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MORPHOLOGICAL AND MOLECULAR IDENTIFICATION, GROWTH PROFILE ANALYSIS, AND BIOCHEMICAL PARAMETER ESTIMATION ON BLUE-GREEN ALGAE

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ABSTRACT

The study of cyanobacteria and blue-green algae species is important to the global scientific community because a significant number of beneficial strains of cyanobacterial species fix atmospheric nitrogen, contributing to the fertility of agricultural soils worldwide, while others act as nuisance microorganisms in aquatic ecosystems due to their involvement in toxic bloom events. Despite their ecological importance and environmental concerns, their identification and taxonomy remain problematic and uncertain, frequently relying on current morphological and physiological studies, which generate confusing classification systems and typically vary under different conditions. As a result, the current study sought to investigate differences in morphological, biochemical, and genotypic features of three cyanobacteria and algal strains isolated from Rameshwaram marine bodies using a polyphasic approach. The strains were characterised using morphometric, genetic (*16S and 18S rRNA*), and biochemical data (Chlorophyll, Carotenoids, Protein, Carbohydrate, Starch). Morphological analysis and sequencing of *16S rRNA* fragments revealed that the strains belonged to the *Oscillatoria nigro-viridis* species, and sequencing of *18S rRNA* fragments revealed that the strains belonged to. *Spirogyra sp.* and *Chlorella vulgaris*. These strains displayed distinct morphological and genetic characteristics, allowing easy assignment to their respective genera. The biochemical characterization revealed that the strains grew the most on the 11^{th} – 13^{th} day.

Keywords: cyanobacteria, blue-green algae, toxic bloom, Rameshwaram, *16S and 18S Rrna*

INTRODUCTION

Cyanoprokaryotes are unique among microorganisms because they contain chlorophyll a and can perform photosynthesis **[1]**. They have gram-negative cell walls with peptidoglycan layers ranging in thickness from 10 to 200 nm **[2]**. Cyanobacteria's dominant pigments are chlorophyll a, chlorophyll c, and phycocyanine, which contribute to their distinctive bluegreen coloration **[3]**. They also contain various colour pigments such as -carotene and xanthophyll. Some cyanobacteria species also contain phycoerythrin pigments, which are one of the phycobilin bilanes and are responsible for red coloration **[4]**. Cyanobacteria can be unicellular or multicellular aggregates that form a colony. Those of unicellular cyanobacteria have bacilli, discoidal, actinoidal, or fibrillary shapes, whereas those of colony forming cyanobacteria have spherical shapes **[5]**. Certain cyanobacteria species have special cells called heterocysts, which are smaller than akinete and are responsible for fixing free nitrogen from the air. These cells are found only in cyanobacteria and are part of the Tallus fibre **[6] [7]**. Cyanobacteria are classified based on morphological characteristics. However, changes in environmental and developmental conditions may cause morphological traits to change. Using elective culture conditions can reduce strain diversity in cultures **[8]**. These factors necessitate the development of molecular techniques for cyanobacterial identification **[9] [10]**. The 16S rRNA gene is the most commonly used marker gene for both microorganism identification and relationship analysis. While the 16S rRNA gene contains many evolutionarily conserved sequences, it also contains many species-specific variable sequences **[11]**. Using PCR to amplify these variable sequences, species identification is possible.

The current study obtained molecular information about the 16S and 18S rRNA genes from a filamentous cyanobacterial strain and blue-green algae isolated from Rameshwaram marine bodies. The genotypic characteristics, as well as morphological and biochemical characteristics, were used to characterise the strains.

MATERIALS AND METHODS

Cyanobacteria and Blue-green algae isolation and culture conditions

The culture was isolated from the water of Rameswaram Lake in Tamil Nadu, India. Under the microscope, the selected sample (strain) was examined to identify the morphological features of green algae and cyanobacteria. The microalgae strains were scaled up by subsequent culturing in BG 11 Medium **[12]**, and the cyanobacteria strains

were culturing in ASN Medium **[13]**, which is a selective growth medium for microalgae and cyanobacteria, and the same medium was used for mass cultivation.

