Biochemical Profiling of Soil blue-green Algae

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***Abstract*—The global demand for sustainable and renewable resources has been escalating due to the depleting fossil fuel reserves and associated environmental issues. This study conducted a comprehensive biochemical analysis of various soil blue-green algae (BGA) isolates to identify potential BGA for biotechnological applications. The research focused on systematically examining the lipid, carbohydrate, protein, and chlorophyll a content of different BGA species under standardized conditions. Species of genera Anabaena and Nostoc showed consistently high yields of valuable biochemical components were prioritized for further development. The study also investigated the optimal environmental conditions required to maximize the production of specific biochemical compounds in the most promising BGA species. The feasibility of using these high-yield BGA species in biofuel production, bioethanol production, nutritional supplements, animal feed, and agricultural enhancements was explored. Major results indicated significant variability in biochemical composition among the different species, with certain BGA species demonstrating particularly high lipid, carbohydrate, protein, and chlorophyll a content. These findings highlighted the potential of BGA for various biotechnological applications, addressing the pressing issues of energy scarcity, environmental degradation, and food security. These results have significant large-scale implications, providing a foundation for the sustainable and eco-friendly utilization of BGA in diverse industrial applications.**

***Index Terms*—Soil blue green algae, biochemical analysis, lipids, proteins, carbohydrates, chlorophyll a.**

I. INTRODUCTION

The global demand for sustainable and renewable resources has been escalating due to the depleting fossil fuel reserves and the environmental issues associated with their use [1]. Traditional energy sources, such as coal, oil, and natural gas, contribute significantly to greenhouse gas emissions, leading to climate change and global warming [2]. Moreover, the growing population and industrial activities intensify the need for alternative sources of energy, food, and materials. Among various potential solutions, algae have gained considerable attention as a versatile and eco-friendly resource. Blue-green algae (BGA), or cyanobacteria, are particularly promising due to their ability to produce a wide range of valuable biochemical compounds, including lipids, carbohydrates, proteins, and chlorophyll [3].

Research efforts have been directed towards exploiting the potential of BGA for various biotechnological applications [4]. Studies have focused on optimizing the cultivation conditions of different algae species to enhance their biomass and biochemical yields. Advances have been made in using BGA for biofuel production, given their ability to produce high lipid content, which can be converted into biodiesel [5]. Additionally, the carbohydrate-rich content of certain algae species has been explored for bioethanol production. The high protein content in BGA has also been investigated for applications in nutrition and animal feed, while their chlorophyll content suggests potential use in biofertilizers and enhancing agricultural productivity [6].

Despite the promising potential of BGA, several limitations remain. One major challenge is the variability in biochemical composition among different species and even within the same species under different environmental conditions [7]. This variability makes it difficult to standardize the production processes and achieve consistent yields of the desired compounds. Additionally, large-scale cultivation and harvesting of BGA pose practical and economic challenges, including the need for significant infrastructure and energy inputs [8]. Moreover, there is limited understanding of the optimal conditions required to maximize the

production of specific biochemical components [7]. The potential environmental impacts of large-scale BGA cultivation also need to be thoroughly assessed.

This study aims to conduct a comprehensive biochemical analysis of various soil blue-green algae isolates. By systematically examining the lipid, carbohydrate, protein, and chlorophyll content of different BGA species, the most promising candidates for biotechnological applications will be identified [9]. This research focuses on conducting thorough biochemical analyses of various BGA isolates to determine their lipid, carbohydrate, protein, and chlorophyll a content under standardized conditions. Species with consistently high yields of valuable biochemical components will be prioritized for further development. The optimal environmental conditions for maximizing the production of specific biochemical compounds in the most promising BGA species will be investigated, and the feasibility of using the identified high-yield BGA species in biofuel production, bioethanol production, nutritional supplements, animal feed, and agricultural enhancements will be explored. This research aims to bridge the existing knowledge gaps and provide a solid foundation for the large-scale utilization of BGA in sustainable biotechnological applications. Through systematic biochemical profiling and optimization studies, the goal is to contribute to the development of eco-friendly solutions that can help mitigate the pressing issues of energy scarcity, environmental degradation, and food security [10].

II. MATERIALS AND METHODS

A. Sample collection

The soil samples were collected from 8 different villages of 8 different talukas in Osmanabad district (2 samples per locations) in the Marathwada region of Maharashtra. About 250 gm. soil samples were collected from the depth of 0-5cm by removing the debris, the upper crust of the soil was collected carefully with the help of forceps and scalped in clean vials [11]. Then collected soil samples were brought to the laboratory in polythene bags, dried at room temperature in diffuse sunlight in shade, then crushed with the help of mortar and pestle, sieved and used for screening.

B. Isolation and screening of BGA

The isolation and culturing of blue-green algae from soil samples were conducted using standard microbial methods, the serial dilution method. Initially, various culture media were used, including BG 11 ± medium [12], Fogg's nitrogen-free medium [13], Chu's 10 medium [14], and Allen and Arnon's medium [15]. The incubation conditions were set at 22 ± 2˚C with a 16/8 light and dark cycle under a light intensity of 5 K lux provided by white, fluorescent light.

After incubation, the growth of blue-green algae in the enriched cultures was compared, and it was observed that BG 11 ± medium yielded the best results. Therefore, for subsequent culturing and subculturing, BG 11 ± medium was preferred. To identify the BGA growth from the enriched cultures, microscopic examination was performed, and identified using standard literature references such as [16], [17], [18], [19], and [20]. Photographs of the algae were taken using a photomicrography unit from the Olympus CH20i microscope. Morphometric studies were conducted using ocular and stage micrometers, and measurements were taken using 10x, 45x, and 100x objectives with a 10x eyepiece. To maintain the cultures, chemically defined nitrogen-free BG 11 media [12] was used as the culturing medium.

