Spirulina Maxima: Characterization And Its Application

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ABSTRACT :

Spirulina maxima,a cyanobacteria, is an aquatic and photosynthetic organism. They are extensively used as food supplements due to their rich content of proteins. *Spirulina maxima* displays valuable nutraceutical properties. Present study is based on the physiochemical characterization of *Spirulina maxima*. Further extraction of photosynthetic pigments i.e. Chlorophyll, Carotenoids and Phycocyanin was carried out.The protein content, anti-oxidant activity and anti-inflammatory activity was evaluated. *Spirulina* was tested to degrade azo dyes which are used in textile industry.

Keywords: *Cyanobacteria, pigments, anti-oxidant, anti-inflammatory, dye degradation.*

I INTRODUCTION:

Spirulina is the general name of filamentous, multicellular, blue-green microalgae belonging to genera, namely *Arthrospira,* which consist of 15 species (Hoseini1, 2003).It is a microscopic and filamentous cyanobacterium that derives its name from the spiral or helical nature of its filaments. These occur naturally in warm, alkaline, salty, brackish lakes, but are also commonly grown by aquaculture and harvested for commercial use (Geitler,L. 1932).There are only a few areas worldwide that have the ideal sunny climate for production of this alga, including Greece (Nigrita, Serres), Japan, India, United States and Spain. (P. D. Karkos, 2011)

Spirulina maxima has attracted people and scientists from all over the world due to its special properties. It has found wide applications in agriculture, food, pharmaceuticals, perfumeries, medicine and science.(Veluchamys,2018). Moreover *Spirulina* can be safely administered to children without any risk and is considered a very suitable food (United Nations World Health Organization, Geneva, Switzerland June 8th, 1993). *Spirulina* has been demonstrated to be an effective dietary source of vitamin A. An investigation in India on preschool children with vitamin A deficiency demonstrated that the bioavailability of carotenes from *Spirulina* was originally harvested from lakes in parts of Africa and Mexico, dried and used as a food but it gained prominence more recently after it was used as a dietary supplement for astronauts on space missions (Mohan, 2014).There are several new peer reviewed scientific studies about *Spirulina's* ability to inhibit viral replication, strengthen both the cellular and humoral arms of the immune system and cause regression and inhibition of cancers.

Present study is based on the physiochemical characterization and properties of *Spirulina maxima* which includes estimation of optimum conditions(pH, temperature, light intensity) required for the growth, extraction and estimation of different pigments, determination of various application such as anti inflammatory property ,antioxidant activity, and dye degredation activity.

II MATERIALS AND METHOD:

Microganism and culture medium:

The cyanobacteria *Spirulina (Arthospira) maxima* was used in this study. The strain was obtained from the Algal Biotechnology Lab, UICT which was previously maintained in modified Zarrouk's media at 27˚ C(Steinbüchel, 2013).

Determination of pH , temperature and light intensity:

Zarrouk's media was prepared of different pH range (pH 3,5,7,8,10).pH was adjusted using 1M NaOH and 1M Hcl. Hundred ml Zarrouk's media was prepared and inoculated with mid exponential phase culture and incubated for a period of 30 days. For determination of the subsequent growth of the culture, cell count using hemocytometer was done after the interval of 10 days.

The growth of the *Spirulina maxima* at different temperature were determined by subjecting the flask with media and culture inoculated at different temperatures (2℃,27℃,37℃,50℃). Hundred ml of inoculated Zarrouk's media and incubated for period of 30 days. Cell count was taken using hemocytometer after the interval of 10 days for determination of subsequent growth of the *S.maxima*.

For determination of optimum light intensity, Erlenmeyer flask with Zarrouk's media inoculated culture from mid exponential phase were covered with different color cellophane sheets. The flasks with cellophane sheets were incubated at 27˚C and cell count using hemocytometer was done after interval of 10 days and the subsequent growth was estimated .Each color has specific wavelength(Table no: 1).

