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RESEARCH ARTICLE

Effect of pH, temperature and salinity on growth and biochemical parameters of *Spirogyra* sp.

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ABSTRACT : Microalgae are considered as the most promising renewable feedstock for bio-fuel production and bio-refineries, due to their advantages of fast growth, efficient carbon dioxide fixation, not competing for arable lands and potable water. *Spirogyra* sp. shows potential for the successful bio-fuel production. In the present study, effect of pH, temperature and salinity on growth and biochemical traits like biomass, lipid, carbohydrate, chlorophyll and protein content of *Spirogyra* sp. were assessed. Most of the traits were found higher in control condition. When *Spirogyra* sp. is grown at pH 7 higher yield of biomass, lipid, chlorophyll, carbohydrate and protein were seen. When isolated *Spirogyra* sp. is grown at 25°C, the higher yields of lipid and chlorophyll content were observed and there is decrease in carbohydrate and protein content. The effect of various concentrations of NaCl on the isolated algal species of *Spirogyra* sp. has showed increased biomass yield at 0.1mM NaCl concentration as compared to control. Initial increase of NaCl concentration from 0.0-0.2 mM decreased the lipid content. The present study signifies that the growth of microalgae not only depends on the temperature, light and nutrient availability, but is also highly affected by the salinity.

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Key Words :
BG-11, Biomass, Bio-fuel, Microalgae, Salinity, *Spirogyra* sp.

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Algae are polyphyletic, non-cohesive assemblages of oxygen which evolves photosynthetic organisms that include seaweeds (macroalgae) and a highly diverse group of microscopic single and multi-cellular organisms (microalgae) (Metting, 1996). Microalgae which are capable of oxidizing inorganic compounds for energy are known as chemoautotrophic or chemolithotrophic. Some species (*i.e.*, heterotrophs) depend on organic compounds for growth, while those of them that depend on light for energy are called photoheterotrophs (photoorganotrophs). Those which are known for oxidizing organic compounds for food are known as chemoheterotrophs (organoheterophs) (Lee, 2008).Microalgae culture is one among the biotechnologies that have recently been developed.

Mass culturing of microalgae on a commercial scale has been performed successfully on species such as C*hlorella*, S*pirulina*, *Scenedesmus*, *Dunaliella salina*, *Haematococcus pluvial* and *Porphyridium cruentum*. Although these micro-organisms are abundant in nature they have not yet made the subject of

scientific investigation. Another reason is that they have genetic ability to produce a wide range of compounds and chemicals that could be of great commercial value (Grossman, 2005). There are probably well over 30,000 species of microalgae, only a few hundred of which are cultured in laboratories at present. A handful of these have been characterized in detail by researchers, who have also estimated the value of their economic potential (Chaumont, 1993). Microalgae biomass is produced in specially engineered facilities, the fundamental design and infrastructure of which are dictated by the growth requirements of the microalgae of interest and the value of the final product. In general microalgae can be produced in open ponds or closed systems, and the culturing methods used can be continuous, semi- continuous or batch (Jorquera *et al.*, 2010).

Microalgae can produce 30 times more oil than any terrestrial oilseed crops for a given surface area. The main biodiesel producing microalgae are *Botryococcus braunii, Chlorella* spp., *Chlorococcum* spp. etc. The biomass of algal species mainly comprises of protein, carbohydrate, and lipids (Spolaore *et al.*, 2006).Number of media compositions for the cultivation of microalgae has been proposed. The elements required for the growth of green algae are N, P, K, Mg, Ca, S, Fe, Cu, Mn and Zn.

The use of microalgae as a fuel feedstock was first proposed over 50 years ago for the production of methane gas. Yielding of biodiesel from microalgae can be 10 to 20 times higher than those obtained from oleaginous seeds or vegetable oils. The oil content in some microalgae can be high and can be induced to produce even higher concentrations of lipids through the implementation of low nitrogen media, varying Fe3+ concentration and increased light intensity (Illman *et al.*, 2000).

Some microalgae have also a convenient fatty acids profile for transesterification and an unsaponifiable fraction which allows for the production of biodiesel with high oxidation stabilityand physical and fuel properties (e.g. density, viscosity, acid value, and heating value) which are comparable to those found in fossil diesel (Rana and Spada, 2007).

In the present study, we investigated the effect of pH, temperature and salinity on green algae *Spirogyra* sp. We have examined these various effects on algal biomass, chlorophyll, lipid, carbohydrate and protein.

