

# Selection of a Nutrient Medium for the First Green Microalgae Isolated In Uzbekistan *Ava Limnothalassea*

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## ABSTRACT

### Keywords

Microalgae, *Ava limnothalassea*, nutrient medium: AF6, Allen, BG-11, Bold basal, Bold-3N, C, "CAM", Carefoot, Chu-10.

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For the first time under the conditions of Uzbekistan, the ability of an algologically pure strain of the green microalga *Ava limnothalassea* to produce wet biomass, total protein, and lipids was studied using traditional culture media widely utilized in scientific literature: modified AF6 (AF6), Allen (Allen), modified BG-11 (BG-11), Bold's Basal Medium (BBM), Bold-3N, modified C, modified "CAM", Carefoot (Cary-L), and Chu-10 (Chu-10). It was noted that these culture media are of significant importance for the isolation of blue-green and green algae, as well as for the analysis of their physiological and biochemical characteristics. At the same time, it was indicated that definitive conclusions regarding the biomass accumulation, protein, and lipid content of the microalga *Ava limnothalassea* cannot be drawn based solely on the use of these media. Despite the substantial scientific and practical significance of the results obtained, the necessity of conducting comprehensive future research to study the properties of the green microalga *Ava limnothalassea* on various culture media designed for cyanobacteria and microalgae is emphasized.

## Introduction

In recent years, amid the accelerating challenges of global climate change and growing concerns over food

security, the search for sustainable alternative sources of environmentally friendly and nutritious food products- and the broad implementation of their efficient use in practice-has become one of the most pressing priorities.

In particular, it calls for extensive scientific research aimed at carbon sequestration, phytoremediation of wastewater, the development of the bioeconomy, and the advancement of renewable energy utilization.

The continuous growth of the global population has led to a sharp increase in demand for food products, while, conversely, the capacity to produce food is steadily declining due to a range of environmental challenges. These include water scarcity, soil erosion, salinization and desertification, climate change, the depletion of soil humus content, the adverse impacts of industrial activity on the environment, rising CO<sub>2</sub> emissions, and other related factors. This situation underscores the necessity of prioritizing the development of a green economy, particularly by focusing on microalgae-based biotechnologies.

Moreover, the depletion of natural resource reserves and declining productivity are giving rise to existential risks [Meeranayak *et al.*, 2020]. It is well recognized that sustainable development requires not only environmental protection but also the maintenance of the dynamic balance of ecosystems and the rational use of resources through innovative and intelligent technologies.

In this context, the concept of “green transformation” has emerged in scientific discourse, with microalgae identified as one of its most sustainable and efficient biological resources [Das *et al.*, 2025].

According to scientific studies, microalgae exhibit a 10-50 times higher efficiency in converting solar energy into chemical energy compared to higher plants [Chew *et al.*, 2020]. Furthermore, the production of one ton of algal biomass can absorb approximately 1,83 tons of CO<sub>2</sub>, thereby contributing to the reduction of carbon emissions while simultaneously enabling the generation of high-biological-value biomass [Sarker *et al.*, 2024]. Products derived from this biomass serve as important raw materials for various sectors of the economy (Table 1).

The results of ongoing research on the utilization of green algae and the study of their key characteristics clearly demonstrate the strategic importance of this biological resource. In particular, the current data available in the AlgaeBase database include 183,214 species and infraspecific taxon names, 24,018 images, 75,994 bibliographic records, and 612,887 distribution records, further underscoring the scientific significance of these organisms [<https://www.algaebase.org>].

In addition, a statistical assessment was conducted to evaluate the scope of scientific publications indexed in major international academic databases. Based on this analysis, large-scale bibliographic and citation databases—such as Lens (Scholar Search Results), Europe PMC Plus (Search Life-Sciences Literature), Google Scholar, CORE (Open Access) and OpenAlex—were examined (Figure 1).

The analysis of the compiled statistical data indicated that the Lens database accounts for the largest share of bibliographic and citation records, representing 74% of the total publications identified. It should be noted that these statistics are subject to change; the reported figures correspond to data available as of 18 February 2026. The results were derived from publications indexed over the most recent five-year period, spanning 2020-2025.

In addition, the search results obtained from these databases using the keywords algae, green algae, nutrient medium for algae, algal cultivation, green algal cultivation and *Ava limnothalassea* are presented in Figure 2.

**According to the statistical data obtained**, a search using the keyword *Ava limnothalassea* yielded only four results in total. Based on the analyzed time frame and the years covered by the selected databases, it can therefore be concluded that, worldwide, only four authors have reported research involving the green alga *Ava limnothalassea*. This finding indicates that cultures belonging to this genus and species remain extremely rare and insufficiently studied.

An analysis of the statistical results further revealed that, in the OpenAlex database alone, a search for the keyword algae returned 34,640 scientific publications; green algae yielded 6,513 publications; nutrient medium for algae produced 1,598 records; algae cultivation resulted in 1,817 publications; and green algal cultivation returned 374 publications (Table 2). In addition, it was noted that beyond these categories—such as journal articles, book chapters, articles in press, review papers, and dissertations—there are also bibliographic and citation records that are not included in the aforementioned counts.

The above-mentioned data indicate that this research addresses a highly relevant and pressing issue. Therefore, for the first time, the present study aims to investigate the growth and development of the green alga *Ava*

*limnothalassea* in different nutrient media, with particular emphasis on its wet biomass production and the formation of primary metabolites.

## Materials and Methods

**Green algal species used in the present study.** The green alga *Ava limnothalassea* represents a culture first isolated from freshwater bodies in Uzbekistan and is currently preserved in the Collection of Unique Microorganisms of the Tashkent Institute of Chemical Technology (authors: N.A. Khojamshukurov, D.R. Bakhranova, and Kh.O. Abdullaev).

**Nutrient media used in the study.** The biomass accumulation, as well as the protein and lipid content of *Ava limnothalassea*, were investigated under standard conditions (temperature 25°C, illumination intensity 25-27 lux, initial inoculum density 10<sup>3</sup> cells/mL). The following nutrient media were used in the experiments (Table 3).

## Results and Discussion

The modified BG-11 nutrient medium is considered a universal medium primarily designed for cyanobacteria, characterized by a relatively high content of nitrogen and mineral components. Based on this medium, Rippka and colleagues isolated more than 150 cyanobacterial strains [Rippka *et al.*, 1976]. The modified BG-11 medium contains a high concentration of nitrogen (1,5 g/l) and is mainly intended for freshwater cyanobacteria. It is widely used for the isolation, cultivation, and maintenance of numerous cyanobacteria, including unicellular species such as *Synechocystis* and *Synechococcus*, filamentous forms such as *Oscillatoria* and *Phormidium*, as well as non-nitrogen-fixing cyanobacteria.

A nitrogen-free variant known as BG-110 is also extensively reported in the literature. In this medium, sodium nitrate (NaNO<sub>3</sub>) is omitted from the composition, making it suitable for the isolation and study of nitrogen-fixing cyanobacteria such as *Anabaena* and *Nostoc*, which are capable of fixing atmospheric nitrogen.

One of the main challenges associated with the use of BG-11 medium, as noted in the literature, is the formation of insoluble precipitates during sterilization due to interactions between phosphates (K<sub>2</sub>HPO<sub>4</sub>) and calcium and magnesium ions. Consequently, the

preparation of this medium requires additional procedural steps, including the separate sterilization of certain components followed by their aseptic combination after cooling. This increases both the complexity of the procedure and the overall cost.

The biomass production, as well as the protein and lipid accumulation of *Ava limnothalassea*, were investigated in BG-11 control medium under standard conditions (temperature 25°C, illumination intensity 25-27 lux, initial inoculum density 10<sup>3</sup> cells/mL) (Figure 3).

During cultivation in BG-11 medium, the *Ava limnothalassea* culture began to exhibit significant biomass accumulation from the fourth day of growth (5,8 g/l). A steady and synchronous increase in biomass was observed between days 4 and 8 (5th day 8.7 g/l, 6th day 11.13 g/l, 7th day 13.3 g/l, 8th day 14.6 g/l). When analyzed in terms of days, it was found that the growth rate of cell biomass during the transition from day 3 to day 4 was 1.6 g/l biomass, while during the transition from day 4 to day 5 and from day 5 to day 6, the biomass index increased by 2.3-2.9 g/l, respectively. Biomass continued to rise consistently until day 8, reaching a maximum value of 14,6 g/l. After day 9, a decline in biomass was observed, which may be attributed to nutrient depletion and physiological aging processes.

