Technology for preparing prototype biopreparations based on microalgae

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Abstract. This article discusses the technology for preparing a prototype biopreparation based on algal microalgae, specifically from *B. braunii* - AnDI-115 and *Chlorococcum infusionum* – AnDI-76. The aim is to cultivate algal objects on an industrial scale, focusing on economic indicators, including reducing the cost of the final product, selecting its preparative form, and facilitating its application process. Based on the exemplary technology of biotechnological production, during the researches, in order to facilitate the process of industrial cultivation of algal objects in the Chu-13 nutrient medium, its economic indicators, including the reduction of the cost of the finished product, the selection of its preparation form and the process of its application Taking into account that when two or more species of microorganisms are grown together, they synthesize unexpected metabolic substances in order to increase the mutual competition and development activity of microbes, B. braunii - AnDI-115 Chlorococcum infusionum - AnDI from algal microalgae grown in Chu-13 nutrient medium From -76, there was an opinion about the technology of preparing a sample biopreparation.

1 Introduction

According to scientific sources, research aimed at cultivating algal objects industrially is planned based on their targeted characteristics. The primary goal is to utilize these characteristics to improve economic indicators, including reducing the cost of the final product, selecting its preparative form, and facilitating its application process. Additionally, determining the safety indicators of algal objects is also crucial. This process is particularly relevant for biopreparations based on cyanobacteria.

The Decrees of the President of the Republic of Uzbekistan, specifically the Presidential Decree No. PF-5853 dated October 23, 2019, regarding the "Approval of the Strategy for the Development of Agriculture in the Republic of Uzbekistan for the Years 2020-2030," the Presidential Decree No. PF-5863 dated October 30, 2019, concerning the "Approval of the Concept for the Protection of the Environment of the Republic of Uzbekistan until 2030," and the Presidential Decree No. PF-60 dated January 28, 2022, on the "Development Strategy of New Uzbekistan for the Years 2022-2026," along with other relevant regulatory and legal documents, serve to implement the tasks outlined within these frameworks.

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Extensive scientific research has been conducted by international organizations, as well as local and foreign scholars, regarding the effective utilization of microalgae in various sectors of the economy, the organization of processes, and the ongoing monitoring of algological, ecological, and biological aspects. This research includes establishing precise requirements for developed products, certification, and the scientific and methodological alignment of systems.

In Uzbekistan, however, there has been a lack of research into the synthesis of phytohormones by microalgae, the impact of these hormones on plant growth and development, the factors affecting phytohormone synthesis, and the selection of nutrient environments that ensure moderate growth and development for producers with high phytohormone synthesis capabilities. Consequently, conducting a screening of microalgae found in local conditions for their phytohormone synthesis potential, evaluating their significance in plant growth and development, establishing production conditions, and implementing the production of algological biopreparations as ecologically clean products hold significant scientific and practical importance.

2 Results and its analysis

One of the key factors determining the economic efficiency in the process of preparing biological fertilizers based on microalgae and cyanobacteria is the cost of the nutrient medium during cultivation, as well as the methods of cultivation and application forms. Therefore, our research focused on selecting nutrient media and cultivation conditions (pH, °C, duration of cultivation, choice of formulation, studying economic efficiency, etc.) that ensure the moderate growth and development of the highly effective microalgae identified.

As a result, based on a typical biotechnology production technology, the research indicates that two microalgae cultivation systems are necessary for the cultivation of *B.braunii*-AnDI-115 and *Ch.infusionum*-AnDI-76 in the *Chu*-13 nutrient medium. Consequently, the possibilities of cultivating both strains in a single growth system were examined during the research. The results obtained are reflected in Table 1.

Upon analyzing the results, it was observed that in the variant where *B. braunii* - AnDI-115 and *Ch. infusionum* - AnDI-76 were cultivated together, the quantity of the *Ch. infusionum* - AnDI-76 culture was significantly higher. Specifically, when *B. braunii* - AnDI-115 was cultivated separately in Chu-13 nutrient medium, the number of viable cells on the third day reached 3.2×10^2, whereas at the same time, the number of cells in the *Ch. infusionum* - AnDI-76 culture was noted to be 4.4×10^2, indicating a substantial quantity.

Table 1. Growth and Development of *B. braunii* - AnDI-115 and *Ch. infusionum* - AnDI-76 Cultures in Chu-13 Nutrient Medium.