Table1: Wavelength of different color

Color	Wavelength(nm)
Red	700-635
Yellow	590-560
Green	560-520
White	300-700

Cultivation

Spirulina maxima culture was axenically grown in the Zarrouk's medium. The sub-culturing of *Spirulina maxima* was carried out in 500ml Zarrouk's medium. The culture were incubated at 27˚C in white light at pH 7.During the growth of the culture the flask was shaken 3-4times/day. Growth of the culture were observed after 15 days of subculturing. *Spirulina maxima* biomass was separated by centrifuging the known volume of sample at 5000rpm for 20 mins. The obtained pellet was then air dried at 75°C for 24 hours . The difference between the final and initial weight help in the determining the dry weight of the *Spirulina maxima* biomass.

Estimation of protein using Lowry method:

The protein content in *Spirulina maxima* was determined by using Lowry method. The standard protein used was Bovine Serum Albumin (2mg/ml).One ml of *Spirulina* water extract (0.1g in 1ml of D/W) was used as unknown sample. (Lowry, 1951)

Extraction of pigments:

Extraction of Chlorophyll, Carotenoids was carried out using Acetone (80%) and Phycocyanin using Phosphate buffer. Chlorophyll , Phycocyanin And Carotenoids extraction was carried out using 0.25g of *Spirulina* powder.The absorbacnce was determined using UV-Spectrophotometer.(Sangeetha.B.,2016)

Anti- Inflammatory activity by inhibition of proteinase activity:

The anti-inflammatory activity of *Spirulina maxima* was studied by setting up an reaction mixture which contains 0.5 ml of trypsin (8.000 armour units), 1ml of 25 MM Tris HCl buffer, pH 7.4 and 1ml of aqueous extract of *Spirulina maxima*(200µg/ml) and standard used was Aspirin(75µg/ml). (Bharathi, 2017)

Anti oxidant activity by DPPH method:

Anti oxidant activity of *Spirulina maxima* was determined using DPPH radical scavenging assay. The stock of 0.2% w/v *Spirulina* water extract,1mM DPPH,95% Ethanol was used. Different concentrations of *Spirulina* water extract was prepared (0.025%, 0.05%, 0.1%) and the reaction mixture was set up which includes 0.3ml of DPPH, 3ml of 95% Ethanol was added in all reaction tubes. In blank 3ml of 95% of ethanol and 0.5ml of D/W was added. The mixture was allowed to incubate for 30 minutes in dark. The absorbance was measured at 517nm.(Anbarasan*, 2011)

Dye degradation activity:

Stock of 50 ppm mono azo(Methyl orange) dye and di azo(Ponceau)dye was prepared. From stock , three different concentration(5ppm, 10ppm,20ppm) of Ponceau, Methyl Orange dye was prepared and inoculated in algal culture.The reductase activity of algae was studied after 3,6 and 9 days of incubation. The absorbance was measured by using UV spectrophotometer in the range of 300nm to 700nm (Omar, 2008)

The percentage of dye degradation was calculated by using an formula :

$$\text{Percentage of dye degradation} = \left(\frac{\text{max. Abs (day 0)} - \text{max. Abs (day6)}}{\text{max. Abs (day0)}} \right) \text{X } 100$$

Where, max- maximum, Abs - Absorbance

Different water samples were collected from different sites the samples collectyed were sea water ,tap water, ground water and for the control sterile Zarrouk's media *(Steinbüchel, 2013)* was used. Five ml of *Spirulina* culture(232.5 cfu/ml) was inoculated in all the water sample containing flask and allow it to incubate at 27℃ and cell count was noted after the interval of 10 days by using Hemocytometer

III RESULT AND DISCUSSION:

Microscopic observation of *Spirulina maxima* was done using the wet mount method .Filamentous rod shaped algae was observed.Figure 3.1

Figure(3.1) Microscopic image of *Spirulina maxima* under high power objective

Determination of pH , temperature and light intensity:

The optimum pH, Temperature and Light intensity for the growth of *Spirulina maxima* was checked optimum growth was found to be between pH range 5-10.The highest cell count of 710 cfu/ml was observed at pH 7 Figure 3.2. Maximum growth was observed at 27℃ which is 680 cfu/ml and no growth

was observed at temperature 2,4,27,55(˚C) Figure 3.3. Less growth was observes in Red, Yellow and Green Light, whereas the maximum growth was seen in white light (300nm- 700nm) respectively on 30thday Figure3.4

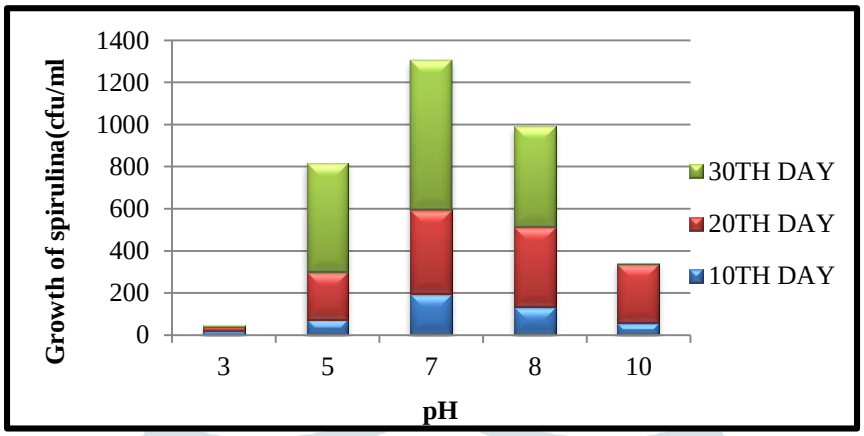


Figure3.2:- Biomass production of *Spirulina maxima* at different pH

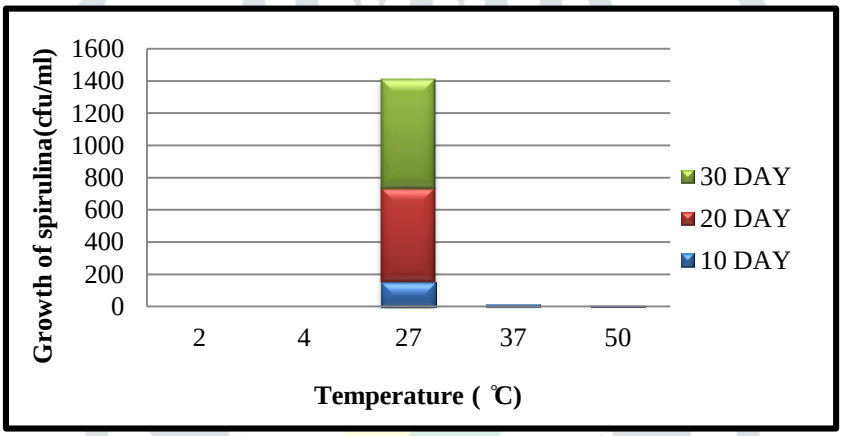


Figure 3.3:- Biomass production of *Spirulina maxima* at different temperatures

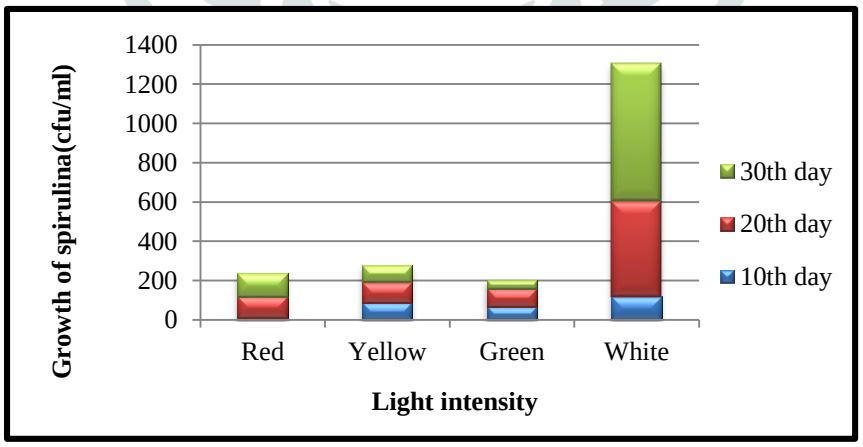


Figure 3.4:- Biomass production of *Spirulina maxima* at different light intensity