C. Isolation of BGA

The isolation of blue-green algae was conducted using standard microbial methods, specifically the serial dilution and pour plate techniques [21]. Approximately 10 grams of sieved soil were dissolved in 100 ml of sterilized BG 11 medium, creating a stock solution. Aseptically, 1 ml of the stock solution was transferred to test tube number 1, which contained 9 ml of BG 11 medium, resulting in a concentration of 10-1. Then, 1 ml of the solution from test tube 1 was transferred to test tube 2, which contained 9 ml of BG 11 medium, creating a concentration of 10-2. This process was repeated for 10-3 and 10-4 dilutions.

Next, 1 ml of each dilution was poured onto agar plates containing solidified BG 11 medium and incubated for 15 days. After the incubation period, bulky masses of algae were observed on the plates with 10-1 and 10-2 concentrations, making it difficult for further isolation. However, the plate with the 10-4 dilution showed almost isolated forms, making it

suitable for further isolation of blue-green algae. This concentration allowed for easier picking of isolated colonies from the plates, which could then be cultured and subcultured in liquid medium.

All isolated colonies were maintained at 28 ± 2˚C under a light intensity of 5 K lux and a 18/8-hour light-dark cycle in pure cultures. These isolates were deposited in the culture room for preservation. The isolated cyanobacterial forms were used for further exploration of their potential applications as biofertilizers.

D. Biochemical analysis of BGA

Biochemical analysis of soil algae involves the determination of various components, including lipids, carbohydrates, crude protein and chlorophyll a. These analyses provide valuable insights into the metabolic activities and nutritional composition of soil algae.

Lipid analysis

The method used for lipid extraction followed a modified protocol originally described by Bligh and Dyer [22]. First, 100 mg of dry biomass was homogenized in a minimal volume of a 1:2 Chloroform: Methanol mixture and transferred into a separating funnel. The volume was adjusted to 37.5 ml with the Chloroform: Methanol mixture and left for an hour, with intermittent shaking. Next, 12 ml of chloroform was added and mixed vigorously. Subsequently, 10 ml of distilled water was added and mixed vigorously again. The mixture was then allowed to stand undisturbed until two clear layers formed. The biomass debris collected at the interphase of the two layers. The lower chloroform layer, containing the lipids, was collected in a preweighed beaker and placed in a water bath at 70°C to evaporate the chloroform completely. After cooling to room temperature, the beaker with the solid lipids was weighed again to determine the weight of the lipids. This weight was then used to calculate the percentage of lipids in the dry biomass.

Carbohydrate analysis

Carbohydrates serve as a major energy source and structural component in algae. Analysing carbohydrate content in soil algae helps assess their energy status and carbohydrate utilization patterns. It provides insights into the carbon metabolism and growth potential of these organisms. The carbohydrates were measured by Phenol-sulphuric acid method [23]. 100 mg of dry cyanobacterial biomass was homogenized in 2 ml of 2.5N HCl and transferred into a test tube. An additional 3 ml of 2.5N HCl was added to make a total volume of 5 ml. The tubes were then placed in a boiling water bath for 3 hours and allowed to cool to room temperature. The contents were decanted into a 150 ml glass beaker. Sodium carbonate crystals were added until effervescence ceased, marking the neutralization step. The final volume was adjusted to 100 ml with distilled water, and 1 ml of this solution was used for estimation.

In a test tube, 1 ml of the carbohydrate solution was mixed with 1 ml of 5% phenol. Immediately, 5 ml of concentrated sulphuric acid was slowly added along the sides of the tube. This mixture was allowed to stand at room temperature for 10 minutes and then vortexed well. It was then kept at room temperature for 30 minutes to develop the color. The absorbance was measured against a control containing 1 ml of distilled water in place of the carbohydrate solution. The absorbance was read at 430 nm and compared to a standard glucose graph prepared with concentrations ranging from 100 to 1000 µg/ml. The concentration obtained was directly calculated as µg/mg of dry biomass, and the final concentration was expressed as a percentage of dry biomass.

Crude protein analysis

Crude protein analysis enables the determination of the total protein content in soil algae. Proteins are essential for various cellular functions and play vital roles in growth, enzymatic activities, and stress responses. Assessing crude protein content helps understand the nutritional quality and physiological state of soil algae populations. The biochemical analysis of blue-green algal species was carried out for their crude protein content by estimation of total nitrogen by Micro-kjeldahl method [24]. Sample aliquots ranging from 25 to 100 µl, containing 0 to 50 µg of nitrogen, were dispensed into digestion tubes. Subsequently, 500 µl of digestion mixture (328 mg of Na2SeO3 dissolved in 95 ml H2SO4 (95-97% w/v) + 5 ml H3PO4 (85% w/v) and 500 µl of H202 solution were added to each tube. The tubes were then placed in a sand bath preheated to 380°C and left for 1 hour. After cooling, 1 ml of distilled water was added to

each tube, followed by the addition of 50 µl of an indicator reagent. Neutralizing solution (NaOH 10M) was slowly added drop by drop until a yellow permanent colour developed.

The volume was adjusted to 5 ml with distilled water and vortexed. Next, 300 µl aliquots were transferred to test tubes and placed in a water bath at 4°C. Sequentially, 1 ml of hypochlorite reagent and 1 ml of phenol reagent were added to each test tube. After thorough mixing, the tubes were incubated for 10 minutes at 40°C. The tubes were then cooled again, and 2 ml of distilled water was added. Absorbance measurements were taken using a spectrophotometer at 580 nm. All samples, blanks, and references were prepared in triplicate. A single batch of distilled water was used for all dilutions. Multiplying total nitrogen value with 6.25 will give crude protein content Boisen, et al. [25].

Chlorophyll a analysis

Chlorophyll a is a pigment crucial for photosynthesis in algae. Quantifying chlorophyll content provides information about the photosynthetic activity and efficiency of soil algae. It helps assess the primary productivity and potential growth capacity of these organisms in the soil environment. Chlorophyll isolation and its quantitative estimation from blue green algal species was carried using method given by Arnon [26]. One gm of blue green algal sample was thoroughly ground in 80% acetone with mortar and pestle. The extract was centrifuged at 5000 rpm for 05 minutes and the supernatant was transferred to a volumetric flask. Complete extraction was done by adding 20 ml of the solvent to pellet and the above process was repeated. The final volume of the supernatant was made up to 100 ml with 80% acetone. The absorbance of the solution was read at 645 nm and 663 nm in a spectrophotometer against the solvent (80% acetone) blank. Amount of chlorophyll-a present in the extract was calculated using following equation.