№	Selected algological objects	The number of cells in the cross-section of days			Cell dry mass. g/L.
		3	7	10	
1	*B.braunii*- AnDI-115	3.2×10^2	6.1×10^4	3.6×10^6	14.2±0.41
2	*Ch.infusionum*-AnDI-76	4.4×10^2	6.4×10^4	3.4×10^6	14.5±0.18
3	*B.braunii*- AnDI-115 + *Ch.infusionum*-AnDI-76	5.1×10^2	4.7×10^4	3.2×10^6	12.6±0.32

Note: The initial number of cells planted in the cultures was 1.6×10^2; the dry mass of the cells and the amount of pigments were determined in the 10-day-old cultures ($P<0.05$).

On the fifth day of cultivation, the *B. braunii*-AnDI-115 culture reached a cell count of 6.1×10^4, while the *Ch.infusionum*- AnDI-76 culture exhibited a cell count of 6.4×10^4, indicating that the growth of *Ch.infusionum* was almost equal to that of *B.braunii-AnDI*-115.

By the fifth day of cultivation, *B. braunii-AnDI*-115 had a viable cell count of 3.6×10^6, while *Ch.infusionum*-AnDI-76 had a count of 3.4×10^6, which was lower compared to *B.braunii*- AnDI-115. It is noteworthy that when cultivated in Chu-13 nutrient medium, the difference in viable cell counts between the two cultures was statistically insignificant.

During the growth of these cultures, the option of cultivating them together with equal initial cell counts revealed the following development indicators. Specifically, on the third day, *B.braunii*-AnDI-115 had 3.2×10^2 viable cells, while *Ch.infusionum*-AnDI-76 had 4.4×10^2. When grown together, the combined culture of *B.braunii*-AnDI-115 and *Ch. infusionum*-AnDI-76 reached a cell count of 5.1×10^2, which was twofold higher compared to B. braunii grown separately and onefold higher than *Ch.Infusionum*-AnDI-76 grown separately. On the seventh day, *B.braunii*-AnDI-115 recorded 6.1×10^4 viable cells, while *Ch.infusionum*-AnDI-76 had 6.4×10^4. The combined culture of *B.braunii*-AnDI-115 and *Ch.infusionum*-AnDI-76 showed a total viable cell count of 4.7×10^4, which was twofold lower than the counts of the separately grown cultures. By the tenth day, *B.braunii*- AnDI-115 reached a viable cell count of 3.6×10^6, while *Ch.infusionum*-AnDI-76 had 3.4×10^6. The total viable cell count for the combined culture was found to be 3.2×10^6 cells/ml.

Upon analysis, it was determined that by the end of the cultivation period, the total number of cells in the combined cultures equaled that of the separately cultivated cultures. However, the overall biomass of the combined cultures was slightly lower than that of the separately cultivated cultures. Specifically, *B.braunii*-AnDI-115 produced a biomass of 14.2 g/l, Ch. infusionum- AnDI-76 produced 14.5 g/l, while the combined culture yielded a total biomass of 12.6 g/l.

3 Conclusion

The results obtained indicate that differences in cell sizes arose as a consequence of competition for nutrient sources during the simultaneous growth of two distinct types of microalgae. It can be concluded that the smaller size of these cells, by several micrometers, may have influenced the overall biomass. Scientific literature indicates that when two or more types of microorganisms are cultivated together in the same environment, their mutual competition can lead to the synthesis of unexpected metabolic compounds (such as antibiotic-like metabolites) or an increase in the quantity of substances they continuously synthesize in response to the external environment (Stirk et al., 2002).

To observe this process, the impact of separately and jointly cultivated algal strains on seed germination was studied in Chu-13 nutrient medium (see Table 2).

The study on the influence of jointly cultivated *B. braunii* - AnDI-115 and *Ch. infusionum* - AnDI-76 cultures on seed germination revealed significant differences in biometric indicators. Specifically, when the *B. braunii* - AnDI-115 and *Ch. infusionum* - AnDI-76 cultures were sown separately, they exhibited average germination rates of 42.11% and 41.61%, respectively. In contrast, the mixed culture of *B. braunii* - AnDI-115 and *Ch. infusionum* - AnDI-76 demonstrated a higher germination rate of 53.42%.