Extraction of pigments:

The Phycocyani(A), Carotenoid(B) and Chlorophyll(C) Figure3.5, content was 6.6x10^{-2}mg/ml , 3.28x10^{-5}mg/ml and 4x10^{-3}mg/ml(Table no:2) respectively in 0.25g of *Spirulina* biomass.

Table no 2:-Extraction of Pigments

Pigments	Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoids	Phycocyanin
Concentration (mg/ml)	2×10^{-3}	2×10^{-3}	4×10^{-3}	3.28×10^{-5}	6.6×10^{-2}

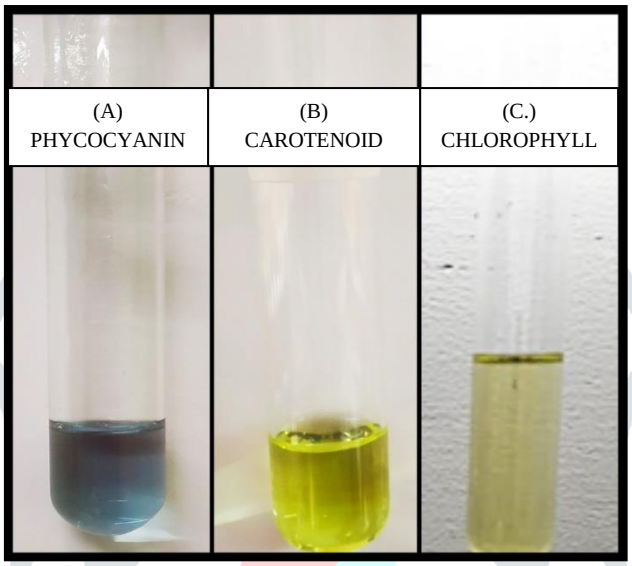


Figure 3.5:-Extraction of pigments Phycocyanin(A),Carotenoid(B), Chlorophyll(C)

Estimation of protein using Lowry method:

The protein content in 0.1g of *Spirulina maxima* extract by Lowry method was found to be 0.32mg/ml Figure3.6

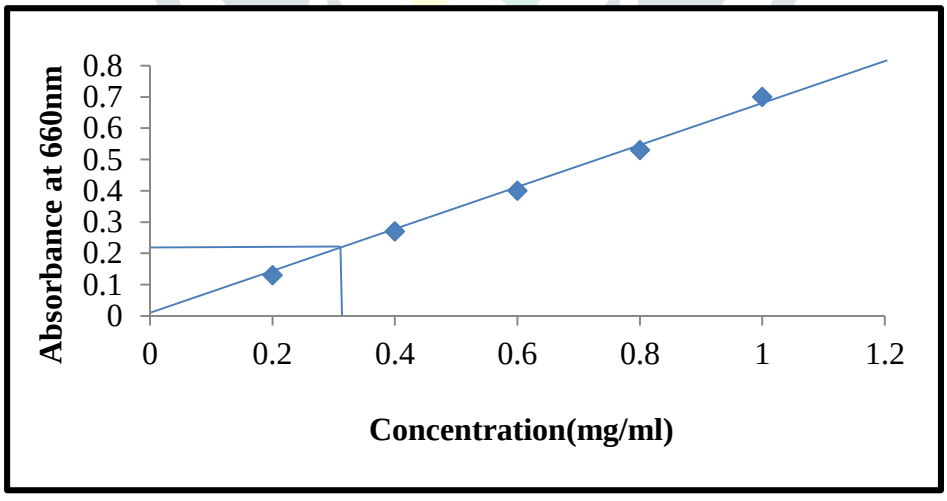
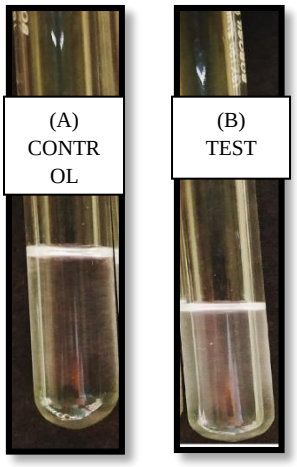