EXPERIMENTAL METHODOLOGY

Collection of sample :

Algae samples were collected from fresh water bodies of college campus (SHIATS), Yamuna river and nearby ponds. It was collected with the help of sterile test tubes. Collection of mass was with a spatula and placing it in a petridish, bottle or conical flask. Culturing of algae was carried out on agar solidified (2%) BG-11 media on sterilized petriplates was grown for one week at 22^0C in culture room. With these different chemical compositions BG-11 media was prepared and also trace metal was added before mixing and it was sterilized by autoclaving at 121^0C at 15 lbs pressure for 15 mins and 2 per cent agar was added for the isolation of algae.

Isolation of algae :

The collected sample was centrifuged in 3 ml distilled water at 5000 rpm for 3 mins. Supernatant is to be removed and pellets to be used and after that pellet is purified. After autoclaving 25 ml of media was poured in sterilized petriplates and left it aside for solidification. Avoid moisture formation in the petriplates. After solidification add inoculum of about 1ml by spreading and streaking method. Now incubate the plates in a growth chamber at 25 ± 1^0C temperature and light intensity of 1.2+0.2 lux in algae culture room for 3 days, after that observe the growing colonies of microalgae.

Maintenance of algae by sub-culturing :

Before transfer of cultures all the transfer pippetes was sterilized, all the glass wares were washed thoroughly and were kept in hot air oven at 180^0C for 20-30 mins. Media was sterilized and liquid media (without agar) was freshly prepared by autoclaving. Flasks of 100 ml was used for transferring culture, fill the flask with freshly prepared media and 1 ml on inoculum from sterilized stock culture and was kept for optimum condition.

Identification of algae :

When colonies start to appear in the petriplates then with the help of sharp object or blade taken out the colony from the cultured microalgae and were centrifuged with DW at 5000 rpm for 3 mins. After centrifugation a drop of aliquot was kept on glass slide and after putting cover slip it was observed under compound microscope (40 X). The different morphological characters considered for identification *i.e.*, chlorophyll present, its cell structure,

spines present on cell, etc.

Effect of green algae with different pH on algal biomass, lipid, protein, carbohydrate and chlorophyll:

The influence of pH on algal growth was also examined. Three different pH values of 6, 7 and 8 were set up by adjusting using 1M HCl and1M NaOH. A control culture was also set up in parallel to which the pH was not adjusted. Freshly prepared media with different adjusted pH was autoclaved and were transferred into 12 sterile test tubes. In each test tubes 1 ml of algal solutions was inoculated and sample was kept up to 21 days.

Effect of temperature on algal biomass, lipid, protein, carbohydrate and chlorophyll:

To determine the effect of temperature the culture was kept at 25°C, 27°C, 30°C and 35°C. As it was found that algae best grow at 25°C so, it was taken as control.

Effect of salinity on algal biomass, lipid, protein, carbohydrate and chlorophyll:

To see the impact of NaCl, the algal species was grown in BG-11 medium modified with varying salt concentrations (0.1 mM to 0.5 mM). 1000 mM NaCl stock solution was prepared and then appropriate dosing was done. To study the effect of salinity on green microalgae the experiments were carried out in 250 ml Erlenmeyer flasks each containing 100 ml of BG-11 medium and control culture in BG-11 media was also run parallel. The medium and flasks were sterilized in a hot air oven for 20 min at 180°C in order to prevent any contamination. The samples were drawn on 15th day and were subjected to analysis for various biochemical parameters.

Dry cell algal biomass was measured as cell density (dry cell weight g/lit.) at OD625 of 11 days old culture. The dry algal biomass was calculated by using the regression equation:

$$Y = 1.014x + 0.249;\ R^2 = 0.965;$$ where, Y (g/l) is cell density and x is A_{625}.

Total lipids content were extracted by mixing methanol and chloroform (1:1v/v) with the algal samples using single method of Bligh and Dyer's method (Bligh and Dyer, 1959).

Chlorophyll content of the algae was estimated spectrophotometrically at 650nm and 665nm by method of Tandeau de Marsac and Houmard (Tandeau and Houmard, 1988). The chlorophyll content was calculated using the following formula and was expressed as µg/ml:

$$\text{Chlorophyll (µg/ml)} = 2.55 \times 10^{-2} A_{650} + 0.4 \times 10^{-2} A_{665} \times 10^{3}$$

Carbohydrate was determined by anthrone reagent method by Dubois and co-workers (Dubois *et al.*, 1956). Protein content was estimated at 660nm by the method of Lowry and co-workers (Lowry *et al.*, 1951).

