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EFFECT OF pH ON GROWTH AND BIOPIGMENT ACCUMULATION OF GREEN ALGA DUNALIELLA SALINA

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ABSTRACT: A study was conducted to investigate the effect of different pH, on the growth and biopigment accumulation of a green alga *Dunaliella salina* isolates from Sambhar salt lake, Rajasthan (India). In order to monitor the impact of pH an algal samples of *D. salina* was exposed to different pH regimes (6 -10 pH) along with control (8.5 pH) over a period of 30 days. Set of experiments was conducted to study and evaluate the optimum growth and biopigment content for *D.salina*. The results of this experiment showed that optimum growth of *D.salina* was found at pH 8. Total chlorophyll content of the algal species decreases as the pH value was increased. While total carotenoids content of the algal species increases as the increases pH values and it was found that the green alga species changed its appearance from green to deep orange. The results indicate that algal species showed diverse response to pH stress.

INTRODUCTION: Algae are one of the earth's most important natural resources that make up the lower phylogenetic echelons of the plant kingdom. Around 70% of earth's surface is covered with water and the phytoplankton especially algae believed to contribute around 85-90% in photosynthesis. They are able to survive in diverse habitats and produce vast array of natural products including proteins, enzymes, bioactive compounds and carotenoids. Humans use algae as food, for production of useful compounds, as biofilters to remove nutrients and other pollutants from wastewaters, to improve water quality, as indicators of environmental change, in space technology, and as laboratory research system.

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An alga is commercially cultivated for Pharmaceuticals, Cosmetics and Aquaculture purpose.

In the present era of biotechnology, scientists are investigating microalgae as a solution for the problems that world is facing today. Microalgae are very diverse [1]. They can create a range of useful products, and there is a variety of ways in which they can be cultivated, manipulated, harvested and utilized [2]. *Spirulina* is now being commercial produced as a food supplement which is very rich in protein content. Many microalgae are being investigated for biofuel production.

In particular, some species have been identified as promising producers of useful lipids for biofuel production. *Dunaliella salina* is one such species, which shows lipid accumulation. *D.salina* is a unicellular species with no cell wall [3]. *Dunaliella* have some advantages such as disruption of cells is much easier than that in other algae because of its cell wall less nature, continuous culture in

laboratory is easy and the growth rate is relatively high and resistance to various environmental conditions is higher than in other algae.

Dunaliella is recognized as the only eukaryotic and photosynthetic organism, which grows in wide range of salt concentration, ranging from 0.05M to saturation 5.0 M [4]. This biflagellate unicellular alga is responsible for most of the primary production in hyper-saline environment worldwide [5]. *Dunaliella* is unique in the absence of a rigid polysaccharide cell wall, and the cells are enclosed only within a thin plasma membrane, which are able to change rapidly their volume and shape in response to hypo or hyper osmotic changes [6]. It does this by maintaining a steadily low intracellular ion concentration[7] and by forming compatible solutes such as glycerol that maintain the structure and volume of the cell [8]. This ability to adapt, and its high metabolic and physiological versatility, has led to its identification as a high potential for large-scale cultivation of beta carotene [9].

A number of studies have revealed that growth[10] and pigment compositions [11] of this alga are affected by various stress conditions. It was found that the β-carotene to chlorophyll a ratio gradually increased with an increase in salinity, and as a result, the algae changed its appearance from green to deep orange [12]. The halotolerant unicellular green algae *Dunaliella salina* have potential to accumulate carotenoids to as much as 13% of the dry weight when grown under growth limiting conditions[13].

pH is the important factor for algal growth as it can affect the activity of different enzymes. In general, different algal species have diverse ranges of tolerance to pH. Variation in pH can affect algal growth in a number of ways. It can change the distribution of carbon- dioxide species and carbon availability, alter the availability of trace metals and essential nutrients, and at extreme pH levels potentially cause direct physiological effects. Although *Dunaliella* species has a wide range of pH tolerance but for its optimum growth and biopigment content pH 8 was found optimum [14]. It was reported that at higher pH, there is a risk of precipitation by calcium salts and flocculation of algal biomass [15]. This can lead to reduction in algal growth and it is necessary to avoid an increase of pH above 9 in *Dunaliella* cultures. The objective of this research study was to develop a procedure to optimum growth and biopigment content locally isolated *D. salina*, as a step towards to indicate enhancing carotenoids induction, and then for β-carotene extraction. This objective was achieved by evaluating the optimum growth and biopigment content of locally isolated *D. salina* under different pH regimes.