Molecular characterization:

DNA was extracted based on the Xanthogenate method and amplification was carried out by (*16S and 18S rRNA*). The sequencing response was carried out at Eurofins Genomics in Bangalore using ABI large color cycle sequencing Biosystems responses (Applied Biosystems).

Biochemical Characterization:

Chlorophyll and Carotenoids estimation was carried out acetone Methods,

RESULTS AND DISCUSSION:

Isolation of Algae and cyanobacterial Isolates

Algae samples were taken and grown in BG11 broth and Cyanobacteria were grown in ASN medium for 5 days at incubation. These isolates were pure cultured and stabbed for furtherwork.

Morphological Studies

The axenic culture obtained was observed under an automated inverted Leica microscope (DMI 3000B). The morphological features were recorded and photomicrographs were taken **(Figure 1)**.

Growth Parameter analysis Chlorophyll Estimation

In *spirogyra,* the estimation of chlorophyll was increased from the 0^{th} day – to the 11^{th} day and it was suddenly decreased from the 13^{th} day. The maximum number of chlorophyll present on the 11^{th} day of the culture. In *chlorella,* the estimation of chlorophyll was increased from the 0^{th} day – to the 11^{th} day and it suddenly decreased from the 13^{th} day. The maximum number of chlorophyll present on the 11^{th} day of the culture. In *oscillatoria,* the estimation of chlorophyll was increased from the 0^{th} day – to the 13^{th} day and it suddenly decreased from the 15^{th} day. The maximum number of chlorophyll present on the 13^{th} day of the culture **(Graph 1)**.

Carotenoids Estimation

In *spirogyra* the estimation of Carotenoids was increased from 0^{th} day – 13^{th} day and itwas suddenly decreased from 15^{th} day. The maximum number of Carotenoids present in 13^{th} day of the culture. In *chlorella* the estimation of Carotenoids was increased from 0^{th} day – 13^{th} day and itwas suddenly decreased from 15^{th} day. The maximum number of Carotenoids present in 13^{th} day of the culture. In *oscillatoria* the estimation of Carotenoids was increased from 0^{th} day – 13^{th} day andit was suddenly decreased from 15^{th} day. The maximum number of Carotenoids present in 13^{th} day of the culture **(Graph 2)**.

Dry Weight

In spirogyra the estimation of Dry

Weight was increased from 0th day – 13th day and itwas suddenly decreased from 15th day. The maximum number of Dry Weight present in 13th day of the culture. In chlorella the estimation of Dry Weight was increased from 0th day – 13th day and itwas suddenly decreased from 15th day. The maximum number of Dry Weight present in 13th day of the culture. In oscillatoria the estimation of Dry Weight was increased from 0th day – 13th day and it was suddenly decreased from 15th day. The maximum number of Dry Weight present in 13thday of the culture **(Graph 3)**.

From growth parameter analysis *spirogyra, chlorella* and *oscillatoria* staring lag phasein 0th day, log phase from 3th day – 9th day stationery phase 11th -13th day and death phase from15th day.

Biochemical characterizationProtein

In *spirogyra* the estimation of protein was increased from 0th day – 13th day and it wassuddenly decreased from 15th day. The maximum number of protein present in 13th day of the culture. In *chlorella* the estimation of protein was increased from 0th day – 13th day and it wassuddenly decreased from 15th day. The maximum number of protein present in 13th day of the culture. In *oscillatoria* the estimation of protein was increased from 0th day – 13th day and it was suddenly decreased from 15th day. The maximum number of protein present in 13th day of the culture **(Graph 4, 5)**.

Carbohydrate

In *spirogyra* the estimation of carbohydrate was increased from 0th day – 13th day and it was suddenly decreased from 15th day. The maximum number of carbohydrate present in 13th day of the culture. In *chlorella* the estimation of carbohydrate was increased from 0th day – 13th day and it was suddenly decreased from 15th day. The maximum number of carbohydrate present in 13th day of the culture. In *oscillatoria* the estimation of carbohydrate was increased from 0th day – 13th day and it was suddenly decreased from 15th day. The maximum number of carbohydrate present in 13th day of the culture **(Graph 6, 7)**.

