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GROWTH ASSESSMENT OF *SPIRULINA PLATENSIS* UNDER PHYSILOGICAL STRESS

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ABSTRACT

Spirulina is a spiral, multicellular, filamentous non-heterocystus, non-nitrogen fixing photosynthetic cyanobacteria, *Arthrospira platensis*, is a whole product of biological origin. *Spirulina* requires an abundant supply of nutrients and tolerant to great variations in salinity and pH. The present study examines the possibility of increasing the growth under stress conditions including different concentration of salinity and pH on *S. platensis.* Different concentrations of NaCl *S. platensis* untreated, 20, 40, 60, 80, and 100 mM grown in for 20 days. *Spirulina platensis* cells, growing photoperiod of 12 hours light/dark provided by fluorescent lamps at a light intensity of 140 µmol photons $m^{-2}s^{-1)}$ in optimal Zarrouk's medium under controlled conditions.

It was found that biomass stimulated at lower concentration 20 mM (380mg DW/250 ml culture), 40 mM (319mg DW/250ml culture), 60mM (261mg DW/250ml culture) but reduce at 80mM (197mgDW/250ml culture) and 100mM (161mg DW/250ml culture) at higher concentration of NaCl. As pH is important for the growth of *Spirulina* for biomass, Different pH levels *viz.* untreated, *6,* 7, 8, 9, 10, the effect of pH changes on the productivity of the blue green alga, effect of pH on growth *S. platensis* would be studied for a period of 20 days. The algal dry weight (DW) was greatly enhanced at pH 9.0 (380mg DW/250 ml culture), pH 8.0 (245mg DW/250 ml culture) The decreased algal production at the low and high pH 6.0 (50mg DW/250 ml culture), pH 10.0 (125mg DW/250 ml culture), algal cells began to collapse and likely resulted in reduction or failure of many cellular pro-cesses at high

KEYWORDS: Spirulina, Growth Curve, Biomass, Salinity, pH

INTRODUCTION

Blue-green algae (Cyanobacteria) are among the most primitive life forms on Earth. Their cellular structure is a simple prokaryote. They share features with plants, as they have the ability to perform photosynthesis. Spirulina are multicellular and filamentous blue green algae that has gained considerable popularity in the health food industry and increasingly as a protein and vitamin supplement to aquacultures diets. It grows in water, can be harvested and processed easily and has very high macro and micro nutrient contents. Spirulina is used for food from time immemorial by tribes living around Chad Lake in Africa. The predominant species of phytoplankton of the lake is Spirulina platensis. The algae Spirulina was eaten in Mexico under the names 'Tecuitlatl' (Farrar, w.v...1996)**.** the commercial interest of thesis protein. However, it is a new development (Dabbah, R...1970) Spirulina can be cultivated and grown in both freshwater and seawater medium (Mary Leema et al., 2010). The commercial production of Spirulina used growth medium with sodium bicarbonate as a carbon source (Kebede, 1997) which counts for at least 60% of all nutrient costs. Sodium bicarbonate is added to replenish the amount of CO2 depleted from the medium due to the algal growth. Adaptation to salinity and temperature are vital to the cultivation of Spirulina. Natural seawater has a highly variable chemical composition

(Gagneux- Moreaux et al., 2007), containing more than 50 known elements and a large number of organic compounds. Economic viability of Spirulina depends on the quantities of biomass produced. Large volumes of media cultures are required in order to operate and maintain commercial farm scale which is done usually using outdoor pond systems (Richmond, 1999; Tredici, 2004).

Salinity issues can either provide a better opportunity or be a risk for some countries. From most microalgae, Spirulina has shown good tolerability through wide range of salinity and one of the ways to maintain good quality of Spirulina culture is by regulating salinity concentration. A. *platensis* (*Spirulina)* was confirmed having high endurance to different salinity level but salt has also been documented to be main growth inhibiting substance and is responsible for inhibiting the electron transport activities of PSI and PSII (Boyer, 1982; Zeng & Vonshak, 1998; Pitman and Läuchli, 2002; Zhang et al., 2010). Other freshwater invasive micro algal species are incapable to grow under saline conditions. It is stated that by manipulating environmental parameters like salinity, the growth of competitors can be limited while improving the specific growth rate of the domain culture species (Roessler, 1990; Rocha *et al*., 2003; Bartley *et al.*, 2013). Spirulina required high nutrient inputs and salt concentrations compared to Scenedesmus and Chlorella. This might be the reason for Spirulina was naturally grown in salt lakes exclusively (Ogawa *et al* 1970). (Tasneem Fatma, 1990) reported that synchronous growth of Spirulina platensis was failed to grow both in liquid and solid media at its higher dilution. It was observed that minimum cell population is necessary to initiate and sustain Spirulina cultures. The culture filtrate had an absorbance of 0.96 on ninth day increased 50 per cent growth against control (0.63 absorbance). Spirulina grows optimally in pH range of 9-11 and there is least chance of contamination of other microbes (Supramaniyan *et al* 1992)

MATERIAL AND METHODS

Microorganisms

Slants of *S. platensis* maintained in Zarrouk's agar media at 4^{o}C, were collected from Department of Biotechnology, SOS, Jiwaji University, Gwalior (MP), India.