The present study was an attempt to observed the effect of different pH regimes on the growth, biomass and biopigment composition of *D. salina*. In this study cells of *D.salina* were transferred from 6pH to 10 pH along with the control (8.5pH) in the ASWM [16] (Artificial Sea Water Medium).

MATERIALS AND METHODS:

Microalgae Source: *Dunaliella salina* was isolated from the Sambhar salt lake, Rajasthan, which is located about 35 km. from Jaipur, (Rajasthan).

Experimental Set Up: To evaluate various pH values for the growth and biopigments of *D.salina*, five different pH values *viz*. pH 6, pH 7, pH 8, pH 9, pH 10 along the control (8.5pH) were experimented upon.

The inorganic media for the above experiment set up remain the same and it was Artificial Sea Water Media (ASWM).The inorganic media was sterilized in autoclave at 121 °C for 20 min before inoculation. Conical flasks of 500 ml capacity were prepared containing 250 ml media and 50 ml sample culture (inoculum). The cultures were incubated at 25 °C in a thermo-statically controlled room, with a 12:12 h light: dark period and at a light intensity of 2,500 lux [17 - 18]. Observations were carried out every week over a period of five weeks. Cultures were shaken manually thrice a day to avoid clumping and accelerate the growth process. Experiment for pH regimes was carried out in triplicates.

Growth Measurement: Growth was followed through optical density, dry weight, and growth rate. Biomass was determined by optical density of cultures at 670 nm using Shimadzu UV/VIS spectrophotometer. The dry weight against standard absorbance unit was followed throughout the experiment period. 50-100 ml sample of culture were filtered on whatman GF/C filters, rinsed with

distilled water and weighed after drying for 24h at 80 °C.

Growth rate was calculated on dry weight basis according to the equation given below.

$$\mu(\text{divisions/day}) = (3.322(\log DW2 - \log DW1)/(t2-t1)$$

where t =time and DW=dry weight. Subscripts denote values at different times [19].

Biopigments Analysis: The Chlorophyll contents of samples were estimated by Parsons and Strickland method [20] and carotenoids by Jensen method [21].

Statistical Analysis: Effect of different salt concentration on growth and biopigment of *D.salina* were compared by one way analysis of variance (ANOVA) [22]. Statistical analysis of data was carried out using MS Office Excel analysis Tool Pak. The significance between pairs of variable means was analysed using least significant difference (LSD) test at 5% level of significance.

RESULTS AND DISCUSSION: The results yielded from this research show that a great variability in different pH regime of *D.salina*.

Effect on Growth: Growth analysis of *D.salina* in various pH values shows different growth patterns [23]. As compare to the other pH values, the best growth of *D.salina* was calculated at 8 pH.

At pH 8, the optical density increased exponentially up to the end of the experiment. It was increased 3.8 times more than the initial value and the dry weight also supported optical density, it increased expontially 4.66 times than initial values. Highest growth rate (*i.e.*0.091divisions/day) was recorded at 8 pH (**Fig. 1: a, b**).

8.5 pH (control) was the next to 8 pH, in terms of growth of the alga. The optical density and dry weight recorded of algal sample were increases, optical density was about 3.3 times and dry weight found 4.81 times than their initial values and growth rate of culture at this pH was just next to 8 pH (0.083divisions/day).

7 pH was the next to 8.5 pH (control), in terms of growth of *D.salina*. The optical density and dry weight calculated of algal sample were increases, O.D. was about 3.0 times and dry weight calculated 4.71times than their initial value and growth rate of culture at this pH was just next to 8.5 pH (*i.e.* 0.073divisions/day). The pH value of 6 was next to 7 pH in supporting and maintaining the growth of the alga. At this pH, optical density increased 2.7 times and dry weight 4.65 times than the initial values and growth rate of cultures at this pH was observed (*i.e.*0.066divisions/day). 9pH was the next to 6 pH, in terms of growth of the alga. The optical density was found increased 2.4 times and dry weight 4.12 times than the initial values and growth rate of alga at this pH was calculated (0.030divisions/day).