Chlorophyll a= 0.0127×A 663-0.00269×A665 (mg/ml)

IV. RESULTS AND DISCUSSION

A. Occurrence of BGA in soil samples

In a study within Osmanabad district, soil samples were collected from different locations with the aim of isolating and identifying blue-green algae using BG 11±N culturing media. Established methods, including serial dilution and the pour plate method, were employed, with the 10-4 dilution proving most effective for isolation. In total, 32 blue-green algae species from 11 genera were successfully isolated and identified in the soil samples as shown in table 1.

Table 1 Occurrence of soil BGA

Soil BGA	T1	T2	T3	T4	T5	T6	T7	T8	Total isolates
Aphanothece microscopica	1	2	1	3	1	0	2	1	11
Aphanothece saxicola	1	1	2	1	2	1	1	1	10
Aphanothece castagnei	3	2	1	2	1	2	2	1	14
Aphanothece pallida	1	0	1	1	2	4	1	2	12
Chroococcus limneticus	1	2	3	1	1	2	1	2	13
Chroococcus schizodermaticus	1	2	1	2	1	1	2	2	12
Chroococcus cohaerens	1	2	1	2	2	3	2	1	14
Chroococcus pallidus	2	2	1	2	1	2	1	1	12
Gloeocapsa compacta	1	0	2	2	1	1	1	2	10
Gloeocapsa magma	2	1	2	0	1	2	1	1	10
Gloeocapsa polydematica	1	1	0	1	2	2	2	2	11
Gloeocapsa stegeophila	1	1	2	1	2	1	1	1	10
Gloeothece samoensis	1	2	1	3	2	1	1	1	12
Oscillatoria angustissima	2	2	5	3	5	4	4	2	27
Oscillatoria chilkensis	2	3	4	5	4	4	3	4	29
Oscillatoria anguina	4	6	4	5	4	5	4	5	37
Oscillatoria sancta	5	5	4	4	5	5	5	5	38
Anabaena laxa	8	7	7	8	6	7	7	6	56
Anabaena naviculoides	8	7	8	8	9	8	8	8	64
Anabaena oryzae	5	6	5	5	6	5	6	8	46
Anabaena spiroides	8	7	7	7	8	7	8	8	60
Anabaena vaginicola	8	7	8	8	9	8	7	8	63

Soil BGA	T1	T2	T3	T4	T5	T6	T7	T8	Total isolates
Nostoc calcicola	9	7	8	6	7	8	6	8	59
Nostoc muscorum	9	9	9	8	9	8	8	7	67
Nostoc punctiforme	9	8	7	8	8	9	9	6	64
Nostoc spongiformae	6	7	7	8	8	9	8	7	60
Nostoc carneum	8	7	8	7	7	6	6	7	56
Scytonema coactile	3	2	1	2	2	3	4	5	22
Calothrix javanica	2	3	4	3	2	3	0	4	21
Gloeotrichia longicauda	1	0	1	1	2	3	2	2	12
Gloeotrichia raciborskii	1	2	2	1	1	2	1	1	11
Westiellopsis prolifica	4	3	2	3	2	2	3	2	21

A. Biochemical analysis of selected BGA

Out of 11 genera, only 5 genera (Aphanothece, Chroococcus, Oscillatoria, Anabaena and Nostoc) were selected for the biochemical analysis as shown in table 2.

Table 2 Biochemical analysis of BGA

Soil BGA	Lipids (%)	Carbohydrates (%)	Proteins (%)	Chlorophyll a (mg/mL)
Aphanothece microscopica	1.28 ± 0.02a	37.99 ± 0.27	31.28 ± 0.07b	0.30 ± 0.01
Aphanothece saxicola	1.24 ± 0.05a	33.85 ± 0.03	32.41 ± 0.05c	0.47 ± 0.01
Aphanothece castagnei	1.35 ± 0.01	34.97 ± 0.03	33.41 ± 0.08a	0.32 ± 0.00
Aphanothece pallida	1.42 ± 0.01	39.21 ± 0.05	33.51 ± 0.06a	0.36 ± 0.02
Chroococcus limneticus	3.15 ± 0.01	32.40 ± 0.08a	32.45 ± 0.04c	0.87 ± 0.00
Chroococcus schizodermaticus	3.54 ± 0.06	31.26 ± 0.08a	36.77 ± 0.27	0.95 ± 0.02
Chroococcus cohaerens	3.63 ± 0.10	27.70 ± 0.03	43.44 ± 0.05	0.88 ± 0.01a
Chroococcus pallidus	3.53 ± 0.07	28.63 ± 0.03	40.26 ± 0.01	0.86 ± 0.01a
Oscillatoria angustissima	4.35 ± 0.02	33.37 ± 0.03	39.48 ± 0.08	1.05 ± 0.01b
Oscillatoria chilkensis	4.22 ± 0.04	32.85 ± 0.02	38.46 ± 0.04	1.03 ± 0.01
Oscillatoria anguina	4.25 ± 0.03	33.17 ± 0.02	37.67 ± 0.08d	1.08 ± 0.00
Oscillatoria sancta	4.51 ± 0.08	32.54 ± 0.10	37.81 ± 0.10d	1.05 ± 0.01b
Anabaena laxa	5.36 ± 0.03	44.29 ± 0.03	50.25 ± 0.01	1.26 ± 0.02
Anabaena naviculoides	5.22 ± 0.01	43.44 ± 0.05b	49.41 ± 0.06	1.32 ± 0.02
Anabaena oryzae	5.71 ± 0.03	43.55 ± 0.05	48.57 ± 0.00	1.38 ± 0.00
Anabaena spiroides	5.16 ± 0.02	44.60 ± 0.08	47.77 ± 0.09	1.46 ± 0.01
Anabaena vaginicola	5.12 ± 0.04	46.66 ± 0.27	45.50 ± 0.06	1.29 ± 0.00
Nostoc calcicola	6.16 ± 0.02	43.44 ± 0.05b	35.29 ± 0.03	1.54 ± 0.01
Nostoc muscorum	7.17 ± 0.02	45.17 ± 0.02	33.35 ± 0.08a	1.67 ± 0.01
Nostoc punctiforme	5.38 ± 0.01	57.69 ± 0.05	32.34 ± 0.05	1.58 ± 0.00c
Nostoc spongiformae	8.77 ± 0.02	40.15 ± 0.03	33.23 ± 0.01	1.56 ± 0.01
Nostoc carneum	10.66 ± 0.02	39.19 ± 0.16	31.86 ± 0.01b	1.58 ± 0.01c