Starch

In *spirogyra,* the estimation of Starch was increased from the 0th day – to the 13th day and it was suddenly decreased from the 15th day. The maximum number of Starch present on the 13th day of the culture. In *chlorella,* the estimation of Starch was increased from the 0th day – to the 13th day and it suddenly decreased from the 15th day. The maximum number of Starch present in 13th day of theculture. In *oscillatoria* the estimation of Starch was increased from 0th day – 13th day and it was suddenly decreased from 15th day. The maximum number of Starch present in 13th day of the culture **(Graph 8, 9)**.

From growth parameter and biochemical analysis, the algae and cyanobacteria have maximum growth on the 11th – 13th day. Therefore, I take the 13th day culture for antioxidant activity.

Anti-oxidant Activity

In *spirogyra*, *chlorella,* and *oscillatoria* the maximum radical scavenging activity occurs at the 250µl concentration of our sample whereas BHT act as a standard for anti-oxidant activity **(Graph 10)**.

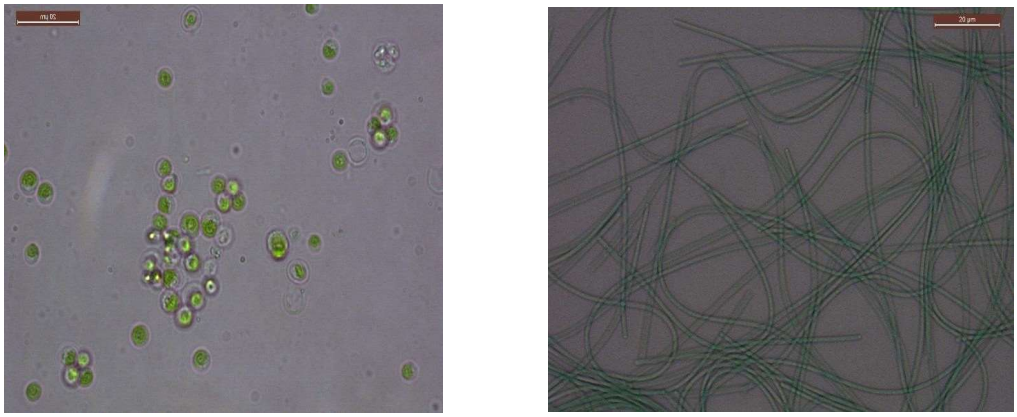
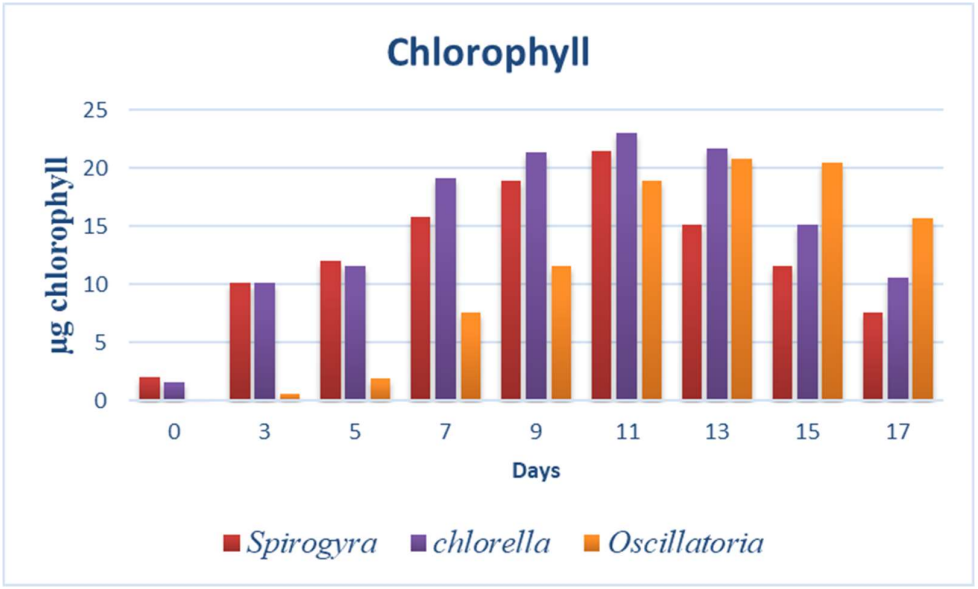
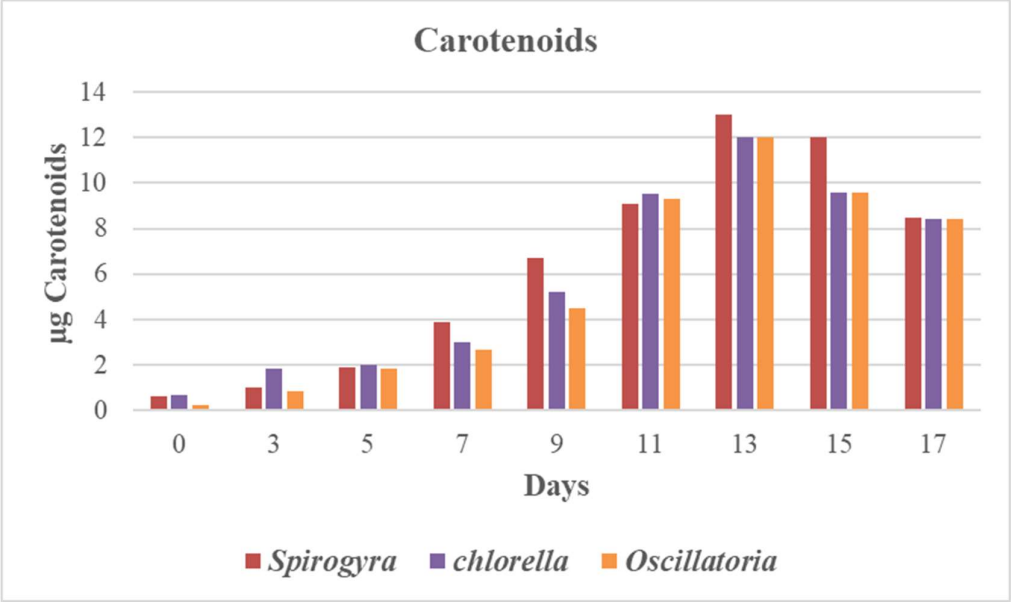