In comparison to all the pH regimes tested, pH 10 was found least effective in promoting the growth of *D. salina*. The optical density and dry weight results were calculated to increases 1.6 times and 3.46 times respectively than the initial values, at the end of experiment. The least growth rate of alga (*i.e.* 0.021 divisions/day) was recorded at this pH value. (**Graph 1: A, B**)

Effect on Biopigment Content: The Biopigment composition of algae correlates with the growth of *D.salina*. The maximum biopigment (Chlorophyll) content were found at 8 pH value. Total chlorophyll content was recorded in culture grown at 8 pH (5.162 µg/ml) followed by control culture at 8.5 pH (4.782µg/ml), 7 pH (4.023µg/ml), 6 pH (2.514µg/ml), 9 pH (1.406µg/ml) and least chlorophyll content was recorded at 10 pH regime (*i.e.* 0.694µg/ml). While total Carotenoids content was observed highest value at 9 pH (5.206µg/ml), followed by 8.5 pH (4.229µg/ml), 8 pH (3.611 µg/ml), 7 pH (2.821µg/ml), 6 pH (2.348 µg/ml) and least carotenoids was found at pH 10 regime (1.475 µg/ml). **Table 1** and **Graph C**.

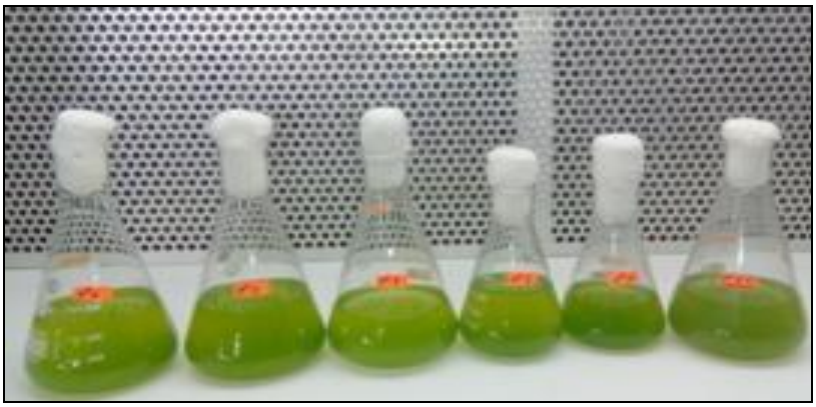
The results demonstrate that at higher stress condition, photosynthesis activity adversely affected, hence the chlorophyll contain was decreased with increase in pH and carotenoids are secondary metabolite, produced by the cell in stress condition as cell protecting process [24]. When the pH values is increased it acts as stress that enhances carotenoids production. It was found that the carotenoids to chlorophyll ratio gradually increased with increases in pH regimes and as a result the alga changed its appearance from green

to deep orange. However cells of *D.salina* adapt to the higher stress conditions due to the presence of glycerol as it has an ability to balance the extracellular osmotic stress [25].

TABLE 1: EFFECT OF VARIOUS pH REGIMES ON BIOPIGMENT COMPOSITION OF *D. SALINA*

pH regimes	Total Chlorophyll (µg/ml)	Total Carotenoids (µg/ml)	Alga color
6 pH	2.514	2.348	Green
7 pH	4.023	2.821	Green
8 pH	5.162	3.611	Green
8.5 pH (Control)	4.782	4.229	Orange
9 pH	1.406	5.206	Orange
10 pH	0.694	1.475	Orange red

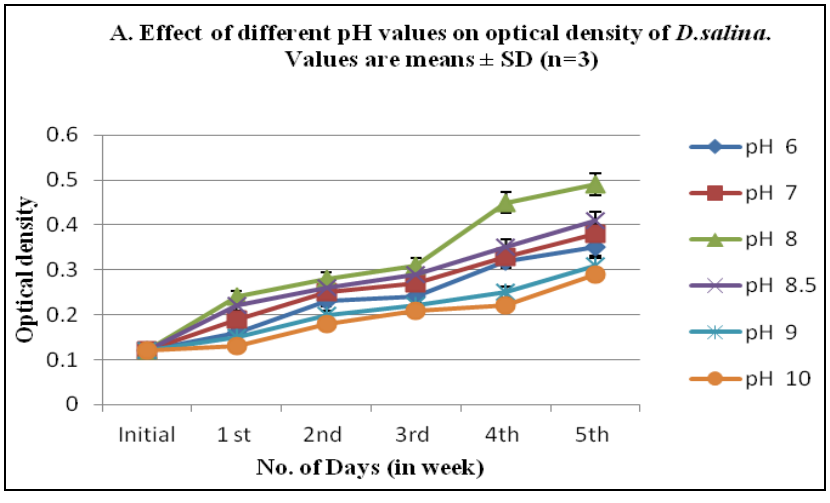
Values are means ± SD(n=3)