EXPERIMENTAL FINDINGS AND DISCUSSION

The Effect of pH, temperature and salinity on growth of *Spirogyra* sp. was simultaneously investigated with different parameters. The following results were obtained during the study.

Isolation and identification :

The algae were isolated based on the characteristics on BG-11 agar medium. The plates were kept at 25±1°C temperature with light intensity of 1.2+0.2 k lux for 3 days. Growth started appearing just after 48 hrs but proper colonies were isolated at 72 hrs for different parameters. During the investigation the following green algae was isolates and identified on BG 11 media which was collected from Allahabad region ,was identified under electric and compound microscope. The algal samples being isolated and were identified as *Spirogyra* sp. (Fig. 1.).

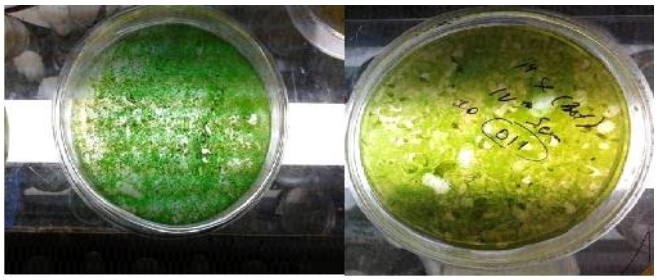

Fig. 1 : Isolated colonies of green algae in blue green-11 agar medium

Evaluation of selected algal isolate under pH conditions :

Spirogyra sp. was grown by adjusting the pH 6, 7 and 8 with 1M HCl and 1M NaOH. pH 7 has better biomass (1.386 g/lit.) and chlorophyll content found to be highest in *Spirogyra* sp. pH 7 showed highest content

in carbohydrate (0.175 mg/ml), lipid (45.666 ml/lit.) and protein (0.026) too (Table 1).

Evaluation of selected algal isolate under different temperature :

At different temperature (25°C, 30°C and 35°C) *Spirogyra* sp. was grown. Biomass (1.52 g/lit.) and chlorophyll (9.53µg/ml) was highest at incubation temperature 25°C. As the temperature was increased algal biomass decreased and chlorophyll content found to be lowest (1.28 µg/ml) at 30°C and slight increased (9.56) at 35°C. Lipid (56.66 ml/lit.), carbohydrate (0.166 mg/ml) and protein (0.026 mg/ml content was also found highest at 25°C and by the increase in temperature protein content decreased and carbohydrate was same at 30°C but decreased at 35°C (Table 2).

Evaluation of selected algal isolate under different NaCl conc. :

Spirogyra sp. was grown on BG-11 medium with varying salt concentration ranges from 0.1 to 0.5 mM stock solution of NaCl. Algal biomass yield was found highest at 0.1 mM NaCl conc.(1.55 g/lit.) as compared to control (1.52 g/lit.) and then it subsequently decreased with the increase in NaCl conc. The initial increase of NaCl conc. from 0.0-0.3 mM decreased the lipid content from 56.66 ml/lit. to 39 ml/lit. The lipid content increased gradually when the NaCl conc. is increased from 0.4-0.5 mM. Total chlorophyll contents decreased as the salt conc. is increased from 0.1-0.5 mM (9.373 µg/ml to 7.643 µg/ml) when compared to control (9.536 µg/ml). Carbohydrate content decreased as the salt conc. increased (0.166 mg/ml to 0.153 mg/ml) from 0.1-0.0.3 mM as compared to control. Decline in total protein contents were at NaCl conc. of 0.1, 0.3 and 0.5 mM and there was highest protein content on control and 0.2 mM. (Fig. 2, 3, 4, 5 and 6).

The hydrogen ion concentration (pH) of the growth medium influences many processes associated with algal growth and uptake of ions (Borowitzka and Borowitzka, 1988). The result of the present study shows that the optimum pH for the algal biomass, lipid, chlorophyll, carbohydrate and protein content of *Spirogyra* sp. is pH 7 (control culture). At this pH the biomass (1.385 g/lit.), lipid content (45.66 ml/lit.), carbohydrate (0.175 mg/ml), chlorophyll (8.95 µg/ml) and protein (0.026 mg/ml) attains its maximum value. Shifting pH to acidic side significantly decreased.