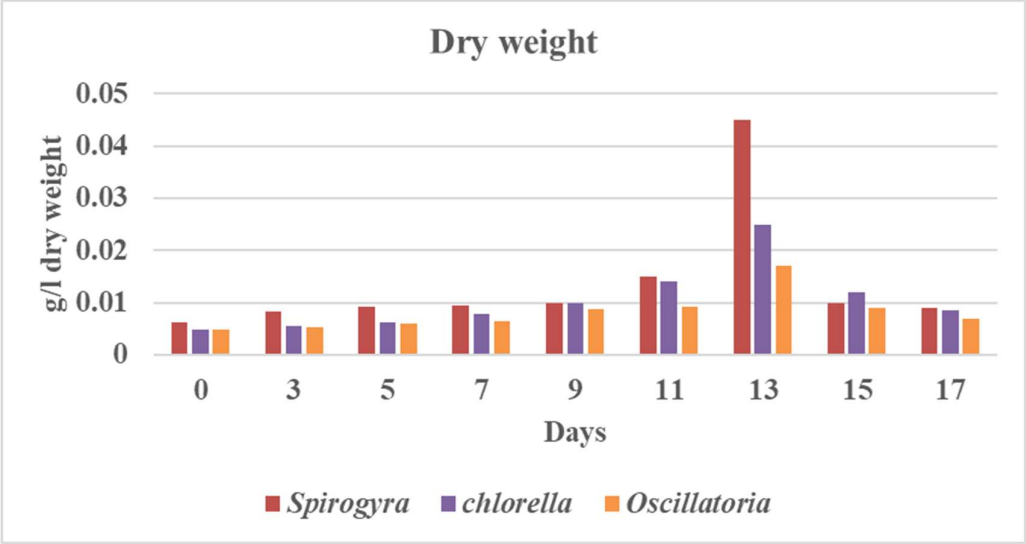
Figure 1: Microscopic image of *Spirogyra, chlorella, and oscillatoria*