Figure 3.6:-Determination of Protein Concentration in *Spirulina maxima*

Anti- Inflammatory activity by inhibition of proteinase activity:

Spirulina shows 60.8% anti-inflammatory activity as compare to aspirin .table no: 3 & Figure3.7.

Table no 3:- Anti –Inflammatory activity



Absorbance at 214nm	Control (aspirin)	Test
	3.193	**1.923**

Figure 3.7:- Anti -Inflammatory activity by proteinase inhibition assay in control(A) and test(B)

Dye degradation activity:-

Spirulina maxima is able to degrade diazo dye rapidly as compare to mono azo dye. Figure 3.8

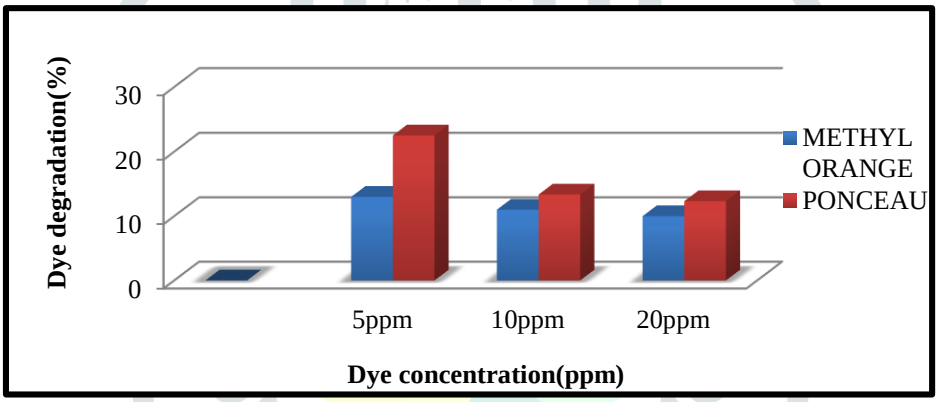


Figure 3.8:-Determination of dye degradation concentration in *Spirulina maxima*

Anti oxidant activity by DPPH method:

Antioxidant activity was determined by using DPPH free radical scavenging assay. It was found that *Spirulina maxima* has antioxidant activity. Figure 3.9

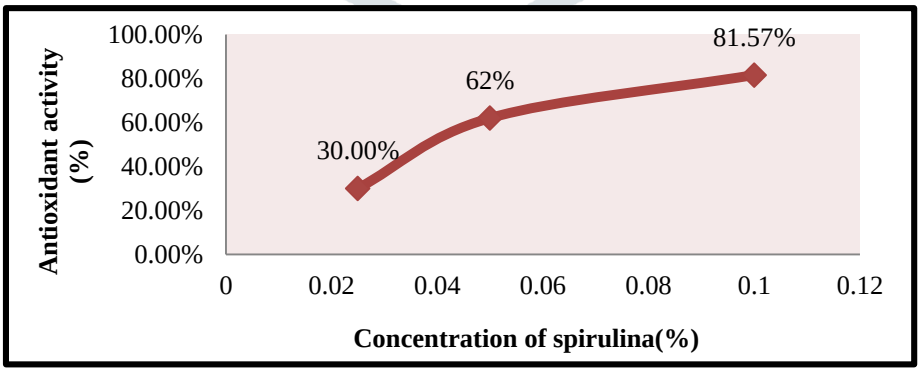


Figure 3.9:-Antioxidant activity shown by *Spirulina maxima*

Growth of *Spirulina maxima* in different water samples:

Growth of *Spirulina maxima* was observed in different water samples. The maximum growth was observed in sea water.Figure3.10.