Inoculum Preparation

Microalgae were cultivated in 4 L Erlenmeyer flasks, with photoperiod of 12 hours light/dark provided by fluorescent lamps at a light intensity of 140 µmol photons.m^{-2}.s^{-1} and temperature of 30 ± 1ºC. Experiments were initiated with 10% (v/v) of inoculum.

Growth Evaluation

Measurement of optical density (O.D.) is particularly suitable for determination of growth of *S. platensis*. The basic advantage of turbidity technique in growth rate measurements is the possibility of taking repeated readings on increase in turbidity of the same batch of the suspension of cells. Growth of *Spirulina* under normal and stress conditions would be assessed spectrophotometrically by measurement of absorbance at 560 nm according to the method of Fatma *et al*., 1994.

Dry Weight Measurement

Algal samples from the different salt concentrations and culture of different pH were filtered under vacuum through 0.45 μm filter membrane and washed several times with distilled water. Then, algal cells were dried at 100°C for

30 min and weighed (Abd El-Baky et al., 2003)..

RESULTS AND DISCUSSIONS

Effect on Growth of *S. Platensis* at Different Concentration of Nacl

Showing in figure 1 and 2 *Spirulina* Salt stress conditions affected algal growth, it was found that biomass stimulated at lower concentration 20 mM (380mg DW/250 ml culture),40 mM (319mg DW/250ml culture),60mM (261mg DW/250ml culture) but reduce at 80mM (197mgDW/250ml culture) and 100mM (161mg DW/250ml culture) at higher concentration as compared to untreated culture..

Effect on Growth of *S. Platensis* at Different pH

Showing in figure 3 and 4 *S. platensis* was grown at different pH (6, 7, 8, 9, 10) in flask culture and monitored and expressed in term of dry weight. The maximum bulk density about 380 mg DW culture was noticed when the pH of culture medium was maintained at 9.0 with medium volume 250ml in a The maximum bulk density was attained on 20th day after the inoculation of culture in medium as compared to untreated culture. The increase in the production of *S.platensis* could have been due to the availability of mire space, oxygen and light to the culture flask.

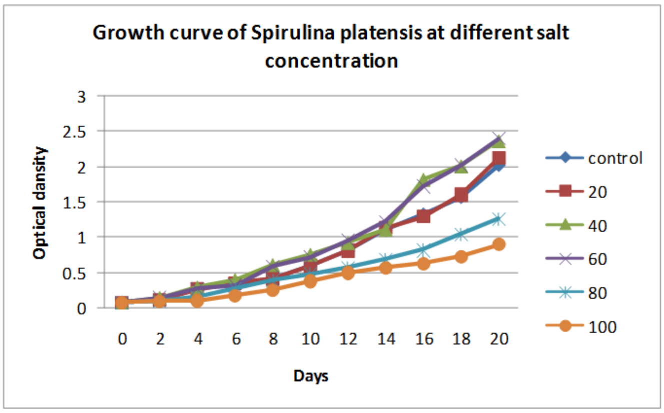

Figure 1: Growth Curve of S. Platensis Cultivated Under Different Salt Concentration (Untreated, 20, 40, 60, 80 and 100Mm Salt Concentration) During 20 Days Incubation Period

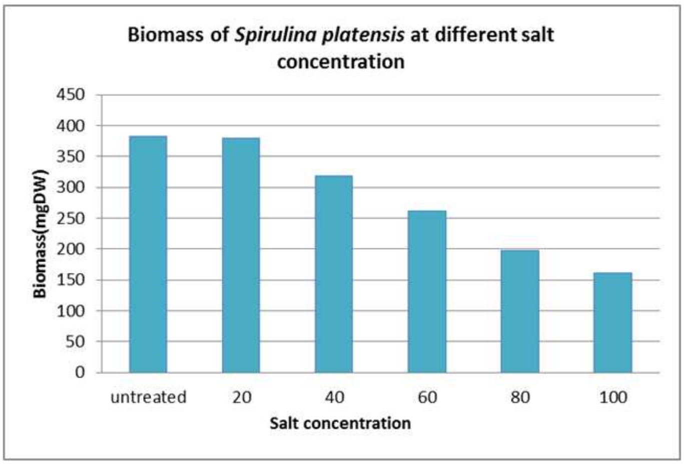

Figure 2: Represented as Dry Weight (mgDW/250ml Culture)