Any mean value followed by ± is standard error of the mean. Any means followed by common superscript alphabets are significantly similar means at 0.05 level of significance within a column.

C. Lipids

The lipid content analysis of various soil blue-green algae isolates revealed a significant range of lipid percentages across different species as shown in figure 1.

The lowest lipid content was observed in Aphanothece saxicola (1.24 ± 0.05%), followed closely by Aphanothece microscopica (1.28 ± 0.02%), Aphanothece castagnei (1.35 ± 0.01%), and Aphanothece pallida (1.42 ± 0.01%) which suggested limited lipid biosynthesis capacity. In the studies given by Abdilah and Troskialina [27], yielded lipid 1.33% using ultrasounds. These lower lipid levels indicated a preference for different metabolic pathways or ecological niches where high lipid storage might not be advantageous.

The Chroococcus genus showed moderate lipid levels with Chroococcus limneticus at 3.15 ± 0.01%, Chroococcus schizodermaticus at 3.54 ± 0.06%, Chroococcus cohaerens at 3.63 ± 0.10%, and

Chroococcus pallidus at 3.53 ± 0.07%, this intermediate lipid accumulation might be linked to their specific environmental adaptations and metabolic requirements. The moderate lipid levels in Chroococcus species could be advantageous for balancing growth and survival in various soil conditions.

The Oscillatoria species demonstrated higher lipid contents, with Oscillatoria angustissima at 4.35 ± 0.02%, Oscillatoria chilkensis at 4.22 ± 0.04%, Oscillatoria anguina at 4.25 ± 0.03%, and Oscillatoria sancta at 4.51 ± 0.08%, the increased lipid production in these species may be attributed to their filamentous morphology and ecological roles in soil ecosystems, potentially offering advantages in terms of energy storage and stress resistance. The higher lipid content in Oscillatoria species may position them as promising candidates for further exploration in biofuel production and other lipid-based applications.

Among the Anabaena species, lipid content was also relatively high, with Anabaena laxa at 5.36 ± 0.03%, Anabaena naviculoides at 5.22 ± 0.01%, Anabaena oryzae at 5.71 ± 0.03%, Anabaena spiroides at 5.16 ± 0.02%, and Anabaena vaginicola at 5.12 ± 0.04% which indicated robust lipid biosynthesis capabilities. These species are known for their nitrogen-fixing abilities, and the relatively high lipid content could be a reflection of their complex metabolic networks, which included both photosynthesis and nitrogen fixation. Anabaena species' ability to produce substantial lipid quantities alongside nitrogen fixation enhanced their potential for integrated agricultural and bioenergy applications.

The Nostoc species exhibited the highest lipid content among the isolates, with Nostoc calcicola at 6.16 ± 0.02%, Nostoc muscorum at 7.17 ± 0.02%, Nostoc punctiforme at 5.38 ± 0.01%, Nostoc spongiformae at 8.77 ± 0.02%, and Nostoc carneum exhibiting the highest lipid content at 10.66 ± 0.02%. The data for Nostoc punctiforme and Nostoc carneum was similar to that of Moten, et al. [28] The significant lipid production in Nostoc species was linked to their resilient extracellular polysaccharide matrix, which provided protection and stability, potentially facilitating greater lipid storage. The high lipid content in Nostoc species underscored their potential for biotechnological applications, including biofuel production, pharmaceuticals, and nutraceuticals. According to Post hoc analysis by Tukeys HSD, Aphanothece Saxicola and Aphanothece microscopica had similarity in the mean values.