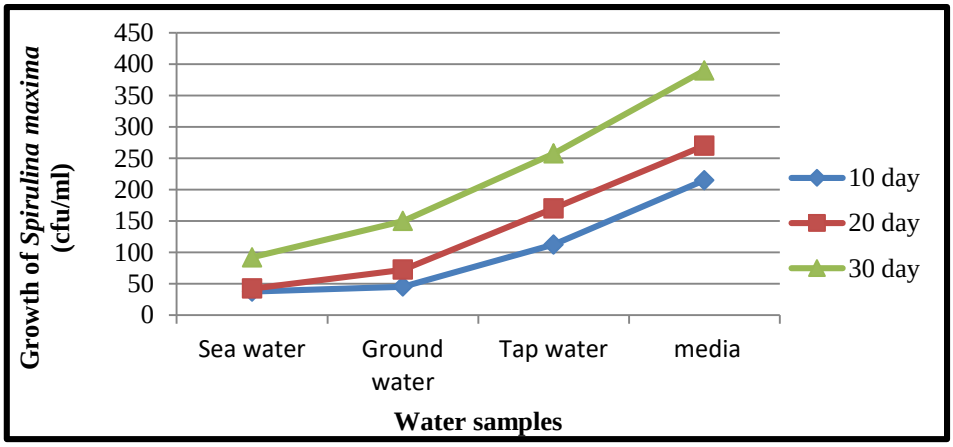


Figure3.10:-Growth of *Spirulina maxima* in different water samples

Present study involves Physical and Chemical characterization of *Spirulina maxima*. Physical characterization includes determination of physical appearance of algae and optimum parameters required for the growth of *Spirulina maxima*. It was found to be filamentous rod shaped algae and optimum conditions required for the growth (maximum biomass) are at pH 7, 27℃ (room temperature) and white light for its optimum growth. Chemical characterization includes extraction of pigments (Chlorophyll, Carotenoids, Phycocyanin) where Phycocyanin content was found to be more as compare to other two pigments. As Phycocyanin is abundantly present in *Spirulina*, hence *Spirulina maxima* can be act as good antioxidant, food coloring agent and good anti- inflammatory agent. Protein estimation was done by Lowry method and protein content in 0.1g/ml of *Spirulina* was found to be 0.32mg/ml. Hence it is rich in protein and can be act as an important source of protein in diet.

Various applications were studied which includes antioxidant activity, anti-inflammatory and dye degradation. Concentration of *Spirulina* increases the antioxidant activity (Figure 3.9). Hence, *Spirulina maxima* can be used as antioxidant agent for the prevention of various disease caused by free radicals in human body. Anti-inflammatory activity of *Spirulina* was tested against standard aspirin (75mg/ml). It was observed that *Spirulina* shows 60.8% anti-inflammatory activity as compare to aspirin. Hence *Spirulina* is an biological product it has less chances of allergic reactions whereas aspirin is an chemical product. It has more chances of allergic reactions (table no 3).

The ability of *Spirulina maxima* to degrade the industrial dyes (azo dyes) was determined. The different concentrations of mono azo dye (5ppm,10ppm,20ppm) and of diazo dye (5ppm,10ppm,20ppm). The mono azo dye used was Methyl orange and diazo dye used was Ponceau. After incubation of dye along with 50ml of culture it was observed that *Spirulina* is able to degrade diazo dye(Figure 3.8).Hence it can be used for the treatment of industrial effluent which contains azo dyes as the main effluents.

Spirulina maxima growth was observed in different water sample that is sea water , ground water, tap water sample. The maximum growth was observed in sea water sample whereas in tap water and ground water the growth observed was less as compared to the sea water, hence sea water can be used for growing of algae and this makes the *Spirulina maxima* cost effective for its use.

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