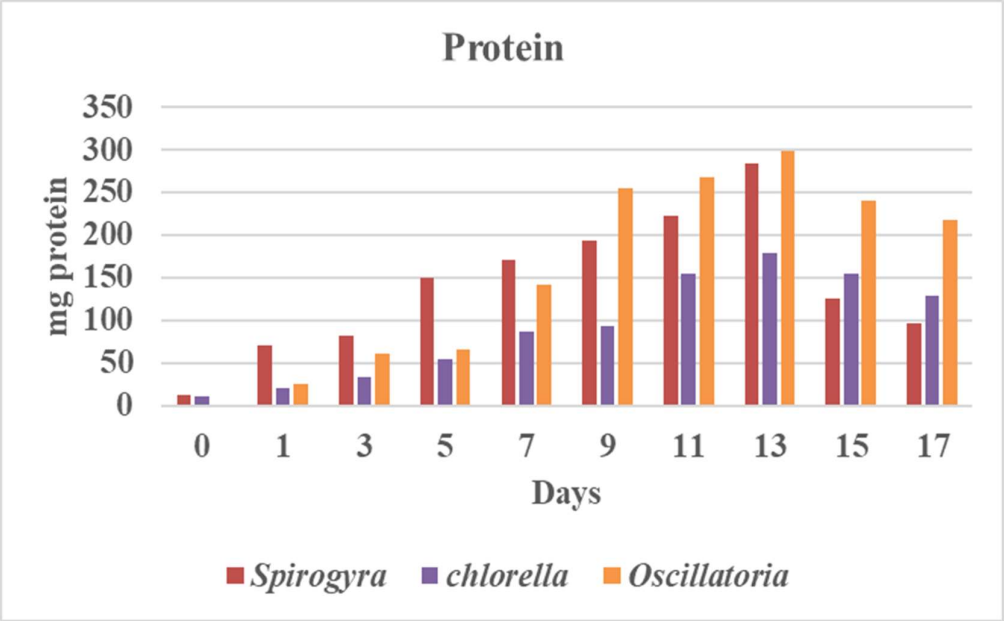
Graph 1: Chlorophyll estimation of *spirogyra, chlorella,* and *oscillatoria*



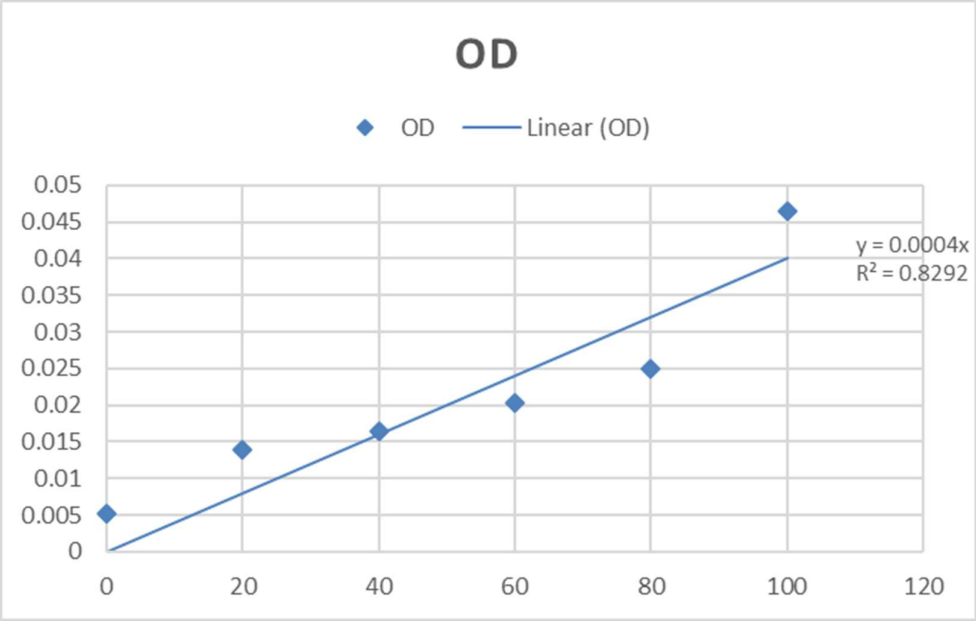
Graph 2: Carotenoids estimation of *spirogyra*, *chlorella* and *oscillatoria*