At the same time, the protein content of the cells initially accounted for 22,2% and gradually decreased over time (2nd day 21.4%, 3rd day 21.0%, 4th day 20.6%). Notably, 4-day, the declining trend in protein content intersected with the increasing trend in lipid accumulation. From day 5 to day 9 of cultivation, the protein content decreased by approximately 1,6%, reaching 17% by day 13.

In contrast, lipid content, which initially constituted 14,3%, began to increase gradually from days 3-4 onward, reaching 20,5%. From day 5 to day 9, lipid accumulation continued to rise steadily (24,5% on day 5, 28% on day 6, 30.1% on day 7, 31,2% on day 8, and 31,7% on day 9). A further increase in lipid content was recorded between days 10 and 13, indicating that enhanced stress conditions in the BG-11 medium stimulated lipid accumulation in the culture.

Subsequent experiments were conducted using the widely applied modified AF6 nutrient medium (AF6) (Figure 3.1). Under standard cultivation conditions (25°C, 25-27 lux illumination, initial cell density 10<sup>3</sup>

cells/ml), the physiological development, biomass productivity, and protein and lipid formation of *Ava limnothalassea* were investigated in AF6 medium.

During the first two days of the experiment, cell adaptation predominated, resulting in relatively slow biomass accumulation, which increased by 3,2 g/l over this period. From day 2 to day 3, and subsequently from day 3 to days 4 and 5, biomass increased sharply to 4,0 g/l, 4,1 g/l, and 6,7 g/l, respectively. Between days 6 and 11, biomass exhibited a gradual upward trend, reaching a maximum value of 16,0 g/l. In the following days, a decline in biomass was observed, decreasing to 12,6 g/l.

The relative protein content showed a steady decreasing trend from the initial days of cultivation, amounting to 36,7% on day 1, 36,0% on day 2 and 34,7% on day 3. This decline became more pronounced as the culture entered the rapid biomass growth phase on day 3. By the end of the experiment, the protein proportion had decreased by 8,6%. In contrast, lipid content initially accounted for 12,3% on day 1 and gradually decreased at a very slow rate throughout the observation period, with an overall reduction of 0,2%.

Compared with the control variant, cultivation in AF6 medium resulted in a 1,4 g/l higher maximum biomass yield and a 12,4% greater relative protein content. This difference can be attributed primarily to the balanced composition of nitrogen sources and macro- and microelements in AF6 medium, which enhanced cell growth and protein synthesis by stimulating metabolic activity directed toward protein biosynthesis, thereby promoting more intensive biomass accumulation. Conversely, the relative lipid content was 18,9% lower than in the control, likely because cellular metabolism was predominantly directed toward growth and protein synthesis, while lipid accumulation remained a secondary process.

It is well established that modified AF6 medium is considered one of the universal culture media [Watanabe *et al.*, 2000]. According to the literature, it has been widely used for the isolation and maintenance of various cyanobacteria, including toxin-producing *Microcystis aeruginosa*, nitrogen-fixing genera such as *Anabaena* and *Dolichospermum*, as well as widely distributed soil and freshwater species such as *Nostoc* and filamentous forms like *Oscillatoria* [Watanabe *et al.*, 2000]. In addition, AF6 medium is extensively employed for the isolation, cultivation, and maintenance of green algae belonging to

the division Chlorophyta, including species of *Chlorella*, *Scenedesmus*, and *Chlamydomonas*. Furthermore, the medium can be further modified-for example, by supplementing it with silicon ( $\text{Na}_2\text{SiO}_3$ )-to support the growth of diatoms (Bacillariophyta) and euglenoids (Euglenophyta). Owing to the presence of EDTA-chelated iron, AF6 medium is also widely used to enhance photosynthetic activity in microalgae.

The growth dynamics and biochemical composition of *Ava limnothalassea* cultivated in Allen (Allen) medium (with BG-11 used as the control) were analyzed under standard conditions (25°C, 25-27 lux illumination, initial inoculum density  $10^3$  cells/mL) (Figure 4.2). According to the obtained results, biomass accumulation in Allen medium proceeded slowly during the first four days of cultivation (day 2 1.8 g/l, day 3 3.2 g/l). On day 4, a marked increase in both biomass (5,2 g/L) and lipid content (21,6%) was observed, coinciding with a gradual decline in protein content (20.1%).

From day 5 onward, the culture entered the exponential growth phase (7,8 g/L on day 5, 10,5 g/L on day 6, and 12,6 g/L on day 7), reaching a maximum biomass of 14,1 g/L on day 8. Subsequently, a decreasing trend was recorded from days 9 to 13, with biomass declining by 5,8 g/L, likely due to nutrient depletion. Throughout cultivation in Allen medium, the protein content showed a gradual decrease. During the first four days, the protein proportion declined by 1,6% (21.3% on the 1st day, 20.9% on the 2nd day, 20.5% on the 3rd day).

The transition from day 4 to day 5 marked the intersection point at which the decreasing protein trend (20,1-19,7%) coincided with increasing biomass and lipid accumulation. Between days 6 and 8, protein content further decreased by 0,4-0,7%, ultimately reaching 16-17% by the end of the experiment.

In contrast, lipid content increased steadily from day 1 to day 3 (15% on day 1, 16% on day 2 and 18,2% on day 3) and rose sharply between days 4 and 8 (21,6% on day 4, 25,5% on day 5, 28,7% on day 6 and 8-day 31.5%). From days 9 to 13, lipid content increased gradually, reaching 32,2%.

This pattern suggests enhanced lipid accumulation under physiological stress conditions in microalgae. Overall, the results indicate that Allen medium is effective for obtaining lipid-rich biomass and demonstrate an inverse correlation between protein and lipid content.

A comparative analysis of growth dynamics and biochemical composition in Allen and BG-11 media under standard conditions showed that microalgae cultivated in Allen medium exhibited a higher growth rate, with maximum biomass reaching 14,1 g/L on day 8. In BG-11 (control) medium, a similar maximum value of 14,6 g/L was recorded, although overall growth intensity was lower. In both media, the intersection of increasing biomass and lipid accumulation with decreasing protein content occurred on day 4. Protein content declined progressively in both media; however, the decrease was more pronounced in Allen medium. Conversely, lipid accumulation was significantly higher in Allen medium, stabilizing at approximately 31-32% between days 9 and 13, whereas lipid enhancement in BG-11 medium was comparatively limited. These findings suggest that Allen medium is more effective than BG-11 control medium for producing biomass enriched in lipids (Figure 4.2).

Allen medium for blue-green algae is primarily used for the cultivation of cyanobacteria and is typically suitable for cultures adapted to alkaline conditions [Watanabe *et al.*, 2000]. It has been widely applied for the isolation, cultivation, and maintenance of various cyanobacteria, including representatives of the unicellular picoplankton genus *Synechococcus* (e.g., *Synechococcus leopoliensis*), colony-forming *Chroococcus* species characterized by mucilaginous capsules, thickly encapsulated *Gloeocapsa* species, and filamentous, motile multicellular forms such as *Oscillatoria* [Watanabe *et al.*, 2000].

In subsequent experiments, the Bold's Basal Medium (BBM) was used to evaluate total biomass production as well as protein and lipid biosynthesis in *Ava limnothalassea* under optimal standard conditions (Figure 4.3). According to the observations of this experiment, microalgal growth during the first 2-3 days proceeded slowly, reaching 4,7 g/L. A sharp increase in biomass of 3,3 g/L was recorded between days 3 and 4. On a day-by-day basis, the growth rate continued to accelerate; specifically, biomass increased by 2,3 g/L between days 5 and 6 and by 1,4 g/L between days 6 and 7. The maximum biomass value of 15,8 g/L was observed on day 9. In the subsequent days, a declining trend was noted, which can be attributed to nutrient depletion and physiological aging processes (Figure 4.3).

In BBM medium, the protein proportion decreased sharply by 2,2% on day 5. Notably, this time point coincided with a marked increase in lipid content by 6,2%. By the end of the experiment, the protein

proportion had declined to 18,7%, suggesting a metabolic shift toward lipid biosynthesis. In BBM medium, lipid content showed a gradual increase from day 1 to day 4, rising by 3,2% overall (13,5% on day 1, 14,1% on day 2, 11,9% on day 3 and 16,7% on day 4). From day 5 to day 7, lipid content increased by 7.5%. Between days 8 and 13, lipid accumulation stabilized, reaching 31,4%. Overall, these findings indicate that BBM medium is more effective than the BG-11 control medium for enhancing biomass production and obtaining lipid-rich biomass. In addition, an inverse relationship between protein and lipid content was observed during cultivation.

