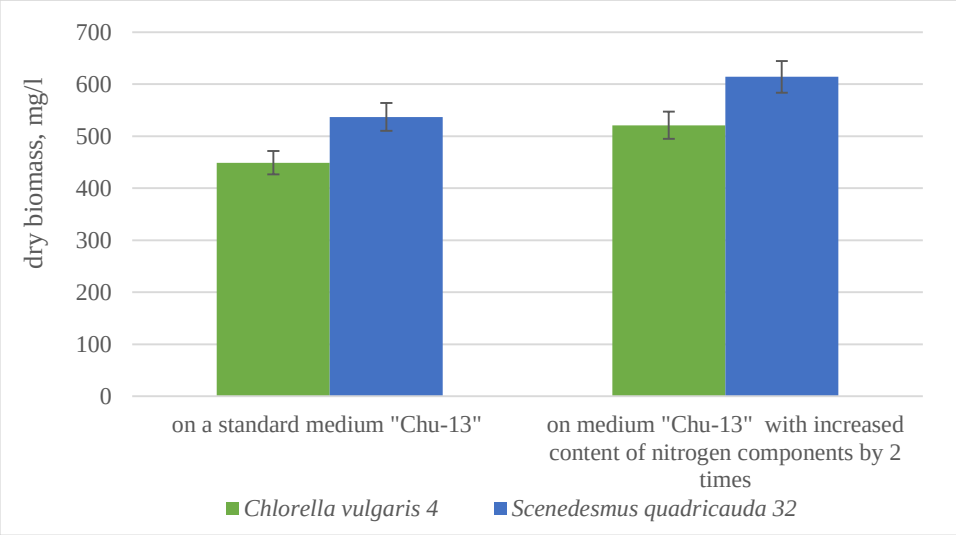

Figure 2. Biomass formation in Chlorella vulgaris 4 and Scenedesmus quadricauda 32 on "Chu-13" medium

Microalgae have a high content of natural proteins that can potentially produce bioactive compounds such as pigments. Almost many species of microalgae contain pigments such as chlorophyll a, chlorophyll b and carotene. On this basis, we compared the formation of chlorophyll and carotenoids in *C. vulgaris* 4 and *S. quadricauda* 32 in standard media "Tamiya" and "Chu-13" and on the same media with increased content of nitrogen components in 2 times during 14 days of cultivation. It was revealed that the content of chlorophyll and carotenoids in *C. vulgaris* 4 and *S. quadricauda* 32 increased in "Tamiya" and "Chu-13" media with increased content of nitrogen components 2 times (Fig.3, 4). Thus, the content of chlorophyll a, chlorophyll b and carotene in the culture of *C. vulgaris* 4 grown on "Tamiya" medium increased by 12.5%, 18.9%, and20%, respectively. While *S. quadricauda* 32 grown on "Chu-13" medium had chlorophyll a content of 20%, respectively. "Chu-13" the content of chlorophyll a, chlorophyll b and carotene increase by 12.3%, 16.6% and 33.3%, respectively. As is known nitrogen source can affect the biomass growth of microalgae, in particular *Chlorella protothecoides*. Thus, significantly different rates of biomass production were achieved when different strains of *C. protothecoides* were grown on media based on nitrate, urea and yeast extract at low, medium and high concentrations [19]. Kim et al, (2016) showed a stimulatory effect on the growth and development of microalgae, in the presence of nitrogen source, yeast extract, possibly as a result of the presence of amino acids, peptides, vitamins and carbohydrates [20]. Consequently, the nitrogen source and its concentration can influence glucose uptake and hence biomass production. Different cultivation strategies have been developed in order to increase the productivity of microalgae pigments. In particular, biotechnological approaches are designed to increase antioxidant metabolites such as chlorophyll and carotenoids. It is known that different strains of *Chlorella* under certain cultivation conditions are able to increase both chlorophyll and carotenoids, components known for their antioxidant properties [21,22].

Many researchers have shown that microalgae, including *Scenedesmus, Chlorella, Botryococcus, Phormidium, Spirulina and Chlamydomonas,* have an excellent ability to bioremediate heavy metals, new synthetic pollutants and pathogens in wastewater [23,24]. Several

species of microalgae such as *Scenedesmus, Chlorella, Euglena, Oscillatoria, Chlamydomonas* and *Ankistrodesmus* proliferate effectively on wastewater [25]. Consequently, microalgae-based methods have recently attracted considerable attention for the treatment of industrial, agro-industrial and livestock contaminants. Microalgae can minimize the risk of eutrophication by removing P and N components [26]. They are considered as multifunctional alternatives to biological treatment, converting unwanted inorganic and organic ingredients into valuable biomass.