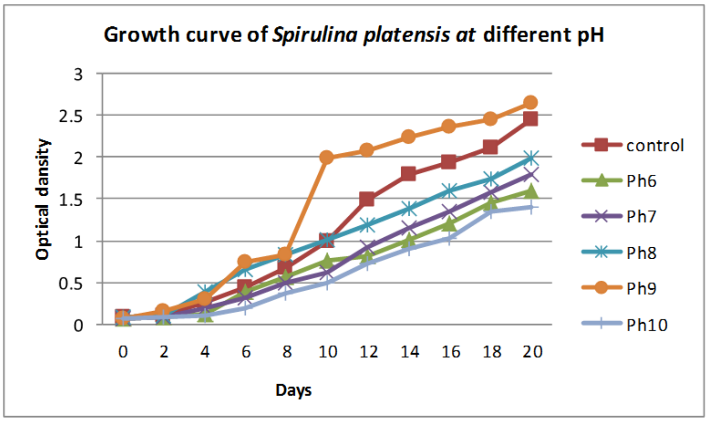

Figure 3: Growth Curve of S. Platensis Cultivated Under Different Ph. (Untreated, Ph6, Ph7, Ph8, Ph9, Ph10) During 20 Days Incubation Period)

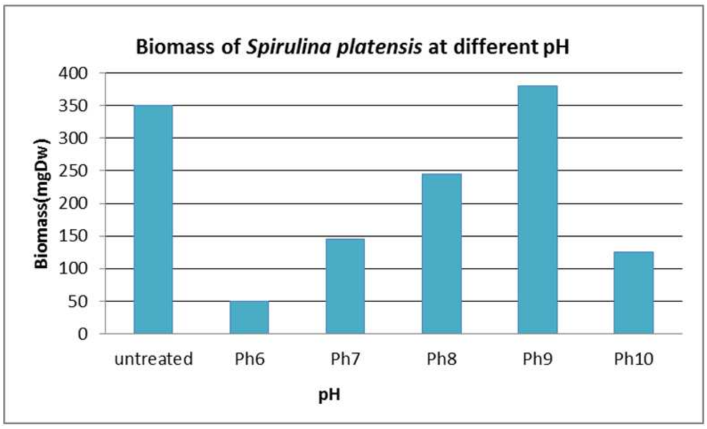

Figure 4: Represented as Dry Weight (mgDW/250ml Culture)

DISSCUSSIONS

Spirulina is an economically important filamentous cyanobacterium. The annual production of the algae is about 10,000 tons which makes it the largest microalgal cultivation industry in the world (Zhang, X., et al 2005). *Spirulina platensis* is naturally found in tropical regions inhabiting alkaline lakes (pH 11) with high concentration of NaCl and bicarbonates. These limiting conditions for other microorganism allow cultivation of microalgae in opened reactors (Harriet *et al.*, 2008), The effect of various concentrations of NaCl on *S. platensis* showed reduced biomass. (Sujatha *et al* 2014). pH is one of the environmental factors which affect the physiological growth, metabolic activities and biomass production of S. platensis. The results demonstrate that S. platensis can adapt to variable pH conditions as suggested earlier (Ogbonda *et al.*, 2007, Celekli *et al*., 2009). pH content on the biomass in *S. platensis* to optimize and improvement for the best culture condition and biomass greatly enhance at pH 9.0(Gaurav *et al*.,2014, Mostafa *et al*.,2016). Found that, the influence of pH for Spirulina platensis growth, protein and Chlorophyll a content were examined and the dry weight of Spirulina platensis was 0.91g/500ml and protein and Chlorophyll a content were 64.3% and 13.2mg/gm respectively at pH 9 (Pandey *et al.*,2010). Biomass concentration in the culture media was calculated as cell dry weight. The combination of 30°C and pH 9.0 gave the highest values. The effect of pH was modulated by temperature and vice versa during biomass production.

This native isolate of *Spirulina sp*. Act as a good source of natural protein that could be easily accepted by rural communities a Salinity has been suggested as a controlling factor for blooms of cyanobacteria in estuaries and is considered as one of the major constraints on species diversity and productivity of natural population of both fresh and marine algae. In a view of environmental fluctuations in which natural population of *Spirulina* occur and the commercial importance of the species, there is a need to study its growth response to variations in salinity and pH under laboratory conditions single cell protein in the form of feed, food and health supplement when properly processed.

CONCLUSIONS

Spirulina platensis showed beneficial properties indicated that, adaptation of the alga to salinity and different pH was characterized by the growth. A high NaCl concentrations resulted in an immediate cessation of growth and a decrease in biomass. In this study, the effects of a wide range of pH were evaluated on the growth of Spirulina platensis.

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