Comparative analysis with the control showed that BBM medium yielded a higher maximum biomass value (15,8 g/L), exceeding the control by 1,2 g/L and Allen medium (14,1 g/L) by 1,7 g/L. Although the protein proportion in BBM medium was 1,9% higher than in the control and 2% higher than in Allen medium, a decreasing trend in protein content was observed across all media. This decline is likely associated with the gradual depletion of nitrogen resources, changes in the carbon-to-nitrogen ratio, and a metabolic shift from structural protein synthesis toward the accumulation of storage compounds. Lipid content in BBM medium was 1,8% lower than in the control and 2,1% lower than in Allen medium, indicating that cellular metabolism in BBM was primarily directed toward growth and biomass accumulation, while lipid biosynthesis became more active under stress or nutrient-limited conditions.

When *Ava limnothalassea* was cultivated in Bold-3N medium under standard conditions, biomass increased gradually by 3,9 g/L during the first five days (Figure 4.4). Between days 5 and 6 and days 6 and 7, biomass increased by 1,8 g/L and 1,9 g/L, respectively. On day 5, the decrease in protein proportion (18,3%) coincided with an increase in biomass (4,4 g/L), and maximum biomass (9,2 g/L) was recorded on day 8. From day 9 to day 13, biomass declined to 4,3 g/L due to nutrient limitation.

In this medium, protein content decreased by 0,7% on day 2, coinciding with the onset of increased lipid accumulation (18%), indicating an intersection between declining protein levels and intensified lipid synthesis. By the end of the experiment, protein content had decreased to 15,4%. Lipid content showed a gradual increase up to day 3 (15,5% on day 1, 15,3% on day 2, and 18% on day 3), followed by a sharp 7,1% rise between days 4 and 7. Thereafter, lipid accumulation

continued gradually, reaching 32.9% by the end of the experiment.

Compared with the control, biomass production in Bold-3N medium was 5,4 g/L lower; it was also 4,9 g/L lower than in Allen medium (14,1 g/L) and 6,3 g/L lower than in BBM medium (15,5 g/L). Protein content was 2,2% lower than in the control, 1,4% lower than in Allen medium (18,6%), and 3,7% lower than in BBM medium (20,9%). The reduction in biomass and protein content can be explained by nitrogen limitation in the medium and a metabolic shift from growth processes toward the synthesis of storage lipids. Conversely, final lipid content was 8,6% higher than in the control, 8,9% higher than in Allen medium, and 6,8% higher than in BBM medium. This increase may be attributed to an elevated carbon-to-nitrogen ratio and intensified stress conditions, which stimulated lipid accumulation as a storage response.

**Bold's Basal Medium (BBM)** is a mineral nutrient medium widely used for the isolation, cultivation, and maintenance of green algae (Chlorophyceae). It has been extensively applied for species belonging to the genera *Chlorella*, *Chlamydomonas*, *Scenedesmus*, and *Chlorococcum*. The nitrogen content of this medium is relatively low, which helps prevent excessively rapid growth and supports the maintenance of physiologically and biochemically stable strains under conditions closer to their natural state.

Bold-3N medium represents a nitrogen-enriched, modified variant of BBM. While BBM contains 2,4 mM nitrogen in the form of  $\text{NaNO}_3$ , Bold-3N is characterized by a higher concentration of 8,82 mM. Bold-3N medium is typically employed for large-scale or industrial cultivation aimed at achieving high biomass yields, whereas BBM is primarily used for morphological studies and strain maintenance of blue-green and green algae. One of the principal differences between the two media is that in Bold-3N the exponential growth phase of algal cells is prolonged, whereas in BBM cultures enter the stationary phase more rapidly, largely due to the lower nitrogen availability.

In subsequent experiments, *Ava limnothalassea* was cultivated in modified C medium (C medium) under standard growth conditions. During the first two days, biomass exhibited a relatively slow increase, reaching 3,9 g/L within this initial period (Figure 5.1).

When *Ava (limnothalassea)* was cultivated in C medium under standard growth conditions, biomass exhibited a

relatively slow increase during the first two days, reaching 3,9 g/L. On day 3, a sharp rise to 4,2 g/L was recorded. A further pronounced increase occurred between days 4 and 5, when biomass reached 14,6 g/L. From day 5 to day 8, growth transitioned from the exponential phase to a slower growth phase, with biomass increasing to 16,0 g/L. This value remained nearly stable until day 12. However, on day 13, biomass declined by 2,2 g/L (Figure 5.1).

In this medium, the relative protein content showed a decreasing trend from the first day of cultivation. A particularly sharp decline of 2,2% was observed on day 4. Thereafter, protein content continued to decrease gradually, reaching 31,3% by the end of the experiment. Lipid content, in contrast, remained relatively constant at approximately 12,2% throughout the entire cultivation period. The stability of lipid content indicates that C medium did not create stress conditions conducive to lipid accumulation and that the metabolism of *Ava (limnothalassea)* was primarily directed toward protein synthesis and cell growth.

Compared with the control, maximum biomass in C medium increased from 14,6 g/L to 16,0 g/L, representing a 1,4 g/L improvement. Moreover, protein content was 16 percentage points higher than the 19% observed in the control medium, confirming more active protein synthesis in C medium. However, lipid content in C medium (12,2%) was 19,0% lower than that recorded in BG-11 (31,21%), further indicating that metabolic processes were predominantly oriented toward protein production. When compared with AF6 and MA media (both 15,9 g/L), C medium showed a slight biomass advantage of 0,1 g/L. Nevertheless, protein content in those media ranged between 31,4% and 31,8%, which was 3-4% lower than in C medium.

Modified C medium is primarily intended for the isolation, cultivation, and maintenance of freshwater cyanobacteria and certain green algae. It is widely used for species of the genus *Microcystis*, including *M. aeruginosa*, known for producing the potent hepatotoxin microcystin [Ichimura, 1971]. Literature sources also report vitamin- and EDTA-enriched modifications of C medium to enhance its stability [Watanabe *et al.*, 2000]. Although primarily designed for *Microcystis*, this medium is also suitable for isolating and maintaining colonial cyanobacteria such as *Merismopedia*, nitrogen-fixing filamentous genera such as *Anabaena* and *Nostoc*, and certain freshwater planktonic species.

In AF6 and MA media, lipid content remained at approximately 12,3%, which was comparable to that observed in C medium. Compared with Bold-3N and CAM media, C medium demonstrated a 3,3-10,1 g/L higher biomass yield. For example, biomass reached 9,2 g/L in Bold-3N and 12,7 g/L in CAM, whereas it attained 16,0 g/L in C medium. Protein content in those media ranged between 17,2% and 17,9%, which was 17-18% lower than in C medium.

Although lipid content in Chu-10 medium reached 34,3-34,6%, biomass production was very low (0,6-0,9 g/L). This indicates that high lipid accumulation does not necessarily correspond to high biomass yield but rather reflects stress-induced lipid synthesis. In contrast, C medium supported high biomass and elevated protein levels despite relatively low lipid content. Overall, C medium proved to be the most effective for achieving high biomass and the highest protein content during eight days of cultivation. The comparatively low lipid accumulation suggests that metabolic processes in C medium were primarily directed toward protein biosynthesis. Therefore, C medium can be considered a suitable option for biotechnological applications aimed at producing protein-rich biomass.

The production of biomass, protein, and lipids by the *Ava limnothalassea* strain was also investigated in modified CAM medium under standard conditions (Figure 3.2). During the first four days, biomass increased slowly (day 1 0.4 g/l, day 2 1 g/l, day 3 2.6 g/l, day 4 3.7 g/l). On day 4, the increasing trends of biomass (4,2 g/L) and lipid content (23,2%) intersected with the declining trend of protein content (19,4%), suggesting a metabolic shift toward lipid biosynthesis. Between days 5 and 8, biomass increased sharply by 6,2 g/L, reaching a maximum of 12,7 g/L. In subsequent days, biomass gradually declined, reaching 6,0 g/L by the end of the experiment. Protein content decreased by 1,5% between days 1 and 4 and continued to decline gradually thereafter, reaching 16,0% by the end of cultivation. Lipid content increased steadily until day 5, reaching 12,1%, and then rose gradually between days 6 and 9 by an additional 2,5%. By the final stage of the experiment, lipid content reached a maximum of 32,5%.