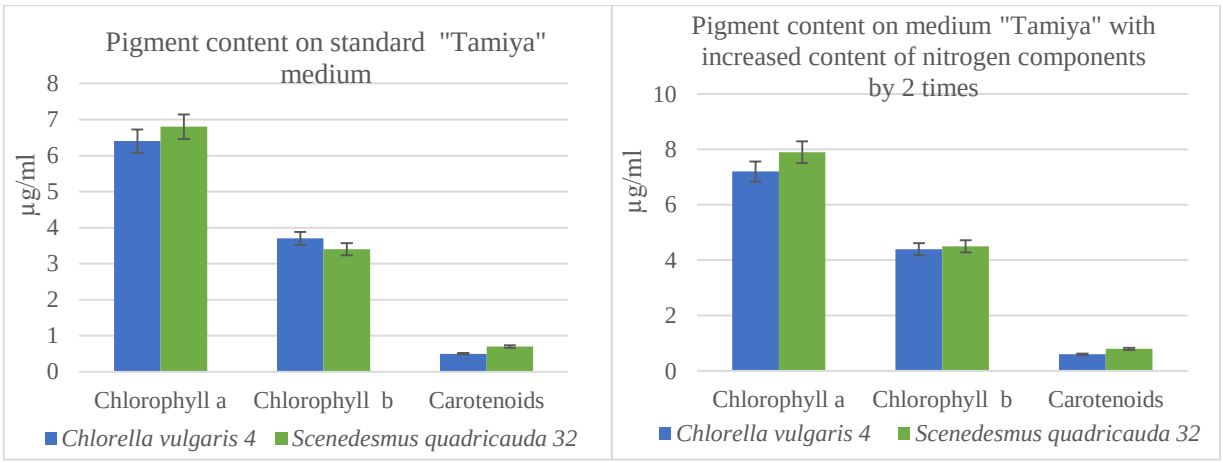

Figure 3. Pigment formation in Chlorella vulgaris 4 and Scenedesmus quadricauda 32 on "Tamiya" medium

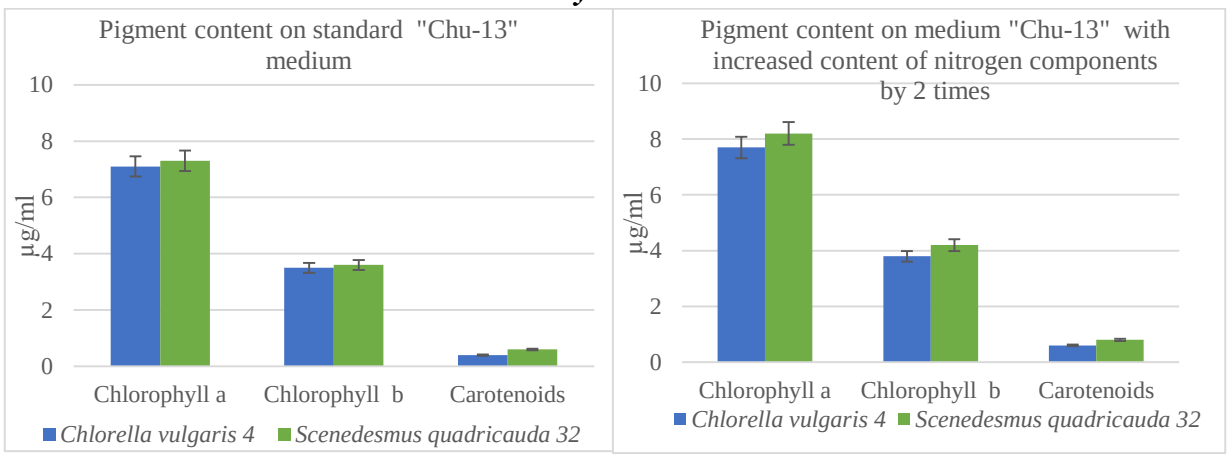

Figure 4. Pigment formation in Chlorella vulgaris 4 and Scenedesmus quadricauda 32 on "Chu-13" medium

CONCLUSION

For the successful production of microalgae biomass, it is very important to work out the technology of obtaining, including optimization of the composition of nutrient medium, cultivation temperature, aeration intensity, etc. Since, cultivation of microorganisms is an important step in industrial and experimental microbiology. The basis of successful cultivation, providing maximum accumulation of biomass, pigments and maintaining the activity of strains, is the selection of nutrient media in accordance with the nutritional and other physiological needs of the microorganism.

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