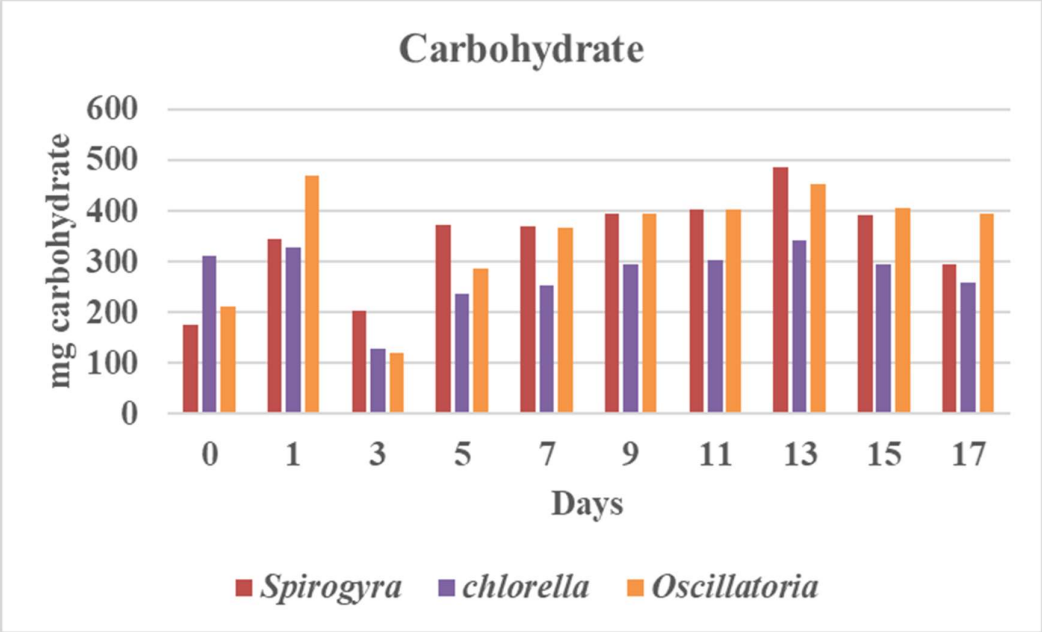
Graph 3: Dry Weight estimation of *spirogyra*, *chlorella* and *oscillatoria*



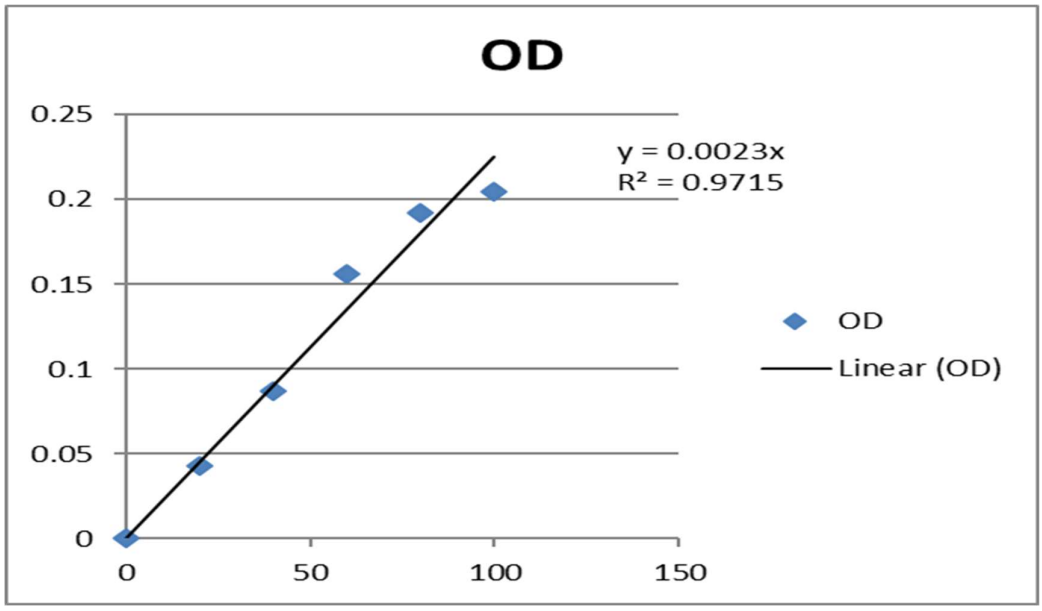
Graph 4: Protein estimation of *spirogyra, chlorella and oscillatoria*