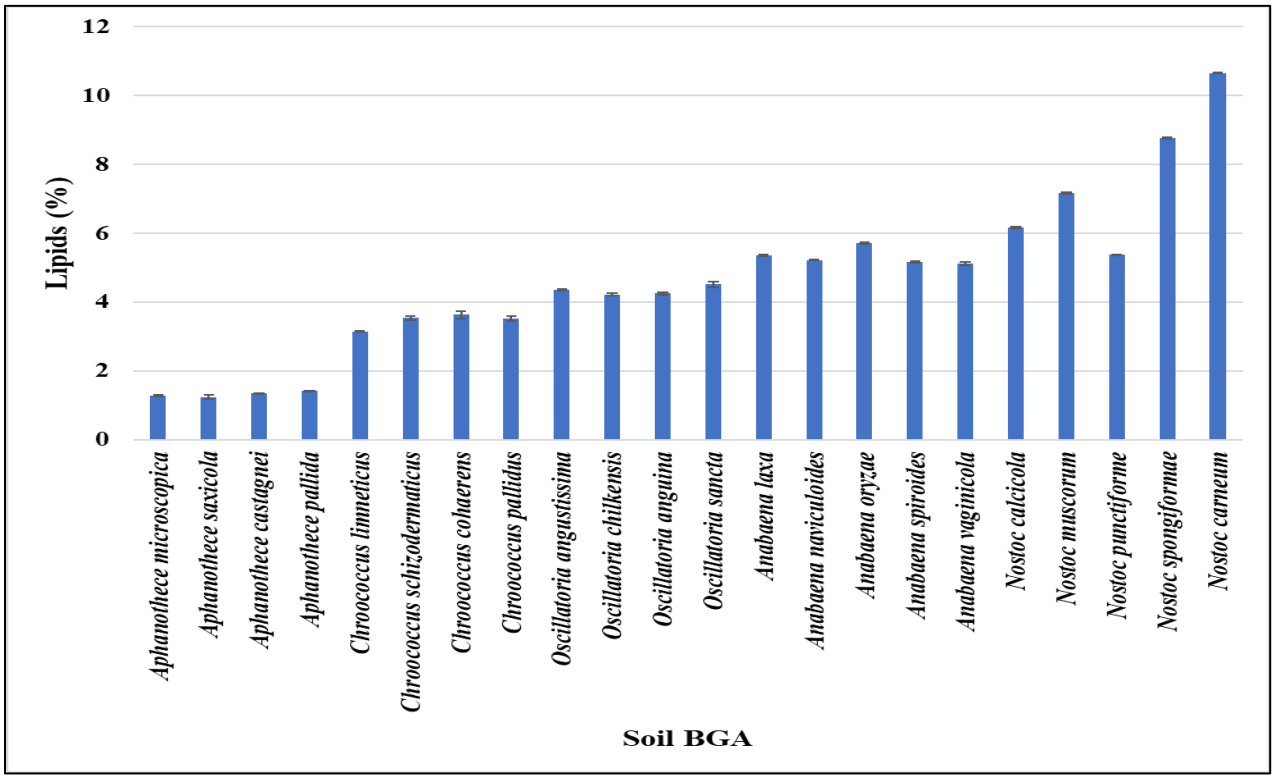

Figure 1 Lipid (%) content in soil BGA

D. Carbohydrates

The carbohydrate content analysis of soil blue-green algae (BGA) isolates revealed substantial variability among different species, reflecting their diverse metabolic strategies and potential ecological roles as shown in figure 2.

Among the Aphanothece species, Aphanothece microscopica exhibited a carbohydrate content of 37.99 ± 0.27%, Aphanothece saxicola had 33.85 ± 0.03%, Aphanothece castagnei showed 34.97 ± 0.03%, and Aphanothece pallida recorded 39.21 ± 0.05%. These moderate carbohydrate levels suggest that Aphanothece species may balance their energy storage between carbohydrates and other biomolecules, contributing to their adaptability in various soil environments [29].

The Chroococcus genus displayed lower carbohydrate levels, with Chroococcus limneticus at 32.4 ± 0.08%, Chroococcus schizodermaticus at 31.26 ± 0.08%, Chroococcus cohaerens at 27.7 ± 0.03%, and Chroococcus pallidus at 28.63 ± 0.03%. These lower carbohydrate contents indicate that Chroococcus species might prioritize other metabolic pathways, such as protein synthesis or secondary metabolite production, over carbohydrate accumulation, suggesting their adaptation to niches where rapid energy storage in the form of carbohydrates is less critical [30].

Oscillatoria species exhibited moderate carbohydrate contents: Oscillatoria angustissima had 33.37 ± 0.03%, Oscillatoria chilkensis at 32.85 ± 0.02%, Oscillatoria anguina at 33.17 ± 0.02%, and Oscillatoria sancta at 32.54 ± 0.1%. The carbohydrate levels in Oscillatoria species indicate their ability to store energy efficiently while maintaining other metabolic functions, allowing them to thrive in diverse soil environments and contributing to their resilience under varying conditions [31].

Anabaena species demonstrated higher carbohydrate levels, with Anabaena laxa at 44.29 ± 0.03%, Anabaena naviculoides at 43.44 ± 0.05%, Anabaena oryzae at 43.55 ± 0.05%, Anabaena spiroides at 44.6 ± 0.08%, and Anabaena vaginicola showing the highest carbohydrate content in this group at 46.66 ± 0.27%. This high carbohydrate content is likely linked to their nitrogen-fixing capabilities, as carbohydrates provide the necessary energy for nitrogen fixation. The ability of Anabaena species to produce substantial amounts of carbohydrates alongside fixing atmospheric nitrogen enhances their ecological role in soil fertility and positions them as valuable candidates for agricultural applications, particularly in sustainable farming practices.

Nostoc species exhibited significant carbohydrate contents, with Nostoc calcicola at 43.44 ± 0.05%, Nostoc muscorum at 45.17 ± 0.02%, Nostoc punctiforme at 57.69 ± 0.05%, Nostoc spongiformae at 40.15 ± 0.03%, and Nostoc carneum at 39.19 ± 0.16%. Among all the isolates, Nostoc punctiforme exhibited the highest carbohydrate content. According to the Post hoc analysis, Chroococcus limneticus and Chroococcus schizodermaticus; Anabaena naviculoides and Nostoc calcicola has similarities in their mean values. The data given by Whitton and Potts [32] showed that species of Nostoc exhibited carbohydrates 57-58% which was similar in Nostoc punctiforme The significant carbohydrate production in Nostoc species can be attributed to their robust extracellular polysaccharide matrix, which provided .protection and structural stability, facilitating efficient carbohydrate storage [33]. The high carbohydrate content in Nostoc species highlighted their potential for various biotechnological applications, including biofuel production, pharmaceuticals, and nutraceuticals, where carbohydrate-rich biomass is a valuable resource.

The carbohydrate content analysis of soil blue-green algae isolates underscored the metabolic diversity within these organisms. The findings suggest that specific BGA species, particularly those from the Anabaena and Nostoc genera, have significant potential for biotechnological applications due to their high carbohydrate production. Further research into optimizing the cultivation conditions and extraction processes for these algae will be essential for harnessing their full potential in sustainable bioproducts and bioenergy production.