Generally, it is believed that the optimum pH differs according to different algal species. Zeinab I. Khalil and co-workers (Zeinab *et al.*, 2010) mentioned that the optimum pH for biomass of pH 7.5 for *Dunaliellabardawil* and for *Chlorella ellipsoidea* the optimum pH was pH 10. They also mentioned that the effect of pH on bothprotein and carbohydrate contents of D. bardawil revealed that pH 7.5 seems to be the most suitable pH for the accumulation of protein and carbohydrates in *Dunaliella* cells. At acidic or alkaline

Table 1: Effect of pH on algal biomass, chlorophyll, carbohydrate, lipid and protein

pH	Algal biomass (g/lit.)	Chlorophyll (µg/ml)	Carbohydrate (mg/ml)	Lipid (ml/lit.)	Protein (mg/ml)
6	0.97	7.596	0.062	26.666	0.021
7	1.386	8.953	0.175	45.666	0.026
8	1.083	7.863	0.136	37.333	0.023
C.D. (P=0.05)	S	S	S	S	S
S.D.	0.194872	0.642394	0.050741	8.875685	0.002739
S.E. ±	0.064957	0.214131	0.016914	2.958562	0.000913

S= Significant

Table 2 : Effect of temperature on algal biomass, chlorophyll, carbohydrate, lipid and protein

Temperature	Algal biomass (g/lit.)	Chlorophyll (µg/ml)	Carbohydrate (mg/ml)	Lipid (ml/lit.)	Protein (mg/ml)
25 ^{0}C	1.52	9.53	0.166	56.66	0.026
30 ^{0}C	1.28	9.08	0.166	52.66	0.016
35 ^{0}C	1.26	9.56	0.153	56.33	0.013
C.D. (P=0.05)	S	NS	S	NS	S
S.D.	0.127584	0.341069	0.005349	5.562773	0.004833
S.E. ±	0.042528	0.11369	0.001783	1.854258	0.001611