Compared with the control (14,6 g/L) and Allen medium (14,1 g/L), biomass in CAM medium was on average 1,9 g/L lower. It was also approximately 3,3 g/L lower than in C (16,0 g/L), AF6 (16,0 g/L), and BBM (15,8 g/L) media, but 3,5 g/L higher than in Bold-3N medium (9,2

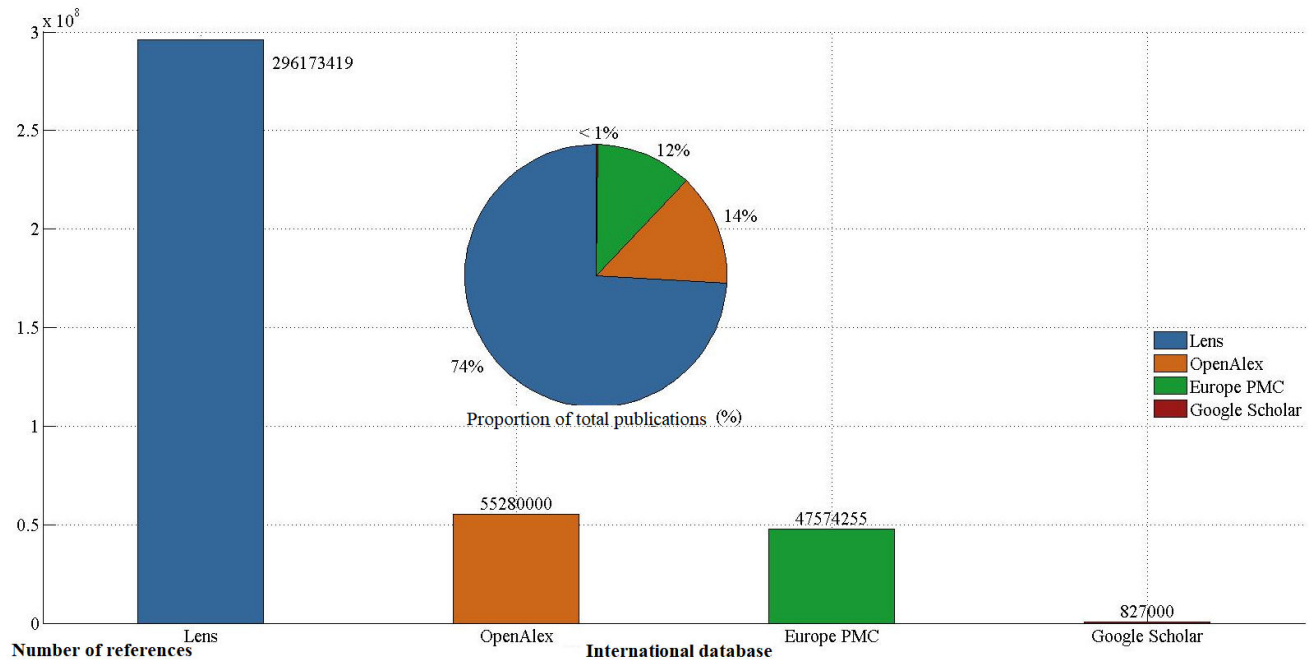
g/L). In terms of protein content, the results showed that, compared with the control, the value was on average 1,0% lower. It was also 3,0% lower than in BBM medium and approximately 0,6% lower than in Bold-3N and Allen media. Conversely, lipid content was higher than in the control (by 0,8%), Allen (0,5%), and BBM (1,6%) media, and markedly higher than in AF6 and C media (by 19,8%).

These differences can be explained by variations in the proportions of nitrogen and other macro- and microelements in the nutrient media, which influence the metabolic pathways of the *Ava limnothalassea* strain. In nutrient-rich media, growth and protein synthesis predominated, whereas under stress conditions, lipid accumulation was more pronounced.

Modified CAM medium represents an improved variant of modified C medium, in which the nitrogen source has been altered. It is specifically designed for freshwater cyanobacteria and certain sensitive algal species [Ichimura and Watanabe, 1974; Watanabe *et al.*, 2000]. In particular, it has been used for the isolation of non-toxic and buoyant strains of *Microcystis*, as well as for the isolation, cultivation, and maintenance of complex cyanobacterial genera such as *Merismopedia* and *Aphanizomenon*, which frequently develop rapidly in aquatic environments. For highly sensitive cyanobacteria that do not exhibit viability in BG-11 medium, modified CAM is considered one of the most suitable media. However, due to the presence of EDTA, this medium should not be exposed to light for prolonged periods, as photochemical reactions may lead to iron precipitation. Therefore, modified CAM medium should be stored in the dark.

When the *Ava limnothalassea* strain was cultivated in Carefoot (Cary-L) medium under standard conditions, analysis of biomass growth dynamics showed relatively slow growth during the first four days (1-day 1,0 g/L, 2-day 1,9 g/L, 3-day 3,5 g/L and 4-day 5,8 g/L ) (Figure 5.3). Between days 4 and 5, biomass growth accelerated sharply, increasing by an additional 2,9 g/L. On days 6 and 7, biomass reached 11,3 and 13,3 g/L, respectively. The maximum biomass value of 14,6 g/L was recorded on day 8. However, in the final days of observation, a declining trend was noted, with biomass decreasing by 7,1 g/L relative to day 8.

**Figure.1** Infographic representation of the total records indexed in the bibliographic and citation databases of Lens, Europe PMC Plus, Google Scholar, CORE, and OpenAlex.



**Figure.2** Search results in the bibliographic and citation databases of Lens, Europe PMC Plus, Google Scholar, CORE, and OpenAlex based on key microalgae-related terms.

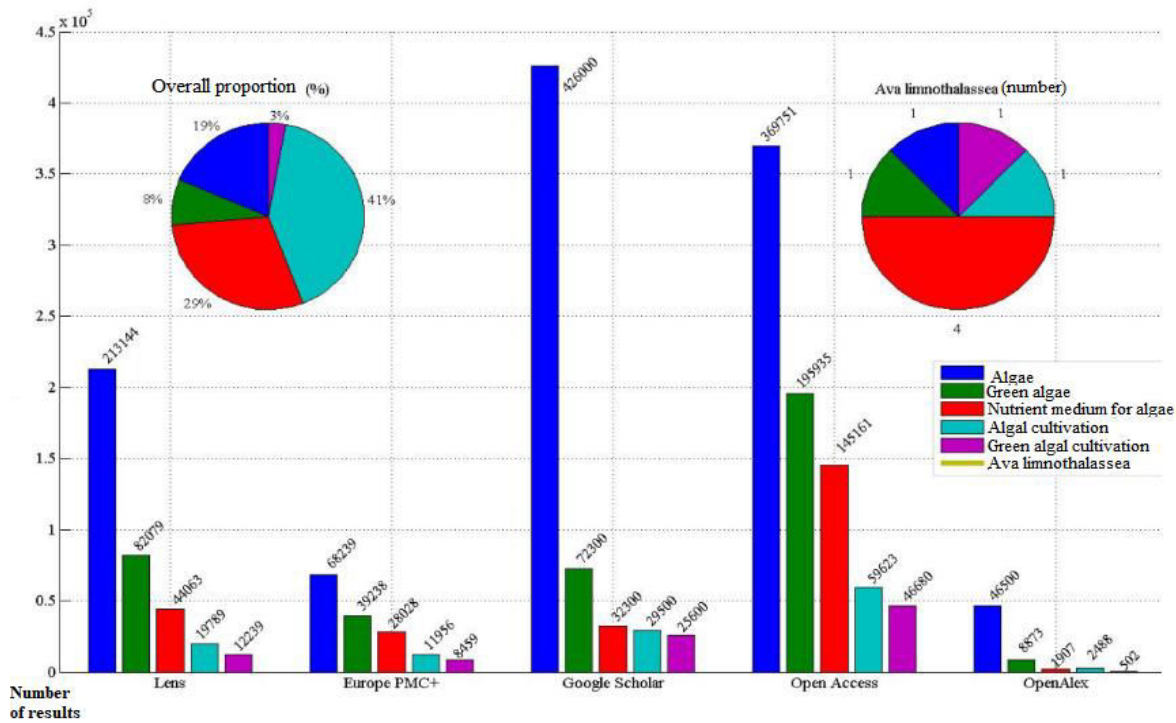


Figure.3 Growth and development of the *Ava limnothalassea* culture in BG-11 (standard) nutrient medium.