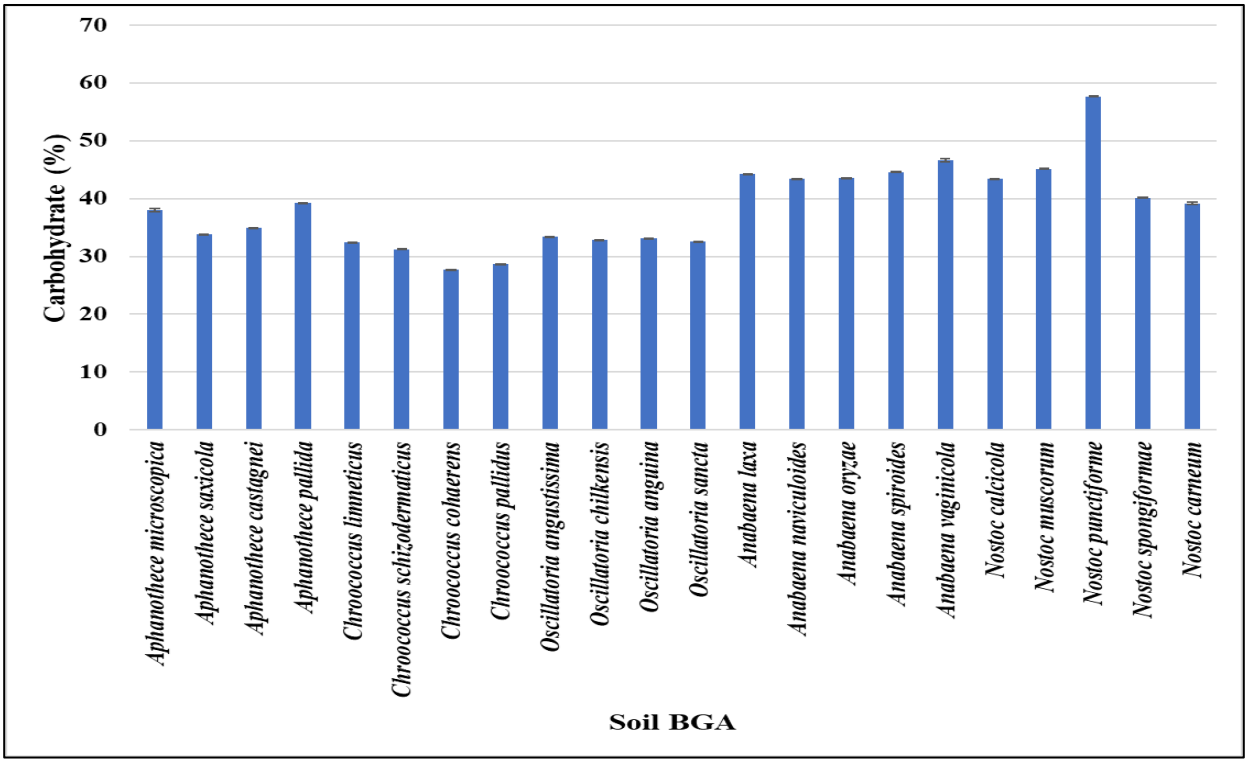

Figure 2 Figure 2 Carbohydrate (%) content in soil BGA

E. Proteins

The protein content analysis of soil blue-green algae (BGA) isolates showed significant variability across different species, reflecting their diverse metabolic strategies and potential ecological roles as shown in figure 3.

The Aphanothece species exhibited relatively moderate protein contents, with Aphanothece microscopica at 31.28 ± 0.07%, Aphanothece saxicola at 32.41 ± 0.05%, Aphanothece castagnei at 33.41 ± 0.08%, and Aphanothece pallida at 33.51 ± 0.06%. These levels indicated a balanced allocation of resources between protein synthesis and other metabolic functions in Aphanothece species [34].

Chroococcus species displayed higher protein levels compared to Aphanothece, with Chroococcus limneticus at 32.45 ± 0.04%, Chroococcus schizodermaticus at 36.77 ± 0.27%, Chroococcus cohaerens at 43.44 ± 0.05%, and Chroococcus pallidus at 40.26 ± 0.01%. The elevated protein content in Chroococcus species suggested a greater emphasis on protein synthesis, which might be crucial for their survival and growth in various soil environments [35].

Oscillatoria species also exhibited substantial protein contents, with Oscillatoria angustissima at 39.48 ± 0.08%, Oscillatoria chilkensis at 38.46 ± 0.04%, Oscillatoria anguina at 37.67 ± 0.08%, and Oscillatoria sancta at 37.81 ± 0.1%. These protein levels indicated that Oscillatoria species invested significantly in protein production, supporting their physiological activities and resilience in diverse conditions [36].

Anabaena species demonstrated the highest protein contents among the analyzed BGAs, with Anabaena laxa at 50.25 ± 0.01%, Anabaena naviculoides at 49.41 ± 0.06%, Anabaena oryzae at 48.57 ± 0%, Anabaena spiroides at 47.77 ± 0.09%, and Anabaena vaginicola at 45.5 ± 0.06%. The high protein content in Anabaena species was likely associated with their nitrogen-fixing capabilities, as proteins were essential for the synthesis of nitrogenase enzymes. This characteristic underscored their potential importance in enhancing soil fertility and their suitability for applications in sustainable agriculture [37].

Nostoc species showed moderate to high protein contents, with Nostoc calcicola at 35.29 ± 0.03%, Nostoc muscorum at 33.35 ± 0.08%, Nostoc punctiforme at 32.34 ± 0.05%, Nostoc spongiformae

at 33.23 ± 0.01%, and Nostoc carneum at 31.86 ± 0.01%. According to the Post hoc analysis, Aphanothece castagnei with Aphanothece pallida and Nostoc muscorum; Aphanothece microscopica and Nostoc carneum; Aphanothece Saxicola and Chroococcus limneticus and Oscillatoria anguina and Oscillatoria sancta were found to have similarity in the mean values. These protein levels, combined with their robust extracellular polysaccharide matrix, suggested that Nostoc species were well-adapted to storing and utilizing proteins effectively, which could be beneficial for various biotechnological applications, including biofertilizers and biostimulants. From a research paper, cyanobacteria exhibit a wide range in their total content, varying from 2.5% to 66.7%, with an average concentration of 36.9% [38].