The *B.braunii-AnDI-115* culture exhibited an average yield that was 17.03% lower compared to the control (IAA, 10^-3 M), while the *Ch.infusionum-AnDI-76* culture showed a 17.53% lower yield. The biopreparation prepared from the mixed culture of *B.braunii-AnDI-115* and *Ch.infusionum-AnDI-76* displayed a 5.94% lower efficacy compared to the control. This indicates that the separately cultivated cultures, particularly *B.braunii-AnDI-115*, showed a 24.89% lower yield (69.28±0.14) compared to the control biopreparation and a 19.96% lower yield compared to the sample biopreparation. The *Ch.infusionum-AnDI-76* culture exhibited a 17.86% lower yield compared to the control and a 12.93% lower yield compared to the sample preparation. Furthermore, the sample biopreparation prepared from

the cultures of *B.braunii-AnDI-115* and *Ch.infusionum-AnDI-76* demonstrated a 4.93% lower yield compared to the control preparation. Thus, the IAA synthesized through chemical means (10^-3 M) ensured a cotton yield of 94.17%, while the biopreparation from the cultures *B.braunii-AnDI-115* and *Ch.infusionum-AnDI-76* provided a yield of 89.24%. This suggests that this biopreparation could be a promising agricultural product. Therefore, in subsequent studies, the synthesis of IAA as the main influencing phytohormone in this biopreparation was examined. Scientific sources indicate that studies on the synthesis of IAA in microorganisms typically use L-tryptophan. Consequently, the synthesis of IAA was investigated in a nutrient medium containing L-tryptophan. The results indicated that the IAA synthesis of *B.braunii-AnDI-115* was lower compared to other cultures. This suggests that the relatively low efficacy of cotton yield may be linked to the IAA synthesis of the *B.braunii-AnDI-115* culture.

Table 2. Impact of Jointly Cultivated *B. braunii* - AnDI-115 and *Ch. infusionum* - AnDI-76 Cultures on Seed Germination in Chu-13 Nutrient Medium.

№	Selected algological objects	"The productivity of cotton seed in the cross-section of days.". %			Biometric indicators of 15-day seedlings		
		3	5	7	Seedling length. cm.	Moist mass of the seedling. g.	Root mass. g
1	*B.braunii* - AnDI-115	42.11±0.33	51.09±0.13	69.28±0.14	5.78±0.23	14.48±0.31	0.10±0.13
2	*Ch.infusionum* - AnDI-76	41.61±0.28	52.42±0.21	76.31±0.37	6.54±0.11	16.34±0.27	0.11±0.14
3	Nazorat (IUK. 10-3M)	59.14±0.14	74.37±0.32	94.17±0.16	13.18±0.08	23.21±0.18	0.23±0.22
4	*B.braunii*- AnDI-115 + *Ch.infusionum*- AnDI-76	53.42±0.23	72.17±0.18	89.24±0.33	11.23±0.17	19.08±0.26	0.20±0.18

Additionally, during the research, it was found that each culture synthesized different amounts of IAA. Specifically, in a nutrient medium with 0.5 mg/mL L-tryptophan, *B.braunii-AnDI-115* produced 5.36 mg/mL of IAA on the 7th day, while in a medium with 1.0 mg/mL L-tryptophan, it produced 7.49 mg/mL, and at 1.5 mg/ml, the yield was 9.12 mg/ml. At 2.0 mg/mL L-tryptophan, the synthesis reached 11.36 mg/ml (Table 3).

Moreover, the highest synthesis occurred in a nutrient medium with 2.50 mg/mL L-tryptophan, yielding 12.17 mg/ml of IAA, while at 3.0 mg/ml, the synthesis decreased to 11.18 mg/mL. This indicates that the maximum IAA synthesis of this culture depends on the concentration of L-tryptophan in the nutrient medium, with the optimal concentration being 2.50 mg/ml.

Table 3. Synthesis of Indole-Acetic Acid by Model Microalgae *(Growth statistics in 7 days).*

№	Samples	L-tryptophan quantity. mg/mL.					
		0.5	1.0	1.5	2.0	2.50	3.0
		IUC quantity. mg/mL.					
1	*B.braunii*-AnDI-115	5.36	7.49	9.12	11.36	12.17	11.18

2	*Ch.infusionum*-AnDI-76	7.18	10.12	13.80	15.43	17.67	15.22
3	*B.braunii*-AnDI-115 + *Ch.infusionum*-AnDI-76	13.37	24.16	28.44	34.18	31.33	29.52

The dependence of the synthesis of IAA (Indole-3-acetic acid) from these microorganisms on the amount of L-tryptophan can also be observed in subsequent studies. In particular, in the culture of *Ch.infusionum-AnDI-76*, at a concentration of 0.5 mg/mL L-tryptophan in the nutrient medium, 7.18 mg/mL of IAA was produced on the 7th day, while at 1.0 mg/mL L-tryptophan, it was 10.12 mg/mL, at 1.5 mg/mL, it was 13.80 mg/mL, and at 2.0 mg/mL L-tryptophan, it reached 15.43 mg/mL. Furthermore, the maximum production of IAA was recorded at 2.50 mg/mL of L-tryptophan, with a yield of 17.67 mg/mL, whereas at 3.0 mg/mL, the synthesis decreased to 15.22 mg/ml.