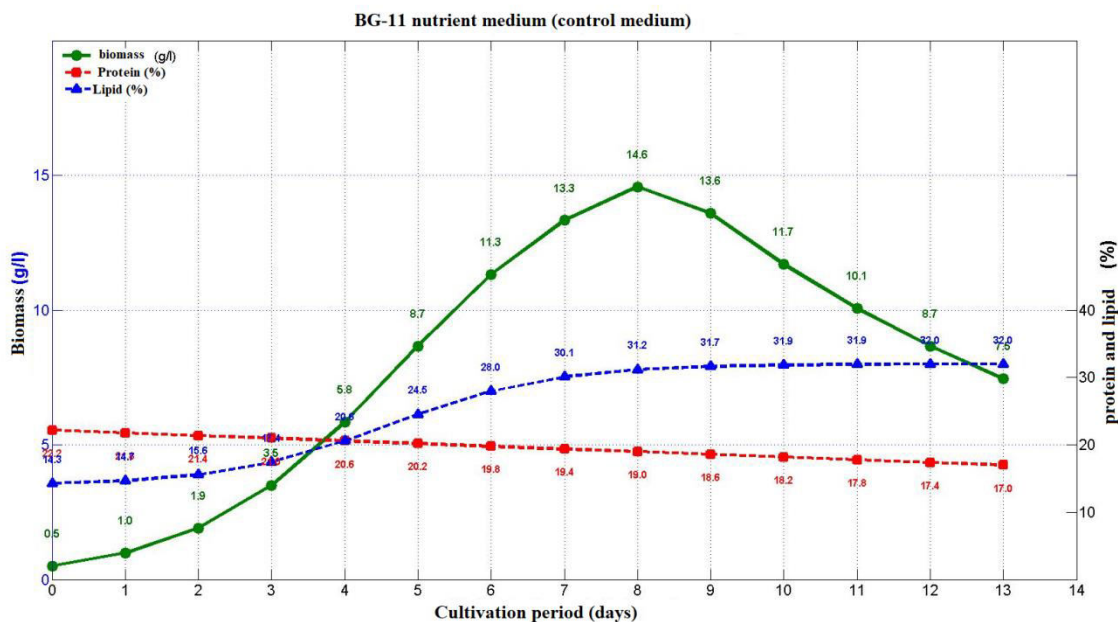


Table.1 Possibilities of using algae mass in economic sectors

Products	Basic compounds	Industrial sector	Source
Hydrocolloids	Agar-agar, alginate, carrageenan	Food industry, textile industry	Algal Technology., 2024
Energy sources	Biodiesel, bioethanol, biogas	Transport and energy sectors	Sarker <i>et al.</i> , 2024
Nutritional supplements	Proteins, vitamins, omega-3 fatty acids	Livestock farming, medicine	Das <i>et al.</i> , 2025
Agricultural products	Biofertilizers, biological control agents, phytohormones	Crop production	Meeranayak <i>et al.</i> , 2020
Biologically active compounds	Astaxanthin, antioxidants	Cosmetics, pharmaceuticals	Chew <i>et al.</i> , 2020

Table.2 Statistical overview of available data on microalgae in scientific databases

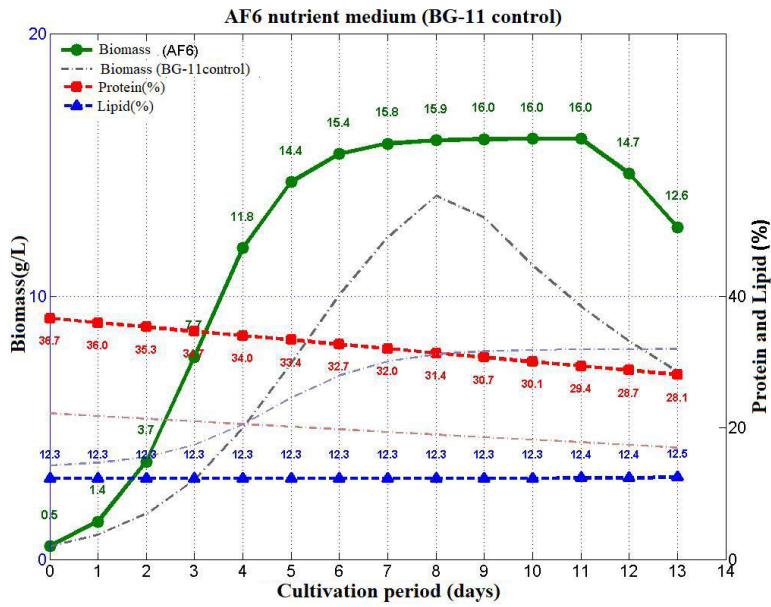
№	International scientific database	Total number of scientific sources (18.02.2026)	Including the number of records retrieved through keyword-based searches (items).					
			Algae	Green algae	Nutrient medium for algae	Algal cultivation	Green algal cultivation	<i>Ava limnothalassea</i>
1	OpenAlex, total:	55280000	46500	8873	1907	2488	502	1
	Including,	Article	34640	6513	1598	1817	374	1
	Book chapter	3750	750	97	271	49	0	
	Article in press	3170	653	61	182	36	0	
	Review article	2979	571	57	114	26	0	
	Dissertation	771	204	44	51	9	0	

**Table.3** Nutrient media used for the cultivation of the green alga *Ava limnothalassea*

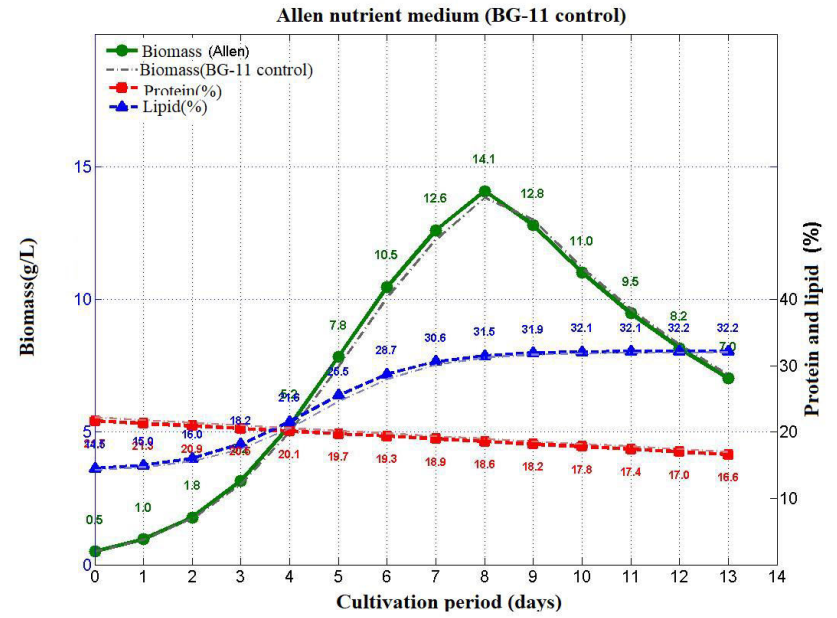
N <sup>o</sup>	Chemical components of the prepared solution	Amount added to the nutrient medium (g/L)	Concentration in Final Medium
<b>Modified AF6 nutrient medium (Kato 1982, Watanabe <i>et al.</i>, 2000)</b>			
	MES buffer (2-(N-morpholino)ethanesulfonic acid)	-	400 mg
	Fe-citrate	2 g	1 ml
	Citric acid	2 g	1 ml
	NaNO <sub>3</sub>	140g	1 ml
	NH <sub>4</sub> NO <sub>3</sub>	22 g	1 ml
	MrSO <sub>4</sub> · 7H <sub>2</sub> O	30 g	1 ml
	KH <sub>2</sub> PO <sub>4</sub>	10 g	1 ml
	K <sub>2</sub> HPO <sub>4</sub>	5 g	1 ml
	CaCl <sub>2</sub> · 2H <sub>2</sub> O	10 g	1 ml
<b>Trace metals solution - 1 mL:</b>			
	Na <sub>2</sub> EDTA · 2H <sub>2</sub> O		5,0 g
	FeCl <sub>3</sub> · 6H <sub>2</sub> O		0,98 g
	MnCl <sub>2</sub> · 4H <sub>2</sub> O		0.18 g
	ZnSO <sub>4</sub> · 7H <sub>2</sub> O		0.11 g
	CoCl <sub>2</sub> · 6H <sub>2</sub> O	20,0 g	1 ml
	Na <sub>2</sub> MoO <sub>4</sub> · 2H <sub>2</sub> O	12,5 g	1 ml
<b>Vitamins:</b>			
	Thiamine HCl (vitamin B <sub>1</sub> )		10 mg
	Biotin (vitamin H)	2,0 g	1 ml
	Pyridoxine · HCl (vitamin B <sub>6</sub> )	1,0 g	1 ml
	Cyanocobalamin (vitamin B <sub>12</sub> )	1,0g	1 ml
<b>Allen medium for blue-green algae (Allen M. B., 1959; Watanabe <i>et al.</i>, 2000)</b>			
	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		1.320 g
	KH <sub>2</sub> PO <sub>4</sub>		0.272 g
	MrSO <sub>4</sub> · 7H <sub>2</sub> O		0.247 g
	CaCl <sub>2</sub>		0.055g
<b>Mixed metal salts solution - 1 mL:</b>			
	Fe-Na-EDTA 3H <sub>2</sub> O	30,6 g	
	H <sub>3</sub> BO <sub>3</sub>	-	2,86 g
	MnCl <sub>2</sub> · 4H <sub>2</sub> O	-	1,79 g
	(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>2</sub> · 4H <sub>2</sub> O	-	0,13 g
	ZnSO <sub>4</sub> · 7H <sub>2</sub> O	-	0,22 g
	CuSO <sub>4</sub> · 5H <sub>2</sub> O	-	0,079 g
	NH <sub>4</sub> VO <sub>3</sub>	-	0,023 g
<b>Modified BG-11 nutrient medium (Allen 1968, Allen and Stanier 1968, Rippka <i>et al.</i>, 1979)</b>			
	Iron citrate solution		1 ml
	Citric acid	6 g	1 ml
	Iron(III) ammonium citrate	6 g	1 ml
	NaNO <sub>3</sub>	-	1.5 g
	K <sub>2</sub> HPO <sub>4</sub> · 3H <sub>2</sub> O	40g	1 ml
	MrSO <sub>4</sub> · 7H <sub>2</sub> O	75g	1 ml