In summary, the protein content analysis of soil blue-green algae isolates highlighted the metabolic diversity and potential ecological roles of these organisms. The findings suggested that specific BGA species, particularly those from the Anabaena and Chroococcus genera, had significant potential for biotechnological applications due to their high protein production. Further research into optimizing the cultivation conditions and extraction processes for these algae would be essential for maximizing their potential in sustainable bioproducts and bioenergy production.

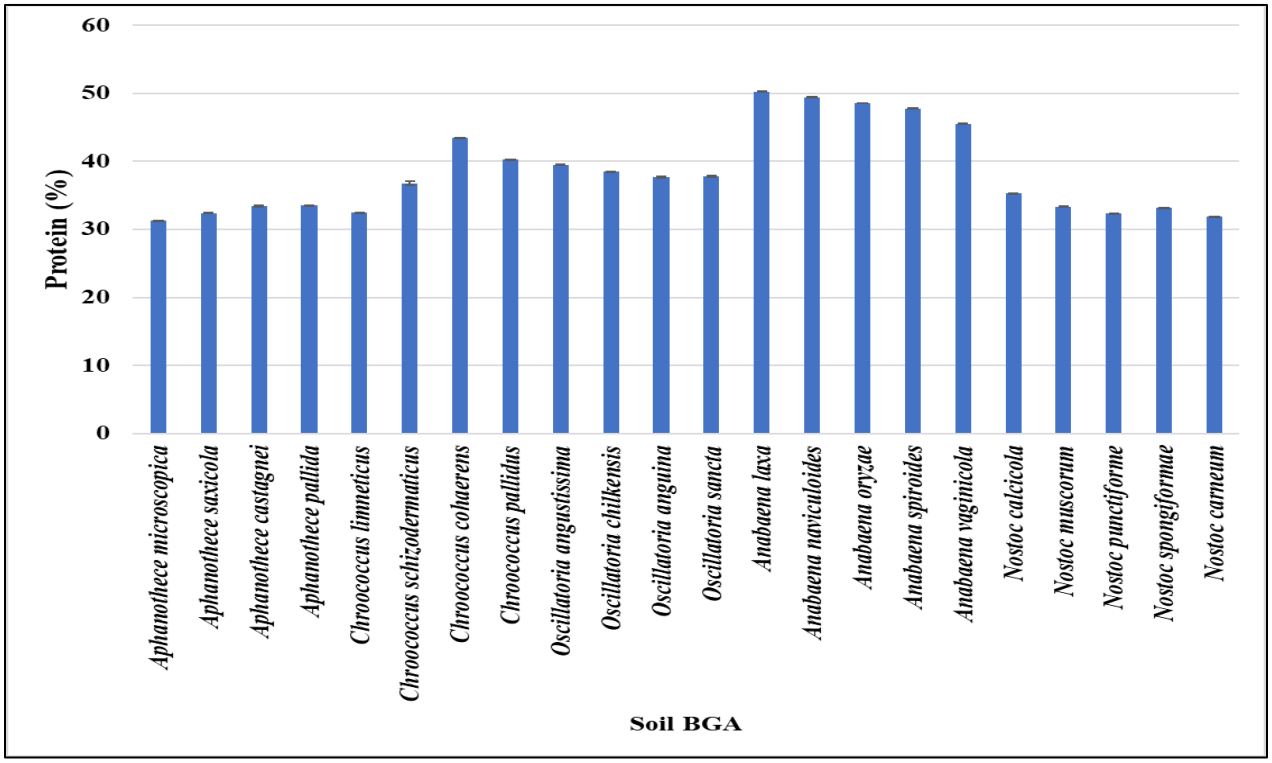

Figure 3 Protein (%) content in soil BGA

F. Chlorophyll a

The analysis of chlorophyll a content in soil blue-green algae (BGA) isolates revealed considerable variation among the species, indicating differences in their photosynthetic capacities as shown in figure 4.

Among the Aphanothece species, Aphanothece microscopica showed the lowest chlorophyll a content at 0.3 ± 0.01 mg/mL, followed by Aphanothece saxicola at 0.47 ± 0.01 mg/mL, Aphanothece castagnei at 0.32 ± 0 mg/mL, and Aphanothece pallida at 0.36 ± 0.02 mg/mL. These values suggest that Aphanothece species may have lower photosynthetic efficiency compared to other BGA.

Chroococcus species exhibited higher chlorophyll a levels, with Chroococcus limneticus at 0.87 ± 0 mg/mL, Chroococcus schizodermaticus at 0.95 ± 0.02 mg/mL, Chroococcus cohaerens at 0.88 ± 0.01 mg/mL, and Chroococcus pallidus at 0.86 ± 0.01 mg/mL. These higher values reflect a greater potential for photosynthesis, which could be advantageous in nutrient-rich environments [39].

The Oscillatoria species demonstrated even higher chlorophyll a content, indicating robust photosynthetic activity. Oscillatoria angustissima had 1.05 ± 0.01 mg/mL, Oscillatoria chilkensis had 1.03 ± 0.01 mg/mL, Oscillatoria anguina had 1.08 ± 0 mg/mL, and Oscillatoria sancta had 1.05 ± 0.01 mg/mL. These results suggest that Oscillatoria species are well-adapted to efficiently capture light energy, which supports their growth and survival in diverse conditions [40].