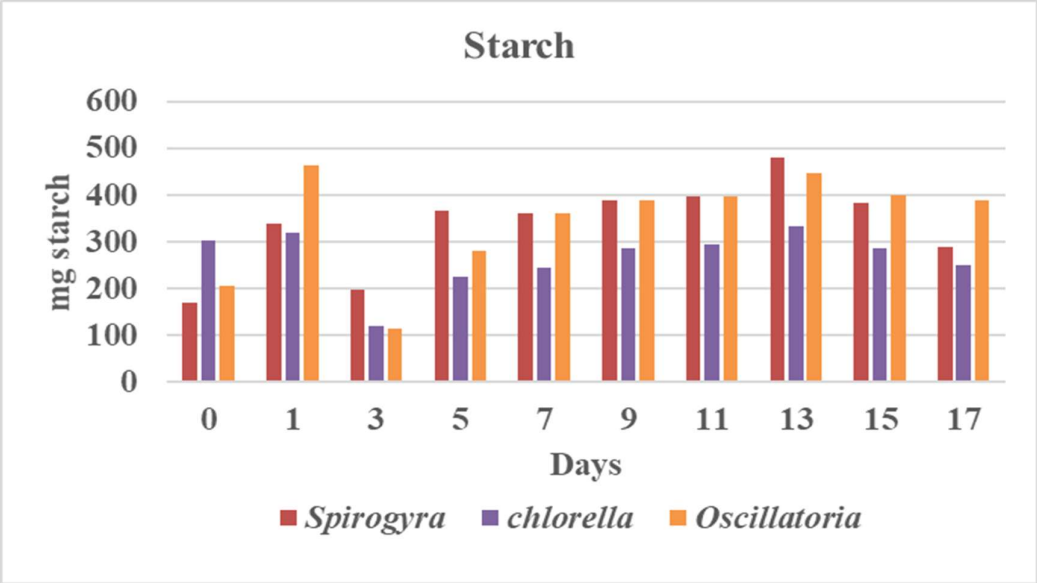
Graph 5: Protein Standard of BSA (bovine serum albumin)