CaCl <sub>2</sub> · 2H <sub>2</sub> O	36 g	1 ml
Na <sub>2</sub> CO <sub>3</sub>	20 g	1 ml
MrNa <sub>2</sub> EDTA · H <sub>2</sub> O	1.0 g	1 ml
<b>Trace metals solution - 1 ml:</b>		
H <sub>3</sub> BO <sub>3</sub>	-	2.860 g
MnCl <sub>2</sub> · 4H <sub>2</sub> O	-	1.810 g
ZnSO <sub>4</sub> · 7H <sub>2</sub> O	-	0.220 g
CuSO <sub>4</sub> · 5H <sub>2</sub> O	79.0 g	1 ml
Na <sub>2</sub> MoO <sub>4</sub> · 2H <sub>2</sub> O	-	0.391 g
Co(NO <sub>3</sub> ) <sub>2</sub> · 6H <sub>2</sub> O	49.4 g	1 ml
<b>Bold's Basal Medium (Bold 1949, Bischoff and Bold 1963)</b>		
Macroelements		
NaNO <sub>3</sub>	25.00 g	10 ml
CaCl <sub>2</sub> · 2H <sub>2</sub> O	2.50 g	10 ml
MrSO <sub>4</sub> · 7H <sub>2</sub> O	7.50 g	10 ml
K <sub>2</sub> HPO <sub>4</sub>	7.50 g	10 ml
KH <sub>2</sub> PO <sub>4</sub>	17.50 g	10 ml
NaCl	2.50 g	10 ml
<b>Alkaline EDTA solution -1 ml</b>		
EDTA	50.00 g	-
KOH	31.00 g	-
<b>Acidified iron solution-1 ml</b>		
FeSO <sub>4</sub> · 7H <sub>2</sub> O	4.98 g	-
H <sub>2</sub> SO <sub>4</sub>	-	1 ml
Boron solution	-	1 ml
H <sub>3</sub> BO <sub>3</sub>	11.42 g	-
<b>Modified C nutrient medium (Ichimura 1971, Watanabe <i>et al.</i>, 2000)</b>		
Tris(hydroxymethyl)aminomethane base	-	0.50 g
KNO <sub>3</sub>	-	0.10 g
Ca(NO <sub>3</sub> ) <sub>2</sub> · 4H <sub>2</sub> O	-	0.15 g
Na <sub>2</sub> β-glycerophosphate · 5H <sub>2</sub> O	50 g	1 ml
MrSO <sub>4</sub> · 7H <sub>2</sub> O	40 g	1 ml
<b>Trace metals- 1 ml:</b>		
Na <sub>2</sub> EDTA	-	1.000 g
FeCl <sub>3</sub> · 6H <sub>2</sub> O	-	0.194 g
MnCl <sub>2</sub> · 4H <sub>2</sub> O	36.00 g	1 ml
ZnCl <sub>2</sub>	10.44 g	1 ml
Na <sub>2</sub> MoO <sub>4</sub> · 2H <sub>2</sub> O	12.62 g	1 ml
CoCl <sub>2</sub> · 6H <sub>2</sub> O	4.04 g	1 ml
<b>Vitamins:</b>		
Thiamine HCl (vitamin B <sub>1</sub> )	-	10 mg
Biotin (vitamin H)	0.1g	1 ml

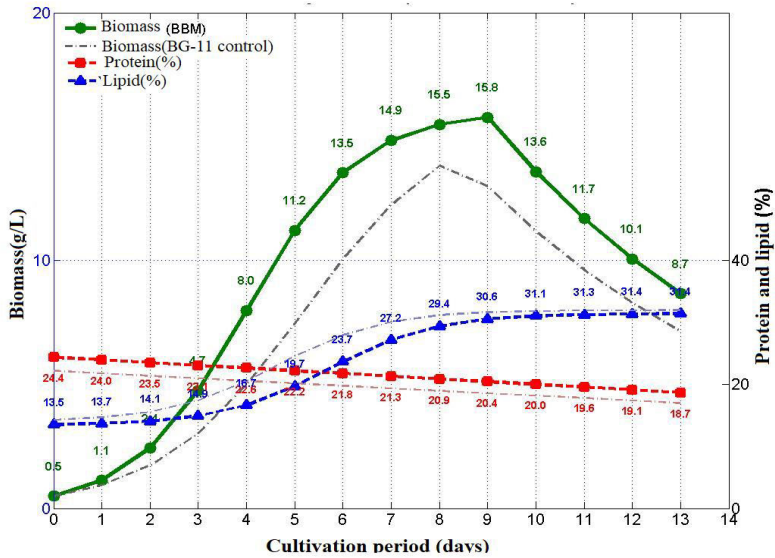
Cyanocobalamin (vitamin B <sub>12</sub> )	0.1 g	1 ml
<b>Modified CAM nutrient medium (Ichimura and Watanabe, 1974; Watanabe <i>et al.</i>, 2000), supplemented with iron-EDTA solution</b>		
HEPES buffer - 2-(4-(2-hydroxyethyl)-1-piperazinyl)ethanesulfonic acid	-	0.40 g
KNO <sub>3</sub>	-	0.10 g
Ca(NO <sub>3</sub> ) <sub>2</sub> · 4H <sub>2</sub> O	20.0 g	1 ml
NH <sub>4</sub> NO <sub>3</sub>	50.0 g	1 ml
Na <sub>2</sub> β-glycerophosphate · 5H <sub>2</sub> O	30.0 g	1 ml
MrSO <sub>4</sub> · 7H <sub>2</sub> O	20.0 g	1 ml
<b>iron-EDTA solution - 1 ml:</b>		
Na <sub>2</sub> EDTA · 2H <sub>2</sub> O	-	0.372 g
FeCl <sub>3</sub> · 6H <sub>2</sub> O	-	0.270 g
<b>Trace metals- 1 ml:</b>		
Na <sub>2</sub> EDTA	-	1.000 g
FeCl <sub>3</sub> · 6H <sub>2</sub> O	-	0.194 g
MnCl <sub>2</sub> · 4H <sub>2</sub> O	36.00 g	1 ml
ZnCl <sub>2</sub>	10.44 g	1 ml
Na <sub>2</sub> MoO <sub>4</sub> · 2H <sub>2</sub> O	12.62 g	1 ml
CoCl <sub>2</sub> · 6H <sub>2</sub> O	4.04 g	1 ml
<b>Vitamins:</b>		
Thiamine · HCl (B <sub>1</sub> )	-	10 mg
Biotin (vitamin H)	0.1 g	1 ml
Cyanocobalamin (vitamin B <sub>12</sub> )	0.1 g	1 ml
<b>Carefoot nutrient medium (Carefoot, 1968), supplemented with a metal salt mixture (Provasoli and Pintner, 1960)</b>		
NaNO <sub>3</sub>	-	0.25 g
K <sub>2</sub> HPO <sub>4</sub>	9.7 g	1 ml
KH <sub>2</sub> PO <sub>4</sub>	22.7 g	1 ml
MrSO <sub>4</sub> · 7H <sub>2</sub> O	4.9 g	1 ml
CaCl <sub>2</sub> · 2H <sub>2</sub> O	16.5 g	1 ml
NaCl	16.5 g	1 ml
<b>Trace metals solution -1 ml:</b>		
Na <sub>2</sub> EDTA · 2H <sub>2</sub> O	-	1.512 g
FeCl <sub>3</sub> · 6H <sub>2</sub> O	-	0.194 g
MnCl <sub>2</sub> · 4H <sub>2</sub> O	-	0.036 g
ZnCl <sub>2</sub>	-	0.010 g
Na <sub>2</sub> MoO <sub>4</sub> · 2H <sub>2</sub> O	-	0.013 g
CoCl <sub>2</sub> · 6H <sub>2</sub> O	3.64 g	1 ml
<b>“Chu 10” nutrient medium (Chu, 1942)</b>		
Ca(NO <sub>3</sub> ) <sub>2</sub>	40.0 g	1 ml
K <sub>2</sub> HPO <sub>4</sub>	5.0 g	1 ml
MgSO <sub>4</sub> · 7H <sub>2</sub> O	25.0 g	1 ml
Na <sub>2</sub> CO <sub>3</sub>	20.0 g	1 ml
Na <sub>2</sub> SiO <sub>3</sub>	25.0 g	1 ml
FeCl <sub>3</sub>	0.8 g	1 ml