Anabaena species showed some of the highest chlorophyll a levels among the BGAs studied, with Anabaena laxa at 1.26 ± 0.02 mg/mL, Anabaena naviculoides at 1.32 ± 0.02 mg/mL, Anabaena oryzae at 1.38 ± 0 mg/mL, Anabaena spiroides at 1.46 ± 0.01 mg/mL, and Anabaena vaginicola at 1.29 ± 0 mg/mL. These high values underscore the efficiency of Anabaena species in photosynthesis, likely contributing to their nitrogen-fixing capabilities and overall ecological success [41]. Nostoc species were found to have the highest chlorophyll content among the analyzed isolates, indicating superior photosynthetic capabilities. Nostoc calcicola had 1.54 ± 0.01 mg/mL, Nostoc muscorum had 1.67 ± 0.01 mg/mL, Nostoc punctiforme had 1.58 ± 0 mg/mL, Nostoc spongiformae had 1.56 ± 0.01 mg/mL, and Nostoc carneum had 1.58 ± 0.01 mg/mL. According to the Post hoc analysis, Chroococcus cohaerens with Chroococcus pallidus; Oscillatoria angustissima with Oscillatoria sancta and Nostoc punctiforme with Nostoc carneum were found to have similarity in the mean values. Studies by Gregor and Maršálek [42] showed similar results as our research. These high levels of chlorophyll a suggest that Nostoc species are highly efficient in capturing light energy, making them ideal candidates for applications in biofertilizers and other biotechnological uses.

In summary, the chlorophyll a content analysis of soil blue-green algae isolates highlighted significant differences in photosynthetic capacity across different genera and species. Anabaena and Nostoc species, in particular, demonstrated high chlorophyll a level, indicating their potential for high photosynthetic efficiency and ecological importance. These findings suggest that species from these genera could be particularly valuable in biotechnological applications requiring efficient light energy capture and conversion, such as in biofuel production and sustainable agriculture. Further research into optimizing growth conditions and harnessing these photosynthetic capabilities will be essential for maximizing their potential benefits.

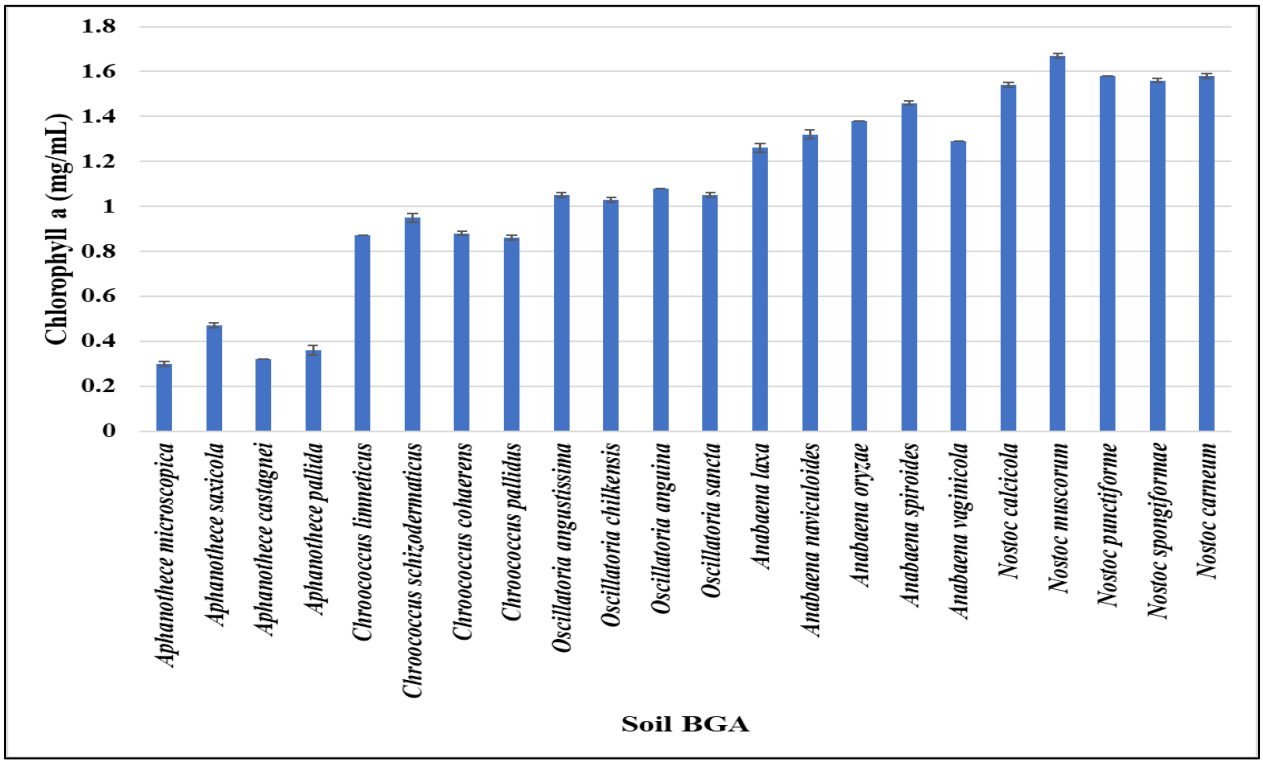

Figure 4 Chlorophyll a (mg/mL) content in soil BGA

V. CONCLUSION

The biochemical analysis of soil blue-green algae (BGA) isolates revealed significant variations in lipid, carbohydrate, protein, and chlorophyll a content across different genera and species. Nostoc species were found to have the highest lipid content, suggesting a strong potential for biotechnological applications, particularly in biofuel production. Carbohydrate analysis showed that certain Nostoc species had the highest content, indicating their potential as a bioresource for bioenergy and bioproducts. Protein analysis revealed Anabaena species with the highest protein content, highlighting their suitability for applications in nutritional supplements and animal feed. Chlorophyll a analysis indicated that Nostoc species had the highest content, suggesting superior photosynthetic efficiency and potential for use in biofertilizers and other agricultural applications.

Overall, Nostoc and Anabaena species emerged as particularly promising genera for various biotechnological applications due to their high biochemical component content. The significant lipid, carbohydrate, protein, and chlorophyll a levels found in these species underscore their versatility and potential in sustainable bioenergy, bioproducts, and agricultural enhancements. Future research should focus on optimizing the cultivation conditions for these algae to maximize their biochemical yields and further explore their industrial applications.

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