NS=Non-significant S= Significant

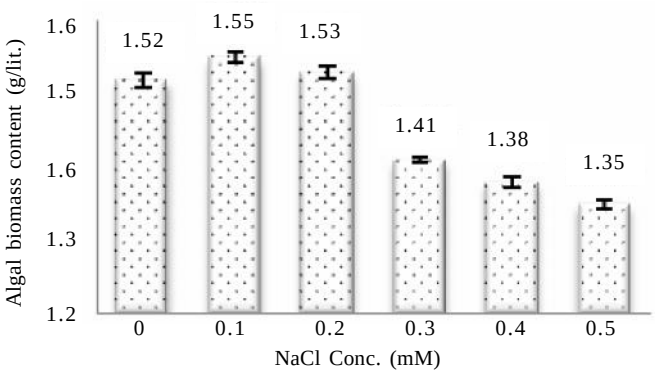

Fig. 2 : Effect of NaCl concentration on algal biomass

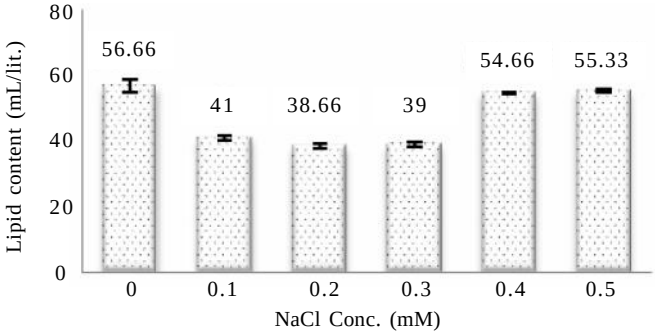

Fig. 3 : Effect of salt concentration on lipid content

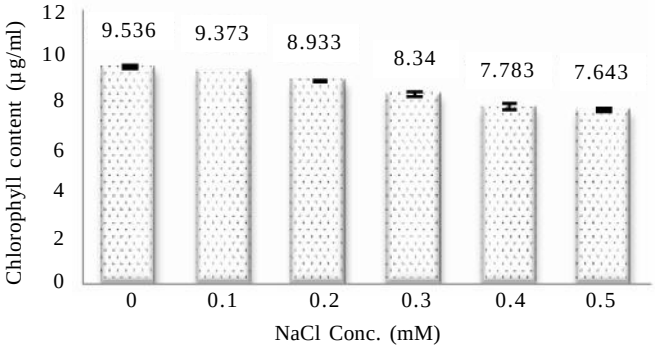

Fig. 4 : Effect of salt concentration on chlorophyll

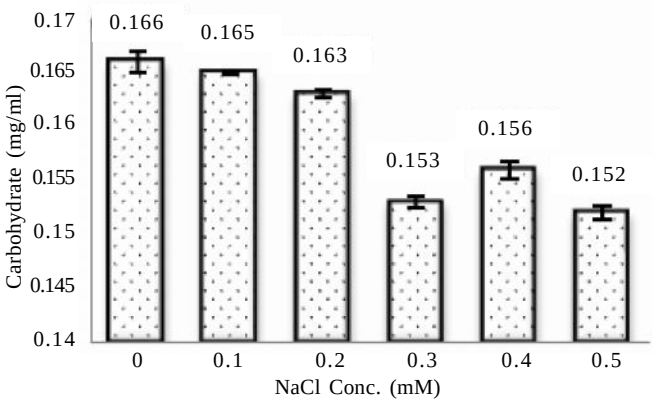

Fig. 5 : Effect of salt concentration on carbohydrate

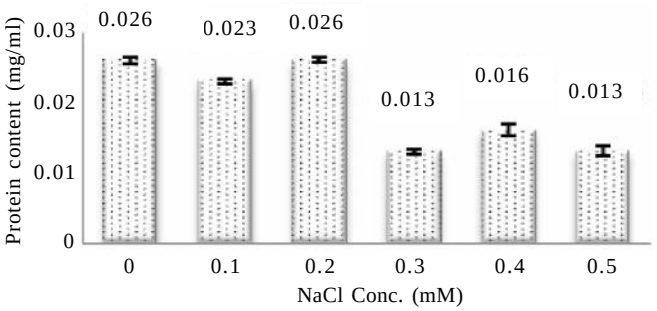

Fig. 6 : Effect of salt concentration on protein content

pH values, the contents of bothprotein and carbohydrates significantly decreased. Same author also favored the accumulation of chlorophyll, the content of the chlorophyll significantly decreased with the higher or lower pH values.

The present study of temperature on *Spirogyra* sp. for growth and biochemical traits showed that best suited temperature for algal biomass is 25°C (control culture). Carbohydrate (0.166-0.153 mg/ml) and protein (0.026-0.013 mg/ml) is decreased with the increase in temperature and chlorophyll content (9.53 µg/ml) was highest at 25°C and lowest (9.08 µg/ml) at 30°C, then sudden increase (9.56 µg/ml) was recorded at 35°C. Lipid content was best seen at 25°C (56.66 ml/lit.) and 35°C (56.33 ml/lit.). The result obtained was founded similar to Anita Kirroliaa and co-workers (Kirroliaa *et al.*, 2012) who seen the effect of incubation temperature on various physiological and biochemical traits on *Chlorococcum* sp. According to their finding Lipid content accumulated, chlorophyll content and algal biomass yield were found highest when *Chlorococcum* sp. was grown at incubation temperature of 25°C at 120 rpm for 11 days. The results indicated that lipid accumulation and chlorophyll contents were favored at 25°C.

Similarly, when these parameters were treated with various salt concentration (NaCl 0.0-0.5 mM) in *Spirogyra* sp. algal biomass (1.55-1.35 g/lit.) and chlorophyll (9.53-7.64 µg/ml) found to be decreased with the increase in salt concentration Lipid favored highest growth at 0.0 mM (56.66 ml/lit.) and decreased with increased concentration but at 0.4 mM the lipid content increased (54.66 ml/lit.) with increase in salt concentration. Carbohydrate (0.166 mg/ml) and protein (0.026 mg/ml) content were found to be highest at 0.0 mM.

The stress condition was also seen in the finding of

Takagi and his coworkers (Takagi and Yoshida, 2006). They found the similar results in *Dunaliella* cells. Total chlorophyll contents decreased as the salt concentration is increased from 0.2 to 1.0mM when compared to control for the culture studied.

Conclusion :

The present study signifies that growth of algae not only depends on light and nutrient availability, but is also affected by pH, temperature and salinity. When *Spirogyra* sp. was subjected to different pH, higher lipid (45.66 ml/lit.), chlorophyll (8.95µg/ml) and biomass (1.38 g/lit.) was observed at pH 7. At 25^0C *Spirogyra* sp. showed highest accumulation than to other temperatures. When *Spirogyra* sp. was subjected to various NaCl concentrations, increased biomass yield was obtained at 0.1 mM NaCl concentration as compared to control and then it subsequently decreases with increase in NaCl concentration. Initial increase of NaCl concentration from 0.0-0.1 mM decreased the lipid accumulation from 56.66 ml/lit. to 41 ml/lit.

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