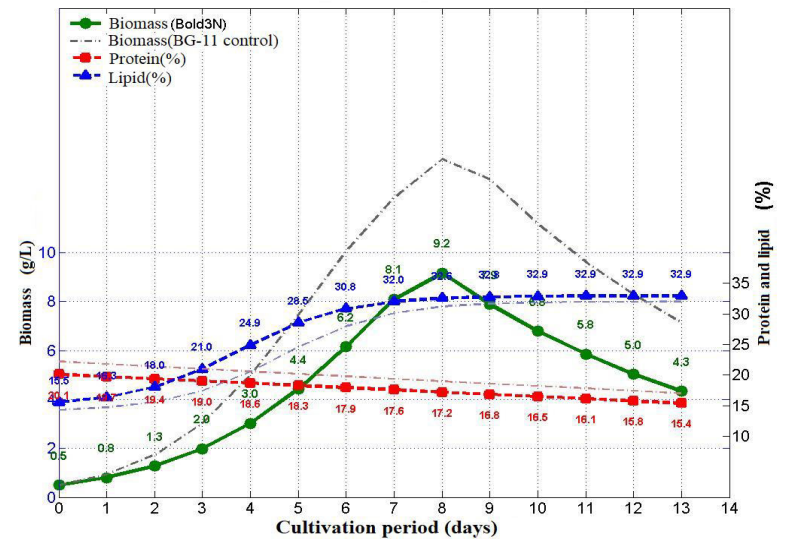
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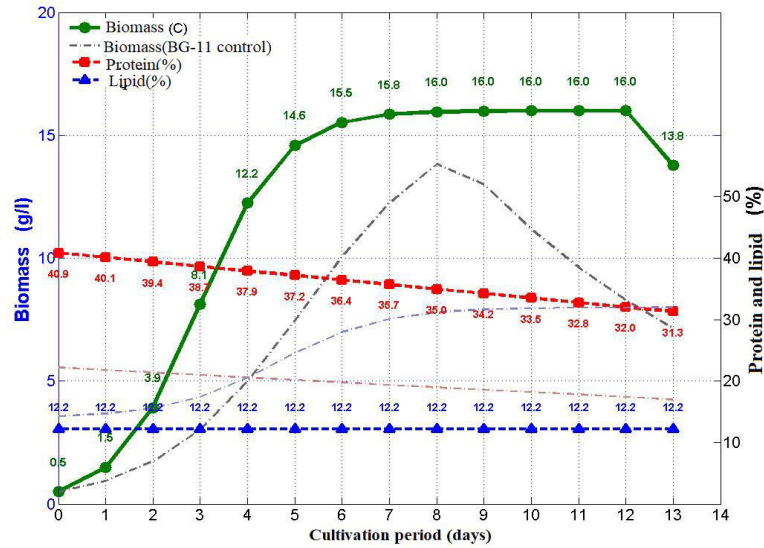


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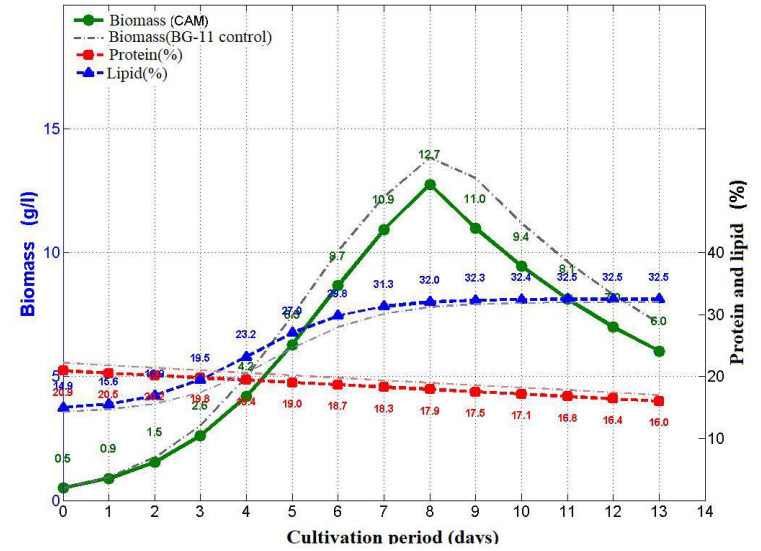


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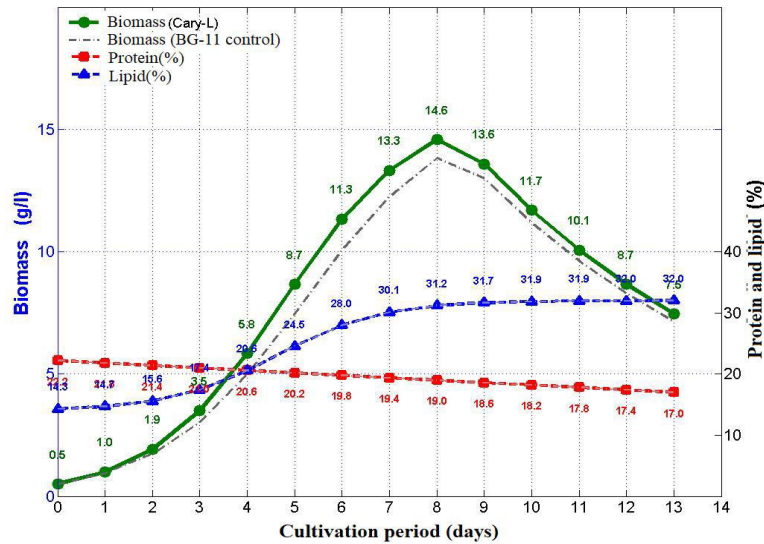
Figure.4 Growth dynamics of the Ava culture under different nutrient media conditions: 1 - AF6; 2 - Allen; 3 - BBM; 4 - Bold-3N.



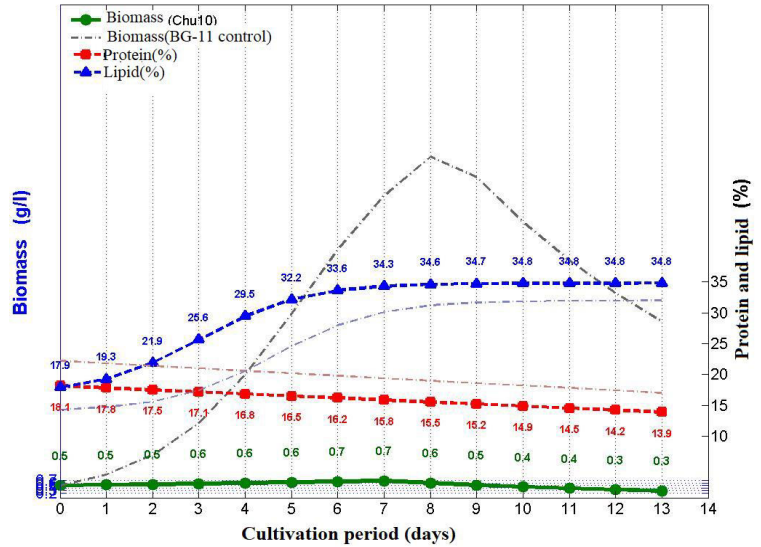
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Figure.5 Growth and development of the Ava culture in different nutrient media: 1 - C; 2 - CAM; 3 - Carefoot; 4 - Chu-10.