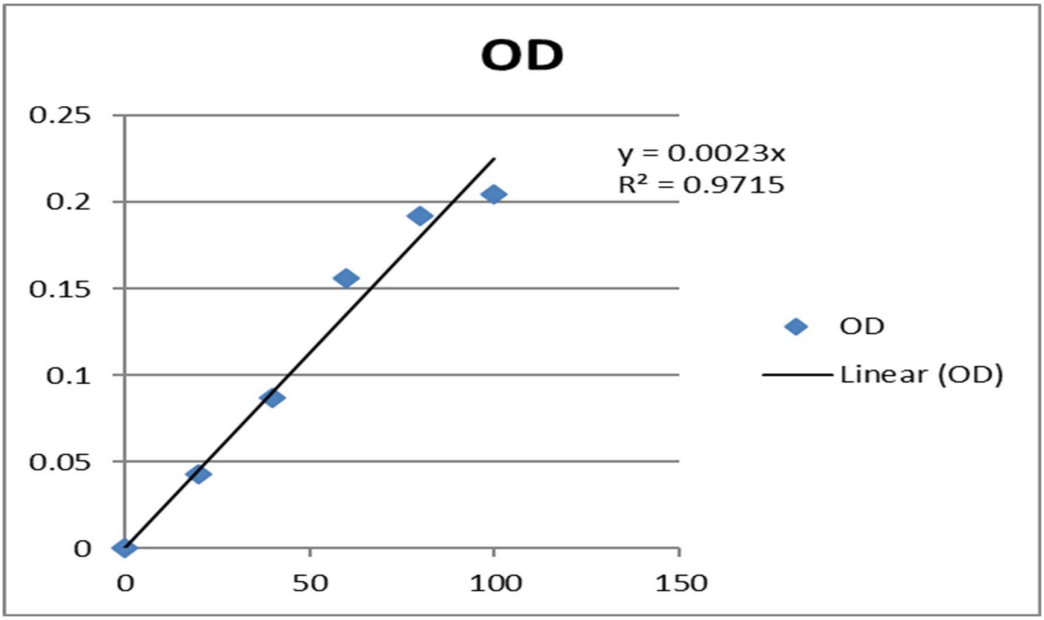
Graph 6: Carbohydrate estimation of *spirogyra, chlorella* and *oscillatoria*



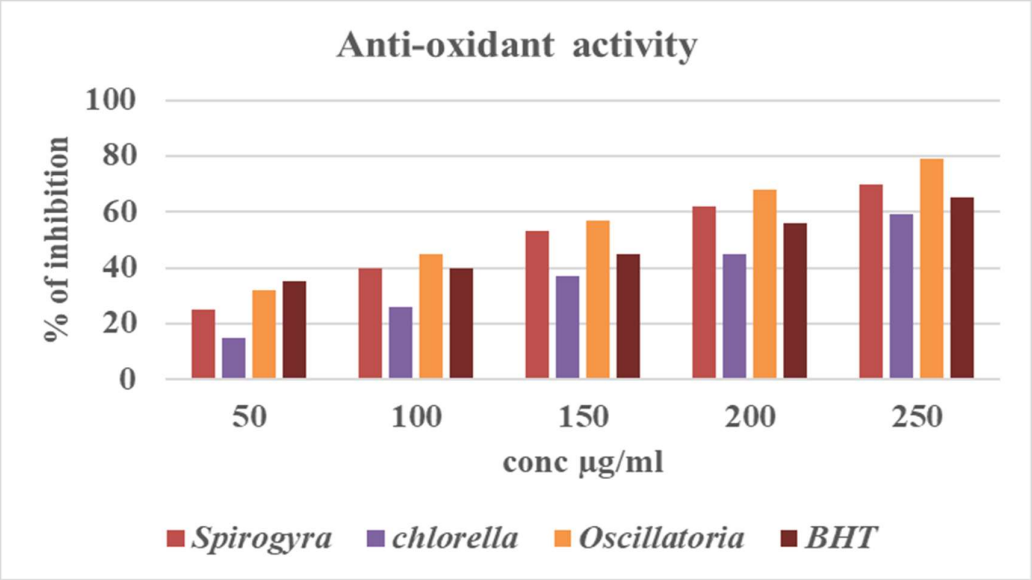
Graph 7: Carbohydrate Standard of Glucose



Graph 8: Starch Carbohydrate estimation of *spirogyra*, *chlorella* and *oscillatoria*



Graph 9: Starch Standard of Glucose



Graph 10: Anti-oxidant of *spirogyra*, *chlorella,* and *oscillatoria*

CONCLUSION

Morphological identification was carried out using a microscope. Growth parameter analysis *spirogyra, chlorella,* and *oscillatoria* staring lag phase in 0^{th} day, log phase from 3rd day – 9^{th}-day stationery phase 11^{th} -13^{th} day, and death phase from 15^{th} day. Biochemical analysis of algae and cyanobacteria have maximum growth on the 11^{th} – 13^{th} day. In *spirogyra, chlorella,* and *oscillatoria* the maximum radical scavenging activity occurs at 2the 50µl concentration of our sample.

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