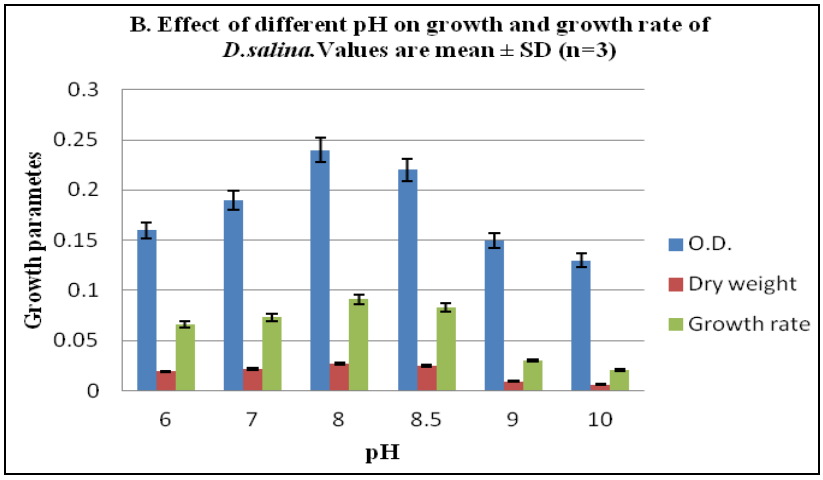


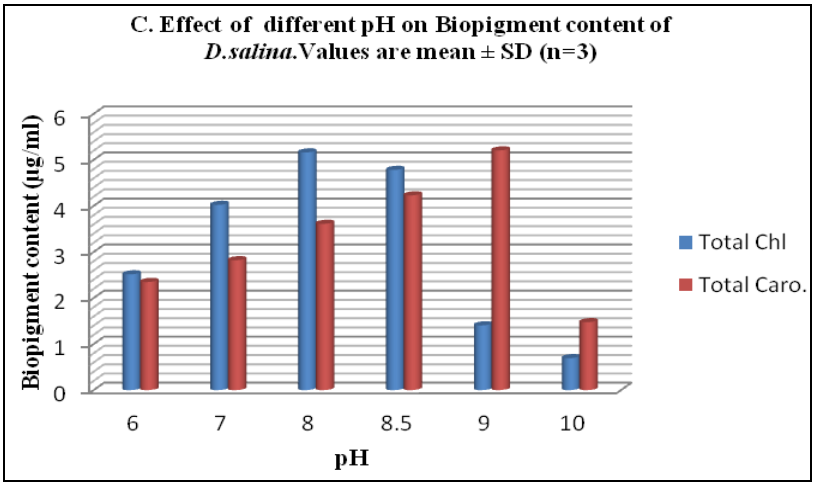
(a) *D.SALINA* GROWN IN DIFFERENT pH REGIMES (6 TO 10) INITIAL GREEN COLOUR OF ALGA

(b) *D.SALINA* SHOWING SLIGHT ORANGE COLOUR CHANGES AT IVTH WEEK OF EXP

FIG. 1: a, b *D.SALINA* GROWN DIFFERENT PH REGIME SHOWING VARIOUS RESPONSE







GRAPH A, B, C: EFFECT OF DIFFERENT pH REGIMES ON THE GROWTH, GROWTH RATE AND BIOPIGMENT CONTENT OF *D. SALINA*

CONCLUSION: On the basis of our findings we concluded that the maximum biomass production with high biopigment content (Chlorophyll) in *Dunaliella salina* were observed in cultures grown at pH 8. While highest Carotenoids accumulation calculated at pH 9. *D. salina* has a wide range of pH tolerance from pH 6 to pH 11 with different growth rates. Growth and Chlorophyll content of the alga supported low pH values and total chlorophyll content of the algal species decreases as the pH value was increased. While Carotenoids accumulation in alga found at higher pH and total carotenoids content of the algal species increases as the increases pH values and it was found that the green alga species changed its appearance from green to deep orange. The alga *Dunaliella* includes many important scientific aspects and application on the biotechnology (β-carotene and glycerol production), physiology (osmoregulation), bioenergy (bioreactor and biofuel production) and pharmaceutical industries. The Sambhar Salt Lake is a natural habitat for *D.salina* and therefore it has a very promising potential as the site of biofuel and β-carotene production in the state of Rajasthan (India). The high salinity, hot climate, abundant sun light and the high temperature present at the lake provide nearly the ideal set of condition for biofuel production from *Dunaliella salina*.

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CONFLICT OF INTEREST: The authors declare that they are no conflict of interest regarding this manuscript.

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