Protein content in Cary-L medium showed a gradual decrease over time. During the first four days, protein content declined by 1,6% (21,6% on day 1, 21,4% on day 2, 21,0% on day 3 and 20,6% on day 4). Moreover in 4-day, the point of decline in protein content intersected with the increasing trend in lipid accumulation (20,6%). By day 8, the relative protein proportion had decreased by 3,2% compared with day 1, reaching 19,0%. In subsequent days, protein content continued to decline, decreasing by an additional 2,0% by the end of the experiment.

In Cary-L medium, lipid content showed a gradual increase from day 1 (14,7%) to day 4 (20,6%), rising by 5,9%. On day 4, the increase in lipid content coincided with an increase in biomass (5,8 g/L), while protein content (20,6%) showed a declining trend. Between days 5 and 8, lipid content increased sharply by 6,7%, reaching 31,2%. By the end of cultivation, this value increased by an additional 0,8%.

These results allowed comparison of Cary-L medium with the control and other nutrient media. In the BG-11 control medium, biomass reached 14,6 g/L, which was equivalent to that observed in Cary-L. However, protein content in the control was 19,0%, and lipid content was 31,21%, indicating that lipid levels were nearly comparable between the two media. In Allen medium, biomass reached 14,1 g/L (0,5 g/L lower than Cary-L), protein content was 18,6% (0,4% lower), and lipid content was 31,5% (0,3% higher). In BBM medium, biomass reached 15,5 g/L (0,9 g/L higher than Cary-L), protein content was 20,9% (1,9% higher), while lipid content was 29,4% (1,8% lower). In Bold-3N medium, biomass was 9,2 g/L (5,4 g/L lower), protein content was 17,2% (1,8% lower), and lipid content was 32,6% (1,4% higher) compared with Cary-L.

In AF6 medium, biomass reached 15,9 g/L (1,3 g/L higher than Cary-L), and protein content was 31,4% (12,4% higher), whereas lipid content was only 12,3%, which was 18,9% lower than in Cary-L. In C medium, biomass reached 16,0 g/L (1,4 g/L higher), protein content was 35,0% (16,0% higher), and lipid content was 12,2% (19,0% lower) compared with Cary-L.

Carefoot (Cary-L) medium is a specialized, vitamin and micronutrient-rich medium designed for the isolation, maintenance, and cultivation of multicellular green algae (Chlorophyta) and certain freshwater microalgae. It is particularly suitable for species such as *Volvox aureus*

and *Volvox carteri*, as well as dinoflagellates of the genus *Peridinium*, especially auxotrophic strains. Its suitability is attributed to the buffering system that stabilizes pH, making it especially useful for the isolation and maintenance of sensitive algae. It is also widely used as an indicator medium for detecting algae in contaminated wastewater.

Under standard cultivation conditions in Chu-10 medium, the physiological development, biomass productivity, and protein and lipid formation of *Avalimnothalasssea* were investigated (Figure 5.4). Biomass remained nearly unchanged throughout the experiment. From day 1 (0,5 g/L) to day 8, biomass increased slightly by 0,1 g/L, reaching 0,6 g/L. By the end of the experiment, biomass declined by 0,3 g/L. This limited growth may be attributed to nutrient constraints or reduced physiological activity.

In Chu-10 medium, initial protein (18,1%) and lipid (17,9%) contents were nearly equal. On day 2, protein decreased by 0,3%, while lipid content increased by 1,4%. By day 8, protein content had declined by 2,6%, reaching 15,5%, and decreased by an additional 1,6% by the end of cultivation. In contrast, lipid content increased sharply from 25,6% on day 3 to 34,3% on day 7 (an increase of 8,7%), reaching 34,6% on day 8 and gradually increasing to 34,8% by the end of the experiment.

Comparative analysis showed that biomass in Chu-10 medium reached only 0,6 g/L on day 8, the lowest value among all tested media. Compared with the control and Cary-L media (14,6 g/L), biomass in Chu-10 was 14,0 g/L lower; compared with Allen (14,1 g/L), it was 13,5 g/L lower; and compared with BBM (15,5 g/L), it was 14,9 g/L lower. These findings clearly indicate that Chu-10 medium is unsuitable for biomass accumulation.

Protein content in Chu-10 medium (15,5%) was 15,9% lower than in AF6 (31,4%), 5,4% lower than in BBM (20,9%), 3,5% lower than in Cary-L (19,0%), and 3,1% lower than in Allen (18,6%). Compared with Bold-3N (17,2%), the difference was 1,7%. However, lipid accumulation in Chu-10 was among the highest observed (34,6%). This value was 3,4% higher than in Cary-L (31,2%) and the control (31,21%), 3,1% higher than in Allen (31,5%), and 5,2% higher than in BBM (29,4%). In contrast, lipid content in AF6 and C media (12,2-12,3%) was 22,0-22,4% lower than in Chu-10. These results indicate that although Chu-10 medium is

unsuitable for biomass production, it may be advantageous for studies aimed at obtaining lipid-rich biomass.

Chu-10 medium is widely used in studies of algal physiology, biochemistry, and ecology and was originally recommended for investigating the mineral requirements of freshwater plankton [Chu, 1942]. Several variants (Chu-1 to Chu-14) have been described in the literature; however, Chu-10, due to its higher mineral content, has been most widely adopted [Stein, 1973]. Nevertheless, it is considered less suitable for large-scale biomass production [Ibrahim *et al.*, 2024].

In conclusion, for the first time under the conditions of Uzbekistan, the wet biomass production, total protein content, and lipid accumulation capacity of the axenic green alga *Ava limnothalassea* were investigated in widely used conventional nutrient media, including modified AF6 (AF6), Allen, modified BG-11 (BG-11), Bold's Basal Medium (BBM), Bold-3N, modified C, modified CAM, Carefoot (Cary-L), and Chu-10. These media are recognized as important tools for the isolation of blue-green and green algae and for the analysis of their physiological and biochemical characteristics. At the same time, it was noted that clear and definitive conclusions regarding biomass, protein, and lipid accumulation of *Ava limnothalassea* cannot be drawn solely on the basis of cultivation in these media.

In particular, the Bold-3N medium, characterized by a threefold higher nitrogen concentration, was found to stimulate cell division and promote enhanced protein synthesis. In contrast, Carefoot (Cary-L), modified C, modified CAM, and Chu-10 media were shown to play a significant role in lipid synthesis. For maintaining the viability of *Ava limnothalassea* and for comprehensive studies of its physiological characteristics, modified AF6 (AF6), Allen, and Bold's Basal Medium (BBM) proved to be especially suitable.

The results demonstrated that *Ava limnothalassea* exhibits tolerance to both neutral and moderately alkaline nutrient media. This finding suggests its potential applicability in the remediation of ecologically unstable and heavily polluted waters, as well as its resilience to rapid fluctuations in pH. Such characteristics highlight its practical significance for industrial-scale cultivation and for the production of raw materials applicable to various sectors of the economy. Despite the scientific and practical relevance of the

findings, further comprehensive investigations are required to evaluate the properties of *Ava limnothalassea* in a broader range of nutrient media commonly used for cyanobacteria and microalgae.

### Author Contributions

Durdona R. Bakhranova: Investigation, formal analysis, writing—original draft. Khurshidbek O. Abdullaev: Validation, methodology, writing—reviewing. Alvina Farooqui:—Formal analysis, writing—review and editing. Dilafruz Kh. Kuchkarova: Investigation, writing—reviewing. Tripathi Gyanendra: Resources, investigation writing—reviewing. Nortoji A. Khujamshukurov: Validation, formal analysis, writing—reviewing.

### Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Declarations

**Ethical Approval** Not applicable.

**Consent to Participate** Not applicable.

**Consent to Publish** Not applicable.

**Conflict of Interest** The authors declare no competing interests.

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