When analyzing the obtained results, it was found that the maximum synthesis of IAA for the cultures *B.braunii-AnDI-115* and *Ch.infusionum-AnDI-76* depended on the concentration of L-tryptophan in the nutrient medium, with an optimal concentration of 2.50 mg/mL for both cultures. When investigating the amount of IAA synthesized by the mixed culture of *B.braunii-AnDI-115* and *Ch.infusionum-AnDI-76*, it was again observed that the synthesis of IAA depended on the concentration of L-tryptophan. Specifically, in the nutrient medium with 0.5 mg/mL of L-tryptophan, 13.37 mg/mL of IAA was produced on the 7th day, while at 1.0 mg/mL, it reached 24.16 mg/mL, at 1.5 mg/mL, it was 28.44 mg/ml, and the maximum production at 2.0 mg/mL L-tryptophan was 34.18 mg/ml.

In contrast to the previously mentioned data, a decrease in IAA production was observed at 2.50 mg/mL of L-tryptophan in the mixed cultures, with 31.33 mg/mL recorded, and at 3.0 mg/ml, the synthesis decreased to 29.52 mg/ml. This indicates that the tendency of the separately grown cultures towards L-tryptophan decreased compared to the mixed cultures, with a maximum indicator of 2.0 mg/ml.

Changes were also noted in some physiological and cultural characteristics of the microorganisms studied in the nutrient medium with L-tryptophan. In particular, it was observed that the cell sizes of the separately grown cultures *B.braunii-AnDI-115* and *Ch.infusionum-AnDI-76* were smaller compared to those grown together, with the dimensions of *B.braunii-AnDI-115* being less than those of *Ch.infusionum-AnDI-76*.

Based on these results, it was found that the dry mass of the cells of the separately cultivated *B.braunii-AnDI-115* culture was 14.2 g/L, while for *Ch.infusionum-AnDI-76*, it was 14.5 g/L. However, when these cultures were grown together, the total biomass was 12.6 g/L, which is significantly lower than that of the separately cultivated cultures, indicating a clear tendency.

When comparing these scientific results with global sources, researchers from the University of Punjab in Pakistan—Mehboob Ahmed, Lucas Stal, and Shahida Hasnain—reported that the synthesis of IAA in cyanobacteria (MMG-9 *Arthrospira platensis*) could be regulated by the nutrient medium containing L-tryptophan, with a maximum endogenous IAA synthesis occurring at a concentration of 1.5 mg/ml.

Additionally, it was determined that the pH of the nutrient medium and light-dark regimes also affected the synthesis of IAA in these cultures, with the synthesis not being observed in darkness. Thus, the observed decrease in biomass associated with IAA synthesis was proven both theoretically and practically.

The testing of sample biopreparations in a production environment was conducted at the "Saifillo Bobo Ziroboti" farm located in the Bukhara region, Bukhara district, as well as at the fields of the "IGH Ziroboti" farm in the same district. The trials utilized the Bukhara-10 (Sardor) variety of cotton, with the biopreparation "Algobiostim" prepared from the strains B. braunii-AnDI-115 and Ch. infusionum-AnDI-76 cultivated in the Chu-13 nutrient medium, applied at a rate of 4.0-4.5 L/ha (30 billion cells/mL). The experimental work was carried out on 2.0 hectares in the first section of the "Saifillo Bobo Ziroboti" farm, while a

control area of 0.5 hectares was established, where no treatment was applied. An additional 0.5 hectares was set aside as a reference plot.

For the reference variant, the Serhosil biopreparation (2.5 L/ha, "Agro Natural Life" LLC, Uzbekistan) was applied. The cotton seedlings were treated three times: when the initial 4-5 true leaves had formed, and during the budding and fruiting stages, by spraying a liquid solution from the leaves. The results of the experiment were obtained by calculating the biometric indicators of the seedlings every 10 days. Traditional Dospekhov methods were used for the data analysis. The sample biopreparation was mixed with 300 L of water at a rate of 4.0/4